WORLD HEALTH ORGANIZATION
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

VOLUME 71
RE-EVALUATION OF SOME ORGANIC CHEMICALS, HYDRAZINE AND HYDROGEN PEROXIDE

1999
I A R C
L Y O N
FRANCE

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IARC MONOGRAPHS

ON THE

EVALUATION OF CARCINOGENIC RISKS TO HUMANS

Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide

VOLUME 71

This publication represents the views and expert opinions
of an IARC Working Group on the
Evaluation of Carcinogenic Risks to Humans,
which met in Lyon,
17–24 February 1998

1999

IARC MONOGRAPHS

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic risk of chemicals to humans involving the production of critically evaluated monographs on individual chemicals. The programme was subsequently expanded to include evaluations of carcinogenic risks associated with exposures to complex mixtures, life-style factors and biological agents, as well as those in specific occupations.

The objective of the programme is to elaborate and publish in the form of monographs critical reviews of data on carcinogenicity for agents to which humans are known to be exposed and on specific exposure situations; to evaluate these data in terms of human risk with the help of international working groups of experts in chemical carcinogenesis and related fields; and to indicate where additional research efforts are needed.

The lists of IARC evaluations are regularly updated and are available on Internet: http://www.iarc.fr/.

This project has been supported by Cooperative Agreement 5 UO1 CA33193 awarded by the United States National Cancer Institute, Department of Health and Human Services. Additional support has been provided since 1986 by the European Commission, since 1993 by the United States National Institute of Environmental Health Sciences and since 1995 by the United States Environmental Protection Agency through Cooperative Agreement Assistance CR 824264.

IARC Library Cataloguing in Publication Data

Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide /
IARC Working Group on the Evaluation of Carcinogenic Risks to Humans
(1999 : Lyon, France).

(IARC monographs on the evaluation of carcinogenic risks to humans ;
71 part 1, part 2 and part 3)

1. Carcinogens – congresses 2. Occupational Exposure – congresses
I. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans
II. Series

ISBN 92 832 1271 1 (NLM Classification: W1)
ISSN 1017-1606

Distributed by IARC*Press* (Fax: +33 4 72 73 83 02; E-mail: press@iarc.fr)
and by the World Health Organization Distribution and Sales, CH-1211 Geneva 27
(Fax: +41 22 791 4857)

PRINTED IN FRANCE

CONTENTS

Part Three B—Few new data

NOTE TO THE READER

The term ‘carcinogenic risk’ in the *IARC Monographs* series is taken to mean the probability that exposure to an agent will lead to cancer in humans.

Inclusion of an agent in the *Monographs* does not imply that it is a carcinogen, only that the published data have been examined. Equally, the fact that an agent has not yet been evaluated in a monograph does not mean that it is not carcinogenic.

The evaluations of carcinogenic risk are made by international working groups of independent scientists and are qualitative in nature. No recommendation is given for regulation or legislation.

Anyone who is aware of published data that may alter the evaluation of the carcinogenic risk of an agent to humans is encouraged to make this information available to the Unit of Carcinogen Identification and Evaluation, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France, in order that the agent may be considered for re-evaluation by a future Working Group.

Although every effort is made to prepare the monographs as accurately as possible, mistakes may occur. Readers are requested to communicate any errors to the Unit of Carcinogen Identification and Evaluation, so that corrections can be reported in future volumes.

IARC WORKING GROUP ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS: RE-EVALUATION OF SOME ORGANIC CHEMICALS, HYDRAZINE AND HYDROGEN PEROXIDE

Lyon, 17–24 February 1998

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PREAMBLE

IARC MONOGRAPHS PROGRAMME ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

PREAMBLE

1. BACKGROUND

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme to evaluate the carcinogenic risk of chemicals to humans and to produce monographs on individual chemicals. The *Monographs* programme has since been expanded to include consideration of exposures to complex mixtures of chemicals (which occur, for example, in some occupations and as a result of human habits) and of exposures to other agents, such as radiation and viruses. With Supplement 6 (IARC, 1987a), the title of the series was modified from *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans* to *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, in order to reflect the widened scope of the programme.

The criteria established in 1971 to evaluate carcinogenic risk to humans were adopted by the working groups whose deliberations resulted in the first 16 volumes of the *IARC Monographs series*. Those criteria were subsequently updated by further ad-hoc working groups (IARC, 1977, 1978, 1979, 1982, 1983, 1987b, 1988, 1991a; Vainio *et al.*, 1992).

2. OBJECTIVE AND SCOPE

The objective of the programme is to prepare, with the help of international working groups of experts, and to publish in the form of monographs, critical reviews and evaluations of evidence on the carcinogenicity of a wide range of human exposures. The *Monographs* may also indicate where additional research efforts are needed.

The *Monographs* represent the first step in carcinogenic risk assessment, which involves examination of all relevant information in order to assess the strength of the available evidence that certain exposures could alter the incidence of cancer in humans. The second step is quantitative risk estimation. Detailed, quantitative evaluations of epidemiological data may be made in the *Monographs*, but without extrapolation beyond the range of the data available. Quantitative extrapolation from experimental data to the human situation is not undertaken.

The term ‘carcinogen’ is used in these monographs to denote an exposure that is capable of increasing the incidence of malignant neoplasms; the induction of benign neoplasms may in some circumstances (see p. 19) contribute to the judgement that the exposure is carcinogenic. The terms ‘neoplasm’ and ‘tumour’ are used interchangeably.

Some epidemiological and experimental studies indicate that different agents may act at different stages in the carcinogenic process, and several mechanisms may be involved. The aim of the *Monographs* has been, from their inception, to evaluate evidence of carcinogenicity at any stage in the carcinogenesis process, independently of the underlying mechanisms. Information on mechanisms may, however, be used in making the overall evaluation (IARC, 1991a; Vainio *et al.*, 1992; see also pp. 26–27).

The *Monographs* may assist national and international authorities in making risk assessments and in formulating decisions concerning any necessary preventive measures. The evaluations of IARC working groups are scientific, qualitative judgements about the evidence for or against carcinogenicity provided by the available data. These evaluations represent only one part of the body of information on which regulatory measures may be based. Other components of regulatory decisions vary from one situation to another and from country to country, responding to different socioeconomic and national priorities. **Therefore, no recommendation is given with regard to regulation or legislation, which are the responsibility of individual governments and/or other international organizations.**

The *IARC Monographs* are recognized as an authoritative source of information on the carcinogenicity of a wide range of human exposures. A survey of users in 1988 indicated that the *Monographs* are consulted by various agencies in 57 countries. About 4000 copies of each volume are printed, for distribution to governments, regulatory bodies and interested scientists. The Monographs are also available from IARC*Press* in Lyon and via the Distribution and Sales Service of the World Health Organization in Geneva.

3. SELECTION OF TOPICS FOR MONOGRAPHS

Topics are selected on the basis of two main criteria: (a) there is evidence of human exposure, and (b) there is some evidence or suspicion of carcinogenicity. The term ‘agent’ is used to include individual chemical compounds, groups of related chemical compounds, physical agents (such as radiation) and biological factors (such as viruses). Exposures to mixtures of agents may occur in occupational exposures and as a result of personal and cultural habits (like smoking and dietary practices). Chemical analogues and compounds with biological or physical characteristics similar to those of suspected carcinogens may also be considered, even in the absence of data on a possible carcinogenic effect in humans or experimental animals.

The scientific literature is surveyed for published data relevant to an assessment of carcinogenicity. The IARC information bulletins on agents being tested for carcinogenicity (IARC, 1973–1996) and directories of on-going research in cancer epidemiology (IARC, 1976–1996) often indicate exposures that may be scheduled for future meetings. Ad-hoc working groups convened by IARC in 1984, 1989, 1991, 1993 and 1998 gave recommendations as to which agents should be evaluated in the IARC Monographs series (IARC, 1984, 1989, 1991b, 1993, 1998a,b).

As significant new data on subjects on which monographs have already been prepared become available, re-evaluations are made at subsequent meetings, and revised monographs are published.

4. DATA FOR MONOGRAPHS

The *Monographs* do not necessarily cite all the literature concerning the subject of an evaluation. Only those data considered by the Working Group to be relevant to making the evaluation are included.

With regard to biological and epidemiological data, only reports that have been published or accepted for publication in the openly available scientific literature are reviewed by the working groups. In certain instances, government agency reports that have undergone peer review and are widely available are considered. Exceptions may be made on an ad-hoc basis to include unpublished reports that are in their final form and publicly available, if their inclusion is considered pertinent to making a final evaluation (see pp. 26–27). In the sections on chemical and physical properties, on analysis, on production and use and on occurrence, unpublished sources of information may be used.

5. THE WORKING GROUP

Reviews and evaluations are formulated by a working group of experts. The tasks of the group are: (i) to ascertain that all appropriate data have been collected; (ii) to select the data relevant for the evaluation on the basis of scientific merit; (iii) to prepare accurate summaries of the data to enable the reader to follow the reasoning of the Working Group; (iv) to evaluate the results of epidemiological and experimental studies on cancer; (v) to evaluate data relevant to the understanding of mechanism of action; and (vi) to make an overall evaluation of the carcinogenicity of the exposure to humans.

Working Group participants who contributed to the considerations and evaluations within a particular volume are listed, with their addresses, at the beginning of each publication. Each participant who is a member of a working group serves as an individual scientist and not as a representative of any organization, government or industry. In addition, nominees of national and international agencies and industrial associations may be invited as observers.

6. WORKING PROCEDURES

Approximately one year in advance of a meeting of a working group, the topics of the monographs are announced and participants are selected by IARC staff in consultation with other experts. Subsequently, relevant biological and epidemiological data are collected by the Carcinogen Identification and Evaluation Unit of IARC from recognized sources of information on carcinogenesis, including data storage and retrieval systems such as MEDLINE and TOXLINE.

For chemicals and some complex mixtures, the major collection of data and the preparation of first drafts of the sections on chemical and physical properties, on analysis,

on production and use and on occurrence are carried out under a separate contract funded by the United States National Cancer Institute. Representatives from industrial associations may assist in the preparation of sections on production and use. Information on production and trade is obtained from governmental and trade publications and, in some cases, by direct contact with industries. Separate production data on some agents may not be available because their publication could disclose confidential information. Information on uses may be obtained from published sources but is often complemented by direct contact with manufacturers. Efforts are made to supplement this information with data from other national and international sources.

Six months before the meeting, the material obtained is sent to meeting participants, or is used by IARC staff, to prepare sections for the first drafts of monographs. The first drafts are compiled by IARC staff and sent before the meeting to all participants of the Working Group for review.

The Working Group meets in Lyon for seven to eight days to discuss and finalize the texts of the monographs and to formulate the evaluations. After the meeting, the master copy of each monograph is verified by consulting the original literature, edited and prepared for publication. The aim is to publish monographs within six months of the Working Group meeting.

The available studies are summarized by the Working Group, with particular regard to the qualitative aspects discussed below. In general, numerical findings are indicated as they appear in the original report; units are converted when necessary for easier comparison. The Working Group may conduct additional analyses of the published data and use them in their assessment of the evidence; the results of such supplementary analyses are given in square brackets. When an important aspect of a study, directly impinging on its interpretation, should be brought to the attention of the reader, a comment is given in square brackets.

7. EXPOSURE DATA

Sections that indicate the extent of past and present human exposure, the sources of exposure, the people most likely to be exposed and the factors that contribute to the exposure are included at the beginning of each monograph.

Most monographs on individual chemicals, groups of chemicals or complex mixtures include sections on chemical and physical data, on analysis, on production and use and on occurrence. In monographs on, for example, physical agents, occupational exposures and cultural habits, other sections may be included, such as: historical perspectives, description of an industry or habit, chemistry of the complex mixture or taxonomy. Monographs on biological agents have sections on structure and biology, methods of detection, epidemiology of infection and clinical disease other than cancer.

For chemical exposures, the Chemical Abstracts Services Registry Number, the latest Chemical Abstracts Primary Name and the IUPAC Systematic Name are recorded; other synonyms are given, but the list is not necessarily comprehensive. For biological agents,

taxonomy and structure are described, and the degree of variability is given, when applicable.

Information on chemical and physical properties and, in particular, data relevant to identification, occurrence and biological activity are included. For biological agents, mode of replication, life cycle, target cells, persistence and latency and host response are given. A description of technical products of chemicals includes trade names, relevant specifications and available information on composition and impurities. Some of the trade names given may be those of mixtures in which the agent being evaluated is only one of the ingredients.

The purpose of the section on analysis or detection is to give the reader an overview of current methods, with emphasis on those widely used for regulatory purposes. Methods for monitoring human exposure are also given, when available. No critical evaluation or recommendation of any of the methods is meant or implied. The IARC published a series of volumes, *Environmental Carcinogens: Methods of Analysis and Exposure Measurement* (IARC, 1978–93), that describe validated methods for analysing a wide variety of chemicals and mixtures. For biological agents, methods of detection and exposure assessment are described, including their sensitivity, specificity and reproducibility.

The dates of first synthesis and of first commercial production of a chemical or mixture are provided; for agents which do not occur naturally, this information may allow a reasonable estimate to be made of the date before which no human exposure to the agent could have occurred. The dates of first reported occurrence of an exposure are also provided. In addition, methods of synthesis used in past and present commercial production and different methods of production which may give rise to different impurities are described.

Data on production, international trade and uses are obtained for representative regions, which usually include Europe, Japan and the United States of America. It should not, however, be inferred that those areas or nations are necessarily the sole or major sources or users of the agent. Some identified uses may not be current or major applications, and the coverage is not necessarily comprehensive. In the case of drugs, mention of their therapeutic uses does not necessarily represent current practice, nor does it imply judgement as to their therapeutic efficacy.

Information on the occurrence of an agent or mixture in the environment is obtained from data derived from the monitoring and surveillance of levels in occupational environments, air, water, soil, foods and animal and human tissues. When available, data on the generation, persistence and bioaccumulation of the agent are also included. In the case of mixtures, industries, occupations or processes, information is given about all agents present. For processes, industries and occupations, a historical description is also given, noting variations in chemical composition, physical properties and levels of occupational exposure with time and place. For biological agents, the epidemiology of infection is described.

Statements concerning regulations and guidelines (e.g., pesticide registrations, maximal levels permitted in foods, occupational exposure limits) are included for some countries as indications of potential exposures, but they may not reflect the most recent situation, since such limits are continuously reviewed and modified. The absence of information on regulatory status for a country should not be taken to imply that that country does not have regulations with regard to the exposure. For biological agents, legislation and control, including vaccines and therapy, are described.

8. STUDIES OF CANCER IN HUMANS

(*a*) *Types of studies considered*

Three types of epidemiological studies of cancer contribute to the assessment of carcinogenicity in humans—cohort studies, case–control studies and correlation (or ecological) studies. Rarely, results from randomized trials may be available. Case series and case reports of cancer in humans may also be reviewed.

Cohort and case–control studies relate the exposures under study to the occurrence of cancer in individuals and provide an estimate of relative risk (ratio of incidence or mortality in those exposed to incidence or mortality in those not exposed) as the main measure of association.

In correlation studies, the units of investigation are usually whole populations (e.g. in particular geographical areas or at particular times), and cancer frequency is related to a summary measure of the exposure of the population to the agent, mixture or exposure circumstance under study. Because individual exposure is not documented, however, a causal relationship is less easy to infer from correlation studies than from cohort and case–control studies. Case reports generally arise from a suspicion, based on clinical experience, that the concurrence of two events—that is, a particular exposure and occurrence of a cancer—has happened rather more frequently than would be expected by chance. Case reports usually lack complete ascertainment of cases in any population, definition or enumeration of the population at risk and estimation of the expected number of cases in the absence of exposure. The uncertainties surrounding interpretation of case reports and correlation studies make them inadequate, except in rare instances, to form the sole basis for inferring a causal relationship. When taken together with case–control and cohort studies, however, relevant case reports or correlation studies may add materially to the judgement that a causal relationship is present.

Epidemiological studies of benign neoplasms, presumed preneoplastic lesions and other end-points thought to be relevant to cancer are also reviewed by working groups. They may, in some instances, strengthen inferences drawn from studies of cancer itself.

(*b*) *Quality of studies considered*

The Monographs are not intended to summarize all published studies. Those that are judged to be inadequate or irrelevant to the evaluation are generally omitted. They may be mentioned briefly, particularly when the information is considered to be a useful supplement to that in other reports or when they provide the only data available. Their

inclusion does not imply acceptance of the adequacy of the study design or of the analysis and interpretation of the results, and limitations are clearly outlined in square brackets at the end of the study description.

It is necessary to take into account the possible roles of bias, confounding and chance in the interpretation of epidemiological studies. By 'bias' is meant the operation of factors in study design or execution that lead erroneously to a stronger or weaker association than in fact exists between disease and an agent, mixture or exposure circumstance. By 'confounding' is meant a situation in which the relationship with disease is made to appear stronger or weaker than it truly is as a result of an association between the apparent causal factor and another factor that is associated with either an increase or decrease in the incidence of the disease. In evaluating the extent to which these factors have been minimized in an individual study, working groups consider a number of aspects of design and analysis as described in the report of the study. Most of these considerations apply equally to case–control, cohort and correlation studies. Lack of clarity of any of these aspects in the reporting of a study can decrease its credibility and the weight given to it in the final evaluation of the exposure.

Firstly, the study population, disease (or diseases) and exposure should have been well defined by the authors. Cases of disease in the study population should have been identified in a way that was independent of the exposure of interest, and exposure should have been assessed in a way that was not related to disease status.

Secondly, the authors should have taken account in the study design and analysis of other variables that can influence the risk of disease and may have been related to the exposure of interest. Potential confounding by such variables should have been dealt with either in the design of the study, such as by matching, or in the analysis, by statistical adjustment. In cohort studies, comparisons with local rates of disease may be more appropriate than those with national rates. Internal comparisons of disease frequency among individuals at different levels of exposure should also have been made in the study.

Thirdly, the authors should have reported the basic data on which the conclusions are founded, even if sophisticated statistical analyses were employed. At the very least, they should have given the numbers of exposed and unexposed cases and controls in a case–control study and the numbers of cases observed and expected in a cohort study. Further tabulations by time since exposure began and other temporal factors are also important. In a cohort study, data on all cancer sites and all causes of death should have been given, to reveal the possibility of reporting bias. In a case–control study, the effects of investigated factors other than the exposure of interest should have been reported.

Finally, the statistical methods used to obtain estimates of relative risk, absolute rates of cancer, confidence intervals and significance tests, and to adjust for confounding should have been clearly stated by the authors. The methods used should preferably have been the generally accepted techniques that have been refined since the mid-1970s. These methods have been reviewed for case–control studies (Breslow & Day, 1980) and for cohort studies (Breslow & Day, 1987).

(*c*) *Inferences about mechanism of action*

Detailed analyses of both relative and absolute risks in relation to temporal variables, such as age at first exposure, time since first exposure, duration of exposure, cumulative exposure and time since exposure ceased, are reviewed and summarized when available. The analysis of temporal relationships can be useful in formulating models of carcinogenesis. In particular, such analyses may suggest whether a carcinogen acts early or late in the process of carcinogenesis, although at best they allow only indirect inferences about the mechanism of action. Special attention is given to measurements of biological markers of carcinogen exposure or action, such as DNA or protein adducts, as well as markers of early steps in the carcinogenic process, such as proto-oncogene mutation, when these are incorporated into epidemiological studies focused on cancer incidence or mortality. Such measurements may allow inferences to be made about putative mechanisms of action (IARC, 1991a; Vainio *et al.*, 1992).

(*d*) *Criteria for causality*

After the individual epidemiological studies of cancer have been summarized and the quality assessed, a judgement is made concerning the strength of evidence that the agent, mixture or exposure circumstance in question is carcinogenic for humans. In making its judgement, the Working Group considers several criteria for causality. A strong association (a large relative risk) is more likely to indicate causality than a weak association, although it is recognized that relative risks of small magnitude do not imply lack of causality and may be important if the disease is common. Associations that are replicated in several studies of the same design or using different epidemiological approaches or under different circumstances of exposure are more likely to represent a causal relationship than isolated observations from single studies. If there are inconsistent results among investigations, possible reasons are sought (such as differences in amount of exposure), and results of studies judged to be of high quality are given more weight than those of studies judged to be methodologically less sound. When suspicion of carcinogenicity arises largely from a single study, these data are not combined with those from later studies in any subsequent reassessment of the strength of the evidence.

If the risk of the disease in question increases with the amount of exposure, this is considered to be a strong indication of causality, although absence of a graded response is not necessarily evidence against a causal relationship. Demonstration of a decline in risk after cessation of or reduction in exposure in individuals or in whole populations also supports a causal interpretation of the findings.

Although a carcinogen may act upon more than one target, the specificity of an association (an increased occurrence of cancer at one anatomical site or of one morphological type) adds plausibility to a causal relationship, particularly when excess cancer occurrence is limited to one morphological type within the same organ.

Although rarely available, results from randomized trials showing different rates among exposed and unexposed individuals provide particularly strong evidence for causality.

When several epidemiological studies show little or no indication of an association between an exposure and cancer, the judgement may be made that, in the aggregate, they show evidence of lack of carcinogenicity. Such a judgement requires first of all that the studies giving rise to it meet, to a sufficient degree, the standards of design and analysis described above. Specifically, the possibility that bias, confounding or misclassification of exposure or outcome could explain the observed results should be considered and excluded with reasonable certainty. In addition, all studies that are judged to be methodologically sound should be consistent with a relative risk of unity for any observed level of exposure and, when considered together, should provide a pooled estimate of relative risk which is at or near unity and has a narrow confidence interval, due to sufficient population size. Moreover, no individual study nor the pooled results of all the studies should show any consistent tendency for the relative risk of cancer to increase with increasing level of exposure. It is important to note that evidence of lack of carcinogenicity obtained in this way from several epidemiological studies can apply only to the type(s) of cancer studied and to dose levels and intervals between first exposure and observation of disease that are the same as or less than those observed in all the studies. Experience with human cancer indicates that, in some cases, the period from first exposure to the development of clinical cancer is seldom less than 20 years; latent periods substantially shorter than 30 years cannot provide evidence for lack of carcinogenicity.

9. STUDIES OF CANCER IN EXPERIMENTAL ANIMALS

All known human carcinogens that have been studied adequately in experimental animals have produced positive results in one or more animal species (Wilbourn *et al.*, 1986; Tomatis *et al.*, 1989). For several agents (aflatoxins, 4-aminobiphenyl, azathioprine, betel quid with tobacco, bischloromethyl ether and chloromethyl methyl ether (technical grade), chlorambucil, chlornaphazine, ciclosporin, coal-tar pitches, coal-tars, combined oral contraceptives, cyclophosphamide, diethylstilboestrol, melphalan, 8-methoxypsoralen plus ultraviolet A radiation, mustard gas, myleran, 2-naphthylamine, nonsteroidal oestrogens, oestrogen replacement therapy/steroidal oestrogens, solar radiation, thiotepa and vinyl chloride), carcinogenicity in experimental animals was established or highly suspected before epidemiological studies confirmed their carcinogenicity in humans (Vainio *et al.*, 1995). Although this association cannot establish that all agents and mixtures that cause cancer in experimental animals also cause cancer in humans, nevertheless, **in the absence of adequate data on humans, it is biologically plausible and prudent to regard agents and mixtures for which there is *sufficient evidence* (see p. 24) of carcinogenicity in experimental animals as if they presented a carcinogenic risk to humans**. The possibility that a given agent may cause cancer through a species-specific mechanism which does not operate in humans (see p. 27) should also be taken into consideration.

The nature and extent of impurities or contaminants present in the chemical or mixture being evaluated are given when available. Animal strain, sex, numbers per group, age at start of treatment and survival are reported.

Other types of studies summarized include: experiments in which the agent or mixture was administered in conjunction with known carcinogens or factors that modify carcinogenic effects; studies in which the end-point was not cancer but a defined precancerous lesion; and experiments on the carcinogenicity of known metabolites and derivatives.

For experimental studies of mixtures, consideration is given to the possibility of changes in the physicochemical properties of the test substance during collection, storage, extraction, concentration and delivery. Chemical and toxicological interactions of the components of mixtures may result in nonlinear dose–response relationships.

An assessment is made as to the relevance to human exposure of samples tested in experimental animals, which may involve consideration of: (i) physical and chemical characteristics, (ii) constituent substances that indicate the presence of a class of substances, (iii) the results of tests for genetic and related effects, including studies on DNA adduct formation, proto-oncogene mutation and expression and suppressor gene inactivation. The relevance of results obtained, for example, with animal viruses analogous to the virus being evaluated in the monograph must also be considered. They may provide biological and mechanistic information relevant to the understanding of the process of carcinogenesis in humans and may strengthen the plausibility of a conclusion that the biological agent under evaluation is carcinogenic in humans.

(*a*) *Qualitative aspects*

An assessment of carcinogenicity involves several considerations of qualitative importance, including (i) the experimental conditions under which the test was performed, including route and schedule of exposure, species, strain, sex, age, duration of follow-up; (ii) the consistency of the results, for example, across species and target organ(s); (iii) the spectrum of neoplastic response, from preneoplastic lesions and benign tumours to malignant neoplasms; and (iv) the possible role of modifying factors.

As mentioned earlier (p. 11), the *Monographs* are not intended to summarize all published studies. Those studies in experimental animals that are inadequate (e.g., too short a duration, too few animals, poor survival; see below) or are judged irrelevant to the evaluation are generally omitted. Guidelines for conducting adequate long-term carcinogenicity experiments have been outlined (e.g. Montesano *et al.*, 1986).

Considerations of importance to the Working Group in the interpretation and evaluation of a particular study include: (i) how clearly the agent was defined and, in the case of mixtures, how adequately the sample characterization was reported; (ii) whether the dose was adequately monitored, particularly in inhalation experiments; (iii) whether the doses and duration of treatment were appropriate and whether the survival of treated animals was similar to that of controls; (iv) whether there were adequate numbers of animals per group; (v) whether animals of each sex were used; (vi) whether animals were allocated randomly to groups; (vii) whether the duration of observation was adequate; and (viii) whether the data were adequately reported. If available, recent data on the incidence of specific tumours in historical controls, as

well as in concurrent controls, should be taken into account in the evaluation of tumour response.

When benign tumours occur together with and originate from the same cell type in an organ or tissue as malignant tumours in a particular study and appear to represent a stage in the progression to malignancy, it may be valid to combine them in assessing tumour incidence (Huff *et al.*, 1989). The occurrence of lesions presumed to be pre-neoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed. If an agent or mixture induces only benign neoplasms that appear to be end-points that do not readily progress to malignancy, it should nevertheless be suspected of being a carcinogen and requires further investigation.

(*b*) *Quantitative aspects*

The probability that tumours will occur may depend on the species, sex, strain and age of the animal, the dose of the carcinogen and the route and length of exposure. Evidence of an increased incidence of neoplasms with increased level of exposure strengthens the inference of a causal association between the exposure and the development of neoplasms.

The form of the dose–response relationship can vary widely, depending on the particular agent under study and the target organ. Both DNA damage and increased cell division are important aspects of carcinogenesis, and cell proliferation is a strong determinant of dose–response relationships for some carcinogens (Cohen & Ellwein, 1990). Since many chemicals require metabolic activation before being converted into their reactive intermediates, both metabolic and pharmacokinetic aspects are important in determining the dose–response pattern. Saturation of steps such as absorption, activation, inactivation and elimination may produce nonlinearity in the dose–response relationship, as could saturation of processes such as DNA repair (Hoel *et al.*, 1983; Gart *et al.*, 1986).

(*c*) *Statistical analysis of long-term experiments in animals*

Factors considered by the Working Group include the adequacy of the information given for each treatment group: (i) the number of animals studied and the number examined histologically, (ii) the number of animals with a given tumour type and (iii) length of survival. The statistical methods used should be clearly stated and should be the generally accepted techniques refined for this purpose (Peto *et al.*, 1980; Gart *et al.*, 1986). When there is no difference in survival between control and treatment groups, the Working Group usually compares the proportions of animals developing each tumour type in each of the groups. Otherwise, consideration is given as to whether or not appropriate adjustments have been made for differences in survival. These adjustments can include: comparisons of the proportions of tumour-bearing animals among the effective number of animals (alive at the time the first tumour is discovered), in the case where most differences in survival occur before tumours appear; life-table methods, when tumours are visible or when they may be considered ‘fatal’ because mortality rapidly follows tumour development; and the Mantel-Haenszel test or logistic regression,

when occult tumours do not affect the animals' risk of dying but are 'incidental' findings at autopsy.

In practice, classifying tumours as fatal or incidental may be difficult. Several survival-adjusted methods have been developed that do not require this distinction (Gart *et al.*, 1986), although they have not been fully evaluated.

10. OTHER DATA RELEVANT TO AN EVALUATION OF CARCINOGENICITY AND ITS MECHANISMS

In coming to an overall evaluation of carcinogenicity in humans (see pp. 26–27), the Working Group also considers related data. The nature of the information selected for the summary depends on the agent being considered.

For chemicals and complex mixtures of chemicals such as those in some occupational situations or involving cultural habits (e.g. tobacco smoking), the other data considered to be relevant are divided into those on absorption, distribution, metabolism and excretion; toxic effects; reproductive and developmental effects; and genetic and related effects.

Concise information is given on absorption, distribution (including placental transfer) and excretion in both humans and experimental animals. Kinetic factors that may affect the dose–response relationship, such as saturation of uptake, protein binding, metabolic activation, detoxification and DNA repair processes, are mentioned. Studies that indicate the metabolic fate of the agent in humans and in experimental animals are summarized briefly, and comparisons of data on humans and on animals are made when possible. Comparative information on the relationship between exposure and the dose that reaches the target site may be of particular importance for extrapolation between species. Data are given on acute and chronic toxic effects (other than cancer), such as organ toxicity, increased cell proliferation, immunotoxicity and endocrine effects. The presence and toxicological significance of cellular receptors is described. Effects on reproduction, teratogenicity, fetotoxicity and embryotoxicity are also summarized briefly.

Tests of genetic and related effects are described in view of the relevance of gene mutation and chromosomal damage to carcinogenesis (Vainio *et al.*, 1992). The adequacy of the reporting of sample characterization is considered and, where necessary, commented upon; with regard to complex mixtures, such comments are similar to those described for animal carcinogenicity tests on p. 18. The available data are interpreted critically by phylogenetic group according to the end-points detected, which may include DNA damage, gene mutation, sister chromatid exchange, micronucleus formation, chromosomal aberrations, aneuploidy and cell transformation. The concentrations employed are given, and mention is made of whether use of an exogenous metabolic system *in vitro* affected the test result. These data are given as listings of test systems, data and references. The Genetic and Related Effects data presented in the *Monographs* are also available in the form of Graphic Activity Profiles (GAP) prepared in collaboration with the United States Environmental Protection Agency (EPA) (see also Waters *et al.*, 1987)

using software for personal computers that are Microsoft Windows® compatible. The EPA/IARC GAP software and database may be downloaded free of charge from *www.epa.gov/gapdb*.

Positive results in tests using prokaryotes, lower eukaryotes, plants, insects and cultured mammalian cells suggest that genetic and related effects could occur in mammals. Results from such tests may also give information about the types of genetic effect produced and about the involvement of metabolic activation. Some end-points described are clearly genetic in nature (e.g., gene mutations and chromosomal aberrations), while others are to a greater or lesser degree associated with genetic effects (e.g. unscheduled DNA synthesis). In-vitro tests for tumour-promoting activity and for cell transformation may be sensitive to changes that are not necessarily the result of genetic alterations but that may have specific relevance to the process of carcinogenesis. A critical appraisal of these tests has been published (Montesano *et al.*, 1986).

Genetic or other activity manifest in experimental mammals and humans is regarded as being of greater relevance than that in other organisms. The demonstration that an agent or mixture can induce gene and chromosomal mutations in whole mammals indicates that it may have carcinogenic activity, although this activity may not be detectably expressed in any or all species. Relative potency in tests for mutagenicity and related effects is not a reliable indicator of carcinogenic potency. Negative results in tests for mutagenicity in selected tissues from animals treated *in vivo* provide less weight, partly because they do not exclude the possibility of an effect in tissues other than those examined. Moreover, negative results in short-term tests with genetic end-points cannot be considered to provide evidence to rule out carcinogenicity of agents or mixtures that act through other mechanisms (e.g. receptor-mediated effects, cellular toxicity with regenerative proliferation, peroxisome proliferation) (Vainio *et al.*, 1992). Factors that may lead to misleading results in short-term tests have been discussed in detail elsewhere (Montesano *et al.*, 1986).

When available, data relevant to mechanisms of carcinogenesis that do not involve structural changes at the level of the gene are also described.

The adequacy of epidemiological studies of reproductive outcome and genetic and related effects in humans is evaluated by the same criteria as are applied to epidemiological studies of cancer.

Structure–activity relationships that may be relevant to an evaluation of the carcinogenicity of an agent are also described.

For biological agents—viruses, bacteria and parasites—other data relevant to carcinogenicity include descriptions of the pathology of infection, molecular biology (integration and expression of viruses, and any genetic alterations seen in human tumours) and other observations, which might include cellular and tissue responses to infection, immune response and the presence of tumour markers.

11. SUMMARY OF DATA REPORTED

In this section, the relevant epidemiological and experimental data are summarized. Only reports, other than in abstract form, that meet the criteria outlined on p. 11 are considered for evaluating carcinogenicity. Inadequate studies are generally not summarized: such studies are usually identified by a square-bracketed comment in the preceding text.

(*a*) *Exposure*

Human exposure to chemicals and complex mixtures is summarized on the basis of elements such as production, use, occurrence in the environment and determinations in human tissues and body fluids. Quantitative data are given when available. Exposure to biological agents is described in terms of transmission and prevalence of infection.

(*b*) *Carcinogenicity in humans*

Results of epidemiological studies that are considered to be pertinent to an assessment of human carcinogenicity are summarized. When relevant, case reports and correlation studies are also summarized.

(*c*) *Carcinogenicity in experimental animals*

Data relevant to an evaluation of carcinogenicity in animals are summarized. For each animal species and route of administration, it is stated whether an increased incidence of neoplasms or preneoplastic lesions was observed, and the tumour sites are indicated. If the agent or mixture produced tumours after prenatal exposure or in single-dose experiments, this is also indicated. Negative findings are also summarized. Dose–response and other quantitative data may be given when available.

(*d*) *Other data relevant to an evaluation of carcinogenicity and its mechanisms*

Data on biological effects in humans that are of particular relevance are summarized. These may include toxicological, kinetic and metabolic considerations and evidence of DNA binding, persistence of DNA lesions or genetic damage in exposed humans. Toxicological information, such as that on cytotoxicity and regeneration, receptor binding and hormonal and immunological effects, and data on kinetics and metabolism in experimental animals are given when considered relevant to the possible mechanism of the carcinogenic action of the agent. The results of tests for genetic and related effects are summarized for whole mammals, cultured mammalian cells and nonmammalian systems.

When available, comparisons of such data for humans and for animals, and particularly animals that have developed cancer, are described.

Structure–activity relationships are mentioned when relevant.

For the agent, mixture or exposure circumstance being evaluated, the available data on end-points or other phenomena relevant to mechanisms of carcinogenesis from studies in humans, experimental animals and tissue and cell test systems are summarized within one or more of the following descriptive dimensions:

(i) Evidence of genotoxicity (structural changes at the level of the gene): for example, structure–activity considerations, adduct formation, mutagenicity (effect on specific genes), chromosomal mutation/aneuploidy

(ii) Evidence of effects on the expression of relevant genes (functional changes at the intracellular level): for example, alterations to the structure or quantity of the product of a proto-oncogene or tumour-suppressor gene, alterations to metabolic activation/inactivation/DNA repair

(iii) Evidence of relevant effects on cell behaviour (morphological or behavioural changes at the cellular or tissue level): for example, induction of mitogenesis, compensatory cell proliferation, preneoplasia and hyperplasia, survival of premalignant or malignant cells (immortalization, immunosuppression), effects on metastatic potential

(iv) Evidence from dose and time relationships of carcinogenic effects and interactions between agents: for example, early/late stage, as inferred from epidemiological studies; initiation/promotion/progression/malignant conversion, as defined in animal carcinogenicity experiments; toxicokinetics

These dimensions are not mutually exclusive, and an agent may fall within more than one of them. Thus, for example, the action of an agent on the expression of relevant genes could be summarized under both the first and second dimensions, even if it were known with reasonable certainty that those effects resulted from genotoxicity.

12. EVALUATION

Evaluations of the strength of the evidence for carcinogenicity arising from human and experimental animal data are made, using standard terms.

It is recognized that the criteria for these evaluations, described below, cannot encompass all of the factors that may be relevant to an evaluation of carcinogenicity. In considering all of the relevant scientific data, the Working Group may assign the agent, mixture or exposure circumstance to a higher or lower category than a strict interpretation of these criteria would indicate.

(*a*) *Degrees of evidence for carcinogenicity in humans and in experimental animals and supporting evidence*

These categories refer only to the strength of the evidence that an exposure is carcinogenic and not to the extent of its carcinogenic activity (potency) nor to the mechanisms involved. A classification may change as new information becomes available.

An evaluation of degree of evidence, whether for a single agent or a mixture, is limited to the materials tested, as defined physically, chemically or biologically. When the agents evaluated are considered by the Working Group to be sufficiently closely related, they may be grouped together for the purpose of a single evaluation of degree of evidence.

(i) *Carcinogenicity in humans*

The applicability of an evaluation of the carcinogenicity of a mixture, process, occupation or industry on the basis of evidence from epidemiological studies depends on the

variability over time and place of the mixtures, processes, occupations and industries. The Working Group seeks to identify the specific exposure, process or activity which is considered most likely to be responsible for any excess risk. The evaluation is focused as narrowly as the available data on exposure and other aspects permit.

The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

Sufficient evidence of carcinogenicity: The Working Group considers that a causal relationship has been established between exposure to the agent, mixture or exposure circumstance and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence.

Limited evidence of carcinogenicity: A positive association has been observed between exposure to the agent, mixture or exposure circumstance and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

Inadequate evidence of carcinogenicity: The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association between exposure and cancer.

If no epidemiological data were available, this is stated.

Evidence suggesting lack of carcinogenicity: There are several adequate studies covering the full range of levels of exposure that human beings are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent, mixture or exposure circumstance and any studied cancer at any observed level of exposure. A conclusion of 'evidence suggesting lack of carcinogenicity' is inevitably limited to the cancer sites, conditions and levels of exposure and length of observation covered by the available studies. In addition, the possibility of a very small risk at the levels of exposure studied can never be excluded.

In some instances, the above categories may be used to classify the degree of evidence related to carcinogenicity in specific organs or tissues.

(ii) *Carcinogenicity in experimental animals*

The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:

Sufficient evidence of carcinogenicity: The Working Group considers that a causal relationship has been established between the agent or mixture and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.

Exceptionally, a single study in one species might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset.

Limited evidence of carcinogenicity: The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; or (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the study; or (c) the agent or mixture increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential, or of certain neoplasms which may occur spontaneously in high incidences in certain strains.

Inadequate evidence of carcinogenicity: The studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect because of major qualitative or quantitative limitations.

If no data from animal experiments were available, this is stated.

Evidence suggesting lack of carcinogenicity: Adequate studies involving at least two species are available which show that, within the limits of the tests used, the agent or mixture is not carcinogenic. A conclusion of evidence suggesting lack of carcinogenicity is inevitably limited to the species, tumour sites and levels of exposure studied.

(*b*) *Other data relevant to the evaluation of carcinogenicity and its mechanisms*

Other evidence judged to be relevant to an evaluation of carcinogenicity and of sufficient importance to affect the overall evaluation is then described. This may include data on preneoplastic lesions, tumour pathology, genetic and related effects, structure–activity relationships, metabolism and pharmacokinetics, physicochemical parameters and analogous biological agents.

Data relevant to mechanisms of the carcinogenic action are also evaluated. The strength of the evidence that any carcinogenic effect observed is due to a particular mechanism is assessed, using terms such as weak, moderate or strong. Then, the Working Group assesses if that particular mechanism is likely to be operative in humans. The strongest indications that a particular mechanism operates in humans come from data on humans or biological specimens obtained from exposed humans. The data may be considered to be especially relevant if they show that the agent in question has caused changes in exposed humans that are on the causal pathway to carcinogenesis. Such data may, however, never become available, because it is at least conceivable that certain compounds may be kept from human use solely on the basis of evidence of their toxicity and/or carcinogenicity in experimental systems.

For complex exposures, including occupational and industrial exposures, the chemical composition and the potential contribution of carcinogens known to be present are considered by the Working Group in its overall evaluation of human carcinogenicity. The Working Group also determines the extent to which the materials tested in experimental systems are related to those to which humans are exposed.

(*c*) *Overall evaluation*

Finally, the body of evidence is considered as a whole, in order to reach an overall evaluation of the carcinogenicity to humans of an agent, mixture or circumstance of exposure.

An evaluation may be made for a group of chemical compounds that have been evaluated by the Working Group. In addition, when supporting data indicate that other, related compounds for which there is no direct evidence of capacity to induce cancer in humans or in animals may also be carcinogenic, a statement describing the rationale for this conclusion is added to the evaluation narrative; an additional evaluation may be made for this broader group of compounds if the strength of the evidence warrants it.

The agent, mixture or exposure circumstance is described according to the wording of one of the following categories, and the designated group is given. The categorization of an agent, mixture or exposure circumstance is a matter of scientific judgement, reflecting the strength of the evidence derived from studies in humans and in experimental animals and from other relevant data.

Group 1 —The agent (mixture) is carcinogenic to humans.
The exposure circumstance entails exposures that are carcinogenic to humans.

This category is used when there is *sufficient evidence* of carcinogenicity in humans. Exceptionally, an agent (mixture) may be placed in this category when evidence of carcinogenicity in humans is less than sufficient but there is *sufficient evidence* of carcinogenicity in experimental animals and strong evidence in exposed humans that the agent (mixture) acts through a relevant mechanism of carcinogenicity.

Group 2

This category includes agents, mixtures and exposure circumstances for which, at one extreme, the degree of evidence of carcinogenicity in humans is almost sufficient, as well as those for which, at the other extreme, there are no human data but for which there is evidence of carcinogenicity in experimental animals. Agents, mixtures and exposure circumstances are assigned to either group 2A (probably carcinogenic to humans) or group 2B (possibly carcinogenic to humans) on the basis of epidemiological and experimental evidence of carcinogenicity and other relevant data.

Group 2A—The agent (mixture) is probably carcinogenic to humans.
The exposure circumstance entails exposures that are probably carcinogenic to humans.

This category is used when there is *limited evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in experimental animals. In some cases, an agent (mixture) may be classified in this category when there is *inadequate evidence* of carcinogenicity in humans, *sufficient evidence* of carcinogenicity in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Exceptionally, an agent, mixture or exposure circumstance may

be classified in this category solely on the basis of *limited evidence* of carcinogenicity in humans.

Group 2B—The agent (mixture) is possibly carcinogenic to humans. The exposure circumstance entails exposures that are possibly carcinogenic to humans.

This category is used for agents, mixtures and exposure circumstances for which there is *limited evidence* of carcinogenicity in humans and less than *sufficient evidence* of carcinogenicity in experimental animals. It may also be used when there is *inadequate evidence* of carcinogenicity in humans but there is *sufficient evidence* of carcinogenicity in experimental animals. In some instances, an agent, mixture or exposure circumstance for which there is *inadequate evidence* of carcinogenicity in humans but *limited evidence* of carcinogenicity in experimental animals together with supporting evidence from other relevant data may be placed in this group.

Group 3—The agent (mixture or exposure circumstance) is not classifiable as to its carcinogenicity to humans.

This category is used most commonly for agents, mixtures and exposure circumstances for which the *evidence of carcinogenicity* is *inadequate* in humans and *inadequate* or *limited* in experimental animals.

Exceptionally, agents (mixtures) for which the *evidence of carcinogenicity* is *inadequate* in humans but *sufficient* in experimental animals may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans.

Agents, mixtures and exposure circumstances that do not fall into any other group are also placed in this category.

Group 4—The agent (mixture) is probably not carcinogenic to humans.

This category is used for agents or mixtures for which there is *evidence suggesting lack of carcinogenicity* in humans and in experimental animals. In some instances, agents or mixtures for which there is *inadequate evidence* of carcinogenicity in humans but *evidence suggesting lack of carcinogenicity* in experimental animals, consistently and strongly supported by a broad range of other relevant data, may be classified in this group.

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GENERAL REMARKS

Since the beginning in 1971 of the *IARC Monographs* Programme on the Evaluation of the Carcinogenic Risk of Chemicals to Man, now the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, some 834 agents have been evaluated, some of them more than once. These monographs and evaluations were published in Volumes 1–70 of the series. During recent years, the volume of biological data that could be relevant to an evaluation process has increased enormously and there has been growing concern that many of the older evaluations are becoming out of date and may require re-evaluation.

In 1979, re-evaluations were made for 54 chemicals, complex mixtures or occupational exposure circumstances for which cancer epidemiological data existed. The results of the meeting were published as Supplement 1 (IARC, 1979). In February 1982 there was another re-evaluation of data on 155 chemicals, groups of chemicals and exposures to complex mixtures that had been considered in Volumes 1–29 of the *IARC Monographs*, for which there were some data on human carcinogenicity. Those re-evaluations were published in Supplement 4 (IARC, 1982). Supplement 4 was subsequently updated as a result of two Working Group meetings, one in December 1986 of which the outcome was Supplement 6 (IARC, 1987a) and another in March 1987, which produced Supplement 7 (IARC, 1987b). The aim of the December 1986 Working Group was to summarize and bring up to date the findings from tests for genetic and related effects for all of the agents that had been evaluated in Volumes 1–42 of the *Monographs* and for which some cancer epidemiological data were available.

The objectives of Supplement 7 were two-fold. The first was to summarize and bring up to date the data on cancer epidemiology and experimental carcinogenesis for all 189 agents that had been evaluated in Volumes 1–42 of the *Monographs* and for which some cancer epidemiological data were available. The second objective was to make overall evaluations of carcinogenicity to humans for all 628 agents that had been evaluated in Volumes 1–42 of the *Monographs.*

Although the number of compounds re-evaluated in this Volume 71 of the *IARC Monographs* is smaller than in previous updatings, the undertaking was more extensive. A total of 121 organic compounds, most of them industrial chemicals, were selected for re-evaluation. Four of these compounds were treated as full length monographs, of the kind to be found in standard volumes of the *IARC Monographs*, i.e., all relevant data were reviewed. These four compounds (acrylonitrile, 1,3-butadiene, chloroprene and dichloromethane) were selected because there was a large quantity of new data and there was specific, widespread interest in having them re-evaluated.

For a number of other chemicals, the starting point for these reviews was the last full summary and evaluation. The summarized statements prepared previously were incorporated with data published since the previous evaluation. These 'mini-monographs' were prepared for those chemicals for which new epidemiological data and/or experimental carcinogenicity data were available that required review and acceptance by the full plenary meeting of invited experts, because changes in the classifications were anticipated.

A third group of chemicals consisted of those for which there was no new epidemiological evidence and, although there might be new experimental carcinogenicity data, these were, in the judgement of the secretariat, unlikely to lead to any change in the evaluation and classification. In these up-dated monographs the previous summary statements, in so far as they existed, are restated and new data are described. In all cases, however, there was a need to describe the toxicity and disposition data, since this was not consistently done in monographs published before the early 1990s.

The full monographs and the mini-monographs appear in Part 1 and Part 2, respectively, and the monograph updates are in Part 3 of this volume. Some of the chemicals were combined into single monographs because there was a close relationship between them. The monograph on 1,3-butadiene also contains the available data on 1,2:3,4-diepoxybutane because this is an important metabolite and a full consideration of the effects of exposure to 1,3-butadiene could not be made without frequent reference to this metabolite, to which human exposure from environmental sources could not be confirmed. Three α-chlorinated toluenes (benzyl chloride, benzal chloride and benzotrichloride) appear in a single mini-monograph along with benzoyl chloride, which is not an α-chlorinated toluene but is inextricably associated with them in industrial processes. Another mini-monograph is on the polychlorophenols and their sodium salts (and contains data on five of them), this forming a natural grouping of the chemicals, as does the combination of *ortho*-, *meta*- and *para*-xylenes.

During the first half of an *IARC Monographs* working group meeting, participants are allocated to specialist subgroups, which review drafts on chemistry and exposure, cancer epidemiology, experimental carcinogenicity and disposition (absorption, distribution, metabolism, excretion) and toxicology. For Volume 71, the same allocation to these subgroups was made, but in addition there was a multidisciplinary subgroup that reviewed all draft documents proposed for update. Wherever this subgroup concluded that a more detailed examination of any particular section was required, that section was passed on to the relevant specialist subgroup and on the recommendation of that specialist subgroup may have been brought to plenary session to consider whether any change in evaluation was appropriate. By this process, an update may have advanced to mini-monograph status. If, however, no such in-depth review was recommended, then the up-dated monograph was reviewed only by the multidisciplinary subgroup and was not reviewed in plenary session by the entire working group.

To minimize repetition in this very large undertaking, certain changes in procedure were introduced. One relates to occupational exposure data in the United States that are

available from the National Occupational Exposure Survey (NOES); another relates to the presentation of data on genetic and related effects. In the case of NOES data, reference is made in each Monograph to this General Remarks section for a short description of the survey. In the case of genetic and related effects data, the experimental evidence is tabulated (unless no new evidence has emerged since the last evaluation) and described in the accompanying text. Previously, there was also a presentation of genetic activity profiles, which summarize pictorially the genetic data already described in the text and in the tables. Since these profiles are now available on the worldwide web, it has been decided to inform readers of the *Monographs* how to access them, but not to print them in this or future volumes.

National Occupational Exposure Survey

Information on the extent of occupational exposure in the United States is available from the National Occupational Exposure Survey (NOES) conducted by the United States National Institute for Occupational Safety and Health (NIOSH). NOES was a nationwide observational survey conducted in a sample of 4490 establishments from 1981 to 1983. The target population was defined as employees working in establishments or job sites in the United States of America employing eight or more workers in a defined list of Standard Industrial Classifications. Generally, these classifications emphasized coverage of construction, manufacturing, transportation, private and business service and hospital industries. The NOES had little or no sampling activity in agriculture, mining, wholesale/retail trade, finance/real estate or government operations. NOES addressed recordable potential exposure that had to meet two criteria: (1) a chemical, physical or biological agent or trade name product had to be observed in sufficient proximity to an employee such that one or two physical phases of that agent or product were likely to come into contact or enter the body of the employee; and (2) the duration of the potential exposure had to meet the minimum duration guidelines, i.e. it must have presented a potential exposure for at least 30 min a week (on an annual average) or be used at least once per week for 90% of the weeks of the work year (NOES, 1997).

Genetic Activity Profiles

The Genetic and Related Effects data presented in the *Monographs* are also available in the form of Graphic Activity Profiles (GAP) prepared in collaboration with the United States Environmental Protection Agency (EPA) (see also Waters *et al.*, 1987) using software for personal computers that are Microsoft Windows® compatible. The EPA/IARC GAP software and database may be downloaded free of charge from *www.epa.gov/gapdb*.

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SUMMARY OF FINAL EVALUATIONS

Agent	Degree of evidence of carcinogenicity		Overall evaluation of carcinogenicity to humans
	Human	Animal	
Acetaldehyde	I	S	2B
Acetamide	ND	S	2B
Acrylic acid	ND	ND	3
Acrylonitrile	I	S	2B
Allyl chloride	I	I	3
Allyl isovalerate	ND	L	3
Aziridine	ND	L	2B*
1,4-Benzoquinone (*para*-quinone)	ND	I	3
1,4-Benzoquinone dioxime	ND	L	3
Benzoyl peroxide	I	L	3
Benzyl acetate	ND	L	3
Bis(2-chloroethyl)ether	I	L	3
1,2-Bis(chloromethoxy)ethane	ND	L	3
1,4-Bis(chloromethoxymethyl)benzene	ND	L	3
Bis(2-chloro-1-methylethyl)ether	ND	L	3
Bis(2,3-epoxycyclopentyl)ether	ND	L	3
Bisphenol A diglycidyl ether	ND	L	3
Bromochloroacetonitrile	ND	I	3
Bromodichloromethane	ND	S	2B
Bromoethane	ND	L	3
Bromoform	ND	L	3
1,3-Butadiene	L	S	2A
1,2:3,4-Diepoxybutane		S	
n-Butyl acrylate	ND	I	3
β-Butyrolactone	ND	S	2B
γ-Butyrolactone	I	ESL	3
Caprolactam	ND	ESL	4
Carbazole	ND	L	3
Carbon tetrachloride	I	S	2B
Catechol	ND	S	2B
α-Chlorinated toluenes and benzoyl chloride (combined exposures)	L		2A
Benzal chloride		L	
Benzotrichloride		S	
Benzyl chloride		S	
Benzoyl chloride		I	
Chloroacetonitrile	ND	I	3
Chlorodibromomethane	ND	L	3

Summary of final evaluations (contd)

Agent	Degree of evidence of carcinogenicity		Overall evaluation of carcinogenicity to humans
	Human	Animal	
Chlorodifluoromethane	I	L	3
Chloroethane	ND	L	3
Chlorofluoromethane	ND	L	3
Chloroprene	I	S	2B
2-Chloro-1,1,1-trifluoroethane	ND	L	3
Cyclohexanone	ND	I	3
Decabromodiphenyl oxide	ND	L	3
Dibromoacetonitrile	ND	I	3
1,2-Dibromo-3-chloropropane	I	S	2B
Dichloroacetonitrile	ND	I	3
Dichloroacetylene	ND	L	3
trans-1,4-Dichlorobutene	ND	I	3
1,2-Dichloroethane	I	S	2B
Dichloromethane	I	S	2B
1,2-Dichloropropane	ND	L	3
1,3-Dichloropropene (technical-grade)	ND	S	2B
1,2-Diethylhydrazine	ND	S	2B
Diethyl sulfate	I	S	2A*
Diglycidyl resorcinol ether (technical-grade)	ND	S	2B
Diisopropyl sulfate	I	S	2B
Dimethylcarbamoyl chloride	I	S	2A*
Dimethylformamide	I	ESL	3
1,1-Dimethylhydrazine	ND	S	2B
1,2-Dimethylhydrazine	ND	S	2A*
Dimethyl hydrogen phosphite	ND	L	3
Dimethyl sulfate	I	S	2A*
1,4-Dioxane	I	S	2B
Epichlorohydrin	I	S	2A*
1,2-Epoxybutane	ND	L	2B*
3,4-Epoxy-6-methylcyclohexylmethyl 3,4-epoxy-6-methylcyclohexane carboxylate	ND	L	3
cis-9,10-Epoxystearic acid	ND	I	3
Ethyl acrylate	ND	S	2B
Ethylene dibromide (1,2-dibromoethane)	I	S	2A*
Glycidaldehyde	ND	S	2B
Hexamethylphosphoramide	ND	S	2B
Hydrazine	I	S	2B
Hydrogen peroxide	I	L	3
Hydroquinone	I	L	3
Isoprene	ND	S	2B
Isopropanol	I	I	3

Summary of final evaluations (contd)

Agent	Degree of evidence of carcinogenicity		Overall evaluation of carcinogenicity to humans
	Human	Animal	
Isopropyl oils	I	I	3
Lauroyl peroxide	ND	I	3
Malonaldehyde (malondialdehyde)	ND	L	3
Methyl acrylate	ND	I	3
2-Methylaziridine (propyleneimine)	ND	S	2B
Methyl bromide	I	L	3
Methyl chloride	I	I	3
4,4′-Methylenediphenyl diisocyanate (industrial preparation)	I		3
Polymeric 4,4′-methylenediphenyl diisocyanate	I		
Mixture containing monomeric and polymeric 4,4′-methylenediphenyl diisocyanate		L	
Methyl iodide	ND	L	3
Methyl methanesulfonate	ND	S	2A*
Morpholine	ND	I	3
1,5-Naphthalene diisocyanate	ND	ND	3
2-Nitropropane	I	S	2B
Pentachloroethane	ND	L	3
Phenol	I	I	3
Phenyl glycidyl ether	ND	S	2B
Polychlorophenols or their sodium salts (combined exposures)	L		2B
2,4-Dichlorophenol		ESL	
2,4,5-Trichlorophenol		I	
2,4,6-Trichlorophenol		L	
Pentachlorophenol		S	
Polyvinyl pyrrolidone	ND	L	3
1,3-Propane sultone	ND	S	2B
β-Propiolactone	ND	S	2B
Resorcinol	ND	I	3
1,1,1,2-Tetrachloroethane	ND	L	3
1,1,2,2-Tetrachloroethane	I	L	3
Tetrafluoroethylene	ND	S	2B
Tetrakis(hydroxymethyl)phosphonium salts	ND	I	3
Toluene	I	ESL	3
Toluene diisocyanates	I	S	2B
Trichloroacetonitrile	ND	I	3
1,1,1-Trichloroethane	I	I	3
1,1,2-Trichloroethane	ND	L	3

Summary of final evaluations (contd)

Agent	Degree of evidence of carcinogenicity		Overall evaluation of carcinogenicity to humans
	Human	Animal	
Triethylene glycol diglycidyl ether	ND	I	3
Tris(2-chloroethyl) phosphate	ND	L	3
1,2,3-Tris(chloromethoxy)propane	ND	L	3
Tris(2,3-dibromopropyl) phosphate	I	S	2A*
Vinyl bromide	ND	S	2A*
Vinylidene chloride	I	L	3
Vinylidene fluoride	ND	I	3
N-Vinyl-2-pyrrolidone	ND	L	3
Xylenes	I	I	3

* Other relevant data were taken into account when making the overall evaluation.
ESL, evidence suggesting lack of carcinogenicity; ND, no epidemiological (or animal) data relevant to the carcinogenicity of the compounds were available; I, inadequate evidence of carcinogenicity; L, limited evidence of carcinogenicity; S, sufficient evidence of carcinogenicity; 2A, probably carcinogenic to humans; 2B, possibly carcinogenic to humans; 3, cannot be classified as to its carcinogenicity to humans; 4, probably not carcinogenic to humans

THE MONOGRAPHS

Part One
Compounds Reviewed in Plenary Sessions
(Comprehensive Monographs)

ACRYLONITRILE

This substance was considered by previous Working Groups, in February 1978 (IARC, 1979) and March 1987 (IARC, 1987a). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

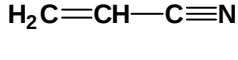
1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 107-13-1

Chem. Abstr. Name: 2-Propenenitrile

Synonyms: AN; cyanoethylene; propenenitrile; VCN; vinyl cyanide

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_3H_3N Relative molecular mass: 53.06

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid (Verschueren, 1996)

(*b*) *Boiling-point*: 77.3°C (Lide, 1995)

(*c*) *Melting-point*: –83.5°C (Lide, 1995)

(*d*) *Density*: d_4^{20} 0.8060 (Lide, 1995)

(*e*) *Spectroscopy data*: Infrared, nuclear magnetic resonance and mass spectral data have been reported (Sadtler Research Laboratories, 1980; Brazdil, 1991)

(*f*) *Solubility*: Soluble in water (7.35 mL/100 mL at 20°C); very soluble in acetone, benzene, diethyl ether and ethanol (Lide, 1995; Budavari, 1996)

(*g*) *Volatility*: Vapour pressure, 13.3 kPa at 23°C; relative vapour density (air = 1), 1.83 (Verschueren, 1996)

(*h*) *Stability*: Flash-point (open cup), 0°C; flammable; polymerizes spontaneously, particularly in the absence of oxygen, on exposure to visible light and in contact with concentrated alkali (Budavari, 1996)

(*i*) *Explosive limits*: Lower, 3.05%; upper, 17.0% (Budavari, 1996)

(*j*) *Octanol/water partition coefficient (P)*: log *P*, 0.25 (Hansch *et al*., 1995)

(*k*) *Conversion factor*: $mg/m^3 = 2.17 \times ppm$[1]

1.1.4 *Technical products and impurities*

Acrylonitrile of 99.5–99.7% purity is available commercially, with the following specifications (ppm by weight, maximum): acidity (as acetic acid), 10; acetone, 75; acetonitrile, 300; acrolein, 1; hydrogen cyanide, 5; total iron, 0.1; oxazole, 10; peroxides (as hydrogen peroxide), 0.2; water, 0.5%; and nonvolatile matter, 100. Hydroquinone monomethyl ether (MEHQ) is added as an inhibitor at concentrations of 35–45 mg/kg (ppm) (Cytec Industries, 1994, 1997). Trade names for acrylonitrile include Acritet, Acrylon, Carbacryl, Fumigrain and Ventox.

1.1.5 *Analysis*

Selected methods for the analysis of acrylonitrile in various matrices are presented in Table 1.

Table 1. Methods for the analysis of acrylonitrile[a]

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Adsorb (charcoal); extract (acetone)	GC/NPD	26 μg/m^3	US Occupational Safety and Health Administration (1990) [Method 37]
	Adsorb (charcoal); extract (acetone in carbon disulfide)	GC/FID	1 μg/sample	Eller (1994) [Method 1604]
Water	Purge (inert gas); trap (Porapak-QS; Chromosorb 101); desorb as vapour (heat to 180°C, backflush with inert gas) onto GC column	GC/FID	0.5 μg/L	US Environmental Protection Agency (1996a) [Method 603] (1986) [Method 8030]
	Add internal standard (isotope-labelled dichloromethane); purge; trap and desorb as above	GC/MS	50 μg/L	US Environmental Protection Agency (1996b) [Method 1624]
Plastics, liquid foods	Dissolve in *ortho*-dichlorobenzene; inject headspace sample	GC/FID	2–20 μg/kg	US Food and Drug Administration (1987)
Solid foods	Cut or mash sample; inject headspace sample	GC/FID	2–20 μg/kg	US Food and Drug Administration (1987)

[a] Abbreviations: GC/FID, gas chromatography/flame ionization detection; GC/MS, gas chromatography/mass spectrometry; GC/NPD, gas chromatography/nitrogen-phosphorus detection

[1] Calculated from: $mg/m^3 = (\text{relative molecular mass}/24.47) \times ppm$, assuming a temperature of 25°C and a pressure of 101 kPa

Exposure to acrylonitrile can be determined by measuring parent acrylonitrile, acrylonitrile metabolites, and adducts. Acrylonitrile metabolites have been measured in blood and urine, but, except for measurement of thiocyanate, these methods have not been developed for routine monitoring of exposed humans (Agency for Toxic Substances and Disease Registry, 1990).

1.2 Production and use

1.2.1 *Production*

Acrylonitrile was first prepared in 1893 by dehydration of either acrylamide or ethylene cyanohydrin with phosphorus pentoxide (Fugate, 1963).

Until 1960, acrylonitrile was produced commercially by processes based on hydrogen cyanide and ethylene oxide or acetylene. The growth in demand for acrylic fibres, starting with the introduction of Orlon by DuPont around 1950, spurred efforts to develop improved process technology for acrylonitrile manufacture. This resulted in the discovery in the late 1950s of a heterogeneous vapour-phase catalytic process for acrylonitrile by selective oxidation of propylene and ammonia, commonly referred to as the propylene ammoxidation process. Commercial introduction of this lower-cost process by Sohio in 1960 resulted in the eventual displacement of all other acrylonitrile manufacturing processes. Today, the ammoxidation process accounts for over 90% of the approximately 4000 thousand tonnes produced worldwide each year. In this process, propylene, ammonia and air react in the vapour phase in the presence of a catalyst (bismuth–iron; bismuth–phosphomolybdate; antimony–uranium; ferrobismuth–phosphomolybdate). Hydrogen cyanide and acetonitrile are the chief by-products formed. Sulfuric acid is used to remove excess ammonia from the reaction mixture, and the nitrile compounds are removed by absorption in water. High-purity acrylonitrile is obtained by a series of distillations (Langvardt, 1985; Brazdil, 1991).

Acrylonitrile was first produced in Germany and the United States on an industrial scale in the early 1940s. These processes were based on the catalytic dehydration of ethylene cyanohydrin. Ethylene cyanohydrin was produced from ethylene oxide and aqueous hydrocyanic acid at 60°C in the presence of a basic catalyst. The intermediate was then dehydrated in the liquid phase at 200°C in the presence of magnesium carbonate and alkaline or alkaline earth salts of formic acid. A second commercial route to acrylonitrile was the catalytic addition of hydrogen cyanide to acetylene. The last commercial plants using these process technologies were shut down in 1970 (Langvardt, 1985; Brazdil, 1991).

Worldwide production of acrylonitrile in 1988 was about 3200 thousand tonnes, with the following breakdown (thousand tonnes): western Europe, 1200; United States, 1170; Japan, 600; the Far East, 200; and Mexico, 60 (Brazdil, 1991). Production in the United States has been reported as (thousand tonnes): 1981, 906; 1984, 1006; 1987, 990; 1990, 1214; 1993, 1129; 1996, 1530. Production in Japan has been reported as (thousand tonnes): 1981, 477; 1984, 523; 1987, 573; 1990, 593; 1993, 594; 1996, 675 (Anon., 1985, 1988, 1991, 1994, 1997).

1.2.2 *Use*

Worldwide consumption of acrylonitrile increased 52% between 1976 and 1988, from 2500 to 3800 thousand tonnes per year. The trend in consumption over this time period is shown in Table 2 for the principal uses of acrylonitrile: acrylic fibre, acrylonitrile–butadiene–styrene (ABS) resins, adiponitrile, nitrile rubbers, elastomers and styrene–acrylonitrile (SAN) resins. Since the 1960s, acrylic fibres have remained the major outlet for acrylonitrile production in the United States and especially in Japan and the Far East. Acrylic fibres always contain a comonomer. Fibres containing 85 wt% or more acrylonitrile are usually referred to as 'acrylics' and fibres containing 35–85 wt% acrylonitrile are called 'modacrylics'. Acrylic fibres are used primarily for the manufacture of apparel, including sweaters, fleece wear and sportswear, and home furnishings, including carpets, upholstery and draperies (Langvardt, 1985; Brazdil, 1991).

The production of ABS and SAN resins consumes the second largest quantity of acrylonitrile. The ABS resins are produced by grafting acrylonitrile and styrene onto polybutadiene or a styrene–butadiene copolymer and contain about 25 wt% acrylonitrile. These products are used to make components for automotive and recreational vehicles, pipe fittings, and appliances. The SAN resins are styrene–acrylonitrile copolymers containing 25–30 wt% of acrylonitrile. The superior clarity of SAN resin allows it to be used in automobile instrument panels, for instrument lenses and for houseware items (Langvardt, 1985; Brazdil, 1991).

The chemical intermediates adiponitrile and acrylamide have surpassed nitrile rubbers as end-use products of acrylonitrile in the United States and Japan. Adiponitrile is further converted to hexamethylenediamine (HMDA), which is used to manufacture nylon 6/6. Acrylamide is used to produce water-soluble polymers or copolymers used for paper manufacturing, waste treatment, mining applications and enhanced oil recovery (Langvardt, 1985; Brazdil, 1991).

Nitrile rubbers, the original driving force behind acrylonitrile production, have taken a less significant place as end-use products. They are butadiene–acrylonitrile copolymers with an acrylonitrile content ranging from 15 to 45%, and find industrial applications in

Table 2. Worldwide acrylonitrile uses and consumption (thousand tonnes)[a]

Use	1976	1980	1985	1988
Acrylic fibres	1760	2040	2410	2520
Acrylonitrile–butadiene–styrene resins	270	300	435	550
Adiponitrile	90	160	235	310
Other (including nitrile rubber, styrene–acrylonitrile resin, acrylamide, and barrier resins)	420	240	390	460

[a] From Brazdil (1991)

areas where their oil resistance and low-temperature flexibility are important, such as in the fabrication of seals (O-rings), fuel hoses and oil-well equipment. New applications have emerged with the development of nitrile rubber blends with poly(vinyl chloride) (PVC). These blends combine the chemical resistance and low-temperature flexibility characteristics of nitrile rubber with the stability and ozone resistance of PVC. This has greatly expanded the use of nitrile rubber in outdoor applications for hoses, belts and cable jackets (Langvardt, 1985; Brazdil, 1991).

Other acrylonitrile copolymers have found specialty applications where good gas-barrier properties are required along with strength and high impact resistance. These barrier resins compete directly with traditional glass and metal containers as well as with poly(ethylene terephthalate) (PET) and PVC in the beverage bottle market. Other applications include packaging for food, agricultural chemicals and medicines (Brazdil, 1991).

A growing specialty application for acrylonitrile is in the manufacture of carbon fibres. These are produced by pyrolysis of oriented polyacrylonitrile fibres and are used to reinforce composites for high-performance applications in the aircraft, defence and aerospace industries. Other minor specialty applications of acrylonitrile are in the production of fatty amines, ion exchange resins and fatty amine amides used in cosmetics, adhesives, corrosion inhibitors and water-treatment resins (Brazdil, 1991).

In the past, acrylonitrile was used with carbon tetrachloride as a fumigant for tobacco and in flour milling and bakery food processing (Suta, 1979). Most pesticides containing acrylonitrile have now been withdrawn (Worthing & Hance, 1991).

1.3 Occurrence

1.3.1 *Natural occurrence*

Acrylonitrile is not known to occur as a natural product.

1.3.2 *Occupational exposure*

According to the 1990–93 CAREX database for 15 countries of the European Union (Kauppinen *et al.*, 1998) and the 1981–83 United States National Occupational Exposure Survey (NOES, 1997), approximately 35 000 workers in Europe and as many as 80 000 workers in the United States were potentially exposed to acrylonitrile (see General Remarks). Occupational exposures to acrylonitrile have been measured in monomer production and in the production of fibres, resins, polymers and other chemical intermediates from acrylonitrile.

(*a*) *Monomer production*

Surveys have reported full-shift personal exposures measured by the companies and the study investigators in four acrylonitrile production plants in the United States (Zey *et al.*, 1989; Zey & McCammon, 1990; Zey *et al.*, 1990a,b). The monomer production operators had 8-h time-weighted average (TWA_8) personal exposures of about 1 ppm [2.2 mg/m^3] or less from about 1978 to 1986, with some TWA_8 levels greater than

10 ppm [22 mg/m^3] (Table 3). In three of these plants, maintenance employees averaged below 0.5 ppm [1.1 mg/m^3], but in one plant the TWA_8 for these workers was about 1.0 ppm [2.2 mg/m^3]. Typical exposures of loaders of acrylonitrile into tank trucks, rail cars or barges varied from about 0.4 to about 6 ppm [0.9–13 mg/m^3]. Respirator use was noted for some of the higher measurements for production and maintenance workers and loaders in these plants. Laboratory technicians in these plants averaged about 0.25 ppm [0.54 mg/m^3] (n = 176; 0.01–2.0 ppm [0.02–4.3 mg/m^3]), except for one plant where the average was 1.00 [2.2 mg/m^3] (n = 57; 0.1–9.4 ppm [0.2–20 mg/m^3]) (not shown in Table 3). Although measurement data were provided by year and several changes were made in these plants to reduce exposure levels, no trends over the years were observed.

Estimates of exposures in these same plants were developed for an epidemiological study (Blair *et al.*, 1998; Stewart *et al*., 1998). For years in which there were exposure measurements, means of the measurements were used (Zey *et al.*, 1989; Zey & McCammon, 1990; Zey *et al.*, 1990a,b). For years in which no measurement data were available, the measurements for each work site were adjusted based on specific conditions in that plant, including changes in the process, in operating and engineering controls, in tasks and other parameters. In the 1950s, two of these plants were in existence and the average estimate for the monomer operators' TWA_8 exposure was 1–2 ppm [2.2–4.4 mg/m^3]. In the 1960s, the average estimate for this job in three of the plants was between 1 and 4 ppm [2.2 and 8.8 mg/m^3] and in the fourth around 15 ppm [33 mg/m^3]. In the 1970s the range of the estimates for the four plants was 0.5–6 ppm [1.1–13 mg/m^3] for this job.

Other chemicals present in acrylonitrile production or in other non-acrylonitrile operations on sites of the companies in the epidemiological study by Blair *et al.* (1998) include acetylene, hydrogen cyanide, propylene, ammonia, acetic acid, phosphoric acid, lactonitrile, hydroquinone, sodium hydroxide, sulfuric acid, acrylamide, acetone cyanohydrin, melamine, methyl methacrylate, *meta*-methylstyrene, urea, methacrylonitrile, butadiene, ammonium hydroxide and ammonium sulfate (Zey *et al.*, 1989, 1990a,b; Zey & McCammon, 1990).

Loaders in a French plant had personal exposure levels of 2.8, 1.9 and 2.4 ppm [6.1, 4.1 and 5.2 mg/m^3] (the latter, a range of 0.1–27 ppm [0.2–59 mg/m^3]) for 30 min to 1.5 h (Cicolella *et al*., 1981). Five-minute to one-hour samples taken near monomer equipment averaged about 20 ppm [44 mg/m^3], with levels up to 56 ppm [122 mg/m^3].

(*b*) *Fibre production*

Full-shift personal samples taken by the companies and the study investigators were reported across a number of jobs over the years 1977–86 in three fibre plants in the United States (Table 3) (Zey & McCammon, 1989; McCammon & Zey, 1990; Zey & Bloom, 1990). The average typical exposures for the operators at the polymerization reactor were 0.9–1.6 ppm [0.4–3.4 mg/m^3], based on more than 450 samples in each plant. Individual measurements were as high as 62 ppm [135 mg/m^3]. Respirators were

Table 3. Full-shift personal occupational exposures in the United States

Process	Job	No. of samples	Mean (mg/m^3)	Range (mg/m^3)	Time period	Reference
Monomer	Monomer production	638	1.1	NG–66	1977–86	Zey *et al.* (1990a)
		110	1.6	0.02–21	1978–86	Zey *et al.* (1990b)
		148	2.5	0.02–81	1977–86[a]	Zey *et al.* (1989)
		254	1.0	NG–25	1977–79, 1986	Zey & McCammon (1990)
	Maintenance	605	0.4	NG–18	1977–86	Zey *et al.* (1990a)
		23	0.6	0.2–1.7	1978–81, 1986	Zey *et al.* (1990b)
		928	2.2	0.04–64	1977–86[a]	Zey *et al.* (1989)
		357	0.7	NG–54	1977–79, 1986	Zey & McCammon (1990)
	Loader	114	5.6	NG–102	1977–86	Zey *et al.* (1990a)
		127	2.8	0.2–39	1978–86	Zey *et al.* (1990b)
		123	12.8	0.04–595	1977–86[a]	Zey *et al.* (1989)
		9	1.2	NG–3.5	1977–79	Zey & McCammon (1990)
Fibre	Polymer	488	3.4	0.4–35	1978–86	Zey & McCammon (1989)
	Dope	13	0.8	0.4–1.6	1978–88	Zey & McCammon (1989)
	Polymer	512	1.9	NG	1977–86	Zey & Bloom (1990)
	Dope, spinning	997	2.0	NG	1977–86	Zey & Bloom (1990)
	Cutting, baling	40	0.6	NG	1978–80, 1986	Zey & Bloom (1990)
	Polymer	645	3.0	NG–96	1979–86	McCammon & Zey (1990)
	Maintenance	7	0.5	0.2–0.8	1986	Zey & McCammon (1989)
		37	1.5	NG	1979–86	Zey & Bloom (1990)
		58	1.0	NG–46	1979–86	McCammon & Zey (1990)
	Tank-farm	93	1.2	0.4–24	1978–83, 86	Zey & McCammon (1989)
		23	1.3	NG	1980–86	Zey & Bloom (1990)

Table 3 (contd)

Process	Job	No. of samples	Mean (mg/m^3)	Range (mg/m^3)	Time period	Reference
Resin	Resin production	126	2.1	0.2–98	1978–86	Bloom & Zey (1990)
	Polymer production	645	0.6	0.2–30	1978–86	McCammon & Zey (1990)
	Compounding	196	0.2	0.2–1.5	1978–86	Bloom & Zey (1990)
	Maintenance	569	0.7	0.2–157	1978–86	Bloom & Zey (1990)
	Tank farm	30	0.5	0.2–11	1978–86	Bloom & Zey (1990)
Adiponitrile	Adiponitrile production	218	1.1	NG–13	1979–86	McCammon & Zey (1990)
Acrylamide	Acrylamide production	77	2.1	0.04–35	1977–86	Zey *et al.* (1989)

NG, not given

[a] For some workers who were wearing respirators, the actual exposure will have been lower than the measured value.

worn in some cases. The dope and spinning operators had exposures averaging below 1 ppm [2.2 mg/m^3]. Lower exposures for these workers occurred in the plants that dried the polymer before the spinning operation, resulting in a lower monomer content in the polymer. Higher exposures occurred in the other plant which had a continuous wet operation without the drying stage. Exposure of maintenance workers averaged 0.2–0.7 ppm [0.5–1.5 mg/m^3]. Tank-farm operators, who are likely also to unload acrylonitrile monomer from trucks, rail cars or barges, had homogeneous exposure levels (0.6–0.7 ppm [1.3–1.5 mg/m^3]) across these plants, as did the laboratory technicians (0.1–0.4 ppm [0.22–0.87 mg/m^3]) (not shown in Table 3).

Estimates of historical exposures were developed for these same plants (Blair *et al.*, 1998; Stewart *et al*., 1998). Using the measurements described above (Zey & McCammon, 1989; McCammon & Zey, 1990; Zey & Bloom, 1990), the average estimate for the polymer reactor operators' TWA_8 exposure in the 1950s and 1960s was about 7 ppm [15 mg/m^3] in one plant and around 15–20 ppm [33–44 mg/m^3] in the other two fibre plants. These levels fell to 3–9 ppm [6.5–19.5 mg/m^3] in the 1970s.

Other chemicals present in the fibres operation or in other operations on sites of the companies in the epidemiological study by Blair *et al.* (1998) include adiponitrile, hexamethylenediamine, polyester, polystyrene, vinyl acetate, *N*,*N*-dimethylacetamide, titanium dioxide, propionitrile, methyl methacrylate, zinc chloride, dyes and vinyl bromide (McCammon & Zey, 1990; Zey & McCammon, 1989; Zey & Bloom, 1990).

Other reports identifying measurement levels in Japan, Canada and Europe did not specify either the duration of the measurement or whether it was a personal or area evaluation. In Japan, Sakurai and Kusumoto (1972) reported that workers in five fibre plants could be divided into groups: one exposed to less than 5 ppm [11 mg/m^3] and the other to less than 20 ppm [44 mg/m^3]. In 1978, the same authors reported personal exposures for six fibre plants of 0.1 ppm [0.2 mg/m^3] (n = 11; 2 plants), 0.5 ppm [1.1 mg/m^3] (n = 37; geometric standard deviation (GSD) = 4.9; 3 plants) and 4.2 ppm [9.1 mg/m^3] (n = 14; GSD = 1.7, 1 plant) (Sakurai *et al*., 1978). Spot area samples gave average levels of 2.1 ppm [4.6 mg/m^3] (n = 116; 2 plants), 7.4 ppm [16 mg/m^3] (n = 394; 3 plants) and 14.1 ppm [31 mg/m^3] (n = 98; 1 plant). Some of the samples showed levels exceeding 100 ppm [220 mg/m^3]. The levels were believed to be representative of typical exposures; past exposures were thought to have been much higher. A third report by these authors indicated that the averages of area samples of two groups of fibre workers were 0.27 ppm (n = 62) and 0.87 ppm (n = 51) [0.59 and 1.9 mg/m^3] (Sakurai *et al*., 1990). These were considered by the authors to be comparable to personal exposures.

In Canadian fibre plants, personal TWA_8 levels were less than 1 ppm [2.2 mg/m^3] for unloading, reactors, wet spinning, maintenance and cleaning and processing in 1980 (Guirguis *et al*., 1984). [These measurements were identified as personal TWA_8 by the authors. It was not clear, however, how the measurements relate to workers' exposures, since they were identified by area descriptors].

In a French fibre plant, short-term measurements (< 2 h) were taken at the grinding (13.5 ppm; range, 2–28 ppm [29; 4.4–61 mg/m^3]), drying (3.4 ppm; 1–7 ppm [7.4;

2.2–15 mg/m^3]) and wringing stations (15.8 ppm; 3–46 ppm [34; 6.5–100 mg/m^3]) (Cicolella *et al*., 1981). These three stations each had local exhaust, but it was identified as being insufficient. An unspecified job had a mean exposure of 3 ppm [6.5 mg/m^3]. Cleaning reactors resulted in exposures of 19 ppm [41 mg/m^3]. A loader had a one-hour measurement of 33 ppm [72 mg/m^3] during loading and 19.8 (1–140 ppm) [43; 2.2–304 mg/m^3] during two disconnections. Sample collection from the reactor was found to result in an average exposure level of 1.7 ppm (0.6–3.4 ppm) [3.7; 13–7.4 mg/m^3] for 10 min.

An average of 3.5 ppm (range, 1.4–9.3 ppm) [7.6; 3–20 mg/m^3] in a Malon production unit was reported in 1973 in Yugoslavia (Orusev *et al*., 1973), and levels of 2.0–3.3 ppm [4.4–7.2 mg/m^3] in the summer and > 0.2 ppm [0.44 mg/m^3] in the winter in Bulgaria in 1976 were reported (Gincheva *et al*., 1977). Levels below 2 ppm [4.4 mg/m^3] were reported in a fibre plant in Portugal (Borba *et al*., 1996).

(*c*) *Resin production*

Personal, full-shift exposures, as measured by the companies and the study investigators, in two resin plants in the United States have been reported (Table 3). At a facility making Barex resin (an acrylonitrile–butadiene resin), the average exposure of the resin operators was about 1 ppm [2.1 mg/m^3], with individual measurements up to 45 ppm [98 mg/m^3] (Zey *et al*., 1990b). The other facility made acrylonitrile–butadiene–styrene (ABS) and styrene–acrylonitrile (SAN) resins and dispersions (Bloom & Zey, 1990). Exposure of resin operators averaged 0.26 ppm [0.6 mg/m^3], with levels up to 14 ppm [30 mg/m^3], while the compounders had lower levels (0.1 ppm [0.2 mg/m^3]). The average for maintenance workers in this plant was about 0.3 ppm [0.7 mg/m^3] and in the tank farm (unloading) about 0.2 ppm [0.5 mg/m^3]. Laboratory technicians had an average exposure of 2.2 ppm [4.8 mg/m^3] (not shown in Table 3). Estimates of exposures in the latter plant were developed as described above (Blair *et al*., 1998; Stewart *et al*., 1998). Based on these data, the TWA_8 exposure for a production labourer in the resin unit was about 7 ppm [15 mg/m^3] in the 1960s and fell to about 3 ppm [6.5 mg/m^3] in the 1970s.

Other chemicals present in the resins operation or in other operations on site in the resin company evaluated in the epidemiological study by Blair *et al*. (1998) include butadiene, styrene, formaldehyde, melamine, maleic anhydride, phosphoric acid and phenol (Zey *et al*., 1990b).

In other reports, it was not clear whether the levels reported were full-shift evaluations or whether they represented personal or area exposures. In Canadian ABS plants, personal TWA_8 exposures were about 1.5 ppm [3.3 mg/m^3] at the reactors, about 1 ppm [2.2 mg/m^3] at the coagulation and drying, compounding and control room areas and about 0.5–0.7 ppm [1.1–1.5 mg/m^3] in the laboratory and during sample-taking and maintenance and cleaning in 1980 (Guirguis *et al*., 1984). [These measurements were identified as personal TWA_8 by the authors. It is not known how the measurements relate to workers' exposures, as they were identified by area descriptors].

In a plastics plant in the Netherlands, a reactor operator performing maintenance work had exposures of 0.3 and 1.8 ppm [0.65 and 3.9 mg/m^3] (Houthuijs *et al*., 1982). The panel operator had a weekly mean of 0.02 ppm [0.04 mg/m^3]. For five unspecified workers, average exposure was about 0.1 ppm [0.2 mg/m^3] (standard deviation (SD), 0.05; range, < 0.02–0.15 ppm) [SD, 0.11; range, 0.04–0.33 mg/m^3] on days when respirators were not in use, and 0.8 ppm [1.7 mg/m^3] (SD, 0.67; range, 0.03–1.8 ppm) [SD, 0.67; range, 0.07–3.9 mg/m^3] when respirators were being worn.

In another study, personal samples were taken of all workers in the polymerization and flocculation process areas of a plant in the United States, where the highest exposure was expected, and of some employees in other areas of the plant (Kutcher, 1978). The sampling duration was 4–6 h and conditions were considered representative of normal conditions. One outside reactor operator had a TWA of 2.9 ppm (n = 12; 0.6–4.3 ppm; total duration, 16.7 h) [6.3; 1.3–9.3 mg/m^3] and another had a TWA of 0.7 ppm [1.5 mg/m^3] (n = 12; total duration, 16.5 h). An inside reactor operator's measured exposure ranged from 0.1 to 0.4 ppm (n = 5). A latex handler had a TWA of 1.0 ppm (n = 12; 0.7–1.3 ppm; 14.7 h [2.2; 1.5–2.8 mg/m^3]). Three flocculation operators had a mean exposure of 3.1 ppm (n = 35; 0.7–9.3 ppm) [6.7; 1.5–20 mg/m^3] and a Banbury operator's measured exposures ranged from 0.1 to 1.2 ppm [0.2–2.6 mg/m^3] (n = 3). A mill operator's exposures ranged from 0.1 to 1.1 ppm [0.2–2.4 mg/m^3] (n = 8) and a packer's from 0.1 to 1.0 ppm [0.2–2.2 mg/m^3] (n = 5). A tank-farm operator had a single measurement of 0.1 ppm [0.2 mg/m^3]. Two laboratory technicians' measurements were 0.1 and 0.2 ppm [0.2 and 0.4 mg/m^3]. During reactor maintenance, acrylonitrile levels of 0.3–0.8 ppm [0.65–1.7 mg/m^3] (n = 6) were found.

In the manufacture of SAN and ABS and polymer dispersions (and also of chemical intermediates) under normal conditions, spot measurements of 5 ppm [11 mg/m^3] were found during 1963–74, and it was assumed that higher levels occurred under some conditions. In 1975–77, monthly readings averaged 1.5 ppm [3.3 mg/m^3] (Thiess & Fleig, 1978). In ABS factories in France, short-term (< 2 h) area measurements averaged 13.5 ppm (0–89 ppm) [29; 0–193 mg/m^3] at an open, unventilated polybutadiene loading operation (Cicolella *et al*., 1981). In the flocculation area, where local exhaust was insufficient, the average level was 2.3 ppm (0.6–4.4 ppm) [5.6; 1.3–9.5 mg/m^3]. Below the reactor during cleaning, the average level was 2.6 ppm (0.8–2.9 ppm) [5.6; 1.7–6.3 mg/m^3]. At an acrylic dispersion plant, loading of an acrylonitrile tank resulted in a mean level of 35.7 ppm (0.4–281 ppm) [77; 0.87–610 mg/m^3]. Workers at Canadian acrylic emulsion facilities in 1980 had personal TWA_8 values of less than 1 ppm [2.2 mg/m^3] at the unloading, reactor and packaging areas and where the product was being used (Guirguis *et al*., 1984). [These measurements were identified as personal TWA_8 by the authors. It is not known how they relate to workers' exposures, as the measurements were identified by area descriptors.]

(*d*) *Rubber and polymer production*

TWA_8 measurements in Canadian nitrile rubber plants averaged 2 ppm [4.4 mg/m^3] at the reactors and in maintenance and cleaning operations, 1.6 ppm [3.5 mg/m^3] at the

coagulation and drying area and 1 ppm [2.2 mg/m^3] during sample taking (Guirguis *et al*., 1984). [These measurements were identified as personal TWA_8 by the authors. It is not known how they relate to workers' exposures as they were identified by area descriptors.]

Short-term (< 6 h) measurements of nitrile rubber workers revealed average levels of 0.1–5.8 ppm [0.2–13 mg/m^3], ranging up to 12.1 ppm [26 mg/m^3] where equipment was hooded but insufficiently ventilated (Cicolella *et al*., 1981). Seven-hour samples at the flocculation area showed levels of 0.4 and 2.2 ppm [0.9 and 4.8 mg/m^3], and at the drying area 0.5 ppm [1.1 mg/m^3] was found. Cleaning the stripper for 3–6 h resulted in levels up to 30 ppm [65 mg/m^3]. The mean for two different individuals collecting samples for 15–20 min were 9.6 and 31.3 ppm [21 and 68 mg/m^3], with measurements up to 78 ppm [170 mg/m^3]. During two 30-min connections for loading or unloading in open air, levels of 1 and 14 ppm [2.2 and 30 mg/m^3] were measured.

Production of butadiene–styrene footwear was found to result in levels of 0.5–5.1 ppm [1.1–11 mg/m^3] acrylonitrile in Russia (Volkova & Bagdinov, 1969). In another rubber footwear plant in the Soviet Union, the mean ambient air concentration of 32 samples was 0.1 ppm (standard error, 0.03) [0.2; SE, 0.065 mg/m^3] over a range of < 0.002–1.0 ppm [0.004–2.2 mg/m^3] (Solionova *et al*., 1992). Seventeen of the samples were below the limit of detection of 0.002 ppm [0.004 mg/m^3]. In a company manufacturing rubber tyres and tubes, levels from two area samples under a Banbury mill were 0.15 ppm [0.33 mg/m^3] acrylonitrile and below the detectable level (Anon., 1976).

(*e*) *Organic chemical synthesis*

Full-shift personal samples on operators in an adiponitrile production unit gave an average of 0.5 ppm [1.1 mg/m^3] acrylonitrile (n = 218; up to 6.1 ppm [13.2 mg/m^3]) (Table 3) (McCammon & Zey, 1990). In an acrylamide operation, the average of the full-shift measurements on the production operators was 1.0 ppm (n = 77; 0.02–16.2 ppm) [2.2; 0.04–35 mg/m^3] (Zey *et al.*, 1989). Supporting operations for both the adiponitrile and acrylamide operations (i.e., maintenance, quality control and unloading) are included in the monitoring data reported for the fibre and monomer operations, respectively (Zey *et al.*, 1989; McCammon & Zey, 1990).

(*f*) *Miscellaneous*

In a thermosetting plastics plant, acrylonitrile levels at a moulding operation were found to be 0.6 ppm [1.3 mg/m^3] (Scupakas, 1968). No acrylonitrile was found at two injection moulding operations of ABS thermoplastics (n = 2) (Anon., 1978, 1980). In an aircraft manufacturer making moulded fibrous glass and plastic (ABS) components, two personal full-shift (6.5 h) samples gave levels of < 0.8 ppm [1.7 mg/m^3] for the oven operator. Two area samples of 3–4 h had levels of less than 1 and less than 1.4 ppm [2.2 and 3 mg/m^3] (Anon., 1979). It was reported that in Canada, minor uses of acrylonitrile, such as coating manufacture, typically resulted in acrylonitrile levels of less than 2 ppm [4.4 mg/m^3] and most were less than 1 ppm [2.2 mg/m^3] (Guirguis *et al*., 1984).

1.3.3 *Air*

Acrylonitrile has not been detected to occur at measurable concentrations in ambient air. Measurable levels of atmospheric acrylonitrile are associated with industrial sources. Mean 24-h acrylonitrile concentrations in atmospheric samples collected within 5 km of factories producing or using acrylonitrile ranged from less than 0.1 to 325 μg/m^3. The occurrence of acrylonitrile was correlated with wind patterns; the highest concentrations were downwind of and in close proximity to the plant. The median concentration of acrylonitrile for 43 measurements in 'source-dominated areas' (i.e., near chemical plants) was 2.1 μg/m^3 (Agency for Toxic Substances and Disease Registry, 1990).

In 1995, industrial releases of acrylonitrile to the environment, as reported to the Toxic Chemical Release Inventory of the United States Environmental Protection Agency, totalled about 2940 tonnes, including 2360 tonnes to underground injection sites and 576 tonnes to the atmosphere (United States National Library of Medicine, 1997a).

1.3.4 *Water*

Acrylonitrile is not a common contaminant of typical surface water or groundwater. In a state-wide survey of 1700 wells in Wisconsin, United States, acrylonitrile was not detected in any sample. Acrylonitrile was detected in 46 of 914 samples of surface water and groundwater taken across the United States, generally at levels less than 10 ppb (μg/L). Levels of acrylonitrile measured in the effluents from a variety of industrial sites (iron and steel factories, textile mills, chemical plants) have ranged from 20 to 4700 ppb (μg/L), resulting in concentrations in nearby rivers ranging from below detection limits to 4300 ppb (μg/L) (Agency for Toxic Substances and Disease Registry, 1990).

1.3.5 *Other*

Residual acrylonitrile has been detected in a limited number of samples of commercial polymeric materials derived from acrylonitrile (United States Consumer Product Safety Commission, 1978); however, current processes for fibre and polymer production are believed to have reduced residual levels significantly (Agency for Toxic Substances and Disease Registry, 1990).

Acrylonitrile has been detected in the smoke of cigarettes at levels of 3.2–15 mg per cigarette (IARC, 1986; Byrd *et al.*, 1990).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for acrylonitrile in several countries are given in Table 4.

Table 4. Occupational exposure limits and guidelines for acrylonitrile[a]

Country	Year	Concentration (mg/m^3)	Interpretation[b]
Australia	1991	4.3 (C2, sk)	TWA
Belgium	1991	4.3 (C2, sk)	TWA
Czechoslovakia	1991	0.5	TWA
		2.5	STEL
Denmark	1993	4 (C, sk)	TWA
Egypt	1993	4.3 (sk)	
Finland	1998	4.3 (sk)	TWA
		9	STEL (15 min)
France	1993	4.3 (C)	TWA
		32.5	STEL (15 min)
Germany	1998	7 (C2)	TRK
Hungary	1991	0.5 (C2, sk)	STEL
India	1993	4.3 (C, sk)	TWA
Japan	1991	4.3 (C2, sk)	TWA
The Netherlands	1993	9 (sk)	TWA
		22	STEL (10 min)
The Philippines	1993	43 (sk)	TWA
Poland	1993	10	TWA
Russia	1991	0.5 (sk)	STEL
Sweden	1991	4.3 (C3, sk)	TWA
		13	STEL
Switzerland	1991	4.3 (C, sk)	TWA
Turkey	1993	43 (sk)	TWA
United Kingdom	1991	4 (MEL, sk)	TWA
United States			
ACGIH (TLV)[c]	1997	4.3 (A2, sk)	TWA
NIOSH (REL)	1997	2.2 (Ca, sk)	TWA
		21.5	Ceiling
OSHA (PEL)	1996	4.3 (sk)	TWA
		43	Ceiling

[a] From International Labour Office (1991); US Occupational Safety and Health Administration (OSHA) (1996); American Conference of Governmental Industrial Hygienists (ACGIH) (1997a,b); US National Library of Medicine (1997b); Deutsche Forschungsgemeinschaft (1998); Ministry of Social Affairs and Health (1998)

[b] TWA, time-weighted average; STEL, short-term exposure limit; TRK, technical exposure limit; MEL, maximum exposure limit; REL, recommended exposure limit; PEL, permissible exposure limit; TLV, threshold limit value; min, minute; A2, suspected human carcinogen; C, suspected of being a carcinogen; C2, probable human carcinogen; C3, suspected of having a carcinogenic potential; Ca, potential occupational carcinogen; sk, skin notation

[c] Countries that follow the ACGIH recommendations for threshold limit values include Bulgaria, Colombia, Jordan, Korea (Republic of), New Zealand, Singapore and Viet Nam

2. Studies of Cancer in Humans

Epidemiological data on acrylonitrile have been reviewed by Koerselman and van der Graaf (1984), Rothman (1994) and Blair and Kazerouni (1997). These reviews were prepared before the publication of several recent reports.

Cohort studies (Table 5)

Kiesselbach *et al*. (1979) conducted a cohort mortality study of 884 male employees at a Bayer AG plant in North Rhine–Westphalia, Germany, who had handled acrylonitrile for at least one year between 1950 and 1 August 1977, in either production or processing. Mortality among these workers through 1 August 1977 was compared with rates for the male population of North Rhine–Westphalia for 1950–77. Sixty workers (6.8%) could not be traced to the closing date of the study. Duration of employment was used as a measure of exposure. The authors indicated that some of the workers also handled butadiene and styrene. Fifty-eight deaths occurred [overall standardized mortality ratio (SMR) 0.5; 95% confidence interval (CI), 0.4–0.7]. The SMR for total cancer was [1.0; n = 20; 95% CI, 0.6–1.5] and the proportionate mortality ratio (PMR) was [1.9]. The SMRs for specific cancers were [0.9] (6 observed, 6.7 expected) [95% CI, 0.3–2.0] for the respiratory tract and [1.3] (4 observed, 3 expected) [95% CI, 0.4–3.4] for stomach.

Thiess *et al*. (1980) evaluated the mortality experience of 1469 workers employed for at least six months at 12 acrylonitrile-using plants in Germany. The cohort was followed through 15 May 1978 for vital status. Tracing of German workers (n = 1081) was very successful (98%), but less so for foreign workers (n = 388) (56%). Mortality in the cohort was compared with local, regional and national mortality rates. SMRs presented here are based on national rates. The follow-up included 15 350 person–years and 89 deaths. Exposure levels were not estimated. The SMRs were [1.3] (27 observed, 20.5 expected) [95% CI, 0.9–1.9] for all cancers, [1.96] (11 observed, 5.9 expected) [95% CI, 0.9–3.3] for lung cancer and [2.3] (4 observed, 1.7 expected) [95% CI, 0.6–6.0] for lymphatic and haematopoietic cancers. SMRs for lung cancer by duration of employment were [2.2] for less than five years (2 observed, 0.9 expected), [3.9] for five to nine years (4 observed, 1.0 expected), and [2.2] for 10 or more years (3 observed, 1.3 expected).

Ott *et al.* (1980) performed a cohort mortality and incidence study of 2904 workers exposed to styrene-based products at four plants of the Dow company and included 100 workers exposed to acrylonitrile. The cohort was followed from 1 January 1940 to 31 December 1975. Mortality rates for the cohort were compared with those in the population of the United States. Vital status was determined for all but 88 (3%) of the workers. Among the acrylonitrile workers, there were one observed death from lung cancer versus 0.5 expected and three cases of leukaemia versus 1.25 expected.

Waxweiler *et al*. (1981) studied 4806 workers employed at a vinyl chloride monomer (IARC, 1987b) plant in the United States between 1942 and 31 December 1973. The

Table 5. Summary of non-overlapping epidemiological studies of workers exposed to acrylonitrile (SMR and 95% CI)

Reference, country	Standardized mortality ratio (observed/expected number)							
	Stomach	Lung	Breast	Brain	Prostate	Lymphatic/ haematopoietic	Liver	Bladder
Kiesselbach *et al*. (1979), Germany	[1.3] (4/3)	[0.9] (6/7) respiratory tract	NA	NA	NA	NA	NA	NA
Thiess *et al*. (1980), Germany	NA	[2.0] (11/5.5)	NA	NA	NA	[2.4] (4/1.6)	[0.8] (1/1.3)	[3.3] (2/0.6)
Delzell & Monson (1982), United States		1.5 (9/5.9)	NA	NA	NA	2.3 (4/1.8)	NA	NA
Mastrangelo *et al*. (1993), Italy	[3.4] (2/0.6)	[0.8] (2/2.6)	NA	[2.5] (1/0.4)	NA	NA	[10.5] (4/0.4)	NA
Swaen *et al*. (1998), Netherlands	0.3 (2/6.7)	1.1 (47/42.8)	NA	1.7 (6/3.4)	0.8 (4/4.8)	NA	1.3 (9/7.1)	1.0 (3/3.1)
Wood *et al*. (1998) (using Du Pont mortality rates), United States	0.5 (3/[6])	0.8 (46/[57.5])	NA	1.1 (6/[5.4])	1.3 (11/[8.5])	0.6 (9/[15])	0.6 (2/[3])	1.2 (4/[3.5])
Blair *et al*. (1998) (using unexposed workers as referents), United States	1.1 (12/10.9)	1.2 (134/111.7)	0.6 (5/8.3)	0.5 (12/24.0)	1.0 (16/16.0)	0.7 (27/38.6)	NA	NA
Benn & Osborne (1998), United Kingdom	1.0 (11/11.4)	1.0 (53/51.5)	NA	NA	NA	0.5 (5/10.0)	1.3 (11/8.8)	NA

NA, not available

cohort was followed for mortality through 1973 with only 73 (1.5%) lost to follow-up. Information on race was not available, but all were assumed to be white because the company indicated that less than 2% of the workforce was non-white. Mortality rates in the cohort were compared with those of the general population of the United States, adjusted for age and calendar time. Company personnel estimated exposures on an ordinal scale from one to five for some 20 chemicals including acrylonitrile. The serially additive expected dose (SAED) model was also used in the analyses (Smith *et al.*, 1980). Cumulative exposure among cases was compared with that of other employees who were under follow-up when the case occurred. Histological information from medical and pathology records was sought on the 45 deaths from lung cancer, obtained for 27 and reviewed by a panel of pathologists. The observed dose of acrylonitrile was lower among the lung cancer cases than among other employees. [Details on the work situation resulting in acrylonitrile exposure were not available and it was not possible to compare SAED results with those from other studies].

Mortality associated with acrylonitrile exposure was evaluated as part of a study of 15 643 male workers in a rubber plant in the United States (Akron, Ohio) (Delzell & Monson, 1982). Included in the analysis were 327 workers who were employed for at least two years in the plant between 1 January 1940 and 1 July 1971, and who had worked in two departments where acrylonitrile was used, i.e., 81 worked only in the nitrile rubber manufacturing operation where exposures to 1,3-butadiene (see this volume), styrene (IARC, 1994a) and vinylpyridine also occurred and 218 only in the department where the latex was coagulated and dried. [No information on levels of exposure to acrylonitrile was provided.] Mortality among these workers was assessed through 1 July 1978 and compared with age- and calendar-time-specific rates for white men in the United States. SMRs were 0.8 (n = 74; 95% CI, 0.7–1.0) for all causes of death, 1.2 (n = 22; 95% CI, 0.8–1.9) for all cancers combined, 1.5 (n = 9; 95% CI, 0.7–2.9) for lung cancer, 4.0 (n = 2; 95% CI, 0.5–14.5) for urinary bladder cancer and 2.3 (n = 4; 95% CI, 0.6–5.8) for cancers of the lymphatic and haematopoietic system. SMRs for lung cancer by duration of employment were [1.0] (4 observed, 3.8 expected) [95% CI, 0.3–2.7] for < 5 years, and [3.3] (5 observed, 1.5 expected) [95% CI, 1.1–7.8] for 5–14 years. No case was observed with duration ≥ 15 years.

Mastrangelo *et al*. (1993) studied a factory in Italy engaged in the manufacture of acrylic fibre for clothing and upholstery using acrylonitrile produced elsewhere. The cohort consisted of 671 workers employed for at least 12 months from the opening of the factory in 1959 through 1988. Follow-up for vital status was through 1990 (no individuals were lost to follow-up). Information on smoking was obtained from medical records. Exposure categories included polymerization (high exposure), fibre manufacture (low exposure) and maintenance (high but discontinuous exposure). [No information on exposure levels was provided.] SMRs were based on general population rates in the Veneto region, adjusted for age, sex and calendar time. SMRs were [1.0] (32 observed) [95% CI, 0.7–1.4] for all causes of death, [3.4] (2 observed) [95% CI, 0.4–12.3] for stomach cancer, [0.8] (2 observed) [95% CI, 0.1–2.9] for lung cancer and [2.6]

(1 observed) [95% CI, 0.1–14.7] for brain cancer. Both lung cancers were among maintenance workers exposed for less than 10 years.

Benn and Osborne (1998) enrolled 3013 male workers employed between 1950 and 1978 in any of six acrylonitrile polymerization and acrylic fibre plants in the United Kingdom. This was an expansion and extended follow-up of a cohort of 1111 workers reported by Werner and Carter (1981). The cohort was expanded by the inclusion of 2498 additional workers employed in polymerization or spinning between 1969 and 1978. Exclusion of 85 workers who could not be traced and 165 workers employed for less than one year left 2763 available for analysis. Exposures were estimated by job and categorized by acrylonitrile exposure and no acrylonitrile exposure. Following discussion with the company personnel, a number of workers from one plant (plant 5) who were classified as spinners in the original study and presumed to have relatively high exposures were reclassified as 'end of the line' workers with minimal or no exposure. [No explanation was provided for why this change was necessary.] The earliest exposure measurements (from the late 1970s) showed means of 0.4–2.7 ppm [0.9–5.9 mg/m^3] (8-h time-weighted average (TWA_8) for the highly exposed polymer workers and spinners. Expected numbers of deaths were derived from rates in the general population in England and Wales, except for a factory in Scotland, for which Scottish rates were used. The cohort contributed 63 058 person–years, of which 72% were from plant 5. SMRs for the cohort were 0.8 (n = 409) [95% CI, 0.76–0.9] for total mortality, 0.9 (n = 121) [95% CI, 0.7–1.1] for total cancer, 1.0 (n = 11) [95% CI, 0.5–1.7] for stomach cancer, 1.0 (n = 53) [95% CI, 0.8–1.3] for lung cancer and 0.5 (n = 5) [95% CI, 0.2–1.2] for lymphatic and haematopoietic cancer. Plant 5 showed deficits for various causes of death, while the other factories combined showed nonsignificant excesses. [This is of concern given the previous problems with assembling the cohort at plant 5.] Persons holding high-exposure jobs had SMRs of 1.7 (n = 7) [95% CI, 0.7–3.4] and 1.4 (n = 27) [95% CI, 0.9–2.1] for stomach and lung cancer, respectively. SMRs among the less exposed and little or not exposed groups were 1.0 (n = 3) [95% CI, 0.2–3.0] and 0.2 (n = 1) [95% CI, 0.0–1.3] for stomach cancer and 0.5 (n = 7) [95% CI, 0.2–1.1] and 1.0 (n = 19) [95% CI, 0.6–1.6] for lung cancer. No duration–response gradient was observed for any cancer. The authors reported that there were five deaths (versus 0.8 expected) from lung cancer (SMR, 6.1 [95% CI, 2.0–14.6]) among workers holding high-exposure jobs and under 45 years of age and an SMR of 2.7 (n = 7 [95% CI, 1.1–5.5]) among those first employed after 1969 who held high-exposure jobs.

Wood *et al*. (1998) studied 2559 male employees exposed to acrylonitrile during Orlon manufacture at Du Pont plants in South Carolina and Virginia, United States. This report subsumed workers previously included in cohorts reported by O'Berg (1980), O'Berg *et al.* (1985) and Chen *et al.* (1987, 1988a), plus new workers exposed to acrylonitrile at these plants since the earlier studies. Mortality was updated through 1991 using the National Death Index and Social Security Administration files. Cancer cases were identified among cohort members during employment using the Du Pont Cancer Registry which records diagnoses among active Du Pont employees. A

common exposure assessment procedure was applied to the work histories of individuals from the two plants. Exposure assessments by job title and work area were in ppm for a 40-h working week and were based on the monitoring data available, process descriptions, production records, work practices and information from employees. Estimates were placed in four exposure categories with arithmetic midpoint values of 0.11 ppm, 1.10 ppm, 11.0 ppm and 30.0 ppm [0.24, 2.4, 24 and 65 mg/m^3]. Cumulative exposures were derived as the summed products of these endpoint values and the time spent in each category and were classified as < 10 ppm–years (n = 879), 10–< 50 ppm–years (n = 746), 50–< 100 ppm–years (n = 391) and ≥ 100 ppm–years (n = 553). SMRs were calculated using mortality rates for the general United States population and deaths among active employees and retirees of the Du Pont company as the referent. Standardized incidence ratios (SIRs) were based on Du Pont cancer incidence rates. The cohort was composed of 2559 workers (1426 in the South Carolina plant and 1143 in the Virginia plant). Vital status as of 1991 was determined for all but 23 workers and death certificates were located for all but two of the 454 presumed decedents. SMRs (based on general population rates) for selected causes of death were 0.7 (n = 454; 95% CI, 0.6–0.8) for all causes, 0.8 (n = 126; 95% CI, 0.6–0.9) for all malignant neoplasms, 0.5 (n = 3; 95% CI, 0.1–1.5) for stomach cancer, 0.8 (n = 46; 95% CI, 0.5–1.0) for lung cancer, 1.1 (n = 6; 95% CI, 0.4–2.5) for brain cancer, 1.2 (n = 4; 95% CI, 0.3–3.0) for bladder cancer, 1.3 (n = 11; 95% CI, 0.6–2.3) for prostate cancer and 0.6 (n = 9; 95% CI, 0.3–1.1) for lymphatic and haematopoietic system cancer. SMRs were similar when mortality rates from Du Pont workers were used for comparison, with the all-cause mortality ratio still less than 1.0 (0.9; 95% CI, 0.8–1.0). Based on general population rates, the relative risk for death from prostate cancer showed an inverse association with latency, duration of exposure, highest ever exposure level and cumulative exposure. Mortality from respiratory cancer was typically less than expected by duration of exposure and across cumulative exposure categories; when analysed by highest ever exposure level, there were no deaths at the lowest level, SMR = 0.7 (n = 3) [95% CI, 0.1–2.0] for moderate level, SMR = 0.7 (n = 21) [95% CI, 0.4–1.1] for the high level and SMR = 1.2 (n = 23) [95% CI, 0.8–1.8] for the very high level. SIRs (based on Du Pont cancer rates) for selected cancers were 0.4 (n = 1; 95% CI, 0.0–2.4) for stomach cancer, 0.8 (n = 17; 95% CI, 0.5–1.3) for lung cancer, 1.6 (n = 12; 95% CI, 0.8–2.8) for prostate cancer, 1.1 (n = 4; 95% CI, 0.3–2.9) for brain cancer, 0.7 (n = 4; 95% CI, 0.2–1.8) for bladder cancer and [1.0] (n = 10; [95% CI, 0.5–1.8]) for lymphatic and haematopoietic cancers. For prostate cancer, the SIR was 0.8 (n = 4) [95% CI, 0.2–2.1] for < 20 years latency and 1.3 (n = 8; 95% CI, 0.6–2.4) for 20 or more years. SIRs for prostate cancer by cumulative exposure were 2.1 (n = 3; 95% CI, 0.4–6.2), 0.5 (n = 1; 95% CI, 0.0–2.8), 0.8 (n = 2; 95% CI, 0.0–2.9), and 2.3 (n = 4; 95% CI, 0.8–5.1) for low (< 10 ppm-years), moderate, high and very high categories of cumulative exposure, respectively. SIRs for respiratory cancer were generally less than 1.0 and showed no patterns by latency, duration, highest exposure level or cumulative exposure.

Swaen *et al*. (1998) studied eight companies in the Netherlands engaged in the production of acrylonitrile, latex rubber (IARC, 1987c), polymers, acrylic fibre, vinylidene/acrylonitrile polymers and acrylamide (IARC, 1994b) and a nitrogen-fixation plant where exposure did not occur. The vital status for this cohort has been recently extended through 1 January 1996 and only results from this recent follow-up are presented here. Criteria for inclusion of workers were exposure (or employment for the comparison plant) for six months before July 1979, male gender, Dutch nationality, residence in the Netherlands and no history of underground coal mining. Data for the study were abstracted by study investigators from the files of six companies, while two companies carried out the data abstraction themselves. The exposed cohort was composed of 2842 workers. Workers (n = 3961) from the nitrogen fixation plant were selected as an unexposed referent population. TWA_8 exposures by year were estimated for job groups with homogeneous exposures by the study industrial hygienist for seven plants. One company provided its own estimates because the study industrial hygienist was not allowed to visit the facility. Exposure estimates were placed in TWA_8 categories with ranges of 0–0.5 ppm, > 0.5–1 ppm, > 1–2 ppm and > 2–5 ppm [0–1.1, > 1.1–2.2, > 2.2–4.3 and > 4.3–10.9 mg/m^3]. No TWA_8 exposures were thought to be greater than 5 ppm. Peak exposures, use of respirators and potential exposure to other chemicals were also evaluated. Vital status was determined for all but 19 workers in the exposed cohort and all but 10 workers in the referent cohort. Cause of death could not be determined for nine decedents in the acrylonitrile cohort and 14 decedents in the referent cohort. SMRs for the exposed and unexposed cohorts were based on mortality rates of the Dutch male population, adjusted for age and calendar time. SMRs from the exposed and unexposed populations were 0.9 (n = 290; 95% CI, 0.8–1.0) and 0.8 (n = 983; 95% CI, 0.77–0.9) for total mortality, 0.9 (n = 97; 95% CI, 0.7–1.1) and 0.8 (n = 332; 95% CI, 0.7–0.9) for all cancers combined, 1.1 (n = 47; 95% CI, 0.8–1.5) and 0.8 (n = 124; 95% CI, 0.6–0.9) for lung cancer, 0.8 (n = 4; 95% CI, 0.2–2.1) and 0.5 (n = 13; 95% CI, 0.3–0.9) for prostate cancer, 1.7 (n = 6; 95% CI, 0.4–3.8) and 0.9 (n = 7; 95% CI, 0.3–1.8) for brain cancer, 1.00 (n = 3; 95% CI, 0.2–2.8) and 1.1 (n = 14; 95% CI, 0.6–1.8) for bladder cancer, and 1.7 (n = 5; 95% CI, 0.5–3.9) and 1.1 (n = 11; 95% CI, 0.6–2.0) for leukaemia. [The Working Group noted that direct comparisons between the exposed and unexposed cohorts were not performed, which might have yielded larger relative risks among the exposed.] SMRs for lung cancer by cumulative exposure were 1.0 (n = 5; 95% CI, 0.3–2.3) for < 1 ppm–year, 1.1 (n = 24; 95% CI, 0.7–1.6) for 1–10 ppm–years and 1.2 (n = 18; 95% CI, 0.7–1.9) for > 10 ppm–years (p for trend = 0.66). For leukaemia, there were no deaths in the lowest cumulative exposure category; SMRs were 0.6 (n = 1; 95% CI, 0.1–3.4) for 1–10 ppm-years and 4.4 (n = 4; 95% CI, 1.2–11.3) for > 10 ppm-years. SMRs for prostate and brain cancer showed no evidence of an exposure–response trend. No clear trend of SMRs with increasing latency was evident. SMRs for lung cancer by peak exposures were 1.2 (n = 15; 95% CI, 0.7–1.9) for no peak, 1.3 (n = 20; 95% CI, 0.8–2.0) for peaks < 10 ppm, 0.7 (n = 8; 95% CI, 0.3–1.2) for peaks 10–< 20 ppm and 1.6 (n = 4; 95% CI, 0.4–4.2) for peaks 20 ppm or greater. SMRs for lung cancer were 1.0

(*n* = 9; 95% CI, 0.5–1.9) among persons using respirators and 1.1 (*n* = 38; 95% CI, 0.8–1.5) among those not using respirators. The SMRs for lung cancer were 1.0 (*n* = 19; 95% CI, 0.6–1.5) among workers not exposed to other carcinogens and 1.2 (*n* = 28; 95% CI, 0.8–1.8) for those with potential exposure to other carcinogens.

Blair *et al*. (1998) conducted a cohort study of 25 460 workers from eight acrylonitrile-producing and -using plants in the United States to evaluate mortality by quantitative level of exposure. The study included 18 079 white men, 4293 white women, 2191 non-white men and 897 non-white women employed from the 1950s through 1983 and followed through 1989 to determine their vital status and cause of death. Vital status was determined for 96% of the cohort; 2038 were believed to be deceased, for which 1919 (94%) death certificates were found. Information on tobacco use was obtained for a sample of workers to assess the potential for confounding. Mortality rates for exposed workers (348 642 person–years) were compared with those for unexposed (196 727 person–years) workers in the cohort, using Poisson regression analysis to minimize the healthy worker effect. An external advisory committee reviewed and approved the protocol and provided guidance and advice to the investigators on all aspects of the study. Compared with rates in the general population of the United States, SMRs for unexposed and exposed were 0.7 (*n* = 702; 95% CI, 0.7–0.8) and 0.7 (*n* = 1217; 95% CI, 0.6–0.7) for total mortality and 0.9 (*n* = 216; 95% CI, 0.8–1.0) and 0.8 (*n* = 326; 95% CI, 0.7–0.9) for total cancer, respectively. Quantitative estimates of inhalation and dermal exposure to acrylonitrile were based on information from production procedures at the plants, interviews with management and labour, monitoring data from the companies, and monitoring conducted by the investigators specifically for the study (Stewart *et al*., 1998). Exposure to chemicals other than acrylonitrile occurred, but numbers of worker–years of exposure were small (≤ 55 000) compared with those for acrylonitrile (348 642). Relative risk (RR) analyses by various indicators of exposure including cumulative (ppm–years), average, peak, intensity, duration and lagged exposure revealed no elevated risk or trends for cancers of the stomach, brain, breast, prostate or lymphatic and haematopoietic system. Cumulative exposure categories were 0 (unexposed), 0.01–0.13 ppm-years, 0.14–0.57 ppm-years, 0.58–1.50 ppm-years, 1.5–8.0 ppm-years and > 8.00 ppm-years. Mortality from lung cancer was elevated among individuals in the highest quintile of cumulative exposure, compared with unexposed workers, and received special analytical attention. RRs and 95% CIs for lung cancer from the lowest to highest quintile of cumulative exposure were 1.1 (*n* = 27; 0.7–1.7), 1.3 (*n* = 26; 0.8–2.1), 1.2 (*n* = 28; 0.7–1.9), 1.0 (*n* = 27; 0.6–1.6) and 1.5 (*n* = 26; 0.9–2.4), respectively. Limiting analysis to 20 or more years since first exposure yielded RRs by quintile of 1.1 (*n* = 11; 0.6–2.2), 1.0 (*n* = 10; 0.5–2.1), 1.2 (*n* = 16; 0.6–2.2), 1.2 (*n* = 16; 0.6–2.1) and 2.1 (*n* = 21; 1.2–3.8). Analyses by duration, average, highest ever and lagged exposures yielded RRs for lung cancer similar to those seen for cumulative exposure, with the highest risks again occurring in the upper quintile. To evaluate RRs for lung cancer at a wider range of cumulative exposure, analyses were also conducted for decile categories. The RR did not continue to increase at higher levels and actually decreased

from the ninth (RR, 1.7) to the tenth (RR, 1.3) decile. There was concern about possible confounding from tobacco use. The RR for ever cigarette smokers compared to never smokers was 3.6 (95% CI, 1.6–8.2). Adjustment for 'ever cigarette use' in the case–cohort analysis changed the RR for lung cancer only slightly (RRs for lowest to highest quintile of cumulative exposure without lagging were 0.3 (n = 5; 95% CI, 0.1–1.0), 0.8 (n = 6; 95% CI, 0.3–1.8), 1.0 (n = 7; 95% CI, 0.4–2.4), 0.9 (n = 13; 95% CI, 0.4–1.9) and 1.6 (n = 9; 95% CI, 0.7–3.3)). Separate analyses for wage and salaried workers, long-term and short-term workers, fibre and non-fibre plants and by individual plants revealed no clear exposure–response pattern. Workers holding maintenance jobs were excluded in one analysis because of concern about exposure to asbestos. The RR in the upper quintile of cumulative exposure dropped from 1.5 (as in the total cohort) to 1.3 when these workers were excluded. There were no deaths from asbestosis or mesothelioma, however. A large proportion of the workers in the study by Collins *et al*. (1989) were also included in this investigation.

3. Studies of Cancer in Experimental Animals

In two unpublished studies described by Maltoni *et al*. (1987), inhalation exposure of male and female Sprague-Dawley rats to 80 ppm (174 mg/m^3) acrylonitrile for 6 h per day on five days per week for two years resulted in increased incidences of glial-cell tumours of the central nervous system (males and females), Zymbal gland tumours (males and females), mammary gland adenocarcinomas (females), small intestine tumours (males) and squamous-cell tumours of the tongue (males). Oral administration of up to 300 mg/L (ppm) acrylonitrile to Sprague-Dawley rats in the drinking-water for two years resulted in significant increased incidences of tumours of the forestomach, tongue, Zymbal gland and brain in males and of the mammary gland, Zymbal gland, tongue, forestomach and brain in females (see also Quast *et al.*, 1981). [The Working Group noted that full reports of these studies have not been published in the peer-reviewed scientific literature.]

3.1 Oral administration

Rat: Two groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were administered acrylonitrile [purity unspecified] at concentrations of 100 or 500 mg/L (ppm) in the drinking-water for life; a group of 51 males and 49 females served as controls. An additional group of 147 males and 153 females was administered drinking-water containing 500 mg/L (ppm) acrylonitrile and selected animals were killed periodically during the study. Body weights and survival were not reported, but were described as lower than those of controls at both doses. At the time of reporting, 215 rats from the 500-ppm dose groups which either died or were killed between months 6 and 18 had been evaluated microscopically for brain lesions. Forty-nine animals [sex not specified] had primary brain tumours described as "similar to ... astrocytomas or anaplastic

astrocytomas". Zymbal gland tumours, stomach and skin papillomas and brain tumours were more frequently seen in acrylonitrile-treated groups than in controls [incidences not given] (Bigner *et al*., 1986). [The Working Group noted the apparent marked brain tumour response in exposed rats, but also that the findings were preliminary; interpretation was hampered by incomplete reporting and a lack of information on control tumour rates.]

A group of 40 male and 40 female Sprague-Dawley rats, 10 weeks of age, was administered 5 mg/kg bw acrylonitrile (99.9% pure) by gavage in olive oil three times per week for 52 weeks. A similar group of 75 males and 75 females was administered olive oil alone and served as controls. Rats were allowed to live out their lifespan and received a complete gross necropsy and histological evaluation at death. Acrylonitrile exposure did not affect body weight gain or survival. No increase in the incidence of tumours at any site was observed (Maltoni *et al*., 1977, 1987). [The Working Group noted the single dose level and the short duration of exposure.]

3.2 Inhalation exposure

Rat: Groups of 30 male and 30 female Sprague-Dawley rats, 12 weeks of age, were exposed to 0, 5, 10, 20 or 40 ppm [0, 11, 22, 43 or 86 mg/m^3] acrylonitrile (99.9% pure) by whole-body inhalation for 4 h per day on five days per week for 52 weeks. Rats were allowed to live out their lifespan and received a complete gross necropsy and histological evaluation at death. Exposure to acrylonitrile did not affect body weight gain or survival. No significant increase in the incidence of tumours at any specific site was observed, but incidence of total benign and malignant tumours, as well as that of total malignant tumours, were increased over those in controls. Glial cell tumours of the brain occurred in two males and one female given 40 ppm, and one male and one female given 20 ppm but not in controls (Maltoni *et al*., 1977, 1987). [The Working Group noted the short duration of exposure and that rats may have tolerated exposure concentrations higher than 40 ppm.]

A group of 54 female Sprague-Dawley rats, 13 weeks of age, was exposed to 60 ppm [130 mg/m^3] acrylonitrile (99.9% pure) by whole-body inhalation for 4 h per day on five days per week for seven weeks. Twenty-two of these rats were pregnant at the start of the study and delivered litters. Male offspring were divided into groups of 67 (A) and 60 (B), and females into groups of 54 (A) and 60 (B). All groups were exposed for 4 h per day on five days per week during the first seven weeks after birth. Subsequently, the duration of exposure was increased to 7 h per day, and breeders and group A offspring were exposed on five days per week for 97 weeks. Group B offspring were similarly exposed for eight weeks. A group of 60 13-week-old female rats (24 pregnant) was exposed to air and served as controls. A total of 158 male and 149 female offspring also served as controls. Rats were allowed to live out their lifespan and received a complete gross necropsy and histological evaluation at death. Exposure to acrylonitrile did not affect body weight gain or survival. The number of rats bearing malignant tumours of any type was increased in all exposed groups. Incidence of malignant mammary tumours was

increased in female offspring exposed for 104 weeks (9/54 exposed versus 8/149 controls), as was that of extrahepatic angiosarcomas (3/54 exposed versus 0/149). The incidence of carcinomas of the Zymbal gland (10/67 exposed versus 2/158 controls) and of benign and malignant hepatocellular tumours (5/67 exposed versus 1/158 controls) was increased in male offspring exposed for 104 weeks. The incidence of glial cell tumours of the brain was increased in both male (11/67 versus 2/158) and female (10/54 versus 2/149) offspring exposed for 104 weeks (Maltoni *et al*., 1987).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

(*a*) *Inhalation exposure*

When volunteers were exposed to an atmosphere containing 20 mg/m^3 acrylonitrile for up to 4 h, retention in the respiratory tract was 46 ± 1.6%, which did not change significantly during the experimental period (Rogaczewska & Piotrowski, 1968).

Workers exposed to 0.13 ppm [0.3 mg/m^3] acrylonitrile vapour in a factory excreted unchanged acrylonitrile in their urine, the amounts being greatest at the end of the exposure period, declining rapidly thereafter until the beginning of the next workday. The amounts excreted in samples taken during the working week or on the following two days off ranged from 2 to 152 μg acrylonitrile/g creatinine (Houthuijs *et al.*, 1982).

(*b*) *Percutaneous absorption*

The percutaneous absorption rate of neat acrylonitrile applied to the forearm of four volunteers was 0.6 mg/cm^2/h (Rogaczewska & Piotrowski, 1968). Therefore, skin uptake of acrylonitrile can contribute to the body burden of this compound.

(*c*) *In-vitro reactions and metabolism in human tissue preparations*

Acrylonitrile is metabolized to the reactive cyanoethylene oxide (CEO) [also called glycidonitrile], mainly by CYP2E1, but also by other forms of human cytochrome P450. Incubation of CEO with human hepatic microsomes, but not cytosolic preparations, significantly increased its basal rate of hydration to the corresponding diol (0.69 nmol/min). This hydration activity was heat-sensitive and potently inhibited by 1,1,1-trichloropropene oxide (IC_{50}, 23 μM), indicating that epoxide hydrolase was the catalyst. The hydration of CEO catalysed by hepatic microsomes from six individuals showed normal saturation kinetics with K_m ranging from 0.6 to 3.2 mM and V_{max} from 8.3 to 18.8 nmol hydration products/min/mg protein. These data show that humans possess a detoxication pathway for CEO that is not active in rodents (see Section 4.1.2(*d*)) (Kedderis & Batra, 1993).

(*d*) *Biomonitoring of human exposure*

Although urinary assays afford a means of monitoring exposure to acrylonitrile, protein adducts are more toxicologically relevant biomarkers of exposure. Like many chemical carcinogens, acrylonitrile forms adducts with the N-terminal valine residues of haemoglobin, which can be cleaved by a modification of the Edman degradation and determined by mass spectrometric techniques, using stable labelled analogues as internal standards (Lawrence *et al*., 1996). This method has revealed far higher levels of the adduct (*N*-(2-cyanoethyl)valine; CEV) in factory workers exposed to acrylonitrile than in matched unexposed controls from the same factory. Exposed workers had CEV levels of 2.0–2.3 nmol/g globin, whereas in a group of control workers in the same factory, the values were 0.03 ± 0.09 nmol CEV/g globin. Levels of acrylonitrile exposure in the workplace were not reported. CEV levels in smoking mothers were about 0.2 nmol/g globin and 0.1 nmol CEV/g globin in their babies. Levels of CEV in nonsmokers were not detectable (Tavares *et al.*, 1996). Bergmark (1997) reported CEV levels of about 0.1 nmol CEV/g globin in smokers.

4.1.2 *Experimental systems*

(*a*) *Absorption and tissue distribution*

After oral administration to rats and mice, acrylonitrile is well absorbed from the gastrointestinal tract, giving rise to detectable amounts of unchanged compound and metabolites in the blood. The principal route of elimination is via the urine, between 77 and 104% of the dose being recovered, with less than 8% of the dose occurring in the faeces (Kedderis *et al*., 1993a). These data confirmed and extended those of Sapota (1982) in rats, which had similar excretion patterns when given 40 mg/kg doses of acrylonitrile labelled with ^{14}C in the cyanide moiety ([1-^{14}C]acrylonitrile) and in the vinyl group ([2,3-^{14}C]acrylonitrile). In both cases, oxidation to $^{14}CO_2$ was a minor pathway (less than 8% of the dose). The elimination pattern was the same after either intraperitoneal or oral dosing (Sapota, 1982) and was unaffected by dose over the range 0–28.8 mg/kg in rats and 0–10 mg/kg in mice (Kedderis *et al*., 1993a). Burka *et al*. (1994) showed that 11% of a 46 mg/kg dose of [2-^{14}C]acrylonitrile was excreted in the expired air of Fischer 344 rats as $^{14}CO_2$.

The tissue distributions of [1-^{14}C]- and [2,3-^{14}C]acrylonitrile (40 mg/kg) were compared in Wistar rats after intraperitoneal and oral administration (Sapota, 1982). The relative distributions of the two labelled forms were very similar and the principal locations of ^{14}C were erythrocytes, liver and kidney. After oral administration, the rate of elimination from tissues was slower for [*cyano*-^{14}C]- than for [1,2-*vinyl*-^{14}C]acrylonitrile. After gavage administration of 46 mg/kg bw [2-^{14}C]acrylonitrile to Fischer 344 rats, radioactivity was well absorbed from the gastrointestinal tract and distributed to all major tissues 24 h after dosing. The highest levels were found in the forestomach, blood and urinary bladder. Prior treatment of rats with phenobarbital had little effect on the pattern of distribution and excretion of ^{14}C, but the CYP inhibitor SKF-525A caused marked changes, with less excretion (less than 40% in urine in 24 h compared with over 60% in

untreated animals) and greater tissue retention, particularly in the blood, liver, kidney, lung, forestomach, glandular stomach, small intestine and urinary bladder (Burka *et al*., 1994).

Higher levels of radioactivity in Sprague-Dawley rats after intravenous administration of [1-^{14}C]acrylonitrile (100 mg/kg bw) were found in blood, liver, duodenum, kidney and adrenal gland than in other tissues (Silver *et al.*, 1987).

Sandberg and Slanina (1980) studied the tissue distribution of [1-^{14}C]acrylonitrile in Sprague-Dawley rats (26 mg/kg bw orally and after intravenous injection) and cynomolgus monkeys (4 and 6 mg/kg bw, orally only) by whole-body autoradiography. In all cases, radioactivity was detected in blood, liver, kidney, lung, adrenal cortex and stomach, the highest levels corresponding to the target organs of toxicity. The same technique was used in a later study of single doses of [2,3-^{14}C]acrylonitrile in rats (Sato *et al*., 1982). Higher concentrations of radioactivity were seen in blood, particularly erythrocytes, lung, liver and kidney, and longer retention was evident in brain and muscle than in other tissues. Most radioactivity was present in the cytosolic fractions prepared from brain, liver and kidney.

Ahmed *et al*. (1982) examined the distribution of [1-^{14}C]acrylonitrile (46.5 mg/kg bw orally) in rats. Some 55% of the dose was recovered in the excreta in 24 h (urine, 40%; faeces, 2%; exhaled as $^{14}CO_2$, 9%; as H^{14}CN, 0.5%; and acrylonitrile, 4.8%). In addition to appreciable retention in the erythrocytes (a feature of the behaviour of metabolically formed thiocyanate noted by Bollard *et al*., 1997), there occurred covalent binding to tissue macromolecules in liver, kidney, spleen, brain, lung and heart. Ahmed *et al.* (1983) also compared the tissue distribution of [1-^{14}C]- and [2,3-^{14}C]acrylonitrile in rats at the same dose level (46.5 mg/kg bw). There was much more covalent binding of radioactive species in all organs examined after administration of [2,3-^{14}C]acrylonitrile, suggesting that metabolites other than thiocyanate play a major role in its retention in the body.

In both rats and mice, the radioactivity derived from orally administered 2-cyano-[1,2-^{14}C]ethylene oxide, the epoxide metabolite of acrylonitrile, was widely distributed to the tissues with no particular accumulation in any organ and was rapidly depleted within 24 h after dosing (Kedderis *et al*., 1993b).

(*b*) *Toxicokinetics*

Gargas *et al*. (1995) have developed a physiological toxicokinetic model of acrylonitrile in rats which includes the behaviour of CEO. In-vitro kinetic studies of the metabolism of both acrylonitrile and CEO showed that epoxidation to CEO is saturable, while glutathione conjugation of acrylonitrile follows first-order kinetics. The model combines these kinetic parameters with tissue partition data to allow simulation of the urinary excretion of acrylonitrile metabolites and the formation of haemoglobin adducts (see below). The model has been further refined by Kedderis *et al*. (1996) to predict the behaviour of acrylonitrile and CEO after inhalation exposure to acrylonitrile.

(*c*) *Metabolic fate of acrylonitrile*

Urinary metabolites of acrylonitrile include *S*-(2-cyanoethyl)mercapturic acid, *N*-acetyl-3-carboxy-5-cyanotetrahydro-1,4-3*H*-thiazine and thiocyanate (Kopecký *et al*., 1979; Langvardt *et al*., 1980; Gut *et al*., 1981; Sapota, 1982). The proportion excreted as thiocyanate by rats is far higher (23% of dose) after oral dosing than after intraperitoneal, intravenous or subcutaneous administration (1–4% of dose; Gut *et al*., 1981). Other metabolites derived from the mercapturic acid pathway include *S*-carboxymethylcysteine, *S*-hydroxyethylmercapturic acid [*N*-acetyl-*S*-(2-hydroxyethyl)cysteine] and thiodiglycolic acid (Müller *et al*., 1987).

The formation of these various products can be rationalized by metabolism along two primary pathways, the products of which undergo extensive secondary and tertiary reactions, leading to a wide range of metabolites in the excreta (Figure 1). The carbon–carbon double bond undergoes epoxidation, mediated predominantly by CYP2E1 in rats and man (Guengerich *et al*., 1981; Kedderis *et al*., 1993c), to yield CEO, or addition of glutathione via a reverse Michael addition in which the sulfur atom is linked with the terminal carbon of acrylonitrile (Van Bladeren *et al*., 1981; Kedderis *et al*., 1995). The primary product of this latter pathway, *S*-(2-cyanoethyl)glutathione, is converted to *S*-(2-cyanoethyl)mercapturic acid and thence to *S*-(2-cyanoethyl)thioacetic acid (Kedderis *et al*., 1993a).

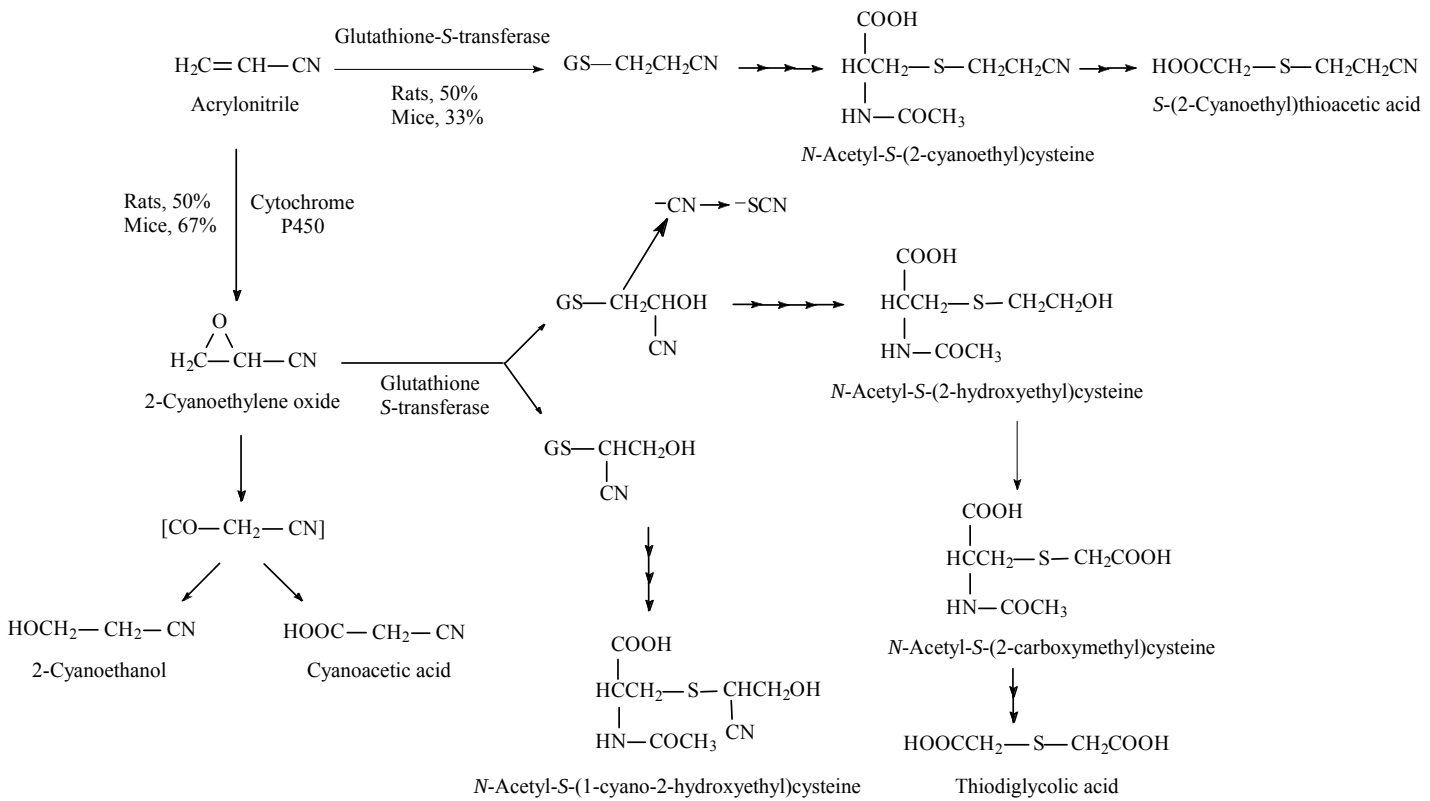
CEO is also a substrate for glutathione conjugation, giving the isomeric conjugates 1- and 2-*S*-glutathionyl-1-cyanoethanol (Van Bladeren *et al*., 1981; Kedderis *et al*., 1993a). The 1-*S*-glutathionyl conjugate is converted to the corresponding cysteinyl conjugate, which is then *N*-acetylated, giving *N*-acetyl-*S*-(1-cyano-2-hydroxyethyl)-cysteine in the urine. The 2-*S*-glutathionyl conjugate loses HCN, which is converted to thiocyanate, and gives rise to the mercapturic acid, *N*-acetyl-*S*-(2-hydroxyethyl)cysteine, which is excreted as such and also undergoes further metabolism to *N*-acetyl-*S*-(2-carboxymethyl)cysteine and thence to thiodiglycolic acid.

The metabolism of CEO gives rise to cyanoacetic acid, 2-cyanoethanol, cyanide, thiocyanate and $^{14}CO_2$ as in-vivo metabolites, which can be rationalized in terms of three distinct reactions: (i) enzymic hydration of the epoxide (Kedderis & Batra, 1993; Kedderis *et al*., 1995) giving a cyanohydrin, glycolaldehyde cyanohydrin, which has been shown *in vitro* to eliminate HCN, this then being converted by rhodanese to thiocyanate, (ii) rearrangement to the α-ketonitrile, pyruvonitrile, which would then be hydrolysed to acetate, the most likely precursor of $^{14}CO_2$, with elimination of cyanide, and (iii) rearrangement to cyanoacetaldehyde, which would then be oxidized to cyanoacetic acid or reduced to 2-cyanoethanol.

(*d*) *Metabolic studies in vitro*

Marked species differences have been reported for the metabolic activation of acrylonitrile to CEO *in vitro*. The V_{max} and V_{max}/K_m for microsomes from mouse liver and lung were approximately four and 13 times greater, respectively, than the kinetic parameters for rat liver and lung microsomes. The rate of CEO production in human liver

Figure 1. The metabolic disposition of acrylonitrile in rats and mice. The percentages given are the fluxes through each pathway after an oral bolus dose of 0.09 mg/kg bw. Modified from Kedderis et al. (1993a)



microsomes was similar to that with rat liver microsomes, but less than that with mouse liver microsomes (Roberts *et al*., 1991).

CEO is relatively stable in pH 7.3 buffer at 37°C ($t_{½}$ = 99 min) and undergoes hydration to the corresponding diol. Incubation of CEO with hepatic microsomes of cytosols from male Fischer 344 rats or B6C3F_1 mice did not enhance its basal rate of hydration, unlike human hepatic microsomes (see Section 4.1.1(*c*)). Prior treatment of rodents with either phenobarbital or acetone induced hepatic microsomal hydration activity towards CEO, whereas treatment with β-naphthoflavone, dexamethasone or acrylonitrile itself was without effect (Kedderis & Batra, 1993).

(*e*) *Influence of dose size and route of administration*

Rats were given acrylonitrile by three separate routes of administration: intravenous, intraperitoneal (both at doses of 0.6, 3 and 15 mg/kg bw) and inhalation (at 4, 20 and 100 ppm [9, 43 and 217 mg/m^3] for 6 h) and the main metabolites were determined in the 0–24-h urine (Tardif *et al*., 1987). The total recovery of metabolites was dose-related, but the pattern of metabolites was dependent upon the route of administration, with more of the mercapturic acids appearing in the urine after intravenous or intraperitoneal dosing, whereas the main metabolite was thiocyanate after inhalation exposure. The relative importance of the various metabolic pathways of acrylonitrile in rats and mice is dose-dependent (Kedderis *et al*., 1993a). The sulfur-containing metabolites derived from the glutathione conjugation of CEO increase in an approximately linear fashion with increasing dose of acrylonitrile in both rats (0–28.8 mg/kg bw) and mice (0–10 mg/kg bw). Differences occur in the patterns of the secondary and tertiary metabolites of the glutathione conjugates of CEO, but these are unlikely to have toxicological significance.

After inhalation exposure, the excretion of thiocyanate and acrylonitrile-derived mercapturic acids by Wistar rats was proportional to the dose over the range 57–271 mg/m^3 (Gut *et al*., 1985).

Burka *et al*. (1994) examined the effect of treatment of rats with phenobarbital and SKF-525A on the urinary metabolite profile of [2-^{14}C]acrylonitrile. Phenobarbital treatment increased the excretion of products attributed to the oxidation of acrylonitrile to CEO, while SKF-525A treatment enhanced the excretion of the mercapturic acid derived from the glutathione conjugation of acrylonitrile itself.

4.2 Toxic effects

4.2.1 *Humans*

Reported toxic effects of acrylonitrile in humans are summarized in Table 6.

(*a*) *Acute toxicity*

(i) *Inhalation exposure*

A 22-year-old chemist, who was exposed to acrylonitrile vapours, developed headache, vertigo, vomiting, tremors, uncoordinated movements and convulsions (Sartorelli, 1966). Vomiting and nausea persisted for 24 h. One day after exposure, slight liver

Table 6. Toxic effects of acrylonitrile in humans

Lesion/dysfunction	Exposure			Reference
	Type	Level	Duration	
Headache, tremor, convulsions	Inhalation	?	Acute	Sartorelli (1966)
Nausea, vomiting, headache, vertigo	Inhalation	35–220 mg/m^3	Acute	Zeller *et al.* (1969)
Dizziness, flushing, nausea, vomiting	Dermal, inhalation	NG	Acute	Vogel & Kirkendall (1984)
Erythema	Dermal	Conc. liquid	Acute	Wilson *et al*. (1948)
Skin burning, blisters	Dermal	Conc. liquid	Acute	Zeller *et al*. (1969)
Headache, poor sleep, chest pain	Inhalation	NG	Months	Zotova (1975)
Headache, weakness, fatigue, nausea, vomiting, nosebleeds, insomnia	Inhalation	NG	Years	Sakurai & Kusumoto (1972); Sakurai *et al*. (1978)
Headache, fatigue, tongue trouble, sweating	Inhalation	NG	Chronic	Kaneko & Omae (1992)
Reduced haemoglobin, other haematological disorders	Inhalation	2.5–5 mg/m^3	Chronic	Shustov (1968)
Blepharoconjunctivitis	Local (vapours?)	NG	Chronic	Delivanova *et al*. (1978)
Gastritis, colitis	Vapours?	5 mg/m^3	Chronic	Enikeeva *et al*. (1976)
Allergic dermatitis	Local	NG	Chronic	Spassovski (1976); Stamova *et al*. (1976)

NG, not given

enlargement and congestion of the oral pharynx, but no disorders of the central nervous system, were noted. After four days, no kidney, liver, cardiac or respiratory abnormalities were detected. Workers exposed to 'mild' concentrations of acrylonitrile in synthetic rubber manufacture developed nausea, vomiting, weakness, nasal irritation and an 'oppressive feeling' in the upper respiratory tract (Wilson, 1944). Headache, fatigue and diarrhoea were observed in some cases, and mild jaundice lasting for several days and accompanied by liver tenderness and low-grade anaemia in a few others. Jaundice lasted for four weeks in one case; this individual complained of lassitude and fatigue after one year. In 16 cases of acute inhalation of acrylonitrile fumes by workers, nausea, vomiting, headache and vertigo were experienced within 5–15 min; none of the workers needed hospitalization (Zeller *et al.*, 1969). Workmen exposed to concentrations varying from 35 to 220 mg/m^3 for 20–45 min during cleaning operations in polymerizers frequently complained of a dull headache, fullness in the chest, irritation of the eyes, nose and

throat, and feelings of apprehension and nervous irritability. Some workmen had 'intolerable itching' of the skin, but no accompanying dermatitis.

(ii) *Dermal exposure*

Zeller *et al*. (1969) reported 50 cases of skin damage resulting from occupational contact with acrylonitrile. A burning sensation developed within 5 min to 24 h followed by a reddening of the area, which often blistered after one day. A male laboratory worker who spilled 'small quantities' of liquid acrylonitrile on his hands developed diffuse erythema on both hands and wrists after 24 h, and blisters on the fingertips by the third day. The hands were slightly swollen, erythematous, itchy and painful. The fingers remained dry and scaly 10 days later (Dudley & Neal, 1942). Wilson *et al*. (1948) observed that direct skin contact led to irritation and erythema followed by scab formation; healing was slow.

In a 24-year-old man, dermal and inhalation exposure to acrylonitrile resulted in dizziness, flushing, nausea and vomiting. Furthermore, increases in serum creatinine phosphokinase, transaminases and myoglobinuria occurred, possibly as a consequence of tissue hypoxia (Vogel & Kirkendall, 1984).

Blisters developed at the sites of contact after 6–8 h in workers who had spilled liquid acrylonitrile on their legs. The skin of two workers who were cleaning apparatus (temperature 50°C) came into contact with 5% acrylonitrile solution; other possible substances in the mixture were not specified. Serious skin burns developed (Babanov, 1957).

Development of allergic dermatitis is possible; a 27-year-old individual developed a rash on his finger following the use for six weeks of a finger splint made from an acrylonitrile–methyl methacrylate copolymer. Patch testing gave positive reactions to the copolymer and 0.1% acrylonitrile (Balda, 1975). In another case report, skin lesions were first observed at the site of contact with liquid acrylonitrile, which then spread rapidly to other neighbouring regions. Several days after contact, the lesions spread rapidly to other parts of the body that had not been exposed, and these extensions were assumed to be an allergic reaction (Hashimoto & Kobayashi, 1961). The occurence of occupational contact dermatitis due to acrylonitrile among five employees in an acrylonitrile production factory was reported by Bakker *et al*. (1991).

(*b*) *Chronic toxicity—occupational exposure*

(i) *General toxicity*

Complaints of poor health, headache, decreased work capacity, poor sleep, irritability, chest pains, poor appetite and skin irritation (during the first months of employment only) came from workers employed in the manufacture of acrylonitrile (Zotova, 1975).

Sakurai and Kusumoto (1972) analysed records from health examinations of acrylonitrile workers at five acrylic fibre factories for a period of about 10 years up to 1970. The prevalence of subjective complaints and abnormal values for some of the liver function tests increased significantly with length of time spent in acrylonitrile-related jobs. It was later commented, however, that the study lacked adequate epidemiological

design, that the exposure levels were not reliably reported and that the findings were based upon routine health examinations (Sakurai *et al.*, 1978). In this latter publication, the results were described of a study of 102 workers whose exposure to acrylonitrile exceeded five years and in 62 matched controls, all of whom had been randomly sampled from six acrylic fibre factories in Japan. The six factories were classified into three groups on the basis of acrylonitrile concentrations in the workplaces. The most highly exposed group had an 8-hour average acrylonitrile concentration of 4.2 ppm [9.1 mg/m^3] by personal sampling, a mean urinary acrylonitrile concentration of 360 µg/L and a mean urinary thiocyanate concentration of 11.4 mg/L. Medical examination, including multiple clinical chemistry measurements and the indocyanine green excretion test, failed to detect any health effect attributable to acrylonitrile.

Babanov *et al*. (1959) reported that workers exposed to acrylonitrile concentrations of 0.6–6 mg/m^3 for approximately three years suffered from headache, insomnia, pains in the heart region, general weakness, decreased working capacity and increased irritability. The vocal cords were inflamed, and pale mucous membranes and skin were seen.

Changes in health status and laboratory tests were not observed in a group of 23 men who had been working for three to five years in an acrylonitrile plant, where exposure levels reached 4.2–7.2 mg/m^3 (Gineeva *et al*., 1977). Stamova *et al*. (1976) studied workers' health in the related polyacrylic fibre plant in which acrylonitrile exposure levels were around 10 mg/m^3, but could fluctuate upwards to 25 mg/m^3. Workers were also exposed to other chemical substances. An increased incidence was found for both skin diseases and various 'neurasthenic' complaints and diseases. Dorodnova (1976) did not find any differences in the gynaecological health status of 410 women working in a polyacrylic fibre plant compared with that of 436 unexposed women. Workers exposed to acrylonitrile were observed from 1950 to 1982 for mortality (Chen *et al*., 1988b). No dose–response relationship was observed between exposure and mortality. Subjective symptoms with significantly high prevalence in acrylonitrile-exposed workers in seven acrylic fibre-manufacturing factories were: headache, tongue trouble, choking lump in throat, fatigability, general malaise, heavy arms and heavy sweating (Kaneko & Omae, 1992). Neurotic status determinations did not reveal any differences between the group of workers investigated and a reference group.

(ii) *Other organs*

Liver

In a study of 102 workers from acrylic fibre factories in Japan, Sakurai *et al*. (1978) did not find any significant abnormality in liver function tests related to acrylonitrile exposure.

Nervous system

Nausea, vomiting, headache and vertigo (Wilson, 1944; Wilson *et al*., 1948; Zeller *et al*., 1969; Zotova, 1975; Sakurai *et al*., 1978) indicate a possible effect of acrylonitrile on the nervous system. Ageeva (1970) reported a significant decrease in an 'epinephrine-

like substance' and an increase in acetylcholine. Depression, lability of autonomic functions (lowered arterial pressure, labile pulse, diffuse dermographia, increased sweating, change in orthostatic reflex) were also observed in workers involved in acrylonitrile production.

4.2.2 *Experimental systems*

(*a*) *Acute toxicity*

In a variety of laboratory animals, acute LD_{50} values for acrylonitrile ranged from 25 to 186 mg/kg bw. For various routes of administration, mice were reported to be more sensitive towards acrylonitrile than rats, guinea-pigs and rabbits. For inhalation exposure, the LC_{50} was determined in most studies for a 4-h period of exposure to be between 140 and 1250 mg/m^3. In these studies, the dog was the most sensitive species, followed by the mouse, rabbit, cat, rat and guinea-pig (McOmie, 1949; Brieger *et al.*, 1952; Jedlicka *et al.*, 1958; Paulet & Desnos, 1961; Graham, 1965; Zeller *et al.*, 1969; Appel *et al.*, 1981).

A number of inhalation studies (Dudley & Neal, 1942; Brieger *et al*., 1952) and studies in which acrylonitrile was administered orally or parenterally (Paulet & Desnos, 1961; Graham, 1965; Paulet *et al*., 1966) have indicated that lethal concentrations or doses of acrylonitrile cause the following sequence of symptoms: excitability and increased breathing frequency, shallow rapid breathing, slow gasping breathing, apnoea and convulsions. Vomiting occurred in cats, dogs and monkeys after inhaling acrylonitrile, and in rats following parenteral administration. Reddening of the skin of the ears, nose and feet (also of the face and genital organs in rhesus monkeys) and mucosa was accompanied by lachrymation, nasal discharge and salivation, not only after inhalation exposure, but also following oral or subcutaneous administration, while hind-leg incoordination, paresis or paralysis were observed in rats after oral administration, and in rabbits after intravenous and intramuscular administration.

Buchter *et al*. (1984) exposed groups of male Wistar rats to acrylonitrile vapours of 4800 ppm [10 400 mg/m^3] for 30 min. Several compounds were given via different routes of administration 10 min after exposure ceased, in order to test their therapeutic efficiency against acute acrylamide poisoning. All untreated animals died. Observations of increased saliva, aqueous humour, spasms and diarrhoea suggested that inactivation of acetylcholinesterase by cyanethylation of serine was involved. In agreement with this hypothesis, atropine sulfate (50 mg/kg bw intravenously) turned out to be a potential antidote. However, *N*-acetylcysteine (300 mg/kg bw intravenously) was the most effective antidote, in line with earlier findings (Hashimoto & Kanai, 1965), demonstrating the excellent potency of thiols in the treatment of acute acrylonitrile toxicity.

Cote *et al*. (1984) and Vodicka *et al*. (1990) observed marked decreases in glutathione levels in a variety of organs such as brain, lung, liver and kidney of rats dosed with acrylonitrile either subcutaneously (75 mg/kg bw) or by inhalation (75–300 mg/m^3). These effects were less pronounced in mouse and hamster tissues. Covalent binding to tissue protein, a dose-dependent decrease in tissue glutathione, and an

increase in blood and brain cyanide levels were observed in acrylonitrile-treated Sprague-Dawley rats (Benz *et al*., 1997a). The authors concluded that depletion of hepatic glutathione is a critical event in acrylonitrile toxicity, exhausting the detoxifying capacity of the liver. The relative potency of various antidotes including L-cysteine, D-cysteine and *N*-acetyl-L-cysteine correlated with their capacity to suppress acrylonitrile metabolism, as monitored by increases in blood cyanide level (Benz *et al*., 1990). However, pretreatment of rats with acrylonitrile for 4 h per day over three consecutive days protected against acute acrylonitrile toxicity without protecting against poisoning by cyanide (Cote *et al*., 1983).

Target organs of acrylonitrile toxicity in experimental animals are listed in Table 7.

(i) *Blood parameters*

Intraperitoneal administration of acrylonitrile to male rats at a dose of 33 mg/kg bw per day for three days decreased serum levels of corticosterone to 30% and prolactin to 40%, but increased follicle-stimulating hormone (FSH) to 200% of control levels, and did not affect luteinizing hormone (LH) (Nilsen *et al*., 1980). In adult male Wistar rats, a single intraperitoneal administration of acrylonitrile of 10 mg/kg bw did not have any effect on serum glutamate-oxaloacetate transferase (SGOT) or serum glutamate-pyruvate transaminase (SGPT) activity, but increased activity of lactate dehydrogenase (LDH) to 200% and of sorbitol dehydrogenase (SDH) to 300% compared with the controls (Noel *et al*., 1978; Duverger-Van Bogaert *et al*., 1978). A 60% increase in serum SDH was found in rats given acrylonitrile at 500 mg/L in the drinking-water for 21 days (Silver *et al*., 1982).

Oral administration of acrylonitrile to male Sprague-Dawley rats at a dose of 80 mg/kg bw led to a significant reduction in packed blood cell volume, mean haemoglobin content per erythrocyte, and number of platelets per litre of blood, 1 h after treatment. Furthermore, significant perturbations of levels of red cell glutathione, 2,3-diphosphoglycerate, adenosine triphosphate, pyruvate and lactate were found (Farooqui & Ahmed, 1983). Gut *et al*. (1984) observed an elevation of blood glucose after a 12-h inhalation exposure of male Wistar rats to 57 mg/m^3 acrylonitrile and higher concentrations.

(ii) *Skin*

Direct application of liquid acrylonitrile to the shaved skin of rabbits immediately induced slight local vasodilatation, without any systemic effect (1–2 mL spread over 100–200 cm^2) or with an increased respiratory rate (3 mL spread over 300 cm^2) (McOmie, 1949). Zeller *et al*. (1969) found that an application of acrylonitrile on a cotton pad to shaved skin of rabbits for 15 min resulted in skin oedema, and for 20 h resulted in slight necrosis.

(iii) *Eye*

Oedema and slight necrosis of the conjunctiva after eight days were observed in rabbits treated with acrylonitrile (Zeller *et al*., 1969).

Table 7. Target organs of acrylonitrile toxicity in mammalian species

Target organ	Species	Type of dysfunction/lesion	Dosage	Duration of treatment	Reference
Skin	Rabbit	Vasodilatation	Dermal application	Acute	McOmie (1949)
		Oedema/necrosis	Dermal application	15 min/20 h	Zeller *et al.* (1969)
Eye	Rabbit	Conjunctivitis	Local application	1 h	McOmie (1949)
		Conjunctivitis/necrosis	Local application	Repeated (over 8 days)	Zeller *et al*. (1969)
Lung	Dog	Pulmonary oedema	100 mg/kg; intravenous	Single dose	Graham (1965)
	Guinea-pig	Pulmonary oedema	100 mg/kg; orally	Single dose	Jedlicka *et al*. (1958)
	Rat	Clara cell hyperplasia	46.5 mg/kg; orally	Single dose	Ahmed *et al*. (1992a)
Forestomach and stomach	Rat	Haemorrhagic gastritis	150 mg/kg; orally	Single dose (after 24 h)	Silver *et al.* (1982)
	Rat	Mucosal erosion and haemorrhage	30 mg/kg; subcutaneous	Single dose	Ghanayem *et al.* (1985)
Adrenals	Rat	Atrophic zona fasciculata	0.05% in drinking-water	21–60 days	Szabo *et al.* (1976)
		Necrosis	150 mg/kg; intravenous	Single dose	Szabo *et. al.* (1981)
Kidney	Rat	Increased urinary volume, glucose	20 mg/kg; orally	Single dose	Rouisse *et al.* (1986)
		Increased urinary *N*-acetyl-D-glucosaminidase	60 mg/kg; orally	Single dose	Rouisse *et al.* (1986)
Liver	Rat	Focal necrosis	150 mg/kg; orally	Single dose	Silver *et al.* (1982)

(iv) *Lung*

Respiratory disturbance and pulmonary oedema were observed in anaesthetized dogs given 100 mg/kg bw intravenously (Graham, 1965) and in guinea-pigs given 100 mg/kg bw orally (Jedlicka *et al*., 1958). After a single oral dose of 46.5 mg/kg bw acrylonitrile, moderate to marked hyperplasia of the Clara cells lining the bronchioles was observed in male Sprague-Dawley rats (Ahmed *et al*., 1992a).

(v) *Forestomach and stomach*

Haemorrhagic gastritis was found in rats necropsied 24 h after administration of acrylonitrile at 150 mg/kg bw in the drinking-water (Silver *et al*., 1982). Pretreatment of rats with cytochrome P450 inducers, such as Arochlor 1254 or phenobarbital, did not modify the extent of stomach/forestomach lesions but markedly increased the ulcerogenic action of acrylonitrile in the duodenum (Szabo *et al*., 1983). Subcutaneous administration of 30 or 50 mg/kg bw acrylonitrile to male Sprague-Dawley rats resulted in dose-dependent superficial mucosal erosion and haemorrhage of the glandular stomach (Ghanayem *et al*., 1985). The occurrence of these lesions was associated with a marked decrease of gastric intracellular reduced glutathione. Sulfhydryl-containing compounds and atropine protected against the acrylonitrile-induced gastric erosions (Ghanayem *et al*., 1985; Ghanayem & Ahmed, 1986).

(vi) *Adrenals*

The effect of lethal doses of acrylonitrile on the adrenals as a model for adrenal apoplexy or haemorrhagic adrenocorticol necrosis was described by Szabo and Selye (1971, 1972) and Szabo *et al*. (1980). After intravenous administration of high doses (150 or 200 mg/kg bw), haemorrhage was observed in both adrenals of most animals, and there was adrenal haemorrhage in some rats following oral administration of 10, 15 or 20 mg/kg bw. Various types of histological damage were observed, particularly in the inner layers of the adrenal cortex but not in the medulla, some of them within 30 min after acrylonitrile administration.

A possible mechanism involving lipid peroxidation in acrylonitrile-induced adrenal injury has been suggested by Silver and Szabo (1982). Szabo *et al*. (1980) investigated the pathogenesis of experimental adrenal haemorrhagic necrosis using various morphological, biochemical and pharmacological methods. Their results suggest that the presence of a functional adrenocortex is necessary for the development of cortical damage. Catecholamine release, endothelial injury in the cortical capillaries and retrograde medullary-cell embolism were suggested as critical events in acrylonitrile-induced adrenocortical necrosis (Szabo *et al*., 1981, 1984).

(vii) *Other organs*

In a human neuroblastoma cell line, Cova *et al*. (1992) found acrylonitrile to be highly toxic, showing an EC_{50} of 72.5 nM for cytotoxicity. The cytotoxic potency of potassium cyanide was 2.5 μM, thus acrylonitrile toxicity in these cells cannot be attributed to its metabolism to cyanide.

Acrylonitrile shows an inhibitory effect on potassium-stimulated respiration of guinea-pig brain cortex slices at 1 mM, but little effect on the liver at the same concentration. A stronger anaesthetic action of acrylonitrile was detected *in vitro* on the sciatic nerve of *Rana nigra maculata*, compared with some other anaesthetic agents (Hashimoto & Kanai, 1965).

In male Fischer 344 rats treated intraperitoneally with 0, 10, 20, 40, 60 or 80 mg/kg bw acrylonitrile, significant increases in urinary volume and glucose were observed 24 h after treatment with 20 mg/kg bw (Rouisse *et al*., 1986). Increased levels of urinary *N*-acetyl-β-D-glucosaminidase were detected after treatment with 60 mg/kg bw acrylonitrile. Symptoms of nephrotoxicity were also observed after a 4-h exposure to 200 ppm [434 mg/m^3] acrylonitrile. Histopathological examination revealed lesions in the proximal tubular region of the kidney.

In Sprague-Dawley rats treated orally with 50, 75, 100 or 150 mg/kg bw acrylonitrile, hepatic nonprotein sulfhydryl concentration was significantly decreased after 30 min (Silver *et al*., 1982). Twenty-four hours after administration of 150 mg/kg bw, focal liver necrosis was observed. In isolated rat hepatocytes, acrylonitrile (1 mM) treatment resulted in the formation of thiobarbituric acid-positive products (a test for malonaldehyde) and in depletion of non-protein sulfhydryl groups, but did not affect markedly the viability of the cells (Nerudová *et al*., 1988).

(*b*) *Chronic toxicity*

Observations have been made on animals treated with acrylonitrile in the drinking-water or food, through inhalation and by dermal application.

Taking into account a variety of toxic end-points of chronic acrylonitrile treatment in Fischer 344 rats, Salsburg (1990) calculated a lowest observable effect level of 3 ppm (mg/L) in drinking water, while 1 ppm acrylonitrile was estimated to be a 'no mean effect level'.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

The teratogenic potential of ingested or inhaled acrylonitrile was investigated by Murray *et al*. (1978). Groups of 29–39 pregnant Sprague-Dawley rats were given acrylonitrile in water at 10, 25 or 65 mg/kg bw per day by gavage from day 6 to day 15 of gestation. A control group of 43 rats was given water. Groups of 30 pregnant rats were exposed for 6 h per day to 0, 40 or 80 ppm [0, 87 or 174 mg/m^3] acrylonitrile by inhalation, during the same period of pregnancy. A dose of 65 mg/kg bw per day caused marked maternal toxicity, significant embryotoxicity and an increased incidence of fetal malformations. Findings of the two studies suggested a teratogenic effect at 25 mg/kg bw per day and at 40 ppm. At 10 mg/kg bw per day and 20 ppm, no embryotoxicity or terato-

genicity was found. There was no apparent correlation between the degree of toxicity seen in the individual dams and the occurrence of malformations in their offspring.

Single intraperitoneal injections of acrylonitrile of 32 mg/kg bw, given on the fifth and seventh days of pregnancy, induced an embryotoxic effect in mice from an inbred strain of AB Jena-Halle, but not in DBA and C57 C1 mice (Scheufler, 1980).

Kankaanpää *et al*. (1979) studied the embryotoxic effects of acrylonitrile using chick eggs, but did not find any clear evidence of teratogenicity.

Exposure of Sprague-Dawley rats to acrylonitrile in drinking-water at a concentration of 500 mg/L (ppm) led to decreased fertility and decreased viability of the young, and the females developed progressive muscular weakness in the hind legs about 16–19 weeks after the weaning of the second litter (Svirbely & Floyd, 1961).

Exposure of Sprague-Dawley rats to acrylonitrile by inhalation in the range of 12–100 ppm [26–220 mg/m^3] for six hours per day on days 6–20 of gestation resulted in fetotoxicity accompanied by overt signs of maternal toxicity at 25 ppm [54 mg/m^3] and higher concentrations. No significant teratogenicity was observed (Saillenfait *et al.*, 1993a).

Willhite (1981a,b) observed skeletal malformations in fetuses after administration of acrylonitrile at 80 mg/kg bw to pregnant hamsters. The histological study of both early embryos and term fetuses revealed mesodermal changes, including a reduction in the number of cells, shrinkage of the cell cytoplasm and enlarged extracellular spaces. In addition, a reduction in mitotic figures and focal necrosis were noted. The affected embryos were smaller and their development was delayed compared with untreated controls. Teratogenic effects were observed only when there was simultaneous maternal toxicity.

In a rat whole-embryo culture system, depletion of glutathione aggravated the embryotoxic and teratogenic effects of acrylonitrile (Saillenfait *et al.*, 1993b).

4.4 Genetic and related effects

4.4.1 *Humans*

There was no difference in the incidence of chromosomal aberrations in the peripheral lymphocytes between 18 workers who had been exposed to acrylonitrile (1.5 ppm [3.3 mg/m^3]) for an average of 15.3 years and 18 workers who had not been exposed to acrylonitrile (Thiess & Fleig, 1978). In another study, it was claimed that there was an excess of chromosomal aberrations (but not sister chromatid exchange) in 10 workers exposed to 2 ppm [4.3 mg/m^3] acrylonitrile, compared with five unexposed subjects. Urine from exposed workers was tested for gene mutagenic potential in *Salmonella typhimurium* TA98, following extraction, concentration and treatment with β-glucuronidase. No activity was found (Borba *et al.*, 1996). [The Working Group noted the inadequate reporting of exposure and cytogenetic data in this study.]

4.4.2 *Experimental systems* (see Table 8 for references)

Acrylonitrile was a subject of a large interlaboratory testing trial, results of which were published in 1985 and which contributed to the substantial quantity of available in-vitro test data.

Table 8. Genetic and related effects of acrylonitrile

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SAF, *Salmonella typhimurium* TM677, forward mutation	+	–	500	Liber (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	0.2% (vapour)	de Meester *et al.* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Lijinsky & Andrews (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	(+)	19	Cerna *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500	Rexroat & Probst (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500	Matsushima *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	500	Zeiger & Haworth (1985)
SA2, *Salmonella typhimurium* TA102, reverse mutation	–	–	1600	Baker & Bonin (1985)
SA2, *Salmonella typhimurium* TA102, reverse mutation	–	–	2500	Matsushima *et al.* (1985)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	–	+	2.5	de Meester *et al.* (1978)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	–	+	0.2% (vapour)	de Meester *et al.* (1979)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	NT	+	372	Duverger-Van Bogaert *et al.* (1981)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	NT	+	372	Duverger-Van Bogaert *et al.* (1982a)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	NT	+	371	Duverger-Van Bogaert *et al.* (1982b)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	100	de Meester *et al.* (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	50	Lijinsky & Andrews (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	9.5	Cerna *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	0.05	Zhurkov *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	167	Zeiger & Haworth (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500	Rexroat & Probst (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	100	de Meester *et al.* (1978)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Lijinsky & Andrews (1980)

Table 8 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500	Rexroat & Probst (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	100	de Meester *et al.* (1978)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	500	Lijinsky & Andrews (1980)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	5000	Zhurkov *et al.* (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2500	Rexroat & Probst (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	100	de Meester *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Lijinsky & Andrews (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	Matsushima *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	Rexroat & Probst (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	3333	Zeiger & Haworth (1985)
SAS, *Salmonella typhimurium* TA1530, reverse mutation	–	+	100	de Meester *et al.* (1978)
SAS, *Salmonella typhimurium* TA102, reverse mutation	–	–	2500	Matsushima *et al.* (1985)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	2500	Matsushima *et al.* (1985)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	1667	Zeiger & Haworth (1985)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	+	+	53	Venitt *et al.* (1977)
EC2, *Escherichia coli* WP2, reverse mutation	+	+	53	Venitt *et al.* (1977)
ECR, *Escherichia coli* WP2 *uvrA polA*, reverse mutation	+	+	5.3	Venitt *et al.* (1977)
ECR, *Escherichia coli* WP2 *lexA*, reverse mutation	–	–	53	Venitt *et al.* (1977)
SCG, *Saccharomyces cerevisiae* D7, gene conversion	+	–	25	Arni (1985)
SCG, *Saccharomyces cerevisiae* JD1, gene conversion	–	+	250	Brooks *et al.* (1985)
SCG, *Saccharomyces cerevisiae* PV-2 and PV-3, gene conversion	–	–	800	Inge-Vechtomov *et al.* (1985)
SCG, *Saccharomyces cerevisiae* D7-144, gene conversion	+	+	0.8	Mehta & von Borstel (1985)
SCG, *Saccharomyces cerevisiae* D7, gene conversion	+	–	20	Parry & Eckardt (1985a)

Table 8 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SCH, *Saccharomyces cerevisiae* D7, homozygosis	–	–	50	Arni (1985)
SCH, *Saccharomyces cerevisiae* PV4a and PV4b, homozygosis	–	–	800	Inge-Vechtomov *et al.* (1985)
SCH, *Saccharomyces cerevisiae* D6 and D61-M, homozygosis	+	+	20	Parry & Eckardt (1985b)
SCH, *Saccharomyces cerevisiae* D61-M, homozygosis	+	NT	199	Zimmermann *et al.* (1985)
SCH, *Saccharomyces cerevisiae* RS112, homozygosis by mitotic recombination or gene conversion	+	+	645	Carls & Schiestl (1994)
ANG, *Aspergillus nidulans*, crossing-over	+	NT	806	Carere *et al.* (1985)
SCF, *Saccharomyces cerevisiae* D5, forward mutation	+	NT	30	Ferguson (1985)
SCF, *Saccharomyces cerevisiae* PV-1, forward mutation	–	–	800	Inge-Vechtomov *et al.* (1985)
SCR, *Saccharomyces cerevisiae* D7, reverse mutation	–	–	50	Arni (1985)
SCR, *Saccharomyces cerevisiae* PV-2 and PV-3, reverse mutation	–	–	800	Inge-Vechtomov *et al.* (1985)
SCR, *Saccharomyces cerevisiae* XV185-14C, reverse mutation	+	(+)	0.8	Mehta & von Borstel (1985)
SCR, *Saccharomyces cerevisiae* RM52, reverse mutation	–	–	800	Mehta & von Borstel (1985)
SCR, *Saccharomyces cerevisiae* D7, reverse mutation	–	–	200	Parry & Eckardt (1985a)
SCR, *Saccharomyces cerevisiae* D61-M, reverse mutation	+	+	20	Parry & Eckardt (1985b)
SZF, *Schizosaccharomyces pombe*, forward mutation	–	–	250	Loprieno *et al.* (1985)
SCN, *Saccharomyces cerevisiae* D6 and D61-M, aneuploidy	+	+	20	Parry & Eckardt (1985b)
SCN, *Saccharomyces cerevisiae* D61-M, aneuploidy	–	NT	792	Zimmermann *et al.* (1985)
SCN, *Saccharomyces cerevisiae* D61-M, aneuploidy	–	NT	2290	Whittaker *et al.* (1990)
TSM, *Tradescantia species*, mutation	(+)	NT	0.5	Schairer *et al.* (1982)

Table 8 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DMG, *Drosophila melanogaster*, genetic crossing over or recombination	–		265 feed	Vogel (1985)
DMG, *Drosophila melanogaster*, genetic crossing over or recombination	–		805 inh	Wuergler *et al.* (1985)
DMM, *Drosophila melanogaster*, somatic mutation (and recombination)	+		424 feed	Fujikawa *et al.* (1985)
DMM, *Drosophila melanogaster*, somatic mutation (and recombination)	+		265 feed	Vogel (1985)
DMM, *Drosophila melanogaster*, somatic mutation (and recombination)	(+)		80 inh	Wuergler *et al.* (1985)
DMN, *Drosophila melanogaster*, aneuploidy	+		2.7 ppm inh	Osgood *et al.* (1991)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		3500 ppm inj	Foureman *et al.* (1994)
DIA, DNA strand breaks/alkali-labile sites, Syrian hamster embryo cells *in vitro*	+	NT	200	Parent & Casto (1979)
DIA, DNA strand breaks/alkali-labile sites, Fischer 344 rat primary hepatocytes *in vitro*	+	NT	66	Bradley (1985)
DIA, DNA strand breaks/alkali-labile sites, Chinese hamster ovary CHO cells *in vitro*	+	+	3710	Douglas *et al.* (1985)
URP, Unscheduled DNA synthesis, male Fischer 344 rat primary hepatocytes *in vitro*	–	NT	530	Probst & Hill (1985)
URP, Unscheduled DNA synthesis, male Fischer 344 rat primary hepatocytes *in vitro*	–	NT	100	Williams *et al.* (1985)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	53	Butterworth *et al.* (1992)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	(+)	(+)	200	Lee & Webber (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	10	Rudd (1983)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	40	Amacher & Turner (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	100	Lee & Webber (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	20	Myhr *et al.* (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	(+)	+	30	Oberly *et al.* (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	?	–	100	Styles *et al.* (1985)

Table 8 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
G51, Gene mutation, mouse lymphoma L5178Y cells, *hprt* locus *in vitro*	(+)	(+)	200	Garner & Campbell (1985)
G51, Gene mutation, mouse lymphoma L5178Y cells, Na^+/K^+ ATPase locus *in vitro*	–	–	200	Garner & Campbell (1985)
GML, Gene mutation, mouse lymphoma P388F cells, *tk* locus *in vitro*	–	+	161	Anderson & Cross (1985)
GIA, Gene mutation, mouse BALB/c 3T3 cells, Na^+/K^+ ATPase locus *in vitro*	NT	+	40	Matthews *et al.* (1985)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	+	5.3	Ved Brat & Williams (1982)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	30	Gulati *et al.* (1985)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	+	106	Natarajan *et al.* (1985)
SIR, Sister chromatid exchange, rat liver RL4 cells *in vitro*	–	NT	5.0	Priston & Dean (1985)
MIA, Micronucleus test, Chinese hamster ovary CHO cells *in vitro*	+	+	1600	Douglas *et al.* (1985)
CIC, Chromosomal aberrations, Chinese hamster Don-6 cells *in vitro*	(+)	NT	5.3	Sasaki *et al.* (1980)
CIC, Chromosomal aberrations, Chinese hamster lung CHL cells *in vitro*	+	NT	18	Ishidate *et al.* (1981)
CIC, Chromosomal aberrations, Chinese hamster liver CH1-L cells *in vitro*	+	NT	12.5	Danford (1985)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	(+)	100	Gulati *et al.* (1985)
CIC, Chromosomal aberrations, Chinese hamster lung CHL cells *in vitro*	+	NT	6.2	Ishidate & Sofuni (1985)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	+	53	Natarajan *et al.* (1985)
CIR, Chromosomal aberrations, rat liver RL4 cells *in vitro*	–	NT	10	Priston & Dean (1985)
AIA, Spindle damage, Chinese hamster liver CH1-L cells *in vitro*	–	NT	25	Parry (1985)
AIA, Aneuploidy, Chinese hamster liver CH1-L cells *in vitro*	–	NT	25	Danford (1985)
TBM, Cell transformation, BALB/c 3T3 mouse cells	(+)	+	7	Matthews *et al.* (1985)

Table 8 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
TCM, Cell transformation, C3H 10T1/2 mouse cells	–	(+)	16	Lawrence & McGregor (1985)
TCM, Cell transformation, C3H 10T1/2 mouse cells	+	NT	6.3	Banerjee & Segal (1986)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	0.01	Barrett & Lamb (1985)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	2	Sanner & Rivedal (1985)
TFS, Cell transformation, Syrian hamster embryo cells, focus assay	+	NT	50	Parent & Casto (1979)
TCL, Cell transformation, NIH/3T3 mouse cells	+	NT	12.5	Banerjee & Segal (1986)
T7S, Cell transformation, SA7/Syrian hamster embryo cells	+	NT	100	Parent & Casto (1979)
DIH, DNA strand breaks, alkali labile site, human bronchial epithelial cells *in vitro*	+	NT	200	Chang *et al.* (1990)
UIH, Unscheduled DNA synthesis, secondary cultures of human mammary epithelial cells *in vitro*	–	NT	53	Butterworth *et al.* (1992)
GIH, Gene mutation, human lymphoblastoid AHH-1 cells *hprt* locus *in vitro*	+	NT	25	Crespi *et al.* (1985)
GIH, Gene mutation, human lymphoblastoid TK6 cells *tk* locus *in vitro*	–	(+)	74	Recio & Skopek (1988)
GIH, Gene mutation, human lymphoblastoid TK6 cells *tk* locus *in vitro*	–	+	40	Crespi *et al.* (1985)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	+	26.5	Perocco *et al.* (1982)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	–	10	Obe *et al.* (1985)
SIH, Sister chromatid exchange, human bronchial epithelial cells *in vitro*	+	NT	150	Chang *et al.* (1990)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	5.3	Cerna *et al.* (1981)
BFA, Bile from Sprague-Dawley rat, *Salmonella typhimurium* TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–		45 iv × 1	Connor *et al.* (1979)
BFA, Urine from NMRI mice or Wistar rats, *Salmonella typhimurium* TA1530, reverse mutation	(+)		30 ip × 1	Lambotte-Vandepaer *et al.* (1980)
UPR, Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	–		75 po × 1	Butterworth *et al.* (1992)

Table 8 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
UVR, Unscheduled DNA synthesis, Fischer 344 rat spermatocytes *in vivo*	–		75 po × 1	Butterworth *et al.* (1992)
SVA, Sister chromatid exchange, C57BL/6 mouse bone-marrow cells *in vivo*	(+)		45 ip × 1	Sharief *et al.* (1986)
MVM, Micronucleus test, NMRI mouse bone-marrow cells *in vivo*	–		30 ip × 1	Leonard *et al.* (1981)
CBA, Chromosomal aberrations, Swiss albino mouse, Sprague-Dawley rat bone-marrow cells *in vivo*	–		40 po × 16	Rabello-Gay & Ahmed (1980)
CBA, Chromosomal aberrations, NMRI mouse bone-marrow cells *in vivo*	–		30 ip × 1	Leonard *et al.* (1981)
CBA, Chromosomal aberrations, mouse bone-marrow cells *in vivo*	–		140 inh	Zhurkov *et al.* (1983)
CBA, Chromosomal aberrations, C57BL/6 mouse bone-marrow cells *in vivo*	–		30 ip × 1	Sharief *et al.* (1986)
CGG, Chromosomal aberrations, mouse, spermatogonia treated *in vivo*, spermatogonia observed	–		140 inh	Zhurkov *et al.* (1983)
DLM, Dominant lethal test, male NMRI mice	–		30 ip × 1	Leonard *et al.* (1981)
DLM, Dominant lethal test, male mice	–		140 inh	Zhurkov *et al.* (1983)
DLR, Dominant lethal test, male Fischer 344 rats	–		60 po × 5	Working *et al.* (1987)
ICR, Inhibition of intercellular communication, Chinese hamster lung V79 cells *in vitro*	(+)	NT	50	Elmore *et al.* (1985)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; inh, inhalation; inj, injection; iv, intravenous; ip, intraperitoneal; po, oral

Acrylonitrile was mutagenic to bacteria, usually but not exclusively in the presence of an exogenous metabolic system. Urine from acrylonitrile-treated rats and mice, but not the bile from rats, also was mutagenic to bacteria, an exogenous metabolic system not being required. In fungi, acrylonitrile produced variable results, positive and negative outcomes being obtained in tests for mutation, gene conversion, mitotic recombination and aneuploidy. It was weakly mutagenic in a single study with the plant, *Tradescantia spp.*, while, in the insect, *Drosophila melanogaster*, it did not induce sex-linked recessive lethal mutations, again in a single study, or genetic crossing over. Consistently positive results were obtained in *D. melanogaster*, however, for somatic cell mutation, and aneuploidy was induced in one study.

In cultured mammalian cells, acrylonitrile induced DNA strand breakage, gene mutation, sister chromatid exchanges and chromosomal aberrations, but not aneuploidy or unscheduled DNA synthesis in rat hepatocytes, at least if the silver grain counting method was used. [Studies using the less reliable scintillation counting method have not been summarized.] Cell transformation was induced in several test systems and gap-junctional intercellular communication was inhibited in one study with Chinese hamster V79 cells.

In studies with human cells *in vitro*, acrylonitrile induced DNA strand breakage in a single study, gene mutations in two studies and sister chromatid exchanges in two of three studies, but not unscheduled DNA synthesis or chromosomal aberrations in single studies.

In rodents treated *in vivo*, acrylonitrile did not induce unscheduled DNA synthesis in hepatocytes or spermatocytes of rats, chromosomal aberrations in the bone marrow of mice or rats, chromosomal aberrations in spermatogonia of mice, micronuclei in the bone marrow of mice, or dominant lethal effects in either rats or mice. Sister chromatid exchanges were, however, induced in mouse bone marrow.

Binding to macromolecules

Peter and Bolt (1981) showed that the time-dependent covalent binding of [2,3-^{14}C]acrylonitrile to rat liver microsomal protein does not necessarily require metabolic activation and noted, in particular, extensive binding to heat-inactivated liver microsomes. Binding was inhibited by a variety of soluble thiol compounds, such as cysteine, glutathione and diethyl dithiocarbamate. Guengerich *et al.* (1981) showed that a substantial level of binding to microsomal protein, but not to DNA, occurred in the absence of NADPH due to direct alkylation by both [1-^{14}C]- and [2,3-^{14}C]acrylonitrile. At least two-thirds of the protein binding was not the result of metabolism. These findings suggest that the protein binding of acrylonitrile may be mediated at least in part by direct alkylation of nucleophilic centres. In the presence of NADPH, irreversible binding to DNA did occur and protein binding was increased. Metabolic activation was also supported by a reconstituted cytochrome P450 system, whereas experiments with human liver microsomal preparations from six people provided no evidence of protein binding and only a very low level of DNA binding in incubations with one of the six preparations tested.

Later experiments showed that most of the label bound to DNA, RNA or polynucleotides after incubation with [2,3-^{14}C]acrylonitrile was removed by chromatography on hydroxyapatite. When [2,3-^{14}C]acrylonitrile was administered to rats, label was also incorporated in the natural bases of RNA. In addition, there was some evidence for modified DNA bases occurring at levels too low to permit identification (Peter *et al.*, 1983). Using a radiometric derivative assay, Hogy and Guengerich (1986) found that DNA alkylation occurred only at very low levels in liver (0.014–0.032 *N7*-(2-oxoethyl)guanine adducts per 10^6 DNA bases) of rats treated with acrylonitrile by intraperitoneal injection. If adducts occurred in the brain, they were at or below the detection level. Nuclear DNA 8-oxodeoxyguanosine levels were increased in the brain, but not in the liver of rats exposed to acrylonitrile, this result being consistent with oxidative damage rather than direct adduct formation (Whysner *et al.*, 1998). A single oral dose of [2,3-^{14}C]acrylonitrile to Sprague-Dawley rats has been reported to lead to association of radioactivity with DNA isolated from brain (Farooqui & Ahmed, 1983), gastric tissue (Abdel-Rahman *et al.*, 1994) and testis (Ahmed *et al.*, 1992b). Unfortunately, the DNA-processing conditions were not stringent enough to eliminate the possibility of covalent binding to contaminating protein. [The Working Group noted that hydrolysis of DNA and identification of any abnormal nucleotides is essential for the demonstration of covalent binding to DNA, particularly for a substance such as acrylonitrile that binds strongly to proteins.]

CEO binds irreversibly to calf thymus DNA and rat microsomal protein, the binding of radioactivity being greater when the ^{14}C label is in carbons 1 and 2, rather than in the nitrile group (Guengerich *et al.*, 1981). 2-Cyano[1,2-^{14}C]ethylene oxide also binds to protein in the liver and brain of rats injected intraperitoneally, but stable binding to DNA or RNA was at or below the limits of detection (Hogy & Guengerich, 1986).

4.4.3 *Mechanistic considerations*

Acrylonitrile is mutagenic, especially after bioactivation by a microsomal system. Since formation of DNA adducts with acrylonitrile *in vitro* is strongly increased by formation of its epoxide, it is very likely that the genotoxicity of acrylonitrile is mediated primarily by this metabolite. The epoxide, therefore, may be implicated in the carcinogenicity of acrylonitrile.

Acrylonitrile is toxic at high dose in several organs, which is possibly related to its glutathione-depleting activity. This toxicity might lead to tumour formation by an indirect mechanism, although no data to support this are evident from the available toxicity data, such as increased cell turnover or DNA labelling.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Acrylonitrile is a monomer used in high volume principally in the manufacture of acrylic fibres, resins (acrylonitrile–butadiene–styrene, styrene–acrylonitrile and others) and nitrile rubbers (butadiene–acrylonitrile). Other important uses are as an intermediate in the preparation of adiponitrile (for nylon 6/6) and acrylamide and, in the past, as a fumigant. Occupational exposures to acrylonitrile occur in its production and use in the preparation of fibres, resins and other products. It is present in cigarette smoke and has been detected rarely and at low levels in ambient air and water.

5.2 Human carcinogenicity data

The potential carcinogenicity of acrylonitrile in occupationally exposed populations has been investigated in several epidemiological studies. Studies carried out in the 1970s and 1980s suggested a possible increased risk of lung cancer among workers exposed to acrylonitrile. However, these were inconclusive because of one or more of the following actual or potential problems: small sample sizes, insufficient length of follow-up, incompleteness of follow-up, inadequate exposure assessment, potential confounding by other occupational carcinogens, and potential confounding by smoking. Consequently, larger and better studies were undertaken, in most cases building upon the same cohorts that had previously been assembled. Four such studies (two in the United States, one in the United Kingdom and one in the Netherlands) were carried out and these now provide the most relevant, informative data on which to base an evaluation. All of the studies made some attempt to establish exposure levels, although for the British study, this was rather cruder than for the others. The two studies from the United States were carried out in similar industries, but the range of cumulative exposure values was quite different between the two, raising questions about the inter-study comparability of methods of exposure assessment. The four studies employed different strategies for comparing exposed with unexposed. While the British study used a classic SMR comparison with national rates, the Dutch study did the same, but also compared the exposed with a different unexposed cohort. One of the studies from the United States compared the exposed with national rates and with rates of mortality and incidence in other plants of the same large company. The other compared the exposed with workers in the same plants who were unexposed to acrylonitrile. Typically, in each study, a number of analyses were carried out, varying comparison groups and other parameters.

There was no significant excess risk for any type of cancer when all exposed workers were compared with unexposed, or with an external comparison population. Further, when the study subjects were subdivided by levels of exposure (cumulative exposure when feasible), for no site but lung was there any hint that risk increased with exposure. For lung cancer, there was an indication that workers with the highest exposures had relative risk estimates greater than 1.0. This finding was strongest in the largest of the studies, which had one of the most intensive exposure assessment protocols, but the other

studies gave either negative or only weakly supportive results. Even in the largest study (where the relative risk in the highest exposure quintile ranged from 1.2 to 1.7 depending on the parameters in the analysis), the finding was not consistently significant; there was no coherent dose–response pattern throughout the range of exposures and the risk in the highest decile of exposure was lower than that in the second highest decile. On balance and given the largely unsupportive findings from the other studies, the evidence from this one study was not considered to be sufficiently strong to conclude that there was a credible association between acrylonitrile and lung cancer. Thus, the earlier indications of an increased risk among workers exposed to acrylonitrile were not confirmed by the recent, more informative studies.

5.3 Animal carcinogenicity data

Acrylonitrile has been tested for carcinogenicity in one study in rats by inhalation with pre- and postnatal exposure. This study confirmed the findings of increased incidences of glial cell tumours of the central nervous system found in several previous studies that had not been fully reported and also found increases in malignant mammary tumours, Zymbal gland carcinomas, benign and malignant hepatocellular tumours and extrahepatic angiosarcomas.

5.4 Other relevant data

Acrylonitrile forms adducts with proteins and glutathione. It also forms DNA adducts *in vitro*, but only after cytochrome P450 bioactivation, most likely through its epoxide metabolite (cyanoethylene oxide), which is also formed *in vivo*. Acrylonitrile–haemoglobin adducts have been detected in exposed workers.

Both acrylonitrile and cyanoethylene oxide can conjugate with glutathione, leading to detoxification of these reactive compounds. At high doses of acrylonitrile, as used in animal studies, glutathione in certain tissues may be depleted. Such glutathione depletion will probably not occur at low-level human exposure.

Acrylonitrile is mutagenic *in vitro*; in *Salmonella* systems, bioactivation (to cyanoethylene oxide) is required, but in *Escherichia coli* and in rodent systems, bioactivation by an added microsomal system is not required. The results of genotoxicity experiments *in vivo* have in most cases been negative, although acrylonitrile is mutagenic in *Drosophila*.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of acrylonitrile.

There is *sufficient evidence* in experimental animals for the carcinogenicity of acrylonitrile.

Overall evaluation

Acrylonitrile is *possibly carcinogenic to humans (Group 2B)*.

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1,3-BUTADIENE

This substance (hereinafter referred to as butadiene) was considered by previous Working Groups, in June 1985 (IARC, 1986; see also correction, IARC, 1987a), March 1987 (IARC, 1987b) and October 1991 (IARC, 1992). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

One of the metabolites of butadiene, 1,2:3,4-diepoxybutane (hereinafter referred to as diepoxybutane), also was previously evaluated by an IARC Working Group (IARC, 1976), and its reevaluation by the present Working Group is included in this monograph.

1. Exposure Data

1.1 Chemical and physical data

Butadiene

1.1.1 *Nomenclature*

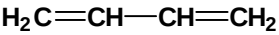
Chem. Abstr. Serv. Reg. No.: 106-99-0

Chem. Abstr. Name: 1,3-Butadiene

IUPAC Systematic Name: 1,3-Butadiene

Synonyms: Biethylene; bivinyl; butadiene; buta-1,3-diene; α,γ-butadiene; *trans*-butadiene; divinyl; erythrene; pyrrolylene; vinylethylene

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_4H_6 Relative molecular mass: 54.09

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless mildly aromatic gas (Budavari, 1996)

(*b*) *Boiling-point*: –4.4°C (Lide, 1995)

(*c*) *Melting-point*: –108.9°C (Lide, 1995)

(*d*) *Density*: d_4^{20} 0.6149 (Lide, 1995)

(*e*) *Spectroscopy data*: Ultraviolet (Grasselli & Ritchey, 1975), infrared (Sadtler Research Laboratories, 1995; prism [893a], grating [36758]), nuclear magnetic resonance and mass spectral data (NIH/EPA Chemical Information System, 1983) have been reported.

(*f*) *Solubility*: Very slightly soluble in water (735 mg/L at 20°C); soluble in ethanol, diethyl ether, benzene and organic solvents; very soluble in acetone (Lide, 1995; Budavari, 1996; Verschueren, 1996)

(*g*) *Volatility*: Vapour pressure, 120 kPa at 0°C (Lide, 1995); 235 kPa at 20°C (Müller & Löser, 1985); relative vapour density (air = 1), 1.87 (Verschueren, 1996)

(*h*) *Stability*: Flash-point, –76°C; very reactive; may form explosive peroxides upon exposure to air; polymerizes readily, particularly if oxygen is present (Lewis, 1993; Budavari, 1996)

(*i*) *Explosive limits*: Lower, 2.0%; upper, 11.5% (Budavari, 1996)

(*j*) *Conversion factor*: $mg/m^3 = 2.21 \times ppm$[1]

Diepoxybutane

1.1.1 *Nomenclature*

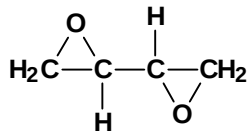
Chem. Abstr. Serv. Reg. No.: 1464-53-5

Chem. Abstr. Name: 2,2′-Bioxirane

IUPAC Systematic Name: 1,2:3,4-Diepoxybutane

Synonym: Butadiene dioxide

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_4H_6O_2$ Relative molecular mass: 86.10

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid (Budavari, 1996)

(*b*) *Boiling-point*: 138°C (Budavari, 1996)

(*c*) *Melting-point*: –19°C (Budavari, 1996)

(*d*) *Solubility*: Miscible with water (hydrolyses) (Budavari, 1996)

(*e*) *Vapour pressure*: 918 Pa at 25°C (United States National Library of Medicine, 1997)

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[1] Calculated from: mg/m^3 = (relative molecular mass/24.47) × ppm, assuming a temperature of 25°C and a pressure of 101 kPa

1.1.4 *Technical products and impurities*

Butadiene is available commercially as a liquefied gas under pressure. The polymerization grade has a minimum purity of 99%, with acetylene as an impurity in the parts-per-million (ppm) range. Isobutene, 1-butene, butane and *cis*-2- and *trans*-2-butene have been detected in pure-grade butadiene (Miller, 1978). Typical specifications for butadiene are: purity, ≥ 99.5%; inhibitor (*tert*-butylcatechol), 50–150 ppm; impurities (ppm max.): 1,2-butadiene, 20; propadiene, 10; total acetylenes, 20; dimers, 500; isoprene, 10; other C_5 compounds, 500; sulfur, 5; peroxides (as H_2O_2), 5; ammonia, 5; water, 300; carbonyls, 10; nonvolatile residues, 0.05 wt% max.; and oxygen in the gas phase, 0.10 vol% max. (Sun & Wristers, 1992). Butadiene has been stabilized with hydroquinone, catechol and aliphatic mercaptans (IARC, 1986, 1992).

1.1.5 *Analysis*

Selected methods for the analysis of butadiene in various matrices are listed in Table 1. Methods of analysis of butadiene in air have recently been evaluated. There appears to be no single preferred method, but newer methods give higher performance. Thermal desorption methods provide high levels of accuracy and precision (Bianchi *et al*., 1997).

The specificity and detection limits of methods for determining simple, small molecules present in packaging material which migrate into packaged goods have been discussed (Vogt, 1988). Butadiene can be determined in plastic polymers, foods and food simulants by chromatographic methods.

Several gas detector tubes are used in conjunction with common colorimetric reactions to detect butadiene. The reactions include the reduction of chromate or dichromate to chromous ion and the reduction of ammonium molybdate and palladium sulfate to molybdenum blue (Saltzman & Harman, 1989).

1.2 Production and use

1.2.1 *Production*

Butadiene was first produced in the late nineteenth century by pyrolysis of petroleum hydrocarbons (Kirshenbaum, 1978). Commercial production started in the 1930s.

Butadiene is manufactured primarily as a coproduct of steam cracking of hydrocarbon streams to produce ethylene in the United States, western Europe and Japan. However, in certain parts of the world (e.g., China, India, Poland and Russia) it is still produced from ethanol. The earlier manufacturing processes of dehydrogenation of *n*-butane and oxyhydrogenation of *n*-butenes have significantly declined in importance and output. Efforts have been made to make butadiene from other feedstocks such as other hydrocarbons, coal, shale oil and renewable sources such as animal and vegetable oil, cellulose, hemicellulose and lignin, but in the United States none of these has moved beyond the research and development stage (Müller & Löser, 1985; Sun & Wristers, 1992).

Steam cracking is a complex, highly endothermic pyrolysis reaction. During the reaction, a hydrocarbon feedstock is heated to approximately 800°C and 34 kPa for less

Table 1. Methods for analysis of butadiene

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Adsorb (charcoal); extract (carbon disulfide)	GC/FID	200 μg/m^3	United States Occupational Safety and Health Administration (1990a)
	Adsorb (charcoal); extract (dichloromethane)	GC/FID	0.2 μg/sample	Eller (1994)
	Adsorb on Perkin-Elmer ATD 400 packed with polymeric or synthetic adsorbent material; thermal desorption	GC/FID	200 μg/m^3	United Kingdom Health and Safety Executive (1992)
Foods and plastic food-packaging material	Dissolve (*N*,*N*-dimethylacetamide) or melt; inject headspace sample	GC/MS-SIM	~1 μg/kg	Startin & Gilbert (1984)
Plastics, liquid foods	Dissolve in *ortho*-dichlorobenzene; inject headspace sample	GC/FID	2–20 μg/kg	United States Food and Drug Administration (1987)
Solid foods	Cut or mash sample; inject headspace sample	GC/FID	2–20 μg/kg	United States Food and Drug Administration (1987)

[a] Abbreviations GC/FID, gas chromatography/flame ionization detection; GC/MS-SIM, gas chromatography/mass spectrometry with single-ion monitoring

than one second, during which carbon–carbon and carbon–hydrogen bonds are broken. As a result, a mixture of olefins, aromatics, tar and gases is formed. These products are cooled and separated into specific boiling-range cuts of C_1, C_2, C_3 and C_4 compounds. The C_4 fraction contains butadiene, isobutene, 1-butene, 2-butene and some other minor hydrocarbons. The overall process yields of butadiene depend on both the process parameters and the composition of feedstocks. Generally, heavier steam-cracking feedstocks produce greater amounts of butadiene. Separation and purification of butadiene from other components is carried out mainly by an extractive distillation process. The most commonly used solvents are acetonitrile and dimethylformamide; dimethylacetamide, furfural and *N*-methyl-2-pyrrolidinone also have been used for this separation. Another commercial process to separate butadiene from other hydrocarbons uses a solution containing cuprous ammonium acetate, which forms a weak copper(I) complex with butadiene (Müller & Löser, 1985; Sun & Wristers, 1992).

Dehydrogenation of *n*-butane via the Houdry process is carried out under partial vacuum (35–75 kPa) at about 535–650°C with a fixed-bed catalyst. The catalyst contains

aluminium oxide and chromium oxide as the principal components. Normal butenes can also be oxidatively dehydrogenated to butadiene in the presence of a high concentration of steam with fairly high selectivity. The reaction temperature is kept below 600°C to minimize over-oxidation, and the reaction pressure is about 34–103 kPa (Müller & Löser, 1985; Sun & Wristers, 1992).

An estimated 3570 thousand tonnes of butadiene were produced worldwide in 1983 (Anon., 1984). By 1989, that figure had risen to an estimated 6620 thousand tonnes, with the following breakdown by global area (thousand tonnes): North America, 1520; South America, 260; western Europe, 1870; eastern Europe, 1490, Africa and the Middle East, 150; and Asia and the Pacific, 1330 (Sun & Wristers, 1992). Production figures by country for the years 1981–96 are presented in Table 2.

Butadiene remains a major industrial commodity in the United States, ranking 36th among all chemicals produced in 1996 (Anon., 1996a). Seven major producers in the United States, with 10 plant locations, had a total annual capacity of 1900 thousand tonnes in 1996 (Anon., 1996b). Available information indicates that butadiene is produced by seven companies each in Japan and Korea; four companies each in France and Germany; three companies in The Netherlands; two companies each in the Czech Republic and the United Kingdom; one company each in Austria, Canada, Finland, Italy, Mexico, Portugal, Romania, Singapore, Spain and Taiwan; and an undisclosed number of companies in Argentina, Brazil, Bulgaria, China, the Commonwealth of Independent States, India, Poland and Saudi Arabia (Anon., 1996b).

Diepoxybutane is not believed to be produced commercially except in small quantities for research purposes (United States National Library of Medicine, 1997).

Table 2. Butadiene production in selected countries from 1981 through 1996 (thousand tonnes)[a]

Country	1981	1984	1987	1990	1993	1996
Canada	126	127	167	192	174	212
China	NR[b]	141	181	258	NR	NR
China (Taiwan)	NR	NR	NR	NR	90	129
France	266	302	307	281	320	344
Germany	NR	753	700	777	879	673
Italy	163	181	NR	NR	NR	NR
Japan	518	627	707	827	809	1025
Korea (Republic of)	NR	NR	NR	168	486	601
United Kingdom	207	258	231	198	NR	NR
United States	1354	1112	1329	1401	1414	1744

[a] From Anon. (1985, 1988, 1991, 1994, 1997); China National Chemical Information Centre (1993)
[b] NR, not reported

1.2.2 *Use*

Butadiene is used primarily in the production of synthetic rubbers, including styrene–butadiene rubber (SBR), polybutadiene rubber (BR), styrene–butadiene latex (SBL), chloroprene rubber (CR) and nitrile rubber (NR). Important plastics containing butadiene as a monomeric component are shock-resistant polystyrene, a two-phase system consisting of polystyrene and polybutadiene; ABS polymers consisting of acrylonitrile, butadiene and styrene; and a copolymer of methyl methacrylate, butadiene and styrene (MBS), which is used as a modifier for poly(vinyl chloride). It is also used as an intermediate in the production of chloroprene, adiponitrile and other basic petrochemicals. The worldwide use pattern for butadiene in 1981 was as follows (%): SBR + SBL, 56; BR, 22; CR, 6; NR, 4; ABS, 4; hexamethylenediamine, 4; other, 4. The use pattern for butadiene in the United States in 1995 was (%): SBR, 31; BR, 24; SBL, 13; CR, 4; ABS, 5; NR, 2; adiponitrile, 12; and other, 9 (Anon., 1996b).

Diepoxybutane has been proposed for use in curing polymers and cross-linking textile fibres (United States National Library of Medicine, 1997).

1.3 Occurrence

1.3.1 *Natural occurrence*

Butadiene is not known to occur as a natural product.

1.3.2 *Occupational exposure*

According to the 1990–93 CAREX database for 15 countries of the European Union (Kauppinen *et al.*, 1998) and the 1981–83 United States National Occupational Exposure Survey (NOES, 1997), approximately 30 000 workers in Europe and as many as 50 000 workers in the United States were potentially exposed to butadiene (see General Remarks).

Potential exposure to butadiene can occur in the following industrial activities: petroleum refining and related operations (production of C_4 fractions containing butadiene, and production and distribution of gasoline), production of purified butadiene monomer, production of various butadiene-based rubber and plastics polymers and other derivatives, and manufacture of rubber and plastics products (tyres, hoses and a variety of moulded objects).

In the descriptions below, the accuracy of the levels of exposure to butadiene may have been affected by the inability to distinguish between butadiene and other C_4 compounds, low desorption efficiency at low concentrations, possible sample breakthrough in charcoal tubes and possible loss during storage in methods used until the mid-1980s (Lunsford *et al.*, 1990; Bianchi *et al.*, 1997). No measurement data are available on levels of exposure to butadiene before the 1970s, when different processes and working conditions (e.g., during the Second World War) would have resulted in exposure levels different from those now prevalent in developed countries.

(*a*) *Petroleum refining and production of crude butadiene*

Exposure data collected in Europe in 1984–85 suggested that gasoline contains a small percentage of butadiene. Levels of exposure of workers in various job groups in the production and distribution of gasoline are shown in Table 3 (see IARC, 1989). Table 4 shows the exposures since 1984 of workers in different areas of petroleum refineries and petrochemical facilities where crude butadiene is produced (usually a C_4 stream obtained as a by-product of ethylene production). Table 5 shows more recent data from crackers of butadiene production plants for the years 1986–93 (ECETOC, 1997).

Table 3. Personal exposures (mg/m^3) to butadiene associated with gasoline during 1984–85 in 13 European countries (540 measurements)

Activity	Arithmetic mean	Range	Exposure duration (TWA)
Production on-site (refining)	0.3	ND–11.4	8 h
Production off-site (refining)	0.1	ND–1.6	8 h
Loading ships (closed system)	6.4	ND–21.0	8 h
Loading ships (open system)	1.1	ND–4.2	8 h
Loading barges	2.6	ND–15.2	8 h
Jetty man	2.6	ND–15.9	8 h
Bulk loading road tankers			
Top loading < 1 h	1.4	ND–32.3	< 1 h
Top loading > 1 h	0.4	ND–4.7	8 h
Bottom loading < 1 h	0.2	ND–3.0	< 1 h
Bottom loading > 1 h	0.4	ND–14.1	8 h
Road tanker delivery (bulk plant to service station)	ND		
Rail car top loading	0.6	ND–6.2	8 h
Drumming	ND		
Service station attendant (dispensing fuel)	0.3	ND–1.1	8 h
Self-service station (filling tank)	1.6	ND–10.6	2 min

From CONCAWE (1987); ND, not detected; TWA, time-weighted average

(*b*) *Monomer production*

Detailed industrial hygiene surveys were conducted in the United States by the National Institute for Occupational Safety and Health in 1985 in four of 10 facilities where butadiene was produced by solvent extraction of C_4 fractions originating as ethylene co-product streams (Krishnan *et al.*, 1987). Levels of butadiene to which workers in various job categories were exposed are summarized in Table 6. Jobs that require workers to handle or transport containers, such as voiding sample cylinders or loading and unloading tank trucks or rail cars, present the greatest potential exposure. Geometric means of full-shift exposure levels for other job categories were below 1 ppm [2.2 mg/m^3]. Short-term samples showed that such activities as open-loop sampling and

Table 4. Eight-hour time-weighted average concentrations of butadiene to which workers in different jobs in petroleum refineries and petrochemical facilities were exposed from 1984 to 1987

Job area	No. of facilities	Arithmetic mean[a]		Range	
		ppm	mg/m^3	ppm	mg/m^3
Production	7	0.24	0.53	0.008–2.0	0.02–4.4
Maintenance	6	0.11	0.24	0.02–0.37	0.04–0.82
Distribution	1	2.9	6.41		
Laboratory	4	0.18	0.40	0.07–0.4	0.16–0.88

From Heiden Associates (1987)
[a] Weighted by number of exposed workers

cylinder voiding were associated with peak exposures of 100 ppm [220 mg/m^3]. Full-shift area samples indicated that ambient concentrations of butadiene were greatest in the rail car terminals (geometric mean, 1.8 ppm [3.9 mg/m^3]) and in the tank storage farm (2.1 ppm [4.7 mg/m^3]).

Exposure data from 15 monomer extraction sites for the year 1995 (Table 7) indicated that in general personal exposure levels were below 5 ppm [11 mg/m^3]. Data from earlier years (1984–93) showed less than 10% of the measured concentrations exceeding 5 ppm [11 mg/m^3] (Table 8) (ECETOC, 1997).

A recent study on biological monitoring for mutagenic effects of exposure to butadiene reported estimated average exposures of 1 ppm [2.2 mg/m^3] for workers in a butadiene monomer plant. Ambient air concentrations in production areas averaged 3.5 ppm [7.7 mg/m^3], while average concentrations of 0.03 ppm [0.07 mg/m^3] were reported for the control area (Ward *et al*., 1996a). Sorsa *et al*. (1996a) reported that 70% of the samples contained below 0.2 ppm [0.4 mg/m^3] butadiene from two plants in Portugal (personal samples) and Finland (area samples), while 5% and 2% of the samples, respectively, were above 10 ppm [22 mg/m^3].

Monitoring in a Finnish plant generally indicated ambient air levels of less than 10 ppm [22 mg/m^3] at different sites (33 samples; mean sampling time, 5.3 h). In personal samples for 16 process workers, the concentrations ranged from < 0.1 to 477 ppm [< 0.22–1050 mg/m^3] (mean, 11.5 ppm [25 mg/m^3]; median, < 0.1 ppm [< 0.22 mg/m^3]; 46 samples; mean sampling time, 2.5 h). The highest concentrations were measured during sample collection. Protective clothing and respirators were used during this operation (Arbetsmiljöfonden, 1991).

Potential exposures in the monomer industry other than to butadiene include extraction solvents and components of the C_4 feedstock. Extraction solvents differ between facilities; some common ones are dimethylformamide, dimethylacetamide, acetonitrile,

Table 5. Personal exposures to butadiene in crackers of butadiene production plants in the European Union

Job category	Year of measurement	Number of people	Number of samples	Personal exposure (ppm)							
				< 1	1–2	2–3	3–4	4–5	5–10	10–25	≥ 25
Unloading, loading, storage	1986–92	210	92	82	3	3	2	0	0	1	0
Distillation (hot)	1986–93	394	392	382	0	3	1	2	0	2	2
Laboratory, sampling	1986–93	132	184	178	2	1	2	1	0	0	0
Maintenance	1986–92	282	371	364	5	0	1	0	0	1	0
Other	1990–92	467	509	487	18	2	1	1	ND[a]	0	0
Total	1986–93	1485	1548	1493	28	9	8	4	0	4	2

From ECETOC (1997)
[a] ND, not detected (detection limit not stated)

Table 6. Eight-hour time-weighted average exposure levels in personal breathing zone samples at four butadiene monomer production facilities, United States, 1985

Job category	No. of samples	Exposure level (ppm [mg/m^3])		
		Arithmetic mean	Geometric mean	Range
Process technician				
Control room	10	0.45 [1.0]	0.09 [0.2]	< 0.02–1.87 [< 0.04–4.1]
Process area	28	2.23 [4.9]	0.64 [1.4]	< 0.08–34.9 [< 0.18–77]
Loading area				
Rail car	9	14.6 [32.4]	1.00 [2.2]	0.12–124 [0.27–273]
Tank truck	3	2.65 [5.9]	1.02 [2.3]	0.08–5.46 [0.18–12.1]
Tank farm	5	0.44 [0.97]	0.20 [0.44]	< 0.04–1.53 [< 0.09–3.4]
Laboratory technician				
Analysis	29	1.06 [2.3]	0.40 [0.88]	0.03–6.31 [0.07–14.0]
Cylinder voiding	3	126 [277]	7.46 [16.5]	0.42–374 [0.93–826]

From Krishnan *et al.* (1987)

Table 7. Personal exposures to butadiene at 15 monomer extraction sites in the European Union in 1995

Job category	Concentration (ppm)	
	Time-weighted averages	Range of values
Production		
Extraction	< 0.01–2	(0–14)
Derivation[a]	1.4–3.4	(0.07–60)
Storage and filling	< 0.02–5	(0–18.1)
Transport	< 0.1–0.7	(0.02–1.2)
Laboratory	0.03–1	(0–13.1)

From ECETOC (1997)

[a] Integrated monomer extraction and styrene–butadiene production on same site

Table 8. Personal exposures to butadiene in extraction units[a] of butadiene production plants in the European Union

Job category	Year of measurement	Number of people	Number of samples	Personal exposures (ppm)							
				< 1	1–2	2–3	3–4	4–5	5–10	10–25	≥ 25
Unloading, loading, storage	1986–93	392	224	178	9	8	7	2	11	22	7
Distillation (hot)	1985–93	256	626	535	20	19	6	11	8	12	15
Laboratory, sampling	1985–93	45	48	29	4	2	2	2	3	5	1
Maintenance	1986–93	248	127	93	14	3	2	1	3	4	7
Other	1984–92	45	10	8	2	0	0	0	0	0	0
Total	1984–93	986	1035	843	49	32	17	16	25	23	30

From ECETOC (1997)
[a] Isolation of butadiene from C_4 stream

β-methoxypropionitrile (Fajen, 1985a), furfural and aqueous cuprous ammonium acetate (United States Occupational Safety and Health Administration, 1990b). Stabilizers are commonly used to prevent formation of peroxides in air and polymerization. No information was available on these other exposures, or on exposures to chemicals other than butadiene that are produced in some facilities, such as butylenes, ethylene, propylene, polyethylene and polypropylene resins, methyl-*tert*-butyl ether and aromatic hydrocarbons (Fajen, 1985b,c).

(*c*) *Production of polymers and derivatives*

Detailed industrial hygiene surveys were conducted in 1986 in five of 17 facilities in the United States where butadiene was used to produce SBR, nitrile–butadiene rubber, polybutadiene rubber, neoprene and adiponitrile (Fajen, 1988). Levels of butadiene to which workers in various job categories were exposed are summarized in Table 9. Process technicians in unloading, in the tank farm, and in the purification, polymerization and reaction areas, laboratory technicians and maintenance technicians were exposed to the highest levels. Short-term sampling showed that activities such as sampling a barge and laboratory work were associated with peak exposures to more than 100 ppm [220 mg/m^3]. Full-shift area sampling indicated that geometric mean ambient concentrations of butadiene were less than 0.5 ppm [1.1 mg/m^3] and usually less than 0.1 ppm [0.22 mg/m^3] in all locations measured at the five plants.

Table 9. Eight-hour time-weighted average exposure levels in personal breathing-zone samples at five plants producing butadiene-based polymers and derivatives, United States, 1986

Job category	No. of samples	Exposure level (ppm [mg/m^3])		
		Arithmetic mean	Geometric mean	Range
Process technician				
Unloading area	2	14.6 [32.27]	4.69 [10.37]	0.770–28.5 [1.7–63.0]
Tank farm	31	2.08 [4.60]	0.270 [0.60]	< 0.006–23.7 [< 0.01–2.4]
Purification	18	7.80 [17.24]	6.10 [13.48]	1.33–24.1 [3.0–53.3]
Polymerization or reaction	81	0.414 [0.92]	0.062 [0.14]	< 0.006–11.3 [< 0.01–5.0]
Solutions and coagulation	33	0.048 [0.11]	0.029 [0.06]	< 0.005–0.169 [< 0.01–4]
Crumbing and drying	35	0.033 [0.07]	0.023 [0.05]	< 0.005–0.116 [< 0.01–0.26]
Packaging	79	0.036 [0.08]	0.022 [0.05]	< 0.005–0.154 [< 0.01–0.34]
Warehouse	20	0.020 [0.04]	0.010 [0.02]	< 0.005–0.068 [< 0.01–0.15]
Control room	6	0.030 [0.07]	0.019 [0.04]	< 0.012–0.070 [< 0.03–0.16]
Laboratory technician	54	2.27 [5.02]	0.213 [0.47]	< 0.006–37.4 [< 0.01–82.65]
Maintenance technician	72	1.37 [3.02]	0.122 [0.27]	< 0.006–43.2 [< 0.01–95.47]
Utilities operator	6	0.118 [0.26]	0.054 [0.12]	< 0.006–0.304 [< 0.01–0.67]

From Fajen (1988)

More recent data are available from 13 of 27 European sites where synthetic rubber and rubber latex were produced and from on-going exposure surveys in an SBR-producing plant in the Netherlands. Less than 10% of the measured concentrations from the European sites exceeded 5 ppm (Table 10). Data from the Netherlands were available from 1976 onwards, although for the earlier surveys the measurement methods used were unknown and therefore the overview is limited to the period 1983–97. No clear trend can be seen for these years, but average exposures were relatively low (arithmetic mean < 3 ppm [6.6 mg/m^3]) (Table 11).

Other data on levels of exposure to butadiene have been collected during health surveys and epidemiological studies (Table 12). At an SBR manufacturing plant in the United States in 1979, the only two departments in which levels were greater than 10 ppm [22 mg/m^3] were the tank farm (53.4 ppm [118 mg/m^3]) and maintenance (20.7 ppm [46 mg/m^3]) (Checkoway & Williams, 1982). In samples taken at one of two United States SBR plants in 1976, levels above 100 ppm [220 mg/m^3] were encountered by technical services personnel (115 ppm [253 mg/m^3]) and an instrument man (174 ppm [385 mg/m^3]) (Meinhardt *et al.*, 1978). Overall mean 8-h time-weighted average (TWA) exposure levels differed considerably between the two plants, however: 1.24 ppm [2.7 mg/m^3] in one plant and 13.5 ppm [30 mg/m^3] in the other (Meinhardt *et al.*, 1982).

A study by the University of Alabama at Birmingham retrospectively assessed historical exposure to butadiene of SBR workers from eight North American plants using elaborate methods. Estimates of 8-h TWA exposures to butadiene were made for a total of 664 plant-specific work area group–year combinations and ranged from 0 to 64 ppm [0–140 mg/m^3]. The median TWA among groups with any butadiene exposure was below 2 ppm in all plants (Macaluso *et al.*, 1996). The same authors also performed an in-depth study to assess the feasibility of improving the exposure estimation procedures in one of the plants (Macaluso *et al.*, 1997). The revised procedures led to exposure estimates that were higher than the original ones, especially during the 1950s and 1960s. Historical exposure profiles of exposed employees in this plant showed average concentrations of 12–16 ppm [26–35 mg/m^3] in the 1940s, 17–25 ppm [38–55 mg/m^3] in the 1950s and a gradual decline to approximately 2 ppm [4.4 mg/m^3] in the 1980s.

A recent biological monitoring study reported average exposures using personal sampling of 0.30, 0.21, and 0.12 ppm [0.66, 0.46 and 0.27 mg/m^3] for the high, intermediate and low exposed groups in an SBR plant in Texas (Ward *et al.*, 1996a). A similar study in Europe reported exposure levels of 0.2–2.0 ppm [0.44–4.4 mg/m^3] in about 50% of the samples and 10% of the samples exceeded 10 ppm [22 mg/m^3] in an SBR plant in Poland (Sorsa *et al.*, 1996b).

The manufacture of butadiene-based polymers and butadiene derivatives implies potential occupational exposure to a number of other chemical agents, which vary according to product and process, including other monomers (styrene, acrylonitrile, chloroprene), solvents, additives (e.g., activators, antioxidants, modifiers), catalysts, mineral oils, carbon black, chlorine, inorganic acids and caustic solutions (Fajen, 1986a,b; Roberts, 1986). Styrene, benzene and toluene were measured in various departments of

Table 10. Eight-hour time-weighted average personal exposures to butadiene in synthetic rubber plants in the European Union (1984–93)

Job category	No. of workers	No. of samples	Personal exposures (ppm)								
			< 0.5	0.51–1	1.01–2	2.01–3	3.01–4	4.01–5	5.01–10	10.01–25	≥ 25
Unloading, loading and storage	132	77	47	1	8	6	3	0	5	5	2
Polymerization	324	147	61	23	25	18	6	4	7	3	0
Recovery	103	165	113	9	9	14	7	4	5	4	0
Finishing	247	120	90	16	3	4	5	1	1	0	0
Laboratory sampling	115	113	68	13	12	6	4	2	3	5	0
Maintenance	141	39	28	1	2	1	1	2	1	2	1
Total	1062	661	407	63	59	49	26	13	22	19	3

From ECETOC (1997)

Table 11. Eight-hour time-weighted average exposure levels of butadiene in personal breathing-zone samples at a plant producing styrene–butadiene polymer in the Netherlands, 1990–97

Year	No. of samples	Exposure level (mg/m^3 [ppm])		
		Arithmetic mean	Range	Method[a]
1990	27	5.45 [2.47]	0.35–69.06 [0.16–31.24]	3M 3500
1991	19	1.11 [0.50]	0.09–2.88 [0.04–1.30]	NIOSH 1024
1992	23	2.79 [1.26]	0.13–11.78 [0.06–5.33]	3M 3520
1993	38	2.87 [1.30]	0.15–13.13 [0.07–5.94]	3M 3520/ NIOSH 1024
1996/97 process operators	20	2.77 [1.25]	0.13–46.62 [0.06–21.10]	3M 3520
1996/97 maintenance workers	14	0.54 [0.24]	0.12–9.89[0.05–4.48]	3M 3520

From Kwekkeboom (1996) and Dubbeld (1998)

[a] Analytical methods used are described by Bianchi *et al*. (1997). Methods 3M 3500 and 3M 3520 involve absorption onto a butadiene-specific activated charcoal, followed by desorption with carbon disulfide or with dichloromethane, respectively, and analysis by direct-injection gas chromatography with flame ionization detection.

a United States SBR-manufacturing plant in 1979: mean 8-h TWA levels of styrene were below 2 ppm [8.4 mg/m^3], except for tank-farm workers (13.7 ppm [57.5 mg/m^3], 8 samples); mean benzene levels did not exceed 0.1 ppm [0.3 mg/m^3], and those of toluene did not exceed 0.9 ppm [3.4 mg/m^3] (Checkoway & Williams, 1982). Meinhardt *et al*. (1982) reported that the mean 8-h TWA levels of styrene were 0.94 ppm [3.9 mg/m^3] (55 samples) and 1.99 ppm [8.4 mg/m^3] (35 samples) in two SBR-manufacturing plants in 1977; the average benzene level measured in one of the plants was 0.1 ppm [0.3 mg/m^3] (3 samples). Average levels of styrene, toluene, benzene, vinylcyclohexene and cyclooctadiene were reported to be below 1 ppm in another SBR plant in 1977 (Burroughs, 1977).

(*d*) *Manufacture of rubber and plastics products*

Unreacted butadiene was detected as only a trace (0.04–0.2 mg/kg) in 15 of 37 bulk samples of polymers and other chemicals synthesized from butadiene and analysed in 1985–86. Only two samples contained measurable amounts of butadiene: tetrahydrophthalic anhydride (53 mg/kg) and vinylpyridine latex (16.5 mg/kg) (JACA Corp., 1987). Detailed industrial hygiene surveys were conducted in 1984–87 in the United States at a rubber tyre plant and an industrial hose plant where SBR, polybutadiene and acrylonitrile–butadiene rubber were processed. No butadiene was detected in any of 124 personal full-shift samples from workers in the following job categories identified as involving potential exposure to butadiene: Banbury operators, mill operators, extruder

Table 12. Eight-hour time-weighted average exposure levels of butadiene measured in two styrene–butadiene rubber manufacturing plants in the United States

Job classification or department	No. of samples	Exposure level		Year of sampling	Reference
		ppm	mg/m^3		
Instrument man	3	58.6	130	1976	Meinhardt *et al.* (1978)
Technical services personnel	12	19.9	43.9		
Head production operator	5	15.5	34.3		
Carpenter	4	7.80	17.2		
Production operator	24	3.30	7.29		
Maintenance mechanic	17	3.15	6.96		
Common labourer	17	1.52	3.36		
Production foreman	1	1.16	2.56		
Operator helper	3	0.79	1.75		
Pipe fitter	8	0.74	1.64		
Electrician	5	0.22	0.49		
Tank farm	8	20.0	44.3	1979	Checkoway & Williams (1982)
Maintenance	52	0.97	2.14		
Reactor recovery	28	0.77	1.7		
Solution	12	0.59	1.3		
Factory service	56	0.37	0.82		
Shipping and receiving	2	0.08	0.18		
Storeroom	1	0.08	0.18		

operators, curing operators, conveyer operators, calendering operators, wire winders, tube machine operators, tyre builders and tyre repair and buffer workers (Fajen *et al.*, 1990).

Personal 8-h TWA measurements taken in 1978 and 1979 in companies where acrylonitrile–butadiene–styrene moulding operations were conducted showed levels of < 0.05–1.9 mg/m^3 (Burroughs, 1979; Belanger & Elesh, 1980; Ruhe & Jannerfeldt, 1980). In a polybutadiene rubber warehouse, levels of 0.003 ppm [0.007 mg/m^3] were found in area samples; area and personal samples taken in tyre plants found 0.007–0.05 ppm [0.016–0.11 mg/m^3] (Rubber Manufacturers' Association, 1984). In a tyre and tube manufacturing plant in the United States in 1975, a cutter man/Banbury operator was reported to have been exposed to butadiene at 2.1 ppm [4.6 mg/m^3] (personal 6-h sample) (Ropert, 1976).

Occupational exposures to many other agents in the rubber goods manufacturing industry were reviewed in a previous monograph (IARC, 1982).

1.3.3 *Air*

According to the United States Environmental Protection Agency Toxic Chemical Release Inventory, industrial releases of butadiene to the atmosphere from manufacturing

and processing facilities in the United States were 4415 tonnes in 1987, 2344 tonnes in 1990 and 1321 tonnes in 1995 (United States National Library of Medicine, 1997).

The United States Environmental Protection Agency (1990) estimated that butadiene is emitted in automobile exhaust at 8.9–9.8 mg/mile [5.6–6.1 mg/km] and comprises about 0.35% of total hydrocarbon exhaust emissions.

Sidestream cigarette smoke contains approximately 0.4 mg butadiene per cigarette, and levels of butadiene in smoky indoor environments are typically 10–20 μg/m^3 (IARC, 1992).

Butadiene is also released to the atmosphere from the smoke of brush fires, the thermal breakdown or burning of plastics and by volatilization from gasoline (Agency for Toxic Substances and Disease Registry, 1992; IARC, 1992).

Reported concentrations of butadiene in urban air generally range from less than 1 to 10 parts per billion [< 2–22 μg/m^3] (IARC, 1992).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for butadiene in several countries are given in Table 13.

2. Studies of Cancer in Humans

Several reviews of the epidemiology of butadiene and cancer have been published, the latest available being by Himmelstein *et al.* (1997). In what follows, ICD codes are given for lymphohaematopoietic cancers in view of the shifting classification with subsequent editions of the International Classification of Diseases.

2.1 Industry-based studies

The most informative industry-based studies of human exposure to butadiene are summarized in Table 14.

In a case–control study nested within a cohort of 6678 male rubber workers in the United States, deaths from cancers at the following sites were compared with a sample of members of the whole cohort (controls): stomach (41 deaths), colorectal (63), respiratory tract (119), prostate (52), urinary bladder (13), lymphatic and haematopoietic (51) and lymphatic leukaemia (14) (McMichael *et al*., 1976). A 6.2-fold increase in risk for lymphatic and haematopoietic cancers (99.9% confidence interval (CI), 4.1–12.5) and a 3.9-fold increase for lymphatic leukaemia (99.9% CI, 2.6–8.0) were found in association with more than five years' work in manufacturing units producing mainly styrene–butadiene rubber during 1940–60. Of the five other cancer sites investigated, only cancer of the stomach was associated with a significant, 2.2-fold increase in risk (99.9% CI, 1.4–4.3). [The Working Group noted that there was no attempt in this study to assess exposure to specific substances; thus, the relevance of the reported findings to the carcinogenicity of butadiene is unknown. A large number of unusually highly significant associations had been reported

Table 13. Occupational exposure limits and guidelines for butadiene[a]

Country	Year	Concentration (mg/m^3)	Interpretation[b]
Australia	1991	22 (C2)	TWA
Belgium	1991	22 (C2)	TWA
Czechoslovakia	1991	20	TWA
		40	Ceiling
Denmark	1993	22 (Ca)	TWA
Finland	1998	2.2	TWA
France	1993	36	TWA
Germany	1998	34 (C1)	TRK
		11	
Hungary	1993	10 (Ca)	STEL
The Netherlands	1996	46	TWA
The Philippines	1993	2200	TWA
Poland	1991	100	TWA
Russia	1991	100	STEL
Sweden	1991	20 (C3)	TWA
		40 (C3)	Ceiling
Switzerland	1991	11 (C)	TWA
Turkey	1993	2200	TWA
United Kingdom	1991	22	TWA
United States			
ACGIH (TLV)[c]	1997	4.4 (A2)	TWA
NIOSH (REL)	1997	(Ca-lfc)	
OSHA (PEL)	1996	2.2	TWA

[a] From International Labour Office (1991); United States Occupational Safety and Health Administration (1996) (OSHA); American Conference of Governmental Industrial Hygienists (1997a,b) (ACGIH); United States National Library of Medicine (1997b); Deutsche Forschungsgemeinschaft (1998); Ministry of Social Affairs and Health (1998)

[b] TWA, time-weighted average; STEL, short-term exposure limit; TRK, technical exposure limit; TLV, threshold limit value; REL, recommended exposure limit; PEL, permissible exposure limit; A2, suspected human carcinogen; C, suspected of being a carcinogen; C1, human carcinogen; C2, probable human carcinogen; C3, suspected of having a carcinogenic potential; Ca, potential occupational carcinogen; lfc, lowest feasible concentration

[c] Countries that follow the ACGIH recommendations for threshold limit values include: Bulgaria, Colombia, Jordan, Korea (Republic of), New Zealand, Singapore and Viet Nam

Table 14. Epidemiological results from the most informative occupational cohorts with exposure to butadiene

Reference	Country	Cohort size/ no. of deaths	Cancer site	Obs. deaths	SMR	95% CI	Comments
Divine & Hartman (1996)	United States	2795/1222	All	282	0.9	0.8–1.0	31 lymphohaematopoietic cancers among those with potentially highest exposure (SMR, 1.7; 95% CI, 1.2–2.4); SMR decreased by duration of employment
			Lymphohaematopoietic	42	1.5	1.1–2.0	
			Leukaemia	13	1.1	0.6–1.9	
Ward *et al*. (1995, 1996b)	United States	364/185	All	48	1.1	0.8–1.4	All 4 lympho/reticulosarcomas had employment ≥ 2 years (SMR, 8.3; 95% CI, 1.6–14.8), as had the stomach cancers (SMR, 6.6; 95% CI, 2.1–15.3), all occurring in the rubber reserve plant
			Lymphosarcoma and reticulosarcoma	4	5.8	1.6–14.8	
			Stomach cancer	5	2.4	0.8–5.7	
			Leukaemia	2	1.2	0.2–4.4	
Delzell *et al*. (1996)	United States and Canada	15 649/3976	All	950	0.93	0.87–0.99	Among so-called 'ever hourly-paid' subjects, there were 45 leukaemia deaths (SMR, 1.4; 95% CI, 1.0–1.9); SMR for hourly subjects having worked for > 10 years and hired ≥ 20 years ago was 2.2 (95% CI, 1.5–3.2) based on 28 leukaemia deaths
			Lymphosarcoma	11	0.8	0.4–1.4	
			Other lymphopoietic	42	1.0	0.7–1.3	
			Leukaemia	48	1.3	1.0–1.7	
Macaluso *et al*. (1996) (overlapping with Delzell *et al*., 1996)	United States and Canada	12 412/3271 exposed to butadiene[a]	Leukaemia deaths by cumulative ppm–years				Including 7 decedents for whom leukaemia was listed as contributory cause of death, Mantel–Haenszel rate ratios adjusted by race and cumulative exposure to styrene were 1.0, 2.0, 2.1, 2.4 and 4.5 for cumulative ppm–years, respectively
			0	8	0.8	[0.3–1.5]	
			< 1	4	0.4	[0.4–1.1]	
			1–19	12	1.3	[0.7–2.3]	
			20–79	16	1.7	[1.0–2.7]	
			≥ 80	18	2.6	[1.6–4.1]	

[a] Derived from Table 3 in the publication, 75% of the total cohort of 16 610 being exposed

between employment in different work sectors of this industry and different diseases, both neoplastic and non-neoplastic. The report did not indicate the numbers of subjects with cancers in different work areas and did not provide sufficient information to assess whether the computations of relative risks and confidence intervals were appropriate.]

The mortality in a cohort of workers who manufactured butadiene monomer in Texas, United States (Downs *et al*., 1987) has been continuously updated and the cohort has also been extended (Divine, 1990; Divine *et al*., 1993). The latest available update was published in 1996 (Divine & Hartman, 1996). The cohort then included 2795 male workers regularly employed for at least six months between 1942 and 1994. Exposure assessment was based on job history and industrial hygiene sampling. The number of workers lost to follow-up was 574 (20.5%), all but 28 (1%) of those were known to be alive as of the end of 1993. A total of 1222 deaths were identified through 1994, and death certificates were obtained for all but 20 of the deaths (1.6%). The standardized mortality ratio (SMR) for all causes of death was 0.88 (95% CI, 0.83–0.93) and that for all cancers (282 deaths) was 0.9 (95% CI, 0.8–1.0). There were 42 deaths from lymphohaematopoietic cancers (ICD-8, 200–209; SMR, 1.5; 95% CI, 1.1–2.0), nine observed deaths from lymphosarcoma and reticulosarcoma (ICD-8, 200; SMR, 1.9; 95% CI, 0.9–3.6), 13 observed deaths from leukaemia (ICD-8, 204–207; SMR, 1.1; 95% CI, 0.6–1.9) and 15 observed from cancer of other lymphatic tissues (ICD-8, 202, 203, 208; SMR, 1.5; 95% CI, 0.9–2.5). The SMRs for the lymphohaematopoietic cancers decreased with length of employment. Subcohort analyses were made for groups with background, low and varied exposure, based on industrial hygiene sampling. The background-exposure group included persons in offices, transportation, utilities and warehouse. The low-exposure group had spent some time in operating units and the varied-exposure group included those with greatest potential exposure in operating units, laboratories and maintenance. There were 11 deaths from lymphatic and haematopoietic cancers (ICD-8, 200–209) in the low-exposure group (SMR, 1.0; 95% CI, 0.5–1.8) and 31 in the varied-exposure group (SMR, 1.7; 95% CI, 1.2–2.4); in both groups, the SMR decreased with duration of employment. For lymphosarcoma and reticulosarcoma, there were two deaths (SMR, 1.1; 95% CI, 0.1–4.0) and seven deaths (SMR, 2.5; 95% CI, 1.0–5.1) in the low- and varied-exposure groups, respectively. For leukaemia, there were three cases (SMR, 0.7; 95% CI, 0.1–2.0) in the low-exposure subgroup and 11 cases in the varied-exposure group (SMR, 1.5; 95% CI, 0.8–2.8). Somewhat elevated SMRs were obtained in the low-exposure group also for cancer of the lung (46 cases, SMR, 1.2; 95% CI, 0.9–1.6) and kidney (6 cases; SMR, 2.1; 95% CI, 0.8–4.7). In the varied-exposure group, there were nine kidney cancers (SMR, 1.9; 95% CI, 0.9–3.7) and 18 prostate cancers (SMR, 1.2; 95% CI, 0.7–1.9), both sites with slightly but insignificantly increasing SMRs with duration of employment (> 10 years). The elevated risk for all the lymphohaematopoietic cancers and their subcategories occurred among persons who were first employed before 1950. As an adjunct to the SMR analyses, modelling was done using a qualitative cumulative exposure score as a time-dependent explanatory variable for all lymphohaematopoietic cancers (ICD-8, 200–209), lymphosarcoma (ICD-8, 200) lymphosarcoma and

other lymphoma (ICD-8, 200, 202), multiple myeloma (ICD-8, 203) and leukaemia (ICD-8, 204–207). None of these cancers was significantly associated with the cumulative exposure score and all risk estimates were close to unity.

A relatively small cohort mortality study included 364 men who were assigned to any of three butadiene production units located within several chemical plants in the Kanawha Valley of West Virginia, United States, including 277 men employed in a rubber reserve plant which operated during the Second World War and produced butadiene from ethanol or from olefin cracking (Ward *et al*., 1995, 1996b). The butadiene production units included in this study were selected from an index developed by the Union Carbide Corporation. Departments included in the study were those where butadiene was a primary product and neither benzene nor ethylene oxide was present. The cohort studied was part of a large cohort (with 29 139 individuals) of chemical workers whose mortality experience had been reported earlier, although without regard to particular exposures (Rinsky *et al*., 1988). Three subjects were lost to follow-up (0.8%). A total of 185 deaths were observed; the SMR for all causes of death was 0.9 in comparison with the general population of the United States. There were seven deaths from lymphatic and haematopoietic cancers (SMR, 1.8; 95% CI, 0.7–3.6), including four cases of lymphosarcoma and reticulosarcoma (SMR, 5.8; 95% CI, 1.6–14.8 with the population of the United States as the reference and persisting in an analysis using county referent rates). The four cases all had duration of employment of two or more years (SMR, 8.3; $p < 0.05$). There were two cases of leukaemia (SMR, 1.2; 95% CI, 0.2–4.4). A non-significant excess of stomach cancer was observed in the overall cohort (5 cases; SMR, 2.4; 95% CI, 0.8–5.7). All five stomach cancer cases occurred among workers employed in the rubber reserve plant for two or more years (SMR, 6.6; 95% CI, 2.1–15.3).

Another relatively small retrospective mortality study, along with prospective morbidity and haematological analyses, was performed for male employees at the Shell Deer Park Manufacturing Complex in the United States (Cowles *et al*., 1994). There were 614 male employees who had worked in jobs with potential exposure to butadiene from 1948 to 1989. Eligible for the cohort were those who had worked for five years or more with potential exposure before 1948 and those who later had achieved five years of exposure or half of their employment duration with potential exposure. Follow-up of mortality was almost complete through 31 December 1989. Those lost to follow-up after 1983 were assumed to be alive. Out of the cohort, 438 were employed in 1982 or later and subject to follow-up also regarding morbidity for the period 1982–89. Industrial hygiene data from 1979 to 1992 showed that most butadiene exposures did not exceed 10 ppm [22 mg/m^3] as an 8-h time-weighted average (TWA), and most were below 1 ppm [2.2 mg/m^3], with an arithmetic mean of 3.5 ppm [7.7 mg/m^3]. Twenty-four deaths occurred during the mortality study period, which provided 7232 person–years of follow-up (average 15 years; range < 1 year to 42 years). For all causes of death, the SMR was 0.5 (95% CI, 0.3–0.7) and for all cancers 0.3 ($n = 4$; 95% CI, 0.1–0.9) by comparison with local (county) rates. Two deaths were

due to lung cancer (SMR, 0.4; 95% CI, 0.1–1.5) and none due to lympho-haematopoietic cancer (1.2 expected). Morbidity events of six days or more for the 438 butadiene employees were compared with the unexposed in the rest of the Shell Deer Park Manufacturing Complex. No cause of morbidity was in excess for this group; the all-cause standardized morbidity ratio was 0.85 (95% CI, 0.77–0.93) and that for all neoplasms was 0.5 (95% CI, 0.2–1.0). [The Working Group noted the relatively scanty information on the material and methods and the unusually low SMR for all causes in this study.]

Bond *et al.* (1992) reported a mortality study on workers engaged in the development and manufacture of styrene-based products, including styrene–butadiene latex production. The person-years of follow-up during 1970–86 for workers in this production were 11 754. By comparison with United States mortality rates, the SMR for all causes of death was 0.9, based on 82 deaths. There were 13 cancers in total (SMR, 0.6), with no site having an SMR exceeding unity. There was one death from haemato-lymphatic cancer (ICD-8, 200–209). [The Working Group noted the unusually low SMR for cancer and the limited information relating to butadiene.]

Delzell *et al*. (1996) and more recently also Sathiakumar *et al*. (1998) evaluated the mortality experience of 15 649 men employed for at least one year at any of eight styrene–butadiene rubber plants in the United States and Canada. Seven of these plants had previously been studied by Matanoski and Schwartz (1987), Matanoski *et al*. (1990a, 1993) and Santos-Burgoa *et al.* (1992), and a two-plant complex studied earlier by Meinhardt *et al*. (1982) and Lemen *et al.* (1990) was also included. Complete work histories were available for 97% of the subjects. About 75% of the subjects were exposed to butadiene and 83% were exposed to styrene. During 1943–91, the cohort had a total of 386 172 person–years of follow-up and 734 individuals were lost to follow-up (5%). A total of 3976 deaths were observed, compared with 4553 deaths expected on the basis of general population mortality rates for the United States or Ontario (SMR, 0.87; 95% CI, 0.85–0.90). Cancer mortality was slightly lower than expected, with 950 deaths (SMR, 0.93; 95% CI, 0.87–0.99). Eleven lymphosarcomas were observed (SMR, 0.8; 95% CI, 0.4–1.4) and 42 other lymphopoietic cancers (SMR, 1.0; 95% CI, 0.7–1.3). These other lymphopoietic cancers included 17 non-Hodgkin lymphomas, 8 Hodgkin's disease, 14 multiple myelomas, one polycythaemia vera and two myelofibrosis. There were slight increases for lymphosarcoma and these other lymphopoietic cancers in some cohort subgroups, but mortality by number of years worked and process group did not indicate any significant association with occupational exposures. There were 48 observed leukaemia deaths in the overall cohort (SMR, 1.3; 95% CI, 1.0–1.7) and among 'ever hourly-paid' subjects there were 45 deaths (SMR, 1.4; 95% CI, 1.0–1.9). The excess was concentrated among 'ever hourly-paid' subjects with 10 or more years of employment and 20 or more years since hire (28 deaths; SMR, 2.2; 95% CI, 1.5–3.2) and among subjects in polymerization (15 deaths; SMR, 2.5; 95% CI, 1.4–4.1), maintenance labour (13 deaths; SMR, 2.7; 95% CI, 1.4–4.5) and laboratories (10 deaths; SMR, 4.3; 95% CI, 2.1–7.9), which were three areas with potential for relatively high exposure to butadiene or styrene monomers.

Nested case–control studies within the United States and Canadian cohort study have been reported on earlier (Matanoski *et al.*, 1990b; Santos-Burgoa *et al.*, 1992). Macaluso *et al*. (1996) reported an additional analysis of leukaemia mortality among 16 610 subjects (12 412 exposed to butadiene) employed at six of the eight North American styrene–butadiene rubber manufacturing plants investigated by Delzell *et al.* (1996) [14 295 workers were included in the Delzell *et al*. analysis and another 2350 workers from plants other than styrene–butadiene rubber manufacturing were not included in Delzell *et al*.]. There were 418 846 person–years of follow-up through 1991 and 58 leukaemia deaths, seven of which were reported as contributory ('underlying') cause of death and included only in analyses using internal comparisons. Retrospective quantitative estimates of exposure to butadiene, styrene and benzene were developed and the estimation procedure entailed identifying work areas within each manufacturing process, historical changes in exposure potential and specific tasks involving exposure, and using mathematical models to calculate job- and time period-specific average exposures. The resulting estimates were linked with the subjects' work histories to obtain cumulative exposure estimates, which were employed in stratified and Poisson regression analyses of mortality rates. Mantel–Haenszel rate ratios adjusted by race, age and cumulative styrene exposure increased with cumulative butadiene exposure from 1.0 in the unexposed category through 2.0, 2.1, 2.4 to 4.5 in the exposure categories < 1, 1–19, 20–79 and ≥ 80 ppm–years, respectively (p for trend = 0.01). The trend of increasing risk with butadiene exposure was still present after exclusion of the unexposed category ($p = 0.03$). The risk pattern was less clear and nonsignificant for styrene exposure (rate ratios, 0.9, 5.4, 3.4 and 2.7 in the exposure categories < 5, 5–9, 10–39 and ≥ 40 ppm–years, respectively; p for trend = 0.14) and the association with benzene was nil after controlling for exposure to butadiene and styrene exposure. Irons and Pyatt (1998) suggested that dithiocarbamates, which were used between the early 1950s and 1965 as stopping agents in the cold polymerization reaction for styrene–butadiene rubber production, might interact with butadiene in causing leukaemia in exposed workers. [The Working Group noted that there is no evidence that dithiocarbamates cause leukaemia and that such an interaction, if demonstrated, would not exclude a contribution of butadiene to the carcinogenic process.]

3. Studies of Cancer in Experimental Animals

3.1 Inhalation exposure

3.1.1 *Mouse*

Groups of 50 male and 50 female $B6C3F_1$ mice, 8–9 weeks of age, were exposed to butadiene (minimum purity, > 98.9%) at concentrations of 625 or 1250 ppm [1380 or 2760 mg/m^3] by whole-body inhalation for 6 h per day on five days per week for 60 weeks (males) or 61 weeks (females). Equal numbers of animals were sham-exposed and served as controls. The study was terminated after 61 weeks because of a high

incidence of lethal neoplasms in the exposed animals. The numbers of survivors at 61 weeks were: males—49/50 control, 11/50 low-dose and 7/50 high-dose; females—46/50 control, 14/50 low-dose and 30/50 high-dose. As shown in Table 15, butadiene produced haemangiosarcomas originating in the heart with metastases to various organs. The incidence of haemangiosarcomas of the heart in historical controls was 1/2372 in males and 1/2443 in females. Other types of neoplasm for which the incidences were significantly increased (Fisher's exact test) in animals of each sex were malignant lymphomas, alveolar–bronchiolar adenomas or carcinomas of the lung and papillomas or carcinomas of the forestomach. Tumours that occurred with significantly increased incidence in females only included hepatocellular adenoma or carcinoma of the liver: 0/50 control, 2/47 ($p = 0.232$) low-dose and 5/49 ($p = 0.027$) high-dose; acinar-cell carcinoma of the mammary gland: 0/50 control, 2/49 low-dose and 6/49 ($p = 0.012$) high-dose; and granulosa-cell tumours of the ovary: 0/49 control, 6/45 ($p = 0.01$) low-dose and 12/48 ($p < 0.001$) high-dose (United States National Toxicology Program, 1984; Huff *et al*., 1985).

Groups of 60 male B6C3F_1 and 60 male NIH Swiss mice, 4–6 weeks of age, were exposed to 0 or 1250 ppm [2760 mg/m^3] butadiene (> 99.5% pure) by whole-body inhalation for 6 h per day on five days per week for 52 weeks. An additional group of 50 male B6C3F_1 mice was exposed similarly to butadiene for 12 weeks and held until termination of the experiment at 52 weeks. The incidence of thymic lymphomas in B6C3F_1 mice was 1/60 control, 10/48 exposed for 12 weeks and 34/60 exposed for 52 weeks and, in NIH Swiss mice, 8/57 exposed for 52 weeks. Haemangiosarcomas of the heart were observed in 5/60 B6C3F_1 mice and 1/57 NIH Swiss mice (Irons *et al*., 1989). [The Working Group noted the absence of reporting on NIH Swiss control mice.]

Table 15. Incidences of tumours in B6C3F_1 mice exposed to butadiene by inhalation for 61 weeks

	Male			Female		
	0	625 ppm	1250 ppm	0	625 ppm	1250 ppm
Haemangiosarcoma of heart (with metastases)	0/50	16/49 ($p < 0.001$)	7/49 ($p = 0.006$)	0/50	11/48 ($p < 0.001$)	18/49 ($p < 0.001$)
Malignant lymphoma	0/50	23/50 ($p < 0.001$)	29/50 ($p < 0.001$)	1/50	10/49 ($p = 0.003$)	10/49 ($p = 0.003$)
Lung: alveolar–bronchiolar adenoma or carcinoma	2/50	14/49 ($p < 0.001$)	15/49 ($p < 0.001$)	3/49	12/48 ($p = 0.01$)	23/49 ($p < 0.001$)
Forestomach papilloma or carcinoma	0/49	7/40 ($p = 0.003$)	1/44 ($p = 0.47$)	0/49	5/42 ($p = 0.018$)	10/49 ($p < 0.001$)

From United States National Toxicology Program (1984); Huff *et al.* (1985)

Groups of 70–90 male and 70–90 female B6C3F$_1$ mice, 6.5 weeks of age, were exposed to butadiene (purity, > 99%) at concentrations of 0, 6.25, 20, 62.5, 200 or 625 ppm [0, 14, 44, 138, 440 or 1380 mg/m^3] for 6 h per day on five days per week for up to two years. Ten animals per group were killed and evaluated after 40 and 65 weeks of exposure. Survival was significantly reduced ($p < 0.05$) in all groups of mice exposed at 20 ppm or higher; terminal survivors were: males: 35/70 control, 39/70 at 6.25 ppm, 24/70 at 20 ppm, 22/70 at 62.5 ppm, 3/70 at 200 ppm and 0/90 at 625 ppm; females: 37/70 controls, 33/70 at 6.25 ppm, 24/70 at 20 ppm; 11/70 at 62.5 ppm; 0/70 at 200 ppm and 0/90 at 625 ppm. As shown in Table 16, exposure to butadiene produced increases in the incidences in both sexes of lymphomas, heart haemangiosarcomas, lung alveolar/ bronchiolar adenomas and carcinomas, forestomach papillomas and carcinomas, Harderian gland adenomas and adenocarcinomas and hepatocellular adenomas and carcinomas. The incidences of mammary gland adenocarcinomas and benign and malignant ovarian granulosa-cell tumours were increased in females (Melnick *et al*., 1990).

Groups of 50 male B6C3F$_1$ mice, 6.5 weeks of age, were exposed to butadiene (purity, > 99%) by whole-body inhalation for 6 h per day on five days per week at 200 ppm [440 mg/m^3] for 40 weeks, 312 ppm [690 mg/m^3] for 52 weeks, 625 ppm [1380 mg/m^3] for 13 weeks, or 625 ppm [1380 mg/m^3] for 26 weeks. After the exposures were terminated, the animals were placed in control chambers for up to 104 weeks. A group of 70 males served as chamber controls (0 ppm). Survival was reduced in all exposed groups; the numbers of survivors at the end of the study were 35 controls, nine exposed to 200 ppm, one exposed to 312 ppm, five exposed to 625 ppm for 13 weeks, and none exposed to 625 ppm for 26 weeks. As shown in Table 17, exposure to butadiene produced increases in the incidence of lymphoma, heart haemangiosarcomas, lung alveolar/bronchiolar adenomas and carcinomas, forestomach papillomas and carcinomas, Harderian gland adenomas and adenocarcinomas, preputial gland carcinomas and kidney tubular adenomas (Melnick *et al*., 1990). [The Working Group noted that this study has also been reported by the United States National Toxicology Program (1992) with additional data analyses.]

Groups of 60 male and 60 female B6C3F$_1$ mice, 8–10 weeks old, were exposed to butadiene [purity unspecified] by whole-body inhalation for a single 2-h period at concentrations of 0, 1000, 5000 or 10 000 ppm [0, 2200, 11 000 or 22 000 mg/m^3]. The mice were then held for two years, at which time all survivors were killed and tissues and organs examined histopathologically. Survival, weight gains and tumour incidences of exposed mice were not affected by butadiene exposure (survival: males—28/60 control, 34/60 low-dose, 44/60 mid-dose, 34/60 high-dose; females—45/60, 36/60, 38/60, 45/60) (Bucher *et al*., 1993). [The Working Group noted the single short duration of exposure.]

3.1.2 *Rat*

Groups of 100 male and 100 female Sprague-Dawley rats, five weeks of age, were exposed to butadiene (minimal purity, 99.2%) by whole-body inhalation at concentrations of 0, 1000 or 8000 ppm [0, 2200 or 17 600 mg/m^3] for 6 h per day on five days

Table 16. Tumour incidences (I) and percentage mortality-adjusted tumour rates (R) in mice exposed to butadiene for up to two years

Tumour	Sex	Exposure concentration (ppm)											
		0		6.25		20		62.5		200		625	
		I	R	I	R	I	R	I	R	I	R	I	R
Lymphoma	M	4/70	8	3/70	6	8/70	19	11/70	25[a]	9/70	27[a]	69/90	97[a]
	F	10/70	20	14/70	30	18/70	41[a]	10/70	26	19/70	58[a]	43/90	89[a]
Heart, haemangiosarcoma	M	0/70	0	0/70	0	1/70	2	5/70	13[a]	20/70	57[a]	6/90	53[a]
	F	0/70	0	0/70	0	0/70	0	1/70	3	20/70	64[a]	26/90	84[a]
Lung, alveolar–bronchiolar adenoma and carcinoma	M	22/70	46	23/70	48	20/70	45	33/70	72[a]	42/70	87[a]	12/90	73[a]
	F	4/70	8	15/70	32[a]	19/70	44[a]	27/70	61[a]	32/70	81[a]	25/90	83[a]
Forestomach, papilloma and carcinoma	M	1/70	2	0/70	0	1/70	2	5/70	13	12/70	36[a]	13/90	75[a]
	F	2/70	4	2/70	4	3/70	8	4/70	12	7/70	31[a]	28/90	85[a]
Harderian gland, adenoma and adenocarcinoma	M	6/70	13	7/70	15	11/70	25	24/70	53[a]	33/70	77[a]	7/90	58[a]
	F	9/70	18	10/70	21	7/70	17	16/70	40[a]	22/70	67[a]	7/90	48
Hepatocellular adenoma and carcinoma	M	31/70	55	27/70	54	35/70	68	32/70	69	40/70	87[a]	12/90	75
	F	17/70	35	20/70	41	23/70	52[a]	24/70	60[a]	20/70	68[a]	3/90	28
Mammary gland, adenocarcinoma	F	0/70	0	2/70	4	2/70	5	6/70	16[a]	13/70	47[a]	13/90	66[a]
Ovary, benign and malignant granulosa-cell tumour	F	1/70	2	0/70	0	0/70	0	9/70	24[a]	11/70	44[a]	6/90	44

From Melnick *et al*. (1990)

[a] Increased compared with chamber controls (0 ppm), $p < 0.05$, based on logistic regression analysis

Table 17. Tumour incidences (I) and percentage mortality-adjusted tumour rates (R) in male mice exposed to butadiene in stop-exposure studies. After exposures were terminated, animals were placed in control chambers until the end of the study at 104 weeks.

Tumour	Exposure									
	0		200 ppm, 40 wk		312 ppm, 52 wk		625 ppm, 13 wk		625 ppm, 26 wk	
	I	R	I	R	I	R	I	R	I	R
Lymphoma	4/70	8	12/50	35[a]	15/50	55[a]	24/50	61[a]	37/50	90[a]
Heart haemangiosarcoma	0/70	0	15/50	47[a]	33/50	87[a]	7/50	31[a]	13/50	76[a]
Lung alveolar–bronchiolar adenoma and carcinoma	22/70	46	35/50	88[a]	32/50	88[a]	27/50	87[a]	18/50	89[a]
Forestomach squamous-cell papilloma and carcinoma	1/70	2	6/50	20[a]	13/50	52[a]	8/50	33[a]	11/50	63[a]
Harderian gland adenoma and adenocarcinoma	6/70	13	27/50	72[a]	28/50	86[a]	23/50	82[a]	11/50	70[a]
Preputial gland adenoma and carcinoma	0/70	0	1/50	3	4/50	21[a]	5/50	21[a]	3/50	31[a]
Renal tubular adenoma	0/70	0	5/50	16[a]	3/50	15[a]	1/50	5	1/50	11

From Melnick *et al*. (1990)

[a] Increased compared with chamber controls (0 ppm), $p < 0.05$, based on logistic regression analysis

per week for 111 weeks (males) or 105 weeks (females). Survival was reduced in low- and high-dose females and in high-dose males; the numbers of survivors were: males—45 control, 50 low-dose and 32 high-dose; females—46 control, 32 low-dose and 24 high-dose. Tumours that occurred at significantly increased incidence in males were pancreatic exocrine adenomas and carcinomas (3 control, 1 low-dose, 10 ($p < 0.05$) high-dose) and interstitial-cell tumours of the testis (0 control, 3 low-dose, 8 ($p < 0.01$) high-dose). Those that occurred at significantly increased incidence (Fisher's exact test) in females were follicular-cell adenomas and carcinomas of the thyroid gland (0 control, 4 low-dose, 11 ($p < 0.001$) high-dose) with a significant, dose-related trend ($p < 0.001$). Tumours that occurred with positive trends (Cochran–Armitage trend test) only in females were sarcomas of the uterus ($p < 0.05$; 1 control, 4 low-dose, 5 high-dose), carcinomas of the Zymbal gland ($p < 0.01$; 0 control, 0 low-dose, 4 high-dose), and benign and malignant mammary tumours ($p \leq 0.001$; 50 control, 79 low-dose and 81 high-dose). Mammary adenocarcinomas were found in 18 control, 15 low-dose and 26 high-dose rats (Owen *et al*., 1987). [The Working Group noted that differences in tumour incidence between groups were not analysed using statistical methods that took into account differences in mortality between control and treated groups.]

3.2 Carcinogenicity of metabolites

1,2-Epoxy-3-butene (epoxybutene)

A group of 30 male Swiss mice was treated with undiluted epoxybutene, the initial monoepoxide metabolite of butadiene, by skin application at a dose of 100 mg three times per week for life. The median survival time was 237 days and four skin tumours were observed (Van Duuren *et al*., 1963). [The Working Group noted that this incidence was similar to that in control groups that were either administered solvents or left untreated.]

1,2:3,4-Diepoxybutane (diepoxybutane)

D,L-Diepoxybutane and *meso*-diepoxybutane induced skin papillomas and squamous-cell carcinomas when applied to the skin of female Swiss mice at a dose of approximately 3 or 10 mg in 100 mg acetone three times per week for life (Van Duuren *et al*., 1963, 1965). Subcutaneous injection of 0.1 mg D,L-diepoxybutane in 0.05 mL tricaprylin once per week for more than one year induced local fibrosarcomas in female Swiss mice; no tumour was observed in three solvent-treated control groups. Similar findings were seen in female Sprague-Dawley rats (Van Duuren *et al*., 1966).

L-Diepoxybutane was administered by intraperitoneal injection (12 injections thrice weekly) to male and female strain A mice at total doses ranging from 1.7 to 192 mg/kg bw in water or tricaprylin. It increased the incidence and multiplicity of lung tumours (Shimkin *et al*., 1966).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

The toxicokinetics and toxicology of 1,3-butadiene have been reviewed recently (ECETOC, 1997; Himmelstein *et al.*, 1997).

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No measured data are available on butadiene in exposed humans.

Metabolites

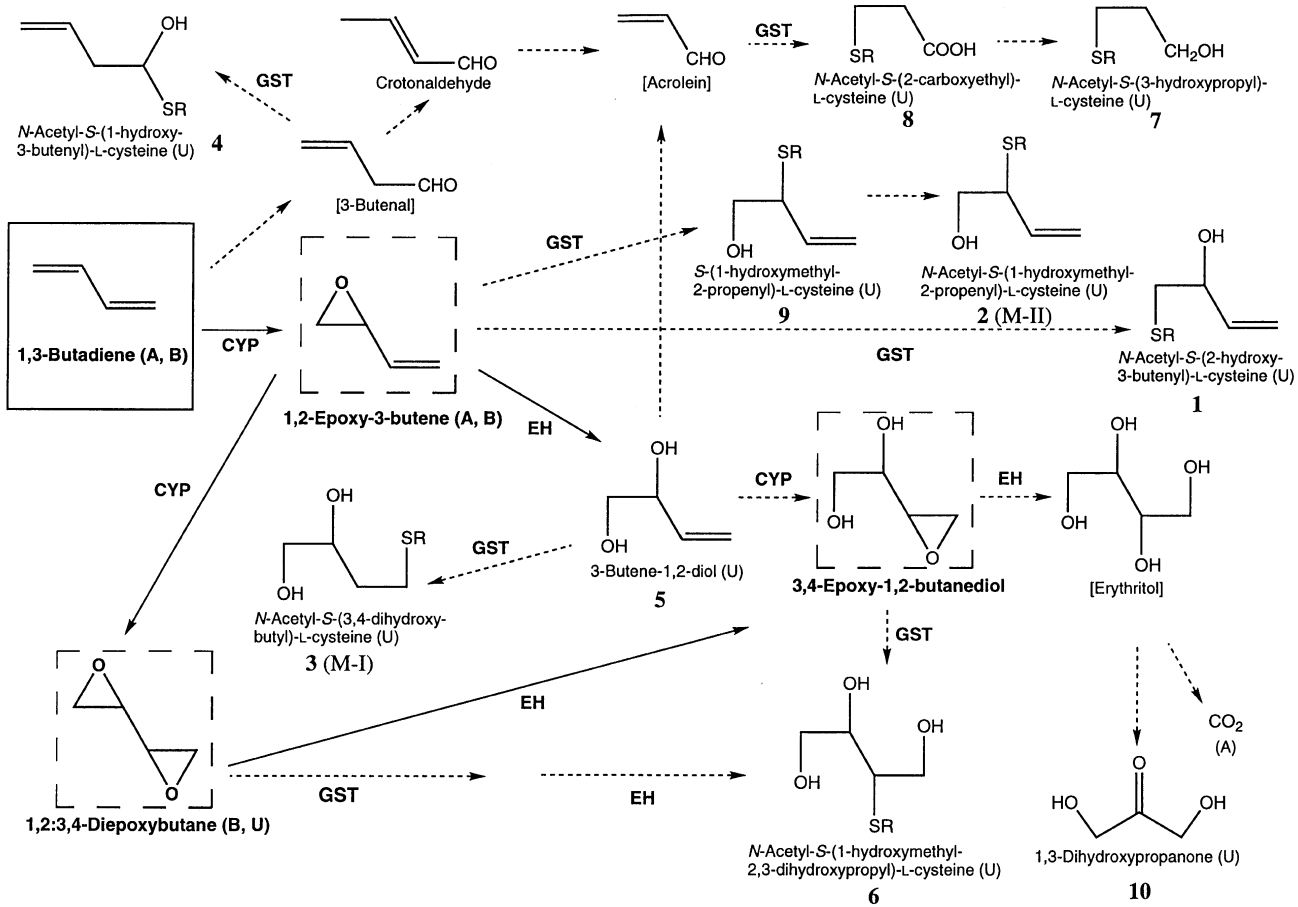
The currently known metabolic pathways of butadiene in man, cynomolgus monkeys, rats and mice are presented in Figure 1.

In seven employees working in production areas with atmospheric concentrations of 3–4 ppm [6.6–8.8 mg/m^3] butadiene over the previous six months, Bechtold *et al.* (1994) detected urinary excretion of the metabolite *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine (M-I, no. 3 in Figure 1) (3.2 ± 1.6 μg/mL) but not of *N*-acetyl-*S*-(1-hydroxymethyl-2-propenyl)-L-cysteine (M-II, no. 2 in Figure 1). In 10 unexposed employees and nine outside controls, urinary M-I concentrations were 0.63 ± 0.19 and 0.32 ± 0.07 μg/mL. M-I was assumed to result from the conjugation of glutathione (GSH) with 3-butene-1,2-diol (butenediol) and M-II from conjugation of GSH with 1,2-epoxy-3-butene (epoxybutene). From the absence of M-II in human urine, it was concluded that epoxybutene is metabolically eliminated in humans predominantly by epoxide hydrolase and not by direct GSH conjugation. Hallberg *et al.* (1997) found the concentration of M-I in urine samples of 24 workers exposed to 2.4 ± 1.8 ppm [5.3 ± 4.0 mg/m^3] butadiene (time-weighted average) to be 2.4 ± 1.9 μg/mL. In 19 controls (butadiene exposure below detection limit of 0.3 ppm [0.66 mg/m^3]), urinary M-I concentrations of 0.69 ± 0.37 μg/mL were measured. In both groups there was no significant difference between smokers and non-cigarette smokers.

Haemoglobin adducts

N-(2-Hydroxy-3-butenyl)valine (HOBVal) as a reaction product of epoxybutene with N-terminal valine in haemoglobin has been found in workers exposed to butadiene. Osterman-Golkar *et al.* (1993) recorded adduct levels of 1.1–2.6 pmol HOBVal/g globin in four nonsmoking workers exposed to about 1 ppm [2.2 mg/m^3] butadiene as estimated from exposure measurements made three to nine months earlier. A haemoglobin binding index of 0.004 pmol HOBVal/(g globin per ppm.h) was estimated from these preliminary results. In nonsmoking workers exposed outside the production area to an environmental butadiene concentration of about 0.03 ppm [0.07 mg/m^3], the adduct levels were below the detection limit of 0.5 pmol HOBVal/g globin. Based upon data from a more recent

Figure 1. Metabolic pathways of butadiene, as deduced from findings in mammals *in vitro* and *in vivo*



A, B, U: metabolites in exhaled air, blood, urine, respectively; CYP, cytochrome P450; GST, glutathione-*S*-transferase; EH, epoxide hydrolase; dashed frame: metabolites forming DNA or haemoglobin adducts; [ ]: proposed metabolites not yet detected**;** dashed lines, assumed pathways; number assignment according to Nauhaus *et al.* (1996)

study (Osterman-Golkar *et al.*, 1996; Sorsa *et al.*, 1996), an even lower binding index of 0.0005 pmol/(g globin per ppm.h) was calculated (Osterman-Golkar & Bond, 1996).

After improving the method to reduce the detection limit to ~0.03–0.05 pmol HOBVal/g globin, Osterman-Golkar *et al*. (1996) measured adduct levels in controls, either five nonsmokers (≤ 0.05 pmol HOBVal/g globin) or four smokers (0.04–0.13 pmol HOBVal/g globin). Similar values were found in laboratory and maintenance workers (≤ 0.06 and ≤ 0.07 pmol HOBVal/g globin in four nonsmokers and three smokers, respectively) exposed to 0.6 ± 0.9 mg/m^3 butadiene. In plant workers exposed to 11.2 ± 18.6 mg/m^3 butadiene, adduct levels were higher (0.2–0.32 and 0.02–0.24 pmol HOBVal/g in three nonsmokers and seven smokers, respectively) [these values were read from a graph]. The mean adduct level given for all 10 workers was 0.16 ± 0.099 pmol HOBVal/g. The authors calculated the amount of butadiene inhaled from the mainstream smoke of 30 cigarettes per day to be equal to that inhaled during an 8-h exposure to 0.1 ppm [0.22 mg/m^3] butadiene. In another plant, measurements of HOBVal were made at two time points (Sorsa *et al*., 1996). In the first investigation, butadiene concentrations at the workplace were > 3 ppm [6.6 mg/m^3], and in the second < 3 ppm. The mean adduct levels were 2 ± 3.6 (n = 12) and 0.54 ± 0.33 pmol HOBVal/g globin (n = 4), respectively. In controls, the mean levels were 0.13 ± 0.35 (n =14) and 0.12 ± 0.05 pmol HOBVal/g globin (n = 8), respectively.

van Sittert and van Vliet (1994) were unable to detect haemoglobin adducts in workers exposed in butadiene manufacture or in smokers.

Pérez *et al*. (1997) found two stereoisomers of *N*-(2,3,4-trihydroxybutyl)valine (THBVal) in haemoglobin resulting from the reaction of 3,4-epoxy-1,2-butanediol (epoxybutanediol) with the N-terminal valine. Theoretically, epoxybutanediol can be formed by oxidation of dihydroxybutene and/or hydrolysis of diepoxybutane. THBVal could also form by direct binding of diepoxybutane to haemoglobin with subsequent hydrolysis of the second epoxide ring. In two workers exposed to a median concentration of 1 ppm butadiene (see Osterman-Golkar *et al*., 1996), the levels of THBVal for one of these isomers were 10 and 14 pmol/g globin, whereas in two control workers the corresponding adduct levels were 1.8 and 3.3 pmol/g globin. These THBVal values were 70-fold higher than corresponding values of HOBVal in the same subjects.

4.1.2 *Experimental systems*

Butadiene

Male Sprague-Dawley rats (Bolt *et al*., 1984) and B6C3F$_1$ mice (Kreiling *et al*., 1986a) were exposed in closed chambers to initial butadiene concentrations in the atmosphere ranging from about 100 to 12 000 ppm [220–26 500 mg/m^3] (rats) or to 5000 ppm [11 000 mg/m^3] (mice) or were treated by intraperitoneal injection of about 1 μL butadiene gas/g bw (rats). The resulting concentration–time courses in the chamber atmosphere revealed linear kinetics below 1000 ppm [2200 mg/m^3] and saturation of metabolism above 2000 ppm [4400 mg/m^3], with maximum rates (μmol/h/kg bw) of 220 in rats and 400 in mice. In the linear range, rates of metabolism per kg body weight were 1.6-fold higher in

mice than in rats. The whole body : air concentration ratio of butadiene at steady state was 0.5 in rats and 1 in mice. Due to metabolic elimination, these values were below the thermodynamic whole body:air partition coefficient, which was determined to be 2.7 in mice and 2.3 in rats. Following induction of metabolizing enzymes by pretreatment of rats with Aroclor 1254, no saturation was observed within the exposure range studied. From these data it was concluded that the rate of butadiene metabolism in the linear range was limited by the uptake from the gas phase into the organism. Metabolism in both species was inhibited effectively by pretreatment with diethyldithiocarbamate. Medinsky *et al*. (1994) also exposed male B6C3F$_1$ mice and Sprague-Dawley rats to butadiene in closed chambers at initial concentrations of up to 5000 ppm. In animals pretreated with pyrazole (32 mg/kg bw) and exposed to initial concentrations of 1200 ppm [2650 mg/m^3] butadiene, metabolism was inhibited completely in rats and the V_{max} was reduced by 87% in mice. Using a dynamic chamber, Leavens *et al*. (1996a) determined the rate of butadiene metabolism in male B6C3F$_1$ mice from the uptake during steady-state exposures (8 h) to 100 or 1000 ppm [220 or 2200 mg/m^3] butadiene. Their value of 246 ± 19 μmol/h/kg bw during exposure to 1000 ppm was close to that of about 270 μmol/h/kg bw given by Kreiling *et al*. (1986) for the same exposure concentration. Bond *et al*. (1986) determined the retention of [1-^{14}C]-butadiene in male B6C3F$_1$ mice and Sprague-Dawley rats exposed via the nose only for 6 h to various concentrations of butadiene. The percentage of ^{14}C retained decreased from 16–20% at 0.14–13 mg/m^3 to 4% at 1800 mg/m^3 in mice and from 17% at 0.14 mg/m^3 to 2.5% at 1800 mg/m^3 in rats, indicating saturation of metabolic elimination. Within the exposure range up to 1800 mg/m^3, the inhaled doses were on average 1.8-fold higher in mice than in rats, when normalized to body surface area. Nose-only exposures (2 h) of male cynomolgus monkeys to [1-^{14}C]butadiene gave much lower retention of butadiene (2.9% at 10.1 ppm [18 mg/m^3], 1.5% at 310 ppm [560 mg/m^3] and 1.7% at 7760 ppm [14 000 mg/m^3]) than in mice and rats. As determined by vacuum-line cryogenic distillation of radioactive compounds, blood concentrations of butadiene in monkeys reached 0.009 μmol/L at 10.1 ppm, 0.6 μmol/L at 310 ppm and 32 μmol/L at 7760 ppm. The resulting blood/air concentration ratios were 0.03, 0.06 and 0.12, respectively, the increase reflecting saturation of butadiene metabolism (Dahl *et al*., 1991). Using headspace gas chromatography, Himmelstein *et al*. (1994) measured the blood concentration of butadiene in male B6C3F$_1$ mice and Sprague-Dawley rats during nose-only exposure (6 h) to butadiene. A steady state was reached in blood after 2 h, giving butadiene concentrations (μmol/L) of 2.4, 37, 58 in mice and 1.3, 18, 37 in rats at 62.5, 625 and 1250 ppm [138, 1380 and 2760 mg/m^3], respectively. These values indicate nearly linear relationships between butadiene concentrations in blood and air, with blood concentrations in mice being about twice those in rats. Blood concentrations declined within minutes after exposure ceased.

Since GSH conjugation is an important pathway in butadiene metabolism, several laboratories have investigated the GSH-depleting effect of butadiene.

Deutschmann and Laib (1989) determined the non-protein sulfhydryl (NPSH) content in lung, liver and heart of male B6C3F$_1$ mice and Sprague-Dawley rats exposed for 7 h to constant butadiene concentrations between 10 and 2000 ppm [22–4400 mg/m^3]. In rats,

hepatic NPSH (% of control) was depleted to 70–80% at 250–1000 ppm and to 40% at 2000 ppm. An NPSH reduction in rat lung to about 80% and 70% was observed only at 1000 and 2000 ppm, respectively, whereas NPSH in rat heart did not change. In mice, hepatic NPSH content began to decrease significantly (70%) at 250 ppm butadiene, and fell to 40% at 1000 ppm and 20% at 2000 ppm. In mouse lung, marked depletion of NPSH (about 50%) occurred at 500 ppm and reached about 10% at 2000 ppm. NPSH content in the heart was reduced to about 75% at 1000 ppm and 30% at 2000 ppm. At conditions of maximum rate of butadiene metabolism (exposure concentration, > 2000 ppm), Kreiling *et al*. (1988) and Laib *et al*. (1990) observed depletion of control levels of hepatic NPSH content in male B6C3F_1 mice to 20% and 4% after 7 h and 15 h, respectively, of butadiene exposure. In contrast, hepatic NPSH content in male Wistar and Sprague-Dawley rats decreased to about 65% and 80%, respectively, after 7 h of exposure; no major change occurred after 15 h.

Following 6 h of exposure to 1250 ppm [2760 mg/m^3] butadiene, Himmelstein *et al*. (1995) found hepatic NPSH to decrease to 57 ± 18% and 62 ± 3% in male B6C3F_1 mice and Sprague-Dawley rats, respectively. In rats exposed to 8000 ppm [17 700 mg/m^3], no further depletion occurred. In lungs of mice, 65% depletion of NPSH was already observed at 62.5 ppm [138 mg/m^3] butadiene, the maximum reduction to 26 ± 13% being reached at 1250 ppm. In rat lung, NPSH was significantly depleted (74 ± 5%) only at 1250 ppm, with a similar value at 8000 ppm.

In male Wistar rats exposed for two weeks (6 h per day, five days per week) to butadiene, urinary mercapturic acids resulting from the conjugation of epoxybutene with GSH were qualitatively analysed by gas chromatography/mass spectrometry after deacetylation as heptafluorobutanoic anhydride derivatives of the cysteine conjugates. The major product formed was assumed to be *S*-(2-hydroxy-3-butenyl)-L-cysteine. Quantitation of the cysteine conjugates as phthaldialdehyde derivatives by high-performance liquid chromatography revealed a nearly linear relationsphip between the amount of cysteine conjugates in afternoon samples [sampling period not given] and the exposure concentration, with a maximum value of about 16 µmol at the highest exposure concentration of 1000 ppm butadiene [2200 mg/m^3] (Osterman-Golkar *et al*., 1991).

Nose-only exposures of B6C3F_1 mice, Sprague-Dawley and Fischer 344/N rats, Syrian hamsters [sexes not specified] to 7600 ppm [14 150 mg/m^3] and male cynomolgus monkeys over 2 h to 8000 ppm [17 700 mg/m^3] [1-^{14}C]butadiene (Sabourin *et al*., 1992) and of male B6C3F_1 mice and Fischer 344/NtacfBR rats over 4 h to 11.7 ppm [26 mg/m^3] butadiene (Bechtold *et al*., 1994) resulted in urinary excretion of two major metabolites identified as *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine (M-I) and *N*-acetyl-*S*-(1-hydroxymethyl-2-propenyl)-L-cysteine (M-II). The ratio of M-I to M-I + M-II was 0.2 in mice, 0.3–0.5 in rats, about 0.4 in hamsters and about 0.9 in monkeys, compared with a value of nearly 1 in humans (see Section 4.1.1). This ratio was positively correlated with the epoxide hydrolase activity in the livers of the different species, suggesting that in these species, as in human metabolism (see Section 4.1.1), hydrolysis of epoxybutene to butenediol precedes the formation of M-I and that M-II is the mercapturate formed from the conjugate of GSH with epoxybutene.

Nauhaus *et al*. (1996) analysed butadiene metabolites in urine of male B6C3F$_1$ mice and Sprague-Dawley rats exposed via the nose only for up to 5 h to 800 ppm [1770 mg/m^3] [1,2,3,4-^{13}C]butadiene. The metabolites identified and their relative quantities are listed in Table 18. Metabolites 1, 2 and 9, derived from epoxybutene via the glutathione pathway, amounted to 70% in mice and 61% in rats, whereas the hydrolytic product butenediol (5) reached only 2.9% in mice and 5% in rats. Metabolite 3, formed by conjugation of butenediol with glutathione, was found in three- to four-fold higher amounts in rats than in mice. Metabolite 6, assumed to be derived from diepoxybutane, was found in small amounts only in mice, as was metabolite 4, which was attributed to the hemithioacetal product of 3-butenal. [Metabolite 6 might also be formed via the oxidation of butenediol to epoxybutanediol.] Metabolites 7 and 8, present only in mouse urine, could not be attributed to a single pathway (via metabolite 3), but the involvement of conversion of butadiene to acrolein has been speculated, too. Rats but not mice excreted 1,3-dihydroxypropanone (10) in urine, probably generated from the postulated erythritol via the pentose phosphate pathway. [The authors assumed erythritol to be derived from diepoxybutane. It might, however, also be formed via the oxidation of butenediol to epoxybutanediol.]

Interaction between butadiene, styrene and benzene

Like butadiene, styrene is metabolized in a first step by cytochrome P450-dependent monooxygenases (Nakajima *et al*., 1994). Co-exposure could therefore lead to mutual influences on the rates of metabolism. Laib *et al*. (1992) co-exposed male Sprague-Dawley rats to butadiene (20, 100, 500, 1000, 3000, 6000 ppm [44, 220, 1100, 2200, 6600, 13 300 mg/m^3] and styrene (0, 20, 100, 250, 500 ppm [0, 85, 430, 1070, 2130 mg/m^3]). Analysing the measured data by means of a toxicokinetic two-compartment model (Filser, 1992), biotransformation rates of both compounds were determined as functions of the exposure concentrations. Whereas butadiene did not affect the metabolic rate of styrene, competitive inhibition of butadiene metabolism by styrene occurred up to a styrene concentration of 90 ppm [380 mg/m^3]. Higher styrene concentrations resulted in only a small additional inhibition. These findings led to the hypothesis that butadiene is metabolized by at least two different cytochrome 450-dependent monooxygenases, only one of which is inhibited by styrene. The presence of several butadiene-metabolizing monooxygenases was later verified by studies *in vitro* (Csanády *et al*., 1992; Duescher & Elfarra, 1994). The lack of inhibition of styrene metabolism by butadiene was attributed to the higher enrichment of inhaled styrene in the body compared to that of inhaled butadiene. Using the data of Laib *et al*. (1992), a physiological toxicokinetic model was developed in order to predict interactions between butadiene and styrene in humans (Filser *et al*., 1993). For low exposure by inhalation to both compounds, biotransformation appeared to be limited by transport to the metabolizing enzymes. Inhibition of butadiene metabolism by styrene in co-exposed people was predicted to occur.

Bond *et al*. (1994) simulated interactions of butadiene with styrene or with benzene in rats using their own physiological toxicokinetic model for butadiene and published

Table 18. Butadiene metabolites in urine of mice and rats exposed to 800 ppm [1770 mg/m^3] [1,2,3,4-^{13}C]butadiene

Metabolite	Percentage of total metabolites	
	Mouse	Rat
1. *N*-Acetyl-*S*-(2-hydroxy-3-butenyl)-L-cysteine	43.9	8.0
2. *N*-Acetyl-*S*-(1-hydroxymethyl-2-propenyl)-L-cysteine	21.6	52.8
3. *N*-Acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine	7.1	26.4
4. *N*-Acetyl-*S*-(1-hydroxy-3-butenyl)-L-cysteine	3.7	Not detected
5. 3-Butene-1,2-diol	2.9	5.0
6. *N*-Acetyl-*S*-(1-hydroxymethyl-2,3-dihydroxypropyl)-L-cysteine	4.6	Not detected
7. *N*-Acetyl-*S*-(3-hydroxypropyl)-L-cysteine	5.4	Not detected
8. *N*-Acetyl-*S*-(2-carboxyethyl)-L-cysteine	4.8	Not detected
9. *S*-(1-Hydroxymethyl-2-propenyl)-L-cysteine	4.7	Not detected
10. 1,3-Dihydroxypropanone	Not detected	5.3

Metabolite numbers correspond to those in Figure 1.

models for styrene (Ramsey & Andersen, 1984) and benzene (Medinsky *et al*., 1989), assuming competitive mutual inhibition of the metabolism of butadiene and styrene and of butadiene and benzene. Whereas the metabolism of butadiene was predicted to be reduced by co-exposure to styrene or benzene, no effect of butadiene on the metabolism of styrene and of benzene was predicted. This was explained by the low solubility of butadiene compared with styrene and benzene.

Leavens *et al.* (1996a, 1997) and Leavens and Bond (1996) further explored the metabolic interactions between butadiene and styrene in male B6C3F$_1$ mice exposed to mixtures of butadiene and styrene by inhalation. At steady state, significant inhibition of butadiene metabolism by styrene was observed with mixtures of 1000 ppm butadiene and 250 ppm styrene, but not with 100 ppm butadiene and 250 ppm styrene. Inhibition by butadiene of styrene metabolism was evidenced by the significant increase in styrene blood concentrations (42% above that in mice exposed to styrene only) in the exposure to 1000 ppm butadiene and 250 ppm styrene. These authors concluded that while exposure to mixtures of styrene and butadiene results in inhibition of metabolism of both styrene and butadiene, interactive effects are seen only at high concentrations that are of little relevance to human exposure.

In order to analyse these observations, Leavens and Bond (1996) developed a physiological toxicokinetic model based on the model of Medinsky *et al*. (1994) for butadiene and the model of Csanády *et al*. (1994) for styrene. As previously found by Laib *et al*. (1992), a reasonable model prediction of the reduced butadiene uptake was obtained only by including two oxidation pathways for both butadiene and styrene, one

catalysed by the same CYP isoenzyme with competitive interaction and another by separate CYP isoenzymes without interaction between the two compounds.

Metabolites in vitro

As in humans, two important metabolites of butadiene are epoxybutene and diepoxybutane (Figure 1).

The half-life of the spontaneous hydration of epoxybutene in water (pH 7) has been calculated using rate constants given in Ross *et al*. (1982) to be 13.7 h and that of diepoxybutane, using rate constants given in Ehrenberg and Hussain (1981), to be 100 h (Gervasi *et al*., 1985).

Epoxybutene is the main first product of the NADPH-dependent metabolism of butadiene in postmitochondrial liver and lung fractions of mouse, rat, monkey and man (Schmidt & Loeser, 1985) and more specifically in the microsomal fraction of mouse liver (Wistuba *et al*., 1989; Elfarra *et al*., 1991; Csanády *et al*., 1992; Duescher & Elfarra, 1992; Recio *et al*., 1992; Sharer *et al*., 1992; Maniglier-Poulet *et al*., 1995), of mouse lung (Csanády *et al*., 1992; Sharer *et al*., 1992), of mouse kidney and testis (Sharer *et al*., 1992), of rat liver (Malvoisin *et al*., 1979; Bolt *et al*., 1983; Wistuba *et al*., 1989; Csanády *et al*., 1992; Cheng & Ruth, 1993; Maniglier-Poulet *et al*., 1995), of rat lung (Csanády *et al*., 1992; Sharer *et al*., 1992), of rat kidney and testis (Sharer *et al*., 1992), of human liver (Csanády *et al*., 1992; Duescher & Elfarra, 1994) and of human lung (Csanády *et al*., 1992). From the correlations with the activity of human liver microsomes to the specific substrates chlorzoxazone (Csanády *et al*., 1992; Duescher & Elfarra, 1994) and coumarin (Duescher & Elfarra, 1994), CYP2E1 and CYP2A6 were concluded to be the major isoenzymes catalysing the oxidation of butadiene, CYP2E1 at low and CYP2A6 at high butadiene concentrations. This was confirmed using microsomal preparations from six human B-lymphoblastoid cell lines, each expressing a particular human cDNA encoding specific CYP isoenzymes (Duescher & Elfarra, 1994).

The rate of butadiene metabolism in diverse cell fractions has been investigated in various species and is characterized by the Michaelis–Menten parameters V_{max} and apparent K_m (K_{mapp}). Three methods have been used to determine these parameters:

(i) loss of butadiene in the headspace of a closed vial due to metabolism in the incubate; analysis using a two-compartment model (Filser *et al*., 1992);

(ii) formation of epoxybutene, measured in the headspace of a closed vial; analysis using a two-compartment model taking into account the further metabolism of epoxybutene (Csanády *et al*., 1992; Recio *et al*., 1992).

(iii) formation of epoxybutene, measured in the incubate without consideration of hydrolysis or vaporization (Malvoisin *et al*., 1979; Elfarra *et al*., 1991; Sharer *et al*., 1992; Cheng & Ruth, 1993; Duescher & Elfarra, 1994; Maniglier-Poulet *et al*., 1995).

The differences in methodology complicate the direct comparison of the results. For physiological toxicokinetic modelling, the data obtained by the first two methods were used.

Tables 19, 20 and 21 present the V_{max}/K_{mapp} values which were used in physiological toxicokinetic models developed for the formation and degradation of epoxybutene and diepoxybutane, all of which were obtained from in-vitro measurements. Although these parameters were obtained in different laboratories, the similarity of the data is striking. Most interestingly, in liver microsomes of NMRI mice, CYP-dependent monooxygenase-mediated oxidation of butadiene was about 10 times lower than in liver microsomes from $B6C3F_1$ mice. However, oxidative metabolism of inhaled butadiene was accurately predicted for conditions *in vivo* using both values. This can be explained by the fact that, over a broad concentration range, the first step in the metabolism of inhaled butadiene is not limited by enzymic capacity but by uptake into the blood and transport through the metabolizing organs (Filser *et al*., 1993). Furthermore, only part of the metabolized inhaled butadiene is systemically available as epoxybutene (Filser & Bolt, 1984; Johanson & Filser, 1993; Csanády *et al*., 1996; Sweeney *et al*., 1997).

Molecular modelling of butadiene oxidation by CYP2E1 has indicated that species differences in the kinetic parameters might be explained by a non-conservative change from Thr-437 to His-437 between rodents and humans and by a conservative change from Ile-438 to Val-438 (Lewis *et al*., 1997).

Table 19. V_{max}/K_{mapp} values of the NADPH-dependent oxidation of butadiene and epoxybutene, as determined in cell fractions and used for physiological toxicokinetic modelling

	V_{max}/K_{mapp} (nmol.L/mg protein/min/mmol)	Reference
Oxidation of butadiene to epoxybutene		
NMRI mouse, liver microsomes	134	Filser *et al*. (1992)
Sprague-Dawley rat, liver microsomes	62	
Human (n = 1), liver microsomes	111	
$B6C3F_1$ mouse		Csanády *et al*. (1992)
Liver microsomes	1295	
Lung microsomes	461	
Sprague-Dawley rat		
Liver microsomes	157	
Lung microsomes	21	
Human		
Liver microsomes (n = 12)	230	
Lung microsomes (n = 5)	75	
Oxidation of epoxybutene to diepoxybutane		
$B6C3F_1$ mouse, liver microsomes	12.8	Csanády *et al*. (1992)
$B6C3F_1$ mouse, liver microsomes	9.2	Seaton *et al*. (1995)
Sprague-Dawley rat, liver microsomes	2.8	
Human (n = 4), liver microsomes	0.15–3.8	

Table 20. V_{max}/K_{mapp} values of the epoxide hydrolase and glutathione *S*-transferase catalysed epoxybutene metabolism, as determined in cell fractions

	V_{max}/K_{mapp} (nmol.L/mg protein/min/mmol)	Reference
Epoxide hydrolase		
NMRI mouse, liver microsomes	13	Kreuzer *et al*. (1991)
Sprague-Dawley rat, liver microsomes	24	
Human (n = 1), liver microsomes	28	
$B6C3F_1$ mouse, liver microsomes	3.6	Csanády *et al*. (1992)
Sprague-Dawley rat, liver microsomes	9.5	
Human (n = 3), liver microsomes	32–38	
Glutathione *S*-transferase		
NMRI mouse, liver cytosol	15	Kreuzer *et al*. (1991)
Sprague-Dawley rat, liver cytosol	11	
Human (n = 1), liver cytosol	8	
$B6C3F_1$ mouse		Csanády *et al*. (1992)
Liver cytosol	14	
Lung cytosol	7.5	
Sprague-Dawley rat		
Liver cytosol	17	
Lung cytosol	2.5	
Human (n = 2), Liver cytosol	4.3	

Liver microsomes from male Sprague-Dawley rats convert butadiene into the *R*- and *S*-enantiomers of epoxybutene (Bolt *et al*., 1983). The ratios of *R*- to *S*-epoxybutene in butadiene-exposed liver microsomes [concentration not specified] were 1 and 1.6 (phenobarbital treatment) in mice [strain not specified], 0.33 and 0.43 (phenobarbital treatment) in rats [strain not specified] and 1.08–1.27 (n = 4) in humans (Wistuba *et al.*, 1989). Exposure of liver microsomes from male Sprague-Dawley rats to 25 000 ppm [55 300 mg/m^3] butadiene gave ratios of *R*- to *S*-epoxybutene that varied with incubation time from about 0.3 at 5 min to about 1 at 30 min (Nieusma *et al.*, 1997). A nearly constant value of 0.75 was determined in liver microsomes from male $B6C3F_1$ mice.

Crotonaldehyde was formed NADPH-dependently as a minor metabolite of butadiene (partial pressure of 48–52 cm Hg = 660 000 ppm) in microsomes obtained from liver, lung or kidney of male $B6C3F_1$ mice (Sharer *et al*., 1992) or human liver (Duescher & Elfarra, 1994), the formation rate being 20–50 times lower than that of epoxybutene. 3-Butenal was suggested as an intermediate metabolite. No crotonaldehyde formation was observed with microsomes from mouse testis or with microsomes of testis, liver, lung or kidney of male Sprague-Dawley rats (Sharer *et al*., 1992).

Table 21. Kinetic constants of epoxide hydrolase- and glutathione *S*-transferase-catalysed metabolism of diepoxybutane in cell fractions

	V_{max} (nmol/mg protein/min)	K_{mapp} (mmol/L)	V_{max}/K_{mapp} (nmol.L/mg protein/min/mmol)
Epoxide hydrolase (Boogaard & Bond, 1996)			
$B6C3F_1$ mouse			
Liver microsomes	32.0 ± 6.0	8.1 ± 1.8	3.93
Lung microsomes	49.8 ± 9.7	7.5 ± 1.7	6.65
Sprague-Dawley rat			
Liver microsomes	52.9 ± 3.5	2.76 ± 0.22	19.2
Lung microsomes	19.3 ± 7.8	7.1 ± 3.4	2.71
Human (n = 6)			
Liver microsomes	155.8 ± 9.8	4.8 ± 0.41	32.5
Lung microsomes	21.7 ± 1.9	2.83 ± 0.37	7.66
Glutathione *S*-transferase (Boogard *et al.*, 1996)			
$B6C3F_1$ mouse			
Liver cytosol	162 ± 16	6.4 ± 1.6	25.3
Lung cytosol	38.5 ± 2.5	1.70 ± 0.37	21.0
Sprague-Dawley rat			
Liver cytosol	186 ± 37	24 ± 6	7.62
Lung cytosol	17.1 ± 3.0	4.2 ± 1.7	4.10
Human (n = 6)			
Liver cytosol	6.4 ± 1.9	2.1 ± 1.4	3.04

Segments from different airway regions or whole airways obtained from male $B6C3F_1$ mice and Sprague-Dawley rats were incubated in headspace vials with 10 000 ppm butadiene gas (34 μmol/L buffer). Epoxybutene formation in tissues from mice was two-fold higher than in rats. The quantity of epoxybutene measured was doubled in the presence of the epoxide hydrolase inhibitor trichloropropene oxide, but remained unchanged following addition of the GSH depletor diethyl maleate, indicating that epoxide hydrolase contributes more than glutathione conjugation to epoxybutene detoxification (Seaton *et al.*, 1996).

Bone-marrow cells of $B6C3F_1$ mice do not contain CYP2E1 (Genter & Recio, 1994). Nevertheless, bone-marrow cells of $B6C3F_1$ mice and humans can oxidize butadiene to epoxybutene, the activity being increased two-fold by 1 mmol hydrogen peroxide/L. The metabolic rate in hydrogen peroxide-fortified lysates of mouse cells (0.0053 nmol/min/mg protein) was two orders of magnitude lower than in mouse and rat liver microsomes (Maniglier-Poulet *et al.*, 1995).

Incubation of butadiene with human myeloperoxidase (from polymorphonuclear leukocytes) in the presence of hydrogen peroxide (1 mmol/L) yielded epoxybutene and small amounts of crotonaldehyde by direct oxygen transfer (Duescher & Elfarra, 1992; Maniglier-Poulet *et al*., 1995). Addition of chloride in the hundred millimolar range led to the formation of 1-chloro-2-hydroxy-3-butene as the major metabolite (Duescher & Elfarra, 1992).

The kinetics of epoxybutene oxidation to diepoxybutane were investigated by Csanády *et al*. (1992) in liver microsomes of male B6C3F$_1$ mice and by Seaton *et al*. (1995) and Krause and Elfarra (1997) in those of male B6C3F$_1$ mice, male Sprague-Dawley rats and human subjects. Similar ratios of V_{max}/K_{mapp} relevant at low epoxybutene concentrations were measured by Csanády *et al*. (1992) and Seaton *et al*. (1995), whereas Krause and Elfarra (1997) found ratios that were one order of magnitude lower than those of Seaton *et al*. (1995) in all three species. [One possible explanation of this difference could be that Krause and Elfarra (1997) determined the kinetic parameters at epoxybutene concentrations that were two to four orders of magnitude higher than those found in the blood of rodents exposed to butadiene under conditions of metabolic saturation. Thus Krause and Elfarra (1997) may have characterized a low-affinity enzyme that is not relevant for in-vivo conditions.] The parameters published by Csanády *et al*. (1992) and Seaton *et al*. (1995) were used for physiological toxicokinetic modelling (see Table 19). [The data of Seaton *et al*. (1995) had to be corrected for hydrolytic loss of diepoxybutane (Sweeney *et al*., 1997).]

Krause and Elfarra (1997) detected NADPH-dependent formation of *meso*- and (±)-diepoxybutane from racemic epoxybutene in mice, rats and humans.

Whereas liver microsomes from male Sprague-Dawley rats formed nonsignificantly higher amounts of diepoxybutane from *R*- than from *S*-epoxybutene, in those of male B6C3F$_1$ mice, the yield was significantly higher from the *S*-isomer than from the *R* (Nieusma *et al*., 1997).

Seaton *et al*. (1995) and Krause and Elfarra (1997) used human B-lymphoblastoid cell lines from the same source each expressing a cDNA of one of eight different human CYP isoenzymes. Epoxybutene at 80 µmol/L was oxidized only by CYP2E1, whereas at 5 mmol/L CYP3A4 was similarly active (Seaton *et al*., 1995). Krause and Elfarra (1997) found CYP2E1 to oxidize epoxybutene at 5 mmol/L nearly four- and six-fold faster than CYP2C9 and 2A6, respectively, whereas in contrast to the Seaton *et al.* (1995) study, CYP3A4 was inactive. Diepoxybutane was hydrolysed in human liver microsomes, the *meso* form being preferred over the two other stereoisomers (Krause & Elfarra, 1997).

In summary, in-vitro results suggest that the rate of cytochrome P450-mediated epoxidation of butadiene to epoxybutene and to diepoxybutane is highest in mice compared with rats and humans and that the rate in humans varies widely (Seaton *et al.*, 1995; see Table 19).

Investigations *in vitro* have demonstrated that epoxybutene is eliminated by microsomal epoxide hydrolase and by cytosolic glutathione *S*-transferase (GST). Epoxide hydro-

lase activity was determined in liver of mouse, rat and man (Kreuzer *et al*., 1991, Csanády *et al*., 1992; Krause *et al*., 1997), in lung of mouse, rat and man (Csanády *et al*., 1992) and in liver of mouse (Recio *et al*., 1992). GST activity was determined in liver of mouse, rat and man (Kreuzer *et al*., 1991; Csanády *et al*., 1992), in lung of mouse, rat and man (Csanády *et al*., 1992), and in liver, lung, testis and kidney of mouse and rat (Sharer *et al*., 1992). Sharer *et al*. (1991) purified π-class GST from human placenta for kinetic studies. The Michaelis–Menten parameters obtained by Kreuzer *et al*. (1991) and Csanády *et al*. (1992) have been used for physiological toxicokinetic modelling (see Table 20).

Hydrolysis of *R*- and *S*-epoxybutene to the respective enantiomer of 3-butene-1,2-diol (butenediol) is nearly completely stereospecific in liver microsomes from male Sprague-Dawley rats, whereas in liver microsomes from male $B6C3F_1$ mice, an inversion of the configuration of 16% (*S*-epoxybutene) and 24% (*R*-epoxybutene) was observed (Nieusma *et al*., 1997).

Epoxybutene is also metabolized by human θ-class GST purified from placenta. Products formed were *S*-(1-hydroxy-3-buten-2-yl)glutathione [*S*-(1-hydroxymethyl-2-propenylglutathione, using the nomenclature of Figure 1] and *S*-(2-hydroxy-3-buten-1-yl)glutathione. The latter product is in 1:1 equilibrium with the relatively stable sulfurane tautomer formed by intramolecular displacement of the hydroxyl group by the sulfur atom (Sharer *et al*., 1991).

Diepoxybutane, like epoxybutene, is eliminated by microsomal epoxide hydrolase in liver and lung of mouse, rat and man (Boogard & Bond, 1996) and by cytosolic GST in liver and lung of mouse and rat and in liver of man (Boogard *et al*., 1996).

In summary, the elimination of epoxybutene and diepoxybutane by GSH conjugation appears to be faster in rodents than in humans. Epoxybutene and diepoxybutane hydrolysis appears to be fastest in humans (see Tables 20 and 21).

Rydberg *et al*. (1996) investigated the reaction of diepoxybutane with valinamide *in vitro* (40°C, pH > 9, 100 h) as a model for the N-terminal valine in haemoglobin. The main products at the lowest diepoxybutane concentration (1 mmol/L) were *N*-(2,3,4-trihydroxybutyl)valinamide and erythritol, formed with similar yields. The amount of a ring-closed pyrrolidine derivative (2,2-*N*,*N*-(2,3-dihydroxybuta-1,4-diyl)valinamide) was three-fold lower. A cross-linked 2,2′-*N*,*N*-(2,3-dihydroxybuta-1,4-diyl)bis-valinamide was detectable at 100 mmol diepoxybutane/L.

Rat θ class GST 5-5 (Thier *et al*., 1995) and human θ class GSTT1-1 (Thier *et al*., 1996), both expressed in *Salmonella typhimurium* TA1535, enhanced the mutagenicity of diepoxybutane but not of epoxybutene. The formation of a reactive glutathione conjugate of the bifunctional diepoxybutane was assumed, possibly a five-membered thialonium ion or a thiiranium (episulfonium) ion. On the other hand, a close correlation was found between the diepoxybutane-dependent induction of sister chromatid exchanges (SCE) (Kelsey *et al*., 1995; Norppa *et al*., 1995; Wiencke *et al*., 1995; Landi *et al*., 1996; Pelin *et al*., 1996) and of micronuclei (Vlachodimitropoulos *et al*., 1997) in human peripheral blood lymphocytes and the homozygous deletion of GSTT1, suggesting detoxification by GSTT1.

Butenediol can be oxidized to 3,4-epoxybutanediol (epoxybutanediol), as has been shown in rat liver microsomes. Incubation for 30 min with butadiene gave concentrations of butenediol and epoxybutanediol which were nearly three-fold and 10-fold, respectively, higher than the corresponding concentration of epoxybutene (Cheng & Ruth, 1993). Epoxybutanediol can, however, also be a product of diepoxybutane hydrolysis.

Kemper and Elfarra (1996) demonstrated the oxidation of butenediol by hepatic alcohol dehydrogenase (ADH), yielding 1-hydroxy-2-butanone as a single stable metabolite; various intermediates have been proposed. For the ADH-dependent oxidation of racemic butenediol in liver cytosol of male B6C3F$_1$ mice, male Sprague-Dawley rats and three humans, saturation kinetics were found. The ratio V_{max}/K_{mapp} was similar in these species. ADH purified from horse liver oxidized butenediol in a stereoselective manner, since V_{max} was about seven times higher for the *S*- than for the *R*-enantiomer.

The fate of epoxybutanediol has not been studied *in vitro*.

Metabolites in vivo

Bolt *et al*. (1983) exposed male Sprague-Dawley rats in a closed system to initial butadiene concentrations of 6000–7000 ppm [13 300–15 500 mg/m^3] and found exhaled epoxybutene to accumulate in the atmosphere up to 2–4 ppm within 15 h. In further studies, animals were exposed for up to 17 h to butadiene concentrations above 2000 ppm [4400 mg/m^3] under conditions of maximum metabolism of butadiene (Filser & Bolt, 1984). Exhaled epoxybutene accumulated in the air of the closed system, reaching a plateau of about 3.7 ppm. Toxicokinetic analysis with a two-compartment model revealed that only 29% of biotransformed butadiene was systemically available as epoxybutene. From these results, the authors deduced the existence of an intrahepatic first-pass effect for epoxybutene formed from butadiene. Using the same experimental design, Kreiling *et al*. (1987) exposed male B6C3F$_1$ mice to butadiene at > 2000 ppm; exhaled epoxybutene accumulated in the atmosphere up to about 10 ppm. From the steady-state concentration of epoxybutene in the atmosphere of the closed chamber containing rats or mice exposed to butadiene under conditions of metabolic saturation and using thermodynamic body/air partition coefficients of 37 for rats (Filser & Bolt, 1984) and 42.5 for mice (Kreiling *et al.*, 1987), the average concentration of epoxybutene in the body was calculated to be 5.5 μmol/L in rats and 17 μmol/L in mice.

In the experiments of Bond *et al*. (1986) described on p. 140, at the end of 6-h exposure to butadiene, blood concentrations of epoxybutene reached values of 0.4 and 4 μmol/L in rats at 130 and 1800 mg/m^3 and 0.7, 0.9 and 15 μmol/L in mice at 13, 130 and 1800 mg/m^3, respectively. In the cynomolgus monkey, Dahl *et al*. (1991) found blood concentrations of only 1.6, 500 and 1100 nmol epoxybutene/L following 2-h exposures to 10, 310 and 7760 ppm [18, 560 and 14 000 mg/m^3] butadiene, respectively, using the same method as Bond *et al*. (1986). [The Working Group noted that due to the unspecific determination of radioactivity in cryogenic traps, contamination of epoxybutene with other metabolic products of butadiene cannot be excluded.]

More recently, concentrations of butadiene epoxides were determined by gas chromatography–mass spectrometry in blood and tissues of B6C3F_1 mice and Sprague-Dawley rats exposed via the nose only to butadiene. Losses of the volatile epoxybutene that may occur in the time between sacrifice and organ dissection have been modelled (Sweeney *et al.*, 1996). These simulations predicted that epoxybutene concentrations in the liver can decrease by orders of magnitude within minutes. Such losses might differ between large and small organs and between those of mouse and rat. However, Himmelstein *et al.* (1994) removed blood from the animal while it was still breathing the exposure atmosphere, so it is unlikely that epoxybutene was lost during sampling. At exposure concentrations of 62.5, 625 and 1250 ppm [138, 1380 and 2760 mg/m^3] butadiene, they found steady-state concentrations (6 h exposure) in blood of 0.56, 3.7 and 8.6 μmol epoxybutene/L in mice and of only 0.07, 0.94 and 1.3 μmol epoxybutene/L in rats. Diepoxybutane reached concentrations of 0.65, 1.9 and 2.5 μmol/L in mice, but was not detected in rats. Bechtold *et al*. (1995) measured epoxybutene concentrations in blood of 0.38 and 0.1 μmol/L in mice and rats, respectively, exposed for 4 h to 100 ppm [220 mg/m^3] butadiene. Diepoxybutane reached 0.33 μmol/L in mice but was not found in rats. Following 6-h exposures to 625 and 1250 ppm butadiene, Himmelstein *et al*. (1995) found epoxybutene concentrations of 0.58 and 0.93 nmol/g (mice) and 0.06 and 0.16 nmol/g (rats) in liver and 2.6 and 3.7 nmol/g (mice) and 0.16 and 0.31 nmol/g (rats) in lung, respectively. Diepoxybutane was detected in mouse lung at concentrations of 0.71 and 1.5 nmol/g tissue at 625 and 1250 ppm butadiene, respectively. Even at 8000 ppm [17 700 mg/m^3] butadiene, no diepoxybutane was detected in rat lung; the detection limit was 0.04 nmol/g.

Thornton-Manning *et al*. (1995a) exposed male mice and rats for up to 4 h to 62.5 ppm [138 mg/m^3] butadiene. Using a highly sensitive method, the authors detected epoxybutene and diepoxybutane in tissues of both species (Table 22). The tissue concentrations of epoxybutene varied considerably between tissues but in general were 3–10 times higher in mice than in rats. With the exception of liver, as the main metabolizing organ, and bone marrow, diepoxybutane reached similar concentrations in all mouse tissues. Corresponding concentrations in rat lung were up to two orders of magnitude lower. The homogeneous distribution of diepoxybutane in the body is also reflected by the similar tissue:hexane partition coefficients determined experimentally (Table 23; Sweeney *et al*., 1997). Thornton-Manning *et al*. (1995b) found tissue concentrations of epoxybutene to be similar in female and male rats exposed for 6 h to 62.5 ppm (Table 22). However, corresponding concentrations of diepoxybutane were three to five times higher in females than in males.

In a further study, Thornton-Manning *et al*. (1997) investigated the disposition of butadiene epoxides in female B6C3F_1 mice and Sprague-Dawley rats following single and repeated (10 days) nose-only exposures (6 h) to 62.5 ppm [138 mg/m^3] butadiene (Table 24). With the exception of lung, tissue and blood concentrations of epoxybutene in rats and mice were higher after repeated exposures. Whereas repeated exposures of rats did not lead to changes in diepoxybutane concentrations, a reduction of up to 30% was observed in mice.

Table 22. Tissue concentrations of epoxybutene and diepoxybutane in rats and mice after inhalation of butadiene

Tissue	Epoxybutene (pmol/g)			
	4 h exposure (Thornton-Manning *et al.*, 1995a)		6 h exposure (Thornton-Manning *et al.*, 1995b)	
	Male mice	Male rats	Male rats	Female rats
Blood	295 ± 27	36 ± 7	25.9 ± 2.9	29.4 ± 2
Liver	8 ± 4	Not detected	n.d.	n.d.
Lung	33 ± 9	Not detected	12.7 ± 5	2.7 ± 4.3
Fat	1302 ± 213	267 ± 14	175 ± 21	203 ± 13
Heart	120 ± 15	40 ± 16	n.d.	n.d.
Spleen	40 ± 19	7 ± 6	n.d.	n.d.
Thymus	104 ± 55	12.5 ± 3.2	n.d.	n.d.
Bone marrow	2.3 ± 1.5[a]	0.2 ± 0.1	9.3; 9.7 (femur)	10.4 ± 1 (femur)
Mammary	n.d.	n.d.	n.d.	57.4 ± 4
	Diepoxybutane (pmol/g)			
Blood	204 ± 15	5 ± 1	2.4 ± 0.4	11.4 ± 1.7
Liver	20 ± 4	Not detected	n.d.	–
Lung	114 ± 37	0.7 ± 0.2	1.4 ± 0.8	4.8 ± 0.7
Fat	98 ± 15	2.6 ± 0.4	1.1 ± 0.1	7.7 ± 1.3
Heart	144 ± 16	3 ± 0.4	n.d.	–
Spleen	95 ± 12	1.7 ± 0.5	n.d.	–
Thymus	109 ± 19	2.7 ± 0.7	n.d.	–
Bone marrow	1.4 ± 0.3[a]	Not detected	1.1; 1.8	7.1 ± 1.3
Mammary	n.d.	n.d.	n.d.	10.5 ± 2.4

$B6C3F_1$ mice and Sprague-Dawley rats inhaled butadiene via the nose only. Three animals were used for each experiment.
n.d., not determined
[a] pmol/mg protein

Inhalation kinetics of epoxybutene were investigated in Sprague-Dawley rats (Filser & Bolt, 1984; Kreiling *et al.*, 1987) and in male $B6C3F_1$ mice (Kreiling *et al.*, 1987) using closed chambers. Animals were exposed to initial concentrations of epoxybutene ranging from 10 to 5000 ppm [22–11 000 mg/m^3] (rats) and 100 to 2000 ppm [220–4400 mg/m^3] (mice). The exhalation of intraperitoneally administered epoxybutene (45.6 µL/kg bw) by rats was also determined (Filser & Bolt, 1984). In rats, first-order kinetics were observed over the whole exposure range. In mice, initial enrichment phases were seen. The further shape of the concentration–time curves was interpreted as showing saturation kinetics. In a

Table 23. Measured partition coefficients of butadiene, epoxybutene and diepoxybutane

	Mouse	Rat	Rat	Man
Butadiene tissue:air	(Medinsky *et al*., 1994)	(Medinsky *et al*., 1994)	(Johanson & Filser, 1993)	(Filser *et al*., 1993)
Blood	1.34	1.49	3.03	1.00
Fat	19.2	22.2	21.9	22.5
Muscle	4.01	1.47	0.73	0.88
Liver	1.35	1.19	0.94	0.68
Lung	1.47	0.92	n.d.	0.48
Kidney	n.d.	n.d.	0.92	0.86
Brain	n.d.	n.d.	0.43	1.05
Spleen	n.d.	n.d.	0.87	n.d.
Epoxybutene tissue:air	(Medinsky *et al*., 1994)	(Medinsky *et al*., 1994)	(Johanson & Filser, 1993)	(Csanády *et al*., 1996)
Blood	36.6	50.4	83.4	93.3
Fat	91.2	138	155	168
Muscle	23.6	19.8	59.9	45.8
Liver	42.1	72.0	53.7	55.3
Lung	56.3	54.7	n.d.	n.d.
Kidney	n.d.	n.d.	70.2	n.d.
Brain	n.d.	n.d.	51.6	n.d.
Diepoxybutane tissue:hexane	(Sweeney *et al*., 1997)			
Blood	0.437			
Fat	0.959			
Muscle	0.795			
Liver	0.615			
Kidney	0.672			

n.d., not determined

later publication, however, it was explained by depletion of GSH at high exposure concentrations, resulting in a loss of GST-mediated detoxification (Johanson & Filser, 1993), on the basis of GSH measurements in tissues of rats and mice exposed to epoxybutene (Deutschmann & Laib, 1989).

Valentine *et al*. (1997) studied the kinetics of epoxybutene and diepoxybutane in blood following intravenous administration to male Sprague-Dawley rats. The following toxicokinetic parameters were obtained for epoxybutene at 71, 143, 286 μmol/kg bw,

Table 24. Tissue concentrations of epoxybutene and diepoxybutane in female mice and rats exposed to butadiene[a]

Tissue	Epoxybutene (pmol/g)			
	Mouse		Rat	
	Single exposure	Multiple exposure	Single exposure	Multiple exposure
Blood	239 ± 24	317 ± 19	44 ± 7	64 ± 8
Lung	~ 25[a]	~ 150[a]	~ 5[a]	Not detected
Mammary	~ 700[a]	~ 1200[a,b]	~ 80[a]	~ 300[a,b]
Fat	~ 1150[a]	~ 1650[a,b]	~ 200[a]	~ 430[a,b]
Femur	~ 56 [a]	Not reported	~ 10[a]	~ 15[a,b]
	Diepoxybutane (pmol/g)			
	Mouse		Rat	
	Single exposure	Multiple exposure	Single exposure	Multiple exposure
Blood	345 ± 33	247 ± 32	14 ± 2	17 ± 2
Lung	219 ± 33	144 ± 13 [b]	5 ± 1	4 ± 0.3
Mammary	265 ± 11	191 ± 17 [b]	11 ± 2	15 ± 1
Fat	203 ± 2	173 ± 11 [b]	8 ± 1	13 ± 0.4 [b]
Ovary	169 ± 13	152 ± 16	6 ± 2	10 ± 7
Femur	214 ± 27	184 ± 19	7 ± 1	9 ± 1

Female B6C3F$_1$ mice and Sprague-Dawley rats were exposed to 62.5 ppm butadiene for 6 h via the nose only, either on one day only or on 10 successive days. Three or four animals were used for each experiment (Thornton-Manning *et al.*, 1997).

[a] Read from diagram

[b] Significantly different from single exposure value, $p \leq 0.05$

respectively: distribution half-lives of 1.4, 1.8, 1.4 min, terminal half-lives of 5.7, 7.0, 8.5 min, systemic clearance of 104, 114, 67 mL/min/kg bw and volume of distribution at steady state of 0.59, 0.58, 0.53 L/kg bw. The corresponding values for diepoxybutane at a dose of 523 μmol/kg bw were: distribution half-life of 2.7 min, terminal half-life of 14 min, systemic clearance of 76 mL/min/kg bw and volume of distribution at steady state of 0.73 L/kg bw. These values were interpreted as demonstrating the similarity of disposition of the two epoxides in rats.

When treated intraperitoneally with epoxybutene (71.3 to 285 μmol/kg bw), male B6C3F$_1$ mice and Sprague-Dawley rats excreted butenediol in urine, the amount within 24 h being less than 1% of the administered dose (Krause *et al.*, 1997).

Conjugation of epoxybutene with GSH in the liver *in vivo* was demonstrated by Sharer and Elfarra (1992) in male Sprague-Dawley rats which excreted *S*-(2-hydroxy-3-

buten-1-yl)glutathione and *S*-(1-hydroxymethyl-2-propenyl)glutathione in a 3:1 ratio in the bile within 60 min following intraperitoneal injection of epoxybutene (14.3–286 μmol/kg bw). The total amount of conjugates excreted was linearly related to dose, indicating no saturation, but accounted for only 7.6 ± 4.2% of the dose.

Following single intraperitoneal administrations of epoxybutene (71.5, 143 or 285 μmol/kg) to male B6C3F_1 mice or Sprague-Dawley rats, diastereomeric pairs of *N*-acetyl-*S*-(2-hydroxy-3-buten-1-yl)-L-cysteine (1 in Figure 1) and *N*-acetyl-*S*-(1-hydroxymethyl-2-propenyl)-L-cysteine (2 in Figure 1) were excreted in the urine within 8 h. In rats, linear dose–response relationships were observed with respect to the excretion of metabolites 1 and 2 (mean, 17% of epoxybutene dose), the amount of metabolite 1 being two to three times higher than that of metabolite 2. In mice, an overproportional increase in the excretion of metabolites 1 and 2 occurred at the highest dose (mean 26%, compared with 7 and 9% at the lower doses, respectively), the amount of metabolite 1 being about one half to one third of that of metabolite 2. The amount per body weight of metabolites 1 and 2 in rats was approximately twice as high as in mice at the lower doses and similar in both species at the high dose (Elfarra *et al*., 1995).

Haemoglobin adducts

Using haemoglobin and epoxybutene, Osterman-Golkar *et al*. (1991) observed the formation of two diastereomeric pairs of adducts to the N-terminal valine of haemoglobin namely *N*-(2-hydroxy-3-buten-1-yl)valine and *N*-(1-hydroxy-3-buten-2-yl)valine. These findings were corroborated by Richardson *et al*. (1996), who incubated erythrocyte suspensions obtained from mice, rats and humans with epoxybutene. The second-order rate constant of adduct formation for the sum of both adducts (HOBVal) was determined *in vitro* at 37°C to be 0.29×10^{-4} L/g globin/h with erythrocytes isolated from mice (Recio *et al*., 1992; value corrected by the same authors to the one quoted here, Osterman-Golkar *et al*., 1993).

In male Wistar rats exposed for two weeks (6 h per day, five days per week) to butadiene, covalent binding of epoxybutene (mainly at C-1) to the N-terminal valine of haemoglobin was observed. Total adduct levels (nmol/g haemoglobin) and the daily average increment (nmol/g haemoglobin) at day 12 were 0.5 and 0.06 at 250 ppm [550 mg/m^3], 1.5 and 0.17 at 500 ppm [1100 mg/m^3] and 3.0 and 0.33 at 1000 ppm [2200 mg/m^3] butadiene. Seventeen days after the end of exposure, the levels had decreased to nearly two thirds of the original values (Osterman-Golkar *et al*., 1991).

Osterman-Golkar *et al*. (1993) observed a linear increase in the HOBVal level up to about 4 nmol/g globin following exposure of male B6C3F_1 mice over four weeks (6 h per day, five days per week) to butadiene (0, 2, 10 and 100 ppm [0, 4, 22 and 220 mg/m^3]). In Sprague-Dawley rats, the increase of HOBVal was linear up to 10 ppm butadiene, amounting to about 0.2 nmol/g globin and reached a value of about 1 nmol/g globin at 100 ppm. The authors also summarized haemoglobin binding indices resulting from butadiene exposure (pmol HOBVal/g globin per ppm.h) in different species as ~0.5 in B6C3F_1 mice, ~0.3 in CD2F_1 mice (from Recio *et al*., 1992), ~0.09 in Wistar rats

(from Osterman-Golkar *et al*., 1991), and ~0.3 and ~0.1 in Sprague-Dawley rats at 0–10 ppm and 10–100 ppm butadiene, respectively. For humans, a value of ~0.004 and an even lower value of 0.0005 have been estimated (see Section 4.1.1).

Albrecht *et al*. (1993) determined HOBVal in female CB6F_1 mice, male and female C3H × 101/EL mice and female Wistar rats exposed (6 h per day for five days) to butadiene concentrations of 0, 50, 200 and 500 ppm [0, 110, 440 and 1100 mg/m^3]. Additionally, animals were exposed to 1300 ppm [2870 mg/m^3] butadiene, with the exception of male C3H × 101/EL mice. In mice, background levels of HOBVal were between 1 and 8 nmol/g globin. Up to 200 ppm butadiene, a steep increase of the HOBVal levels was observed, reaching values between 10 and 16 nmol/g globin. At higher butadiene concentrations, the slope of the curve flattened and at 1300 ppm, the HOBVal value reached about 25 nmol/g globin. No significant strain or sex difference was observed. In rats, background levels were between 1.3 and 2.2 nmol/g globin. Following exposure, the adduct levels were distinctly lower than in mice. The slope of the dose–response curve between 0 and 200 ppm, reaching a level of about 3 nmol/g globin, was somewhat steeper than between 200 and 1300 ppm, at which the level reached about 5 nmol/g globin.

Pérez *et al.* (1997) exposed male Wistar rats for five consecutive days (6 h per day) to constant butadiene concentrations of 0, 50, 200 and 500 ppm [0, 110, 440 and 1100 mg/m^3]. On day 6, animals were killed; the levels of HOBVal were 0.6, 21, 88 and 180 pmol/g.

Osterman-Golkar *et al.* (1998) investigated the dose–response relationships for adduct formation and persistence in rats and mice during long-term low-level exposure to butadiene by inhalation. Values reported by Osterman-Golkar *et al*. (1993) were also recalculated. HOBVal levels were measured in male B6C3F_1 mice and Sprague-Dawley rats following exposure to 0, 2, 10 or 100 ppm [0, 4, 22 or 220 mg/m^3] butadiene for 6 h per day on five days per week for one, two, three or four weeks. The increase and decrease, respectively, of the adduct levels during and three weeks after the end of the four-week exposure indicated that adducts are chemically stable *in vivo* and that elimination follows the turnover of red blood cells. Adduct levels increased linearly with butadiene concentration in mice, whereas a deviation from linearity between 10 and 100 ppm butadiene (decrease in slope) was observed in rats. Blood concentrations of epoxybutene estimated from haemoglobin adduct levels were in general agreement with those reported in mice and rats exposed to 62.5 ppm butadiene, indicating that HOBVal adduct levels can be used to predict blood concentrations of epoxybutene in rats and mice.

After intraperitoneal administration of epoxybutene (10, 20, 40 and 60 mg/kg bw) to male B6C3F_1 mice and Sprague-Dawley rats, HOBVal levels increased with dose approximately linearly in rats and sublinearly in mice. At the highest dose, the binding efficiency in mice was twice that in rats, HOBVal levels reaching about 950 and 460 pmol/g globin in mice and rats, respectively (Richardson *et al*., 1996).

Tretyakova *et al*. (1996) exposed female and male B6C3F_1/CrlBR mice and Crl:CDBR rats to 1000 ppm [2200 mg/m^3] butadiene (6 h per day, 5 days per week, for

13 weeks). Two isomers of HOBVal were found [not further specified], the level of isomer I being 1.3–1.5-fold that of isomer II. HOBVal levels (means of isomer I up to 11 190 and of isomer II up to 8660 pmol/g globin in female mice) were three to four times higher in mice than in rats, the mean levels in females being about twice those in males.

N-(2,3,4-Trihydroxybutyl)valine (THBVal) in haemoglobin is regarded as a reaction product of epoxybutanediol with N-terminal valine. This adduct could, however, form by direct binding of diepoxybutane to haemoglobin with subsequent hydrolysis of the second epoxide (see Section 4.1.1). Two isomers of this adduct were found in male Sprague-Dawley rats 24 or 48 h following intraperitoneal treatment with epoxybutene (78.3 mg/kg bw), epoxybutanediol (30 and 60 mg/kg bw) or diepoxybutane (16.7 and 33.4 mg/kg bw) or after exposure to butadiene. Adduct levels were reported only for 'adduct II'. Compared with a control level of about 2 pmol/g globin, THBVal reached a maximum level of 2800 pmol/g globin after 33.4 mg/kg bw diepoxybutane. As calculated from THBVal levels, diepoxybutane had higher haemoglobin binding indices (pmol THBVal/g globin per μmol/kg bw) of 9.3 and 7.2 (at 16.7 and 33.4 mg/kg doses, respectively) than epoxybutanediol (3.4 and 4.0 at 30 and 60 mg/kg doses, respectively) and epoxybutene (0.07). In Wistar rats killed one day after exposure (6 h per day, for five days) to 0, 50, 200 or 500 ppm [0, 110, 440 or 1100 mg/m^3] butadiene, the highest THBVal levels of 1190 pmol adduct/g globin were found at 200 ppm (controls: 9 pmol THBVal/g globin). The binding index (pmol THBVal/g globin per ppm × h) decreased from 0.5 at 50 ppm to 0.04 at 500 ppm. Parallel determination of the levels of HOBVal determined in the same rats were three-fold (500 ppm) to about 32-fold (50 ppm) lower than the THBVal levels (Pérez *et al*., 1997).

Physiological toxicokinetic models

Physiological toxicokinetic (or pharmacokinetic) models represent descriptions of biological systems and can be used to describe the behaviour of chemicals in the intact animal. Such models have been used to predict the disposition of butadiene and metabolites in rats, mice, and humans. For the case of rats and mice, these predictions can be compared with experimental data. In some cases (see below), the models successfully describe (and accurately predict) the disposition of butadiene and metabolites. Human physiological toxicokinetic model predictions normally cannot be verified due to lack of experimental data.

Several models have been developed to simulate the absorption, distribution, metabolism and excretion of butadiene, some of its metabolites and its adducts to haemoglobin in mouse, rat and man. Critical aspects are discussed in Csanády *et al.* (1996) and in Himmelstein *et al*. (1997). Basically, the models consist of a number of compartments representing diverse tissues and organs, several of which are grouped together. These compartments are linked by blood flow. The main differences between models are the number of metabolizing and nonmetabolizing compartments, the mechanisms of metabolism, the metabolites taken into consideration, and the values of the biochemical,

physiochemical and physiological parameters. The first group of parameters is represented by apparent Michaelis constants, maximum rates of metabolism, tissue concentrations of GSH and turnover rates. The second group consists of the blood:air, tissue:air and tissue:blood partition coefficients of butadiene and selected metabolites. The structure of the tissue compartments, blood flow rates and alveolar ventilation belong to the third group.

Physiological toxicokinetic models have been presented describing the behaviour of inhaled butadiene in the human body. Partition coefficients for tissue:air and tissue:blood, respectively, had been measured directly using human tissue samples or were calculated based on theoretical considerations. Parameters of butadiene metabolism were obtained from in-vitro studies in human liver and lung cell constituents and by extrapolation of parameters from experiments with rats and mice *in vivo* (see above). In these models, metabolism of butadiene is assumed to follow Michaelis–Menten kinetics.

By means of an apparent Michaelis constant (K_{mapp}) together with a maximum rate (V_{max}) of butadiene metabolism both obtained with human liver microsomes (Filser *et al.*, 1992), Filser *et al.* (1993) constructed a human model which was later extended by Csanády *et al*. (1996) for the butadiene metabolites epoxybutene and diepoxybutane. For butadiene and epoxybutane, the required human tissue:air partition coefficients were measured using autopsy material (Table 23). Filser *et al.* (1993) investigated the influence of styrene co-exposure on butadiene metabolism by assuming competitive interaction. Simulations for a 70-kg man exposed over 8 h to 5 or 15 ppm [11 or 33 mg/m^3] butadiene indicated total amounts of butadiene metabolized of 0.095 and 0.285 mmol, respectively, reduced by about 19% and 37% as a result of co-exposure to 20 and 50 ppm styrene, respectively. No influence of butadiene on styrene metabolism was noted.

Kohn and Melnick (1993) and Medinsky *et al.* (1994) used in their models values of K_{mapp} and V_{max} which had been determined by Csanády *et al.* (1992) with microsomes from human liver and lung. The tissue:blood partition coefficients used by Kohn and Melnick (1993) were theoretically derived and were 5–10 times higher than those derived from the tissue:air partition coefficients measured by Filser *et al.* (1992) in human tissues and by Johanson and Filser (1993) and by Medinsky *et al.* (1994) in rodent tissues. Simulations of human exposure to butadiene under workplace conditions (8 h per day, five days per week) indicated that high accumulation in fat would occur, with levels increasing about three-fold during the week. For their model, Medinsky *et al.* (1994) used either their own partition coefficients determined experimentally in mouse tissues or for comparison those which had been published by Kohn and Melnick (1993). Simulations of concentration–time courses in fat tissue resulting from human exposure to 54 ppm [120 mg/m^3] butadiene for 6 h yielded about three-fold lower peak concentrations and an area under the concentration–time curve (AUC) several times lower when the mouse values were used. With the latter values, which are close to those obtained by Filser *et al.* (1992) in human tissues, no suggestion of accumulation during the working week was found.

Internal burdens of epoxybutene in humans resulting from exposure to butadiene were predicted from models by Kohn and Melnick (1993), Johanson and Filser (1996) and Csanády *et al.* (1996) and were compared with simulations for rats and mice. In the model of Kohn and Melnick (1993), metabolic parameters were incorporated which had been obtained by Csanády *et al.* (1992) by measuring butadiene and epoxybutene oxidation and epoxybutene hydrolysis in human liver and lung microsomes *in vitro*, and conjugation of epoxybutene with glutathione in human liver and lung cytosol. Tissue:blood partition coefficients were theoretically derived. The body burden of epoxybutene following exposure to 100 ppm butadiene for 6 h was predicted to be 0.056 μmol/kg in humans.

Johanson and Filser (1996) used metabolic parameters which had been obtained for enzymic butadiene oxidation (Filser *et al.*, 1992) and epoxybutene hydrolysis (Kreuzer *et al*., 1991) in human liver microsomes and for enzymic conjugation of epoxybutene with glutathione in human liver cytosol (Kreuzer *et al.*, 1991). Tissue:air partition coefficients had been determined experimentally for butadiene in human tissues (Filser *et al.* 1993a) and for epoxybutene in rat tissues (Johanson & Filser, 1993). For an eight-hour exposure to 10 ppm butadiene, the model predicted a blood concentration of epoxybutene of about 0.08 μmol/L in a man (Johanson & Filser, 1996). Csanády *et al*. (1996) simulated an exposure to 10 ppm (22 mg/m^3) butadiene over 8 h and predicted an AUC of epoxybutene in blood of 0.27 μmol.h/L. Most of the model parameters used by these authors were identical to those of Johanson and Filser (1996). Tissue:air partition coefficients for epoxybutene in humans used by Csanády *et al*. (1996) were measured with human tissue samples (Table 23). The values suggest an almost homogeneous distribution of epoxybutene in the body, with about twofold enrichment in fat tissue. The models of Johanson and Filser (1996) and Csanády *et al*. (1996) predict AUCs of epoxybutene in humans about one order of magnitude higher than those from the model of Kohn and Melnick (1993). The main reason for this difference might lie in the very high theoretically derived fat:air partition coefficient for butadiene which was used by the latter authors, leading to prediction of storage of inhaled butadiene in fat tissue, resulting in reduced availability for biotransformation to epoxybutene during the time span of a single exposure over 6 h.

Physiological toxicokinetic models for experimental systems

Models presented for mice and rats (Evelo *et al*., 1993; Filser *et al*., 1993; Johanson & Filser, 1993; Kohn & Melnick, 1993; Bond *et al*., 1994; Medinsky *et al*., 1994; Csanády *et al*., 1996; Sweeney *et al.*, 1997) predicted, species specifically, similar toxicokinetic behaviour of butadiene. The only exception was the first model of Kohn and Melnick (1993), which contained much higher theoretically derived partition coefficients than the experimentally determined ones, leading to prediction of butadiene storage in fat tissue. In a second, extended version, the authors used average values of the partition coefficients determined experimentally by Johanson and Filser (1993) and Medinsky *et al*. (1994).

The influence of metabolism in the lung with respect to the toxicokinetics of butadiene was simulated in the models of Evelo *et al*. (1993), Kohn and Melnick (1993), Medinsky *et al*. (1994) and Sweeney *et al.* (1997). The model of Evelo *et al*. (1993) yielded the surprising result that the total metabolic activity in lung of mice exposed to 1 ppm [2.2 mg/m^3] butadiene in air would be nearly equal to that in liver. Experimental data confirming this model prediction have not been published. Under similar conditions, the ratios of lung to liver metabolic activity in rats and humans were around 0.2 and 0.08, respectively. This ratio decreased in all species by 30–50% at 1000 ppm [2200 mg/m^3] exposure. The simulations indicated a strong first-pass effect of butadiene in the lung at low concentrations. The model of Kohn and Melnick (1993) predicted that most butadiene (85–95%) would be metabolized in the liver of the three species, whereas metabolism in the lung accounted for only 4% in mice and 1% in rats and humans. From model simulations of their own closed-chamber uptake data, Medinsky *et al*. (1994) suggested that lung metabolism of butadiene might be important for the total body clearance in mice but not in rats. At lower butadiene concentrations, lung metabolism was predicted to become more important relative to metabolism in the liver, which was attributed to the limitation of hepatic metabolism by the blood flow through the liver.

Physiological toxicokinetic models of butadiene metabolite disposition

Epoxybutene was included as the first metabolite of butadiene in several models. The models of Medinsky *et al*. (1994) and Kohn and Melnick (1996) overpredicted the burden of epoxybutene in rodents two- to three-fold, since it was assumed that biotransformed butadiene would become fully systemically available as epoxybutene. Under the same assumption, the model of Kohn and Melnick (1993) yielded reasonable simulations of experimental data, but the predicted rates of butadiene metabolism in this model were much lower than those which had been determined experimentally (Bolt *et al*., 1984; Kreiling *et al*., 1986; Leavens *et al*., 1996a). In the model of Sweeney *et al*. (1997), epoxybutene formation was reasonably simulated by adjusting the systemic availability of epoxybutene to measured data. It was assumed that only a fraction of the butadiene metabolized was transformed to epoxybutene. Further intermediates formed within the first step of butadiene catabolism not leading to epoxybutene were postulated. The fraction of butadiene oxidized to epoxybutene was estimated to be 0.19 in mice and 0.24 in rats. This fraction is consistent with the 'extraction factor' of 0.29 reported by Filser and Bolt (1984). These authors interpreted their findings as indicative of an intrahepatic first-pass effect for the epoxybutene formed, since only 29% of this metabolite entered systemic circulation. This effect was considered in the models of Johanson and Filser (1993, 1996) and Csanády *et al*. (1996), in which the liver was modelled as consisting of cytosol containing GST and endoplasmic reticulum containing a cytochrome P450–epoxide hydrolase complex. Such a complex was proposed to explain the biotransformation of a series of olefinic hydrocarbons including naphthalene *in vitro* and *in vivo* (Oesch, 1973). Evidence supporting the existence of such a complex comes from the demonstration that the rat microsomal epoxide hydrolase (mEH) and a CYP2B1–mEH

fusion protein, in which the CYP2B1 membrane anchor signal sequence replaced the N-terminal 20 amino acid residues of mEH, could be co-translationally inserted into dog pancreas microsomes, whereas truncated mEH, in which the N-terminal 20 amino acids were deleted, was not co-translationally inserted (Friedberg *et al.*, 1994). The biochemical parameters of butadiene and epoxybutene metabolism incorporated in the model of Johanson and Filser (1993, 1996) were derived from in-vitro data for butadiene (Filser *et al*., 1992) and epoxybutene (Kreuzer *et al*., 1991). The model overpredicted epoxybutene formation by a factor of about two.

In the model of Csanády *et al*. (1996), the biochemical parameters for butadiene in rats and mice were obtained by fitting model simulations to in-vivo data of Bolt *et al*. (1984) and Kreiling *et al*. (1986). The biochemical parameters for epoxybutene were identical to those of Johanson and Filser (1993, 1996). This model accurately predicted experimental data on epoxybutene. The most advanced models are those of Csanády *et al*. (1996) and Sweeney *et al*. (1997), since they can simulate both epoxybutene and diepoxybutane as metabolites of butadiene. The tissue:blood partition coefficients for diepoxybutane were estimated by Csanády *et al*. (1996) to have a value of 1 for all tissues. Sweeney *et al.* (1997) obtained tissue:blood partition coefficients from in-vitro measurements (Table 23). Both models yielded good predictions for mice and rats for both metabolites. For humans, no measured data have been reported against which the predictions could be validated. In addition, the model of Csanády *et al*. (1996) predicted accurately the measured haemoglobin adduct levels (Osterman-Golkar *et al*., 1993; Albrecht *et al*., 1993) of epoxybutene in rodents following exposure to butadiene. None of the models published has included the formation and elimination of epoxybutanediol.

4.1.3 *Comparison of rodent and human data*

By comparing butadiene metabolites in urine of butadiene-exposed mice and humans, Bechtold *et al*. (1994) concluded that in humans epoxybutene was metabolically eliminated predominantly by epoxide hydrolase. In rats, GSH conjugation and hydration pathways were about equal and in mice direct GSH conjugation was more important.

No measured data have been published on the burden of butadiene and its epoxy metabolites in exposed humans that can be used for comparison with the rodent data. However, the models of Kohn and Melnick (1993), Johanson and Filser (1996) and Csanády *et al*. (1996) predict significantly lower body burdens of epoxybutene, based on data derived from human tissues (Tables 19, 20 and 21).

The partition coefficients measured in rodent and human tissue samples (Table 23) suggest that there would be no substantial difference between rodents and humans with respect to distribution of butadiene and its metabolite epoxybutene.

Model predictions have been made of the disposition of butadiene and epoxybutene in rodents and humans. Kohn and Melnick (1993) predicted that the cumulative body burden of epoxybutene after a 6-h exposure to 100 ppm butadiene '(area under the epoxybutene versus time curve from 0 to 6 h)' in humans would be 7- and 35-fold lower than in rat and mouse, respectively. For a 12-h exposure to 10 ppm butadiene, the model

of Johanson and Filser (1996) predicted the internal dose of epoxybutene in humans to be only 3.3- and 5.3-fold lower than in rat and mouse, respectively. Similar results were obtained with the model of Csanády *et al*. (1996) for exposure to 10 ppm butadiene over 8 h: the AUCs of epoxybutene in blood were 3.7- and 4.8-fold lower in humans than in rat and mouse, whereas the AUCs of butadiene in blood were about three-fold lower in humans than in both rodent species.

Osterman-Golkar *et al.* (1993) summarized data on the formation of HOBVal, the adduct of epoxybutene at the N-terminal valine of haemoglobin, in rodents exposed experimentally and in subjects exposed at the workplace to butadiene. The binding indices (pmol HOBVal/g globin per ppm.h) were ~0.5 in $B6C3F_1$ mice, ~0.3 in $CD2F_1$ mice (Recio, 1992), ~0.09 in Wistar rats (Osterman-Golkar *et al*., 1991), ~0.3 and ~0.1 in Sprague-Dawley rats at 0–10 ppm and 10–100 ppm [0–22 and 22–220 mg/m^3] butadiene, respectively, and, as a preliminary value, ~0.004 in humans. [The latter value was estimated assuming an average exposure concentration of 1 ppm [2.2 mg/m^3], but the exposure concentrations were mostly below this value (Osterman-Golkar, 1993; Sorsa *et al*., 1996).] In a later publication, Osterman-Golkar *et al*. (1996) reported a median HOBVal level of 0.16 pmol/g globin in 10 workers exposed to a median butadiene concentration of 2.1 mg/m^3 [0.93 ppm]. Considering a ratio of 3:1 of the C-1:C-2 isomers of the epoxybutene-valine adducts at the N-terminal valine of haemoglobin (Richardson *et al.*, 1996) and assuming a workplace exposure of 8 h per day for five days, a binding index of $(4/3) \times 0.16/(0.93 \times 8 \times 63) \sim 0.0004$ pmol HOBVal/(g globin per ppm.h) can be calculated. An identical low binding index was calculated from data given in a review by Osterman-Golkar and Bond (1996).

Taking all these data together, it can be concluded that exposure of humans to butadiene leads to lower body burdens of the reactive metabolite epoxybutene than in similarly exposed rats and mice. No comparative data are available concerning the intermediate diepoxybutane. Only limited data have been published on the adducts of epoxybutanediol at the N-terminal valine of haemoglobin resulting in *N*-(2′,3′,4′-trihydroxybutyl)valine (Pérez *et al*., 1997).

4.2 Toxic effects

4.2.1 *Humans*

Butadiene

The toxic effects of combined exposures to butadiene and other agents (e.g., styrene, chloroprene, hydrogen sulfide, acrylonitrile) have been reviewed (Parsons & Wilkins, 1976). Concentrations of several thousand parts per million of butadiene irritate the skin, eyes, nose and throat (Carpenter *et al*., 1944; Wilson *et al*., 1948; Parsons & Wilkins, 1976).

Several studies have been reported on the effects of occupational exposure to butadiene, mainly from the USSR and Bulgaria. Few are substantiated by details on the atmospheric concentration or duration of exposure, and control data are generally not provided. The effects reported include haematological disorders (Batkina, 1966; Volkova & Bagdinov, 1969), kidney malfunction, laryngotracheitis, irritation of the upper respiratory tract,

conjunctivitis, gastritis, various skin disorders, a variety of neurasthenic symptoms (Parsons & Wilkins, 1976) and hypertension and neurological disorders (Spasovski *et al*., 1986).

Checkoway and Williams (1982) reported minimal changes in haematological indices among eight workers exposed to about 20 ppm [44 mg/m^3] butadiene, 14 ppm [60 mg/m^3] styrene and 0.03 ppm [0.1 mg/m^3] benzene, compared with those among 145 workers exposed to less than 2 ppm [4.4 mg/m^3] butadiene, 2 ppm [8.5 mg/m^3] styrene and 0.1 ppm [0.3 mg/m^3] benzene. Changes included a slight decrease in haemoglobin level and a slight increase in red-cell mean corpuscular volume. [The Working Group considered that these changes cannot be interpreted as an effect of butadiene on the bone marrow, particularly as alcohol intake was not evaluated.]

Diepoxybutane

Diepoxybutane is highly irritant to the eyes and respiratory tract of accidentally exposed workers (IARC, 1976).

4.2.2 *Experimental systems*

Butadiene

LC_{50} values for butadiene were reported to be 270 000 mg/m^3 in mice exposed for 2 h and 285 000 mg/m^3 in rats exposed for 4 h; after 1 h of exposure, rats were in a state of deep narcosis (Shugaev, 1969). Oral LD_{50} values of 5.5 g/kg bw for rats and 3.2 g/kg bw for mice have been reported (United States National Toxicology Program, 1984).

In female rats exposed to 1–30 mg/m^3 butadiene for 81 days, morphological changes were observed in liver, kidney, spleen, nasopharynx and heart (G.K. Ripp, reported in Crouch *et al*., 1979). In groups of 24 rats exposed to 600–6700 ppm [1300–14 800 mg/m^3] butadiene for 7.5 h per day on six days per week for eight months, no adverse effect was noted, except for a slight retardation in growth at the highest concentration (Carpenter *et al*., 1944). Rats exposed to 2200–17 600 mg/m^3 butadiene for 6 h per day on five days per week for three months showed no treatment-related effect other than increased salivation in females (Crouch *et al*., 1979).

Groups of 110 male and 110 female CD Sprague-Dawley rats were exposed to atmospheres containing 0, 1000 or 8000 ppm [0, 2200 or 17 600 mg/m^3] butadiene for 6 h per day on five days per week. The study was terminated when it was predicted that survival would drop to 20–25% (105 weeks for females, 111 weeks for males). Ten animals of each sex from each group were killed at 52 weeks. Treatment was associated with changes in clinical condition and lowering of body weight gain during the first 12 weeks, then nonsignificant changes, reduced survival and increases in certain organ weights and in the incidence of uncommon tumour types (for details, see Section 3.1.2). Increased mortality in the high-dose males was accompanied by an increase in the severity of nephropathy (Owen *et al*., 1987; Owen & Glaister, 1990).

$B6C3F_1$ mice exposed to 0, 625 or 1250 ppm [0, 1380 or 2760 mg/m^3] butadiene for 6 h per day on five days per week for 60–61 weeks had increased prevalence of atrophy of the ovary and testis, atrophy and metaplasia of the nasal epithelium, hyperplasia of the

respiratory and forestomach epithelium and liver necrosis (see also Section 3.1.1) (United States National Toxicology Program, 1984).

Haematological changes in male B6C3F$_1$ mice exposed to 62.5, 200 or 625 ppm [138, 440 or 1380 mg/m^3] butadiene for 6 h per day on five days per week for 40 weeks included decreased red blood cell count, haemoglobin concentration and packed red cell volume and increased mean corpuscular volume. Similar changes occurred in female mice exposed to 625 ppm butadiene (for details, see Section 3.1.1) (Melnick *et al*., 1990).

The role of murine retroviruses in induction of leukaemias and lymphomas following inhalation of butadiene was evaluated in a series of studies reviewed by Irons (1990). Exposure of groups of male B6C3F$_1$ mice, which have the intact ecotropic murine leukaemia virus, to 1250 ppm [2750 mg/m^3] butadiene for 6 h per day on six days per week for 6–24 weeks resulted in decreases in the number of circulating erythrocytes, in total haemoglobin and in haematocrit and an increase in mean corpuscular volume. Leukopenia, due primarily to a decrease in the number of segmented neutrophils, and an increase in the number of circulating micronuclei were observed (Irons *et al*., 1986a). Persistent immunological defects were not detected after this treatment (Thurmond *et al*., 1986). Exposure of male NIH Swiss mice, which do not possess intact endogenous ecotropic murine leukaemia virus, produced similar results (Irons *et al*., 1986b).

A further study was conducted to examine the expression and behaviour of endogenous retroviruses in these strains during the preleukaemic phase of butadiene exposure. Chronic exposure of B6C3F$_1$ mice to butadiene (1250 ppm [2760 mg/m^3]) for 6 h per day on five days per week for 3–21 weeks increased markedly the quantity of ecotropic retrovirus recoverable from the bone marrow, thymus and spleen. Expression of other endogenous retroviruses (xenotropic, MCF-ERV) was not enhanced. No virus of any type was found in similarly treated NIH Swiss mice (Irons *et al*., 1987a).

Enhanced susceptibility to butadiene-induced leukaemogenesis as a result of an ability to express the retrovirus was suggested by the finding that exposure to 1250 ppm butadiene for one year resulted in a 57% incidence of thymic lymphoma in B6C3F$_1$ mice (with expression of the virus) and a 14% incidence in NIH Swiss (without viral expression) (Irons *et al*., 1989).

Groups of 70 male and 69 female B6C3F$_1$ mice were exposed to 0, 6.25, 20, 62.5, 200 or 625 ppm [0, 14, 44, 138, 440 or 1380 mg/m^3] butadiene for 6 h per day on five days per week for up to 103 weeks. Groups of 10 males and 10 females were killed at 40 and 65 weeks. Ovarian atrophy was noted in female mice at 65 weeks of exposure (after completion of the reproductive life of this species) at 20 ppm and higher. Testicular atrophy occurred after 65 weeks in the male mice at 625 ppm (Melnick & Huff, 1992).

The effect of butadiene exposure on arteriosclerotic plaque development was assessed in white leghorn cockerels exposed for 6 h per day on five days per week for 16 weeks to 20 ppm (44 mg/m^3) butadiene. Plaque frequency and size in the abdominal aorta wall were determined for butadiene-exposed animals and controls. Plaques were larger for butadiene-exposed animals than for corresponding air controls and the authors concluded that butadiene exposure markedly accelerated arteriosclerotic plaque development. Since

one epidemiological study has suggested a link between death from arteriosclerotic heart disease and chronic occupational exposure to butadiene, the authors suggested that their animal model could be used to further investigate this disease (Penn & Snyder, 1996).

Diepoxybutane

Diepoxybutane is highly toxic to rats (oral LC_{50} 78 mg/kg bw), mice and rabbits. Among surviving animals, there was eye, skin and respiratory tract damage. Intramuscular injection of rabbits with 25 mg/kg bw produced leukopenia and lymphopenia. However, once weekly gavage dosing of rats for one year with 5 mg D,L-diepoxybutane was not toxic (IARC, 1976).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Butadiene

The reproductive and developmental toxicity of butadiene has been reviewed (Melnick & Huff, 1992; Christian, 1996).

Fertility was reported to be unimpaired in mating studies in rats, guinea-pigs and rabbits exposed to 600, 2300 or 6700 ppm [1300, 5000 or 14 800 mg/m^3] butadiene by inhalation for 7.5 h per day on six days per week for eight months (Carpenter *et al*., 1944). [The Working Group noted the incomplete reporting of this study].

Pregnant Sprague-Dawley rats (24–28 per group) and Swiss (CD-1) mice (18–22 per group) were exposed to atmospheric concentrations of 0, 40, 200 or 1000 ppm [0, 88, 440 or 2200 mg/m^3] butadiene for 6 h per day on days 6–15 of gestation and killed on gestation day 18 (mice) or 20 (rats). Subsequently, the uterine contents were evaluated; individual fetal body weights were recorded, and external, visceral and skeletal examinations were performed. In rats, maternal toxicity was observed in the 1000-ppm group in the form of reduced extragestational weight gain and, during the first week of treatment, decreased body weight gain. Under these conditions, there was no evidence of developmental toxicity. Maternal toxicity was observed in mice given 200 and 1000 ppm butadiene, while 40 ppm and higher concentrations of butadiene caused significant exposure-related reduction in the mean body weights of male fetuses. Mean body weights of female fetuses were reduced at the 200 and 1000 ppm exposure levels. No increased incidence of malformations was observed in either species. The frequency of fetal variations (supernumerary ribs, reduced sternebral ossification) was significantly increased in mice exposed to 200 and 1000 ppm. In a study of sperm-head morphology, groups of 20 male B6C3F$_1$ mice were exposed to atmospheric concentrations of 0, 200, 1000 or 5000 ppm [0, 440, 2200 or 11 000 mg/m^3] butadiene for 6 h per day for five consecutive days. Small, concentration-related increases in the frequency of abnormal sperm morphology were seen five weeks after exposure (the only time of examination) (Hackett *et al*., 1987; Morrissey *et al*., 1990).

[The Working Group noted that sequential examinations were not conducted after exposure to determine the effect of butadiene on all stages of gamete development].

Female Sprague-Dawley rats were exposed to 0, 200, 1000 or 8000 ppm [0, 440, 2200 or 17 700 mg/m^3] butadiene for 6 h per day for 10 days on days 6–15 of gestation. Maternal body weight gain was significantly reduced at all exposure concentrations, with weight loss at 8000 ppm. Uterine implantation was unaffected. At 8000 ppm, there was a significant reduction in fetal body weights, delay in ossification of the ribs (wavy ribs) and the thoracic centra and incomplete ossification of the sternum. There were no teratogenic effects that were significant or outside the historical control range. The no-observed-effect level (NOEL) was reported as 200 ppm for maternal toxicity and 1000 ppm for developmental effects (Christian, 1996).

Epoxybutene

Groups of 10 female B6C3F$_1$ mice and 10 Sprague-Dawley rats were administered 0.005, 0.02, 0.09, 0.36 or 1.43 mmol/kg bw [0.35, 1.4, 6.3, 25 or 100 mg/kg bw] epoxybutene in sesame oil by intraperitoneal injection daily for 30 days. There was a 10% body weight decrement among the highest-dose mice at the end of the experiment, but there was no body weight effect in rats. Ovarian and uterine weights also were reduced in mice at the highest dose, with an accompanying reduction in the number of developing follicles and absence of primordial follicles, but there was no effect in rats (Doerr *et al.*, 1996).

Diepoxybutane

There are two reports of reproductive toxicity of diepoxybutane in experimental systems. In the first study, groups of 10 female B6C3F$_1$ mice and 10 Sprague-Dawley rats were administered 0.002, 0.009, 0.036, 0.14 or 0.29 mmol/kg bw [0.17, 0.78, 3.1, 12, 25 mg/kg bw] diepoxybutane in sesame oil by intraperitoneal injection daily for 30 days. Diepoxybutane exposure depressed growth at the two highest doses in both rats and mice. Since rats were extremely sensitive to the high-dose diepoxybutane treatment (0.29 mmol/kg), the diepoxybutane was administered to rats at this dose for only 25 days. At day 25, body weights of these rats were 50% of controls and only four of ten rats were alive at day 30. Animals in this group exhibited signs of severe gastrointestinal toxicity as evidenced by diarrhoea. Ovarian toxicity was determined by assessing reproductive organ weights and ovarian follicle number. Although diepoxybutane was ovotoxic in both species, it was more potent in mice than rats. At a dose of 0.14 mmol/kg bw, the ovary was depleted of 83% of the small follicles and 52% of the growing follicles in mice. Only 31% and 40% of these follicle populations were depleted in rats at that dose. A decrease in ovarian and uterine weights with increasing dose was observed in mice at the 0.14 and 0.29 mmol/kg bw doses. Similar observations were also seen in rats (Doerr *et al.*, 1996).

The effects of diepoxybutane on male reproductive cells were investigated by flow cytometric and histological description of testicular cell populations and alterations of chromatin packaging. Male B6C3F$_1$ mice were treated with a single intraperitoneal

injection of diepoxybutane in saline at doses of 8.5, 17, 26, 34, 43 and 52 mg/kg bw. Groups were killed at intervals of 7, 14, 21, 28 and 35 days after treatment. One group was injected with 78 mg/kg bw for observation at three weeks. The treated animals did not show any clinical signs of toxicity on daily observation. Cytotoxic damage to all post-stem cell stages was assessed by alterations in relative ratios of haploid, diploid, and tetraploid testicular cells and by the reduction of relative percentages of cell populations. Dose-dependent reductions of tetraploid cells, round spermatids and elongated spermatids were detected at 7, 21 and 28 days, respectively, reflecting cytotoxic damage on the differentiating spermatogonia compartment. The dose necessary to reduce the number of differentiating spermatogonia to half the control value was 55 mg/kg bw. Stem cells were not affected by this treatment. Depletion of spermatids and reduction of the secondary spermatocyte layers were noted in the seminiferous tubules (Spano *et al.*, 1996).

4.4 Genetic and related effects

The genetic toxicology of butadiene and of its major metabolites, epoxybutene and diepoxybutane, has been reviewed (Adler *et al*., 1995; Jacobson-Kram & Rosenthal, 1995). Additional information is available in a more recent review of the toxicology and epidemiology of butadiene (Himmelstein *et al*., 1997) and a compilation of publications (Adler & Pacchierotti, 1998).

Butadiene

4.4.1 *Humans*

In a small pilot study, the *hprt* locus mutation frequencies in lymphocytes of eight male workers from a high-exposure area in a butadiene production plant in Texas, USA, were compared with those of five (four male and one female) low-exposure workers and six male control area personnel (Ward *et al.*, 1994, 1996a). All subjects were non-smokers. Butadiene concentrations were measured in both area and personal samples, which gave values of 3.5 ± 7.25 ppm [7.7 ± 16.6 mg/m^3] and 0.03 ± 0.03 ppm [0.07 ± 0.07 mg/m^3] for the high-exposure (production) and the control areas, respectively. The levels in the majority of the production area samples were below 1 ppm. Urinary concentrations (mean ± S.D. in ng/mg creatinine) of the butadiene metabolite, 1,2-dihydroxy-4-(*N*-acetylcysteinyl)butane (*N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine, using the nomenclature of Figure 1 (Metabolite 3)), were 1690 ± 201.3, 355 ± 250 and 580 ± 191 for the high- and low-exposure area and control area personnel, respectively. The *hprt* locus mutation frequencies ($\times 10^{-6}$ ± standard deviation (SD)), determined by an autoradiographic technique, were 3.99 ± 2.81, 1.20 ± 0.51 and 1.03 ± 0.12, respectively, in the three groups. The value for the high-exposure group was significantly higher ($p < 0.05$) than those for the other groups. A second study was conducted at the same plant eight months later in which exposures were determined from 8-h personal breathing zone air samplers (Ward *et al.*, 1996a). Three exposure groups were compared (high, intermediate and low, there being no control group), for which the average butadiene concentrations were 0.30 ± 0.59, 0.21 ± 0.21 and 0.12 ± 0.27 ppm, respectively

[0.66 ± 1.30, 0.46 ± 0.46 and 0.27 ± 0.60 mg/m^3]. The corresponding urinary concentrations of 1,2-dihydroxy-4-(*N*-acetylcysteinyl)butane were 761 ± 245, 596 ± 155 and 684 ± 176 ng/mg creatinine. The frequencies of *hprt* locus mutations were 5.33 ± 3.76, 2.27 ± 0.99 and 2.14 ± 0.97, respectively, in the three groups. The value for the high-exposure group was significantly higher ($p < 0.05$) than those for the other groups.

Preliminary data from an on-going population study of rubber plant workers exposed to butadiene and styrene (16 high-exposure (including five smokers) versus nine low-exposure (including three smokers)) are also available (Ward *et al.*, 1996a). Passive badge dosimeters were used to measure butadiene and styrene concentrations in the air. The butadiene detection limit was 0.25 ppm [0.55 mg/m^3] over an 8-h period. Half of the 40 samples collected in the high-exposure areas exceeded the detection limit and 11 were greater than 1 ppm [2.2 mg/m^3]. None of the samples collected in low-exposure areas exceeded the detection limit. The styrene concentration averaged 25% of that of butadiene and only one sample from the high-exposure area had > 1 ppm styrene. The frequencies of *hprt* locus mutations for the non-smokers were 7.47 ± 5.69 and 1.68 ± 0.85 for the high- and low-exposure groups, respectively, and, for the smokers, 6.24 ± 4.37 and 3.42 ± 1.57, respectively. The values for the high-exposure groups were significantly higher ($p < 0.01$) than those for the low-exposure groups.

The *hprt* mutation frequency was also evaluated in two studies using the T-lymphocyte clonal assay. The mutation frequency for 41 workers (15 male, 26 female) exposed to butadiene (1–3.5 ppm [2.2–7.7 mg/m^3]) at a polybutadiene rubber production facility in China was not significantly different from that of the 38 (14 male, 24 female) controls. Mutation frequency decreased with cloning efficiency, increased with age and was moderately higher in women than in men. After adjustment for age, sex and cloning efficiency by multiple regression analysis, the mean mutation frequency was 32% higher in exposed workers than in controls, but this difference was not significant ($p = 0.13$) and was due largely to the greater values among exposed women (Hayes *et al.*, 1996). The *hprt* locus mutation frequencies were measured in blood samples collected twice (in 1993 and 1994) from 19 workers exposed to butadiene and 19 matched controls from a butadiene production plant in the Czech Republic (Tates *et al.*, 1996). Three exposed and three control subjects were the same in 1993 and 1994. Personal passive dosimetry was performed in 1993 and twice in 1994 on the day preceding blood sampling. About half of the 1993 samples were lost, so that five exposed and 13 control lymphocyte samples remained for analysis. The mean exposure level in 1994 was 1.76 ± 4.20 ppm (SD) [3.9 ± 9.3 mg/m^3] and tabulated individual exposure levels ranged from < 0.024 ppm to 10.2 ppm [0.053 and 22.6 mg/m^3]. Using the clonal assay (Tates *et al.*, 1994), the geometric mean of *hprt* mutation frequencies ($\times 10^{-6}$ ± SD) adjusted for cloning efficiency, age and smoking were, respectively, 7.85 ± 7.09 and 10.14 ± 9.16 in pooled (1993 plus 1994) exposed and control subjects. The difference was not significant. A similar result was obtained for the 1994 subjects alone. There was no difference between adjusted geometric mean mutation frequencies of exposed and unexposed non-smokers or between exposed and unexposed smokers.

Cytogenetic analysis of peripheral blood lymphocytes of butadiene production workers showed that occupational exposure to butadiene (median concentration, 1–3.5 ppm [2.2–7.7 mg/m^3]) did not induce chromosomal aberrations, micronuclei, sister chromatid exchanges, DNA strand breaks or alkali-labile sites (Comet assay). These results were obtained from workers in three butadiene production facilities in the United States (Legator *et al*., 1993; Kelsey *et al*., 1995; Hallberg *et al*., 1997), one in Portugal and one in the Czech Republic (Sorsa *et al*., 1994). Lymphocyte cultures from control and exposed subjects from two of these study groups were also irradiated with γ-rays in a challenge assay and chromosomal damage was assessed. The results indicated that butadiene exposure reduced DNA repair competence of the cells (Au *et al*., 1995; Hallberg *et al*., 1997).

As part of the same study in the Czech Republic factory described above (Tates *et al.*, 1996), analysis of chromosomal aberrations in lymphocytes from 1994 subjects indicated that the percentage of aberrant cells was slightly, but significantly, enhanced in exposed subjects compared with the controls (3.11 ± 1.33 and 2.03 ± 1.53, respectively, $p < 0.01$), these data being very similar to those from the earlier study conducted in the same factory (Sorsa *et al.*, 1994), which did not provide evidence for a clastogenic effect (2.9 ± 1.5 and 2.1 ± 1.4, respectively). Frequencies of micronuclei in cytochalasin-B blocked binucleate lymphocytes in 1994 exposed and unexposed workers were not significantly different and there was no evidence for differences in the levels of DNA damage, as provided by the single-cell gel electrophoresis assay.

4.4.2 *Experimental systems* (see Table 25 for references)

In all of the following tests, exposure was to gaseous butadiene unless otherwise indicated. Butadiene induced gene mutations in *Salmonella typhimurium* strains TA100 and TA1535 in the presence of phenobarbital- or 5,6-benzoflavone-induced rat liver S9. It was also weakly mutagenic to TA1535 in the presence of Aroclor 1254-induced rat liver S9, uninduced rat S9 or uninduced mouse S9, but was not mutagenic with uninduced human S9. Mutations were induced in strain TA1530 in the presence of phenobarbital- or Aroclor 1254-induced rat liver S9 but not uninduced S9. Butadiene was not mutagenic to other *Salmonella* strains or to *Escherichia coli*.

Butadiene did not induce somatic cell mutation and recombination or sex-linked recessive lethal mutation in *Drosophila melanogaster*.

Butadiene did not cause DNA single-strand breaks in mouse alveolar macrophage cultures, and was not active in the L5178Y mouse lymphoma ($tk^{+/-}$) assay. A weak positive response was reported for induction of sister chromatid exchanges in Chinese hamster ovary CHO cells exposed to butadiene dissolved in ethanol in the presence of Aroclor 1254-induced rat liver S9. In the same laboratory, sister chromatid exchanges were induced weakly in human whole blood lymphocytes after butadiene dissolved in ethanol was added to the culture medium in the presence or in the absence of Aroclor-1254-induced rat liver S9. In a second study, in which S9 from a variety of sources including mouse and human was used, no sister chromatid exchange was induced in human lymphocyte cultures after exposure to gaseous butadiene.

Table 25. Genetic and related effects of butadiene

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1300 ppm	Arce *et al*. (1990)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	1080 ppm	Araki *et al*. (1994)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	–	+	86 ppm	de Meester *et al*. (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	(+)	650 ppm	Arce *et al*. (1990)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	216 ppm	Araki *et al*. (1994)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1080 ppm	Araki *et al*. (1994)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1300 ppm	Arce *et al*. (1990)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1080 ppm	Araki *et al*. (1994)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	1300 ppm	Arce *et al*. (1990)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	–	–	1080 ppm	Araki *et al*. (1994)
DMM, *Drosophila melanogaster*, somatic mutation or recombination	–		10000 ppm inh	Victorin *et al*. (1990)
DMX, *Drosophila melanogaster*, sex-linked recessive leathal mutations	–		500 ppm inh	Foureman *et al*. (1994)
DIA, Single-strand breaks, NMRI mouse alveolar macrophages *in vitro*	–	NT	40 ppm	Walles *et al*. (1995)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	–	650 ppm	McGregor *et al*. (1991)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	(+)	1.35	Sasiadek *et al*. (1991a)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	–	2160 ppm	Arce *et al*. (1990)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	+	108	Sasiadek *et al*. (1991b)
DVA, DNA cross-links, B6C3F$_1$ mouse liver *in vivo*	+		450 ppm inh 7 h	Jelitto *et al*. (1989)
DVA, DNA cross-links, B6C3F$_1$ mouse liver *in vivo*	–		2070 ppm inh 8 h/d, 7 d	Ristau *et al*. (1990)
DVA, DNA cross-links, B6C3F$_1$ mouse lung, liver *in vivo*	+		250 ppm inh 7 h	Vangala *et al*. (1993)
DVA, DNA single-strand breaks, B6C3F$_1$ mouse liver *in vivo*	+		2000 ppm inh 7 h/d, 7 d	Vangala *et al*. (1993)
DVA, DNA single-strand breaks, NMRI mouse lung and liver *in vivo*	+		200 ppm inh 16 h	Walles *et al*. (1995)
DVA, DNA cross-links, Sprague-Dawley rat liver *in vivo*	–		550 ppm inh 7 h	Jelitto *et al*. (1989)
DVA, DNA cross-links, Sprague-Dawley rat liver *in vivo*	–		1240 ppm inh 8 h/d, 7 d	Ristau *et al*. (1990)
DVA, DNA cross-links, Sprague-Dawley rat liver, lung *in vivo*	–		2000 ppm inh 7 h	Vangala *et al*. (1993)
DVA, DNA single-strand breaks, Sprague-Dawley rat liver *in vivo*	+		2000 ppm inh 7 h/d, 7 d	Vangala *et al*. (1993)

Table 25 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
DVA, DNA strand breaks, CD-1 mouse liver, bone marrow or testis *in vivo*	–		130 ppm inh 6 h/d, 4 wk	Anderson *et al.* (1997)
DVA, DNA damage, CD-1 mouse testicular cells *in vivo*	+[c]		125 ppm inh 6 h	Brinkworth *et al.* (1998)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		4000[c] inh	Arce *et al*. (1990)
UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		4000[d] inh	Arce *et al*. (1990)
UVM, Unscheduled DNA synthesis, B6C3F$_1$ mouse hepatocytes *in vivo*	–		11600[c] inh	Arce *et al*. (1990)
UVM, Unscheduled DNA synthesis, B6C3F$_1$ mouse hepatocytes *in vivo*	–		11600[d] inh	Arce *et al*. (1990)
GVA, Gene mutation, *lac*Z mouse bone marrow *in vivo*	+		625 ppm inh 6 h/d, 5 d/wk, 1 wk	Recio *et al*. (1992)
GVA, Gene mutation, B6C3F$_1$ mouse T-lymphocytes, *hprt* locus *in vivo*	+		625 ppm inh 6 h/d, 5 d/wk, 4 wk	Cochrane & Skopek (1993)
GVA, Gene mutation, B6C3F$_1$ mouse T lymphocytes, *hprt* locus *in vivo*	+		625 ppm inh 6 h/d, 5 d/wk, 2 wk	Cochrane & Skopek (1994)
GVA, Gene mutation, *lac*I mice *in vivo*	+		62.5 ppm inh 6 h/d, 5 d/wk, 4 wk	Sisk *et al*. (1994)
GVA, Gene mutation, B6C3F$_1$ mouse T-lymphocytes, *hprt* locus *in vivo*	+		1300 ppm inh 6 h/d, 5 d/wk, 1 wk	Tates *et al*. (1994)
GVA, Gene mutation, *lac*I mice *in vivo*	+		1250 ppm inh 6 h/d, 5 d/wk, 4 wk	Recio & Meyer (1995)
GVA, Gene mutation, (102/E1 × C3H/E1)F$_1$ mouse splenocytes, *hprt* locus *in vivo*	+[c]		500 ppm inh 6 h/d, 5 d	Tates *et al.* (1998)
GVA, Gene mutation, CD-1 mouse splenocytes, *hprt* locus *in vivo*	–		1300 ppm inh 6 h/d, 5 d/wk, 4 wk	Tates *et al.* (1998)
MST, Mouse spot test, female T-stock mice	+		500 ppm inh 6 h/d, 5 d/wk, 1 wk	Adler *et al*. (1994)
SVA, Sister chromatid exchange, B6C3F$_1$ mouse bone marrow *in vivo*	+		116 ppm inh 6 h	Cunningham *et al*. (1986)

Table 25 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SVA, Sister chromatid exchange, Sprague-Dawley rat bone marrow *in vivo*	–		4000 ppm inh 6 h	Cunningham *et al*. (1986)
SVA, Sister chromatid exchange, B6C3F_1 mouse bone marrow *in vivo*	+		7 ppm inh 6 h/d, 5 d/wk, 2 wk	Tice *et al*. (1987)
MVM, Micronucleus test, B6C3F_1 mouse bone marrow *in vivo*	+		116 ppm inh 6 h	Cunningham *et al*. (1986)
MVM, Micronucleus test, B6C3F_1 mouse peripheral blood *in vivo*	+		70 ppm inh 6 h/d, 5 d/wk, 2 wk	Tice *et al*. (1987)
MVM, Micronucleus test, B6C3F_1 mouse peripheral blood *in vivo*	+		7 ppm inh 6 h/d, 5 d/wk, 13 wk	Jauhar *et al*. (1988)
MVM, Micronucleus test, NMRI mouse bone marrow *in vivo*	+		35 ppm inh 23 h	Victorin *et al*. (1990)
MVM, Micronucleus test, CB6F_1 mice *in vivo*	+		50 ppm inh 6 h/d, 5 d/wk	Autio *et al*. (1994)
MVM, Micronucleus test, (102/E1 × C3H/E1)F_1 mice *in vivo*	+		50 ppm inh 6 h/d, 5 d/wk	Adler *et al*. (1994)
MVM, Micronucleus test, (102 × C3H) mice *in vivo*	+		200 ppm inh 6 h/d, 5 d/wk	Xiao & Tates (1995)
MVM, Micronucleus test, (102/E1 × C3H/E1)F_1 mouse splenocytes *in vivo*	+		130 ppm inh 6 h/d, 5 d	Stephanou *et al.* (1998)
MVM, Micronucleus test (102/E1 × C3H/E1)F_1 mouse spermatids *in vivo*	+		250 ppm inh 6 h/d, 5 d	Tommasi *et al.* (1998)
MVR, Micronucleus test, Sprague-Dawley rat bone marrow *in vivo*	–		4000 ppm inh 6 h/d, 2 d	Cunningham *et al*. (1986)
MVR, Micronucleus test, Sprague-Dawley rats *in vivo*	–		500 ppm 6 h/d, 5 d/wk	Autio *et al*. (1994)
CBA, Chromosomal aberrations, B6C3F_1 mouse bone marrow *in vivo*	+		1500 ppm inh 6 h	Irons *et al*. (1987b)
CBA, Chromosomal aberrations, NIH mouse bone marrow *in vivo*	+		1500 ppm inh 6 h	Irons *et al*. (1987b)
CBA, Chromosomal aberrations, B6C3F_1 mouse bone marrow *in vivo*	+		700 ppm inh 6 h/d, 5 d/wk, 2 wk	Tice *et al*. (1987)
AVA, Aneuploidy, B6C3F_1 mouse bone marrow *in vivo*	–		1500 ppm inh 6 h	Irons *et al.* (1987b)
AVA, Aneuploidy, NIH mouse bone marrow *in vivo*	–		1500 ppm inh 6 h	Irons *et al.* (1987b)

Table 25 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
COE, Chromosomal aberrations, (102/E1 × C3H/E1)F_1 mouse embryo *in vivo*	+		130 ppm inh 6 h/d, 5 d	Pachierotti *et al.* (1998)
DLM, Dominant lethal test, male CD-1 mice	+		233 ppm inh 6 h/d, 5 d/wk, 1 wk	Morrissey *et al.* (1990)
DLM, Dominant lethal test, CD-1 mice	+		1250 ppm inh 6 h/d, 5 d/wk, 10 wk	Anderson *et al.* (1993)
DLM, Dominant lethal test, CD-1 mice	–		6250 ppm inh 6 h	Anderson *et al.* (1993)
DLM, Dominant lethal test, (102/E1 × C3H/E1)F_1 mice	+		1300 ppm inh 6 h/d, 5 d/wk, 1 wk	Adler *et al.* (1994)
DLM, Dominant lethal test (102/E1 × C3H/E1)F_1 mice	+		500 ppm inh 6 h/d, 5 d	Adler *et al.* (1998)
DLM, Dominant lethal test, CD-1 mice	+		65 ppm inh 6 h/d, 5 d/wk, 4 wk	Anderson *et al.* (1998)
DLM, Dominant lethal test, CD-1 mice	+		125 ppm inh 6 h/d, 5 d/wk, 10 wk	Brinkworth *et al.* (1998)
DLR, Dominant lethal test, Sprague-Dawley rats	–		1250 ppm inh 6 h/d, 5 d/wk, 10 wk	Anderson *et al.* (1998)
MHT, Mouse (C3H/E1) heritable translocation test	+		1300 ppm inh 6 h/d, 5 d/wk, 1 wk	Adler *et al.* (1995)
MHT, Mouse (102/E1 × C3H/E1)F_1 heritable translocation test	+		500 ppm inh 6 h/d, 5 d	Adler *et al.* (1998)
BVD, Binding to DNA, male B6C3F_1 mouse or male Wistar rat liver *in vivo*	+		13 ppm inh 4–6.6 h	Kreiling *et al.* (1986b)
BVD, Binding to DNA at N7 of guanine, male B6C3F_1 mouse liver *in vivo*	+		450 ppm inh 7 h	Jelitto *et al.* (1989)
BVD, Binding to DNA at N7 of guanine, male B6C3F_1 mouse liver *in vivo*	+		NG	Bolt & Jelitto (1996)
BVD, Binding to DNA at N^6 of adenine, mouse lung *in vivo*	+		200 ppm inh 6 h/d, 5 d/wk, 1 wk	Koivisto *et al.* (1996)
BVD, Binding to DNA at N7 of guanine, male Wistar rat liver *in vivo*	–		550 ppm inh 7 h	Jelitto *et al.* (1989)

Table 25 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
BVD, Binding to DNA at N7 of guanine, male Wistar rat liver *in vivo*	–		NG	Bolt & Jelitto (1996)
BVD, Binding to DNA at N^6 of adenine, rat lung *in vivo*	+		200 ppm inh 6 h/d, 5 d/wk, 1 wk	Koivisto *et al*. (1996)
BVD, Binding to DNA at N7 of guanine, male Sprague-Dawley rat liver *in vivo*	+		200 ppm inh 6 h/d, 5 d/wk, 1 wk	Koivisto *et al*. (1997)
BVD, Binding to DNA at N7 of guanine, mouse testis and lung *in vivo*	+		200 ppm 6 h/d, 5 d	Koivisto *et al*. (1998)
BVP, Binding to protein, male B6C3F_1 mouse or male Wistar rat liver *in vivo*	+		13 ppm inh 4–6.6 h	Kreiling *et al.* (1986b)
SPM, Sperm morphology, CD-1 mice *in vivo*	+		1165 ppm inh 6 h/d, 5 d/wk, 1 wk	Morrissey *et al*. (1990)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw /day, inh, inhalation exposure; NG, not given
[c] Exposed 6 h on day 1, 3 h on day 2, livers sampled 2 h later
[d] Exposed 6 h on day 1 and 2, livers sampled 18 h later

DNA–DNA and DNA–protein cross-links were formed in the livers and lungs of mice exposed to butadiene at 250, 500 or 1000 ppm [550, 1100 or 2200 mg/m^3] for 7 h. Exposures of up to 2000 ppm [4400 mg/m^3] for 8 h per day for seven days did not induce cross-links in the liver or lung DNA of rats. Single-strand breaks were induced in mouse and rat liver DNA following exposure to 2000 ppm for 7 h per day for seven days and in mouse lung and liver following a 16-h exposure to 200 ppm [440 mg/m^3] of butadiene.

Unscheduled DNA synthesis was not evident in the livers of Sprague-Dawley rats or B6C3F$_1$ mice after exposure to 10 000 ppm [22 000 mg/m^3] butadiene for 6 h per day for two days.

Mutations were induced at the *hprt* locus in mice exposed to butadiene for 6 h per day on five days per week at 625 ppm [1380 mg/m^3] for two weeks or at 1300 ppm [2760 mg/m^3] for one week. Butadiene was mutagenic in the mouse spot test (500 ppm [1100 mg/m^3] 6 h per day for five days) and in two transgenic mouse models. Exposure to 62.5 or 1250 ppm [138 or 2760 mg/m^3] butadiene for 6 h per day on five days per week for four weeks increased the frequency of mutations induced at A:T base pairs in bone marrow of *lac*I mice, while exposure to 625 ppm for 6 h per day for five days increased the *lac*Z mutation frequency in lung but not liver or bone marrow of the MutaMouse®.

Butadiene increased the frequency of sister chromatid exchanges and micronuclei in mouse but not rat bone marrow. Micronucleus frequency also increased in peripheral erythrocytes and splenocytes. Butadiene also induced chromosomal aberrations in mouse bone marrow, and dominant lethal mutations, heritable translocations and sperm-head abnormalities in mice. It did not induce aneuploidy in bone marrow cells *in vivo*.

In a study by Sisk *et al.* (1994), male B6C3F$_1$ *lacI* transgenic mice were exposed by inhalation to 0, 62.5, 625 or 1250 ppm [0, 138, 1380 or 2760 mg/m^3] butadiene for four weeks (6 h per day, five days per week). Animals were killed 14 days after the last exposure and *lacI* mutants were recovered from the DNA according to established protocols. A 2.5- and 3-fold increase in the *lacI* mutant frequency was observed in the bone marrow of mice exposed to 625 or 1250 ppm butadiene, respectively, compared with air-exposed control mice. DNA sequence analysis of *lacI* mutants recovered from the bone marrow of mice exposed to 625 ppm butadiene demonstrated that there was a shift in the spectrum of base substitution mutations at A:T base pairs in butadiene-exposed mice (6/26, 23%), compared to air control mice (2/45, 4%). Recio and Meyer (1995) examined the *lacI* mutational spectrum in the bone marrow of mice exposed to 1250 ppm butadiene in the above study. DNA sequence analysis of *lacI* mutants revealed an increase in mutations at A:T base pairs (9/49, 20%) similar to that observed by Sisk *et al.* (1994).

Recio *et al.* (1998) also examined the *lacI* mutagenicity and mutational spectrum in the spleen of mice exposed to butadiene in the above study. The authors reported three- and four-fold increases in the *lacI* mutant frequency in mice exposed to 625 or 1250 ppm butadiene, respectively, compared with air control mice. DNA sequence analysis of *lacI* mutants recovered from the spleen of mice exposed to 1250 ppm butadiene once again revealed an increase in mutations at A:T base pairs (10/57, 18%) in butadiene-exposed mice compared with air control mice (3/41, 7%). In addition, an increased frequency of

G:C→A:T transitions occurred at non-5′CpG-3′ sites in butadiene-exposed mice. The increased frequency of specific mutations at G:C base pairs was not observed in bone marrow from the same animals; there seem therefore to be tissue-specific differences in the butadiene mutational spectrum.

To examine the effect of exposure time on the *lacI* mutant frequency in butadiene-exposed mice, Recio *et al.* (1996) exposed male B6C3F$_1$ *lacI* transgenic mice by inhalation to 625 or 1250 ppm butadiene for 6 h per day for five days. Mice were killed 14 days following the last exposure and mutant frequency in the bone marrow was determined. The authors reported a five-fold increase in the *lacI* mutant frequency in mice exposed to 625 ppm butadiene compared with air control mice. These results demonstrated that there was little difference in the bone marrow *lacI* mutant frequency between a short-term exposure and the long-term exposure used in the previous study.

Butadiene metabolites

Epoxybutene

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 26 for references)

Epoxybutene was mutagenic to bacteria in the absence of an exogenous metabolic activation system. It did not induce DNA strand-breaks in mouse splenocytes nor unscheduled DNA synthesis in mouse or rat hepatocytes. In one study, it did increase the frequency of sister chromatid exchanges in Chinese hamster ovary CHO cells *in vitro*. However, it had no effect on sister chromatid exchanges or chromosomal aberrations in rat or mouse splenocytes, nor did it induce micronuclei in rat spermatids. Gene mutations at the *tk* and *hprt* loci were observed in human TK6 cells treated with epoxybutene and sister chromatid exchanges were induced in human lymphocyte cultures. In single studies, treatment with epoxybutene *in vivo* induced *hprt* mutations in mouse splenic T cells, and sister chromatid exchanges and chromosomal aberrations in mouse bone marrow. Micronucleus frequencies were also elevated in splenocytes and spermatids of mice and rats following in-vivo exposure to epoxybutene.

Epoxybutanediol

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental data* (see Table 27 for references)

In a single study, epoxybutanediol induced gene mutations in *Salmonella typhimurium* strain TA100 in the presence or absence of an exogenous metabolic activation system. It did not induce micronuclei in Sprague-Dawley rat spermatids *in vitro*.

A marginal response was reported for induction of micronuclei in the bone marrow of rats exposed for 48 h to epoxybutanediol. A positive response was observed in the

Table 26. Genetic and related effects of epoxybutene

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	350	de Meester *et al*. (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	26	Gervasi *et al*. (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	(+)	175	Adler *et al*. (1997)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	+	NT	175	de Meester *et al*. (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	1750	de Meester *et al*. (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	140	Thier *et al*. (1995)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	NT	8750	de Meester *et al*. (1978)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	8750	de Meester *et al*. (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	8750	de Meester *et al*. (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	105	Gervasi *et al*. (1985)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	+	NT	NG	Hemminki *et al*. (1980)
KPF, *Klebsiella pneumonia*, forward mutation	+	NT	70	Voogd *et al*. (1981)
DIA, DNA single-strand breaks, CD-1 mouse splenocytes *in vitro*	–	NT	65	Kligerman *et al*. (1996)
DIA, DNA single-strand breaks, CD rat splenocytes *in vitro*	–	NT	65	Kligerman *et al*. (1996)
URP, Unscheduled DNA synthesis, Sprague-Dawley rat hepatocytes *in vitro*	–	NT	1000	Arce *et al*. (1990)

Table 26 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
UIA, Unscheduled DNA synthesis, B6C3F$_1$ mouse hepatocytes *in vitro*	–	NT	1000	Arce *et al*. (1990)
SIC, Sister chromatid exchange, Chinese hamster cells *in vitro*	+	+	0.07	Sasiadek *et al*. (1991a)
SIM, Sister chromatid exchange, CD-1 mouse splenocytes *in vitro*	–	NT	65	Kligerman *et al*. (1996)
SIR, Sister chromatid exchange, CD rat splenocytes *in vitro*	–	NT	65	Kligerman *et al*. (1996)
MIA, Micronucleus test, Sprague-Dawley rat spermatids *in vitro*	–	NT	70	Sjoblom & Lahdetie (1996)
CIM, Chromosomal aberrations, CD-1 mouse splenocytes *in vitro*	–	NT	65	Kligerman *et al*. (1996)
CIR, Chromosomal aberrations, CD rat splenocytes *in vitro*	–	NT	65	Kligerman *et al*. (1996)
GIH, Gene mutation, human TK6 cells, *tk* locus *in vitro*	+	NT	17.5	Cochrane & Skopek (1993)
GIH, Gene mutation, human TK6 cells, *hprt* locus *in vitro*	+	NT	10.5	Cochrane & Skopek (1993)
GIH, Gene mutation, human TK6 cells, *hprt* locus *in vitro*	+	NT	28	Steen *et al*. (1997b)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	1.75	Sasiadek *et al*. (1991b)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	35	Wiencke & Kelsey (1993)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	3.5	Uuskula *et al*. (1995)
DVA, DNA strand breaks, CD-1 mouse testis *in vivo*	(+)		120 ip × 1	Anderson *et al*. (1997)

Table 26 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
DVA, DNA strand breaks, Sprague-Dawley rat bone marrow *in vivo*	+		80 ip × 1	Anderson *et al*. (1997)
GVA, Gene mutation, B6C3F_1 mouse splenic T cells, *hprt* locus *in vivo*	+		100 ip × 3	Cochrane & Skopek (1993)
GVA, Gene mutation, B6C3F_1 mouse splenic T cells, *hprt* locus *in vivo*	+		60 ip × 3	Cochrane & Skopek (1994)
GVA, Gene mutation, (102/El × C3H/El)F_1 mouse splenocytes, *hprt* locus *in vivo*	+[c]		100 ip × 3	Tates *et al*. (1998)
GVA, Gene mutation, (102/El × C3H/El)F_1 mouse splenocytes, *hprt* locus *in vivo*	–		100 ip × 1	Tates *et al*. (1998)
GVA, Gene mutation, Lewis rat splenocytes, *hprt* locus *in vivo*	–		100 ip × 1	Tates *et al*. (1998)
SVA, Sister chromatid exchange, C57BL/6 mouse bone marrow *in vivo*	+		25 ip × 1	Sharief *et al*. (1986)
MVM, Micronucleus test, (102 × C3H)F_1 mouse splenocytes *in vivo*	+		40 ip × 1	Xiao & Tates (1995)
MVM, Micronucleus test, (102 × C3H)F_1 mouse spermatids *in vivo*	+		40 ip × 1	Xiao & Tates (1995)
MVM, Micronucleus test, (102/El × C3H/El)F_1 mouse bone marrow *in vivo*	+		20 ip × 1	Adler *et al.* (1997)
MVM, Micronucleus test, CD-1 mouse bone-marrow *in vivo*	+		40 ip × 1	Anderson *et al.* (1997)
MVM, Micronucleus test, BALB/c mouse lymphocytes *in vivo*	+		25 ip × 1	Russo *et al*. (1997)
MVM, Micronucleus test, BALB/c mouse spermatids *in vivo*	(+)		73 ip × 1	Russo *et al*. (1997)
MVR, Micronucleus test, Lewis rat spermatids *in vivo*	+		40 ip × 1	Xiao & Tates (1995)
MVR, Micronucleus test, Lewis rat splenocytes *in vivo*	+		80 ip × 1	Xiao & Tates (1995)
MVR, Micronucleus test, Sprague-Dawley rat bone-marrow *in vivo*	(+)		120 ip × 1	Anderson *et al.* (1997)

Table 26 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
MVR, Micronucleus test, Sprague-Dawley rat spermatids *in vivo*	+		13 ip × 1	Lahdetie *et al*. (1997)
MVR, Micronucleus test, Sprague-Dawley rat bone marrow *in vivo*	–		78 ip × 1	Lahdetie & Grawe (1997)
CBA, Chromosomal aberrations, C57BL/6 mouse bone marrow *in vivo*	+		25 ip × 1	Sharief *et al*. (1986)
DLM, Dominant lethal test, (102/El × C3H/El)F_1 mice *in vivo*	–		120 ip × 1	Adler *et al.*, 1997
BID, Binding (covalent) to DNA, salmon testis *in vitro*	+	NT	NG	Citti *et al*. (1984)
BID, Binding (covalent) to DNA, calf thymus *in vitro*	+	NT	21 700	Tretyakova *et al*. (1997)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw /day; ; ip, intraperitoneal; NG, not given

Table 27. Genetic and related effects of epoxybutanediol

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	100	Adler *et al*. (1997)
MIA, Micronucleus test, Sprague-Dawley rat spermatocytes treated, spermatids scored *in vitro*	–	NT	10	Sjoblom & Lahdetie (1996)
MVM, Micronucleus test, (102/El × C3H/El)F_1 mouse bone marrow *in vivo*	+		120 ip × 1	Adler *et al*. (1997)
MVR, Micronucleus test, Sprague-Dawley rat bone marrow *in vivo*	(+)		30 ip × 1	Lahdetie & Grawe (1997)
MVR, Micronucleus test, Sprague-Dawley rat spermatogonia treated *in vivo*, spermatids scored	–		30 ip × 1	Lahdetie *et al*. (1997)
MVR, Micronucleus test, Sprague-Dawley rat spermatocytes treated *in vivo*, spermatids scored	+		30 ip × 1	Lahdetie *et al*. (1997)
DLM, Dominant lethal test, (102/El × C3H/El)F_1 mice *in vivo*	–		240 ip × 1	Adler *et al*. (1997)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw /day; ip, intraperitoneal dose

spermatids after treatment of rat spermatocytes, but not of spermatogonia. Epoxybutanediol induced micronuclei in (102/E1 × C3H/E1)F_1 mouse bone marrow samples 24 h after intraperitoneal injection, but no dominant lethal effects were induced in mice.

Diepoxybutane

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 28 for references)

Diepoxybutane was genotoxic *in vitro* without the addition of exogenous metabolic activation. In bacteria, it induced prophage, DNA repair and gene mutations (positive in *Salmonella* strains TA100 and TA1535 but not TA1537, TA1538 or TA98). The insertion of a rat GST 5-5+ or human GSTT1 plasmid vector in TA1535 increased the activity of diepoxybutane as much as 10-fold. It also induced mutation, gene conversion and mitotic recombination and crossing-over in yeast and reverse mutation in fungi. Diepoxybutane caused both somatic and sex-linked recessive lethal mutations as well as small chromosomal deletions and heritable translocations in *Drosophila melanogaster*.

Costa *et al*. (1997) reported that DNA–protein cross-links were produced by diepoxybutane in cultured human lymphoma cells. DNA cross-links were induced in mouse hepatocytes, but DNA strand breaks and/or alkali-labile sites were not detected in mouse or rat splenocytes *in vitro*. Unscheduled DNA synthesis was induced in Syrian hamster but not rat primary hepatocytes. Diepoxybutane enhanced gene mutations in Chinese hamster ovary CHO and lung V79 cells (*hprt* locus) and in mouse lymphoma L5187Y cells at the *tk* locus. It induced dose-related increases in the frequency of sister chromatid exchanges in CHO cells and in mouse and rat splenocyte cultures and, in a single study, it induced micronuclei in rat spermatids *in vitro*. It also induced chromosomal aberrations in rat and mouse splenocytes and in rat liver epithelial cell cultures. Gene mutations at the *tk* and *hprt* loci were induced in human TK6 cell cultures and dose-related increases were induced by diepoxybutane in sister chromatid exchanges in cultures of human lymphocytes from healthy donors and from patients with a variety of solid tumours, but not from Fanconi's anaemia homozygotes or heterozygotes. A bimodal distribution of sensitivity to induction of sister chromatid exchanges by diepoxybutane was observed in lymphocytes from healthy donors: lymphocyte populations from donors with GSTT1 null genotype showed greater sensitivity to diepoxybutane than those from donors with the GSTT1 gene. No correlation was seen between GSTM1 genotype and sister chromatid exchange induction by diepoxybutane. Chromosomal aberrations were induced in cultures of skin fibroblasts from Fanconi's anaemia heterozygotes, in primary lymphocytes from Fanconi's anaemia homo- and heterozygotes, and in lymphoblastoid cell lines from normal donors, Fanconi's anaemia homo- and heterozygotes and patients with xeroderma pigmentosum and ataxia telangiectasia. Positive results were also reported in one of two studies using lymphocytes from healthy donors. Diepoxybutane caused a weak increase in the frequencies of chromosomal

Table 28. Genetic and related effects of diepoxybutane

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
PRB, Prophage, induction, *Bacillus megaterium*	+	NT	NG	Lwoff (1953)
PRB, Prophage, induction, *Pseudomonas pyocyanea*	+	NT	NG	Lwoff (1953)
PRB, Prophage induction, *Escherichia coli* K-12	+	NT	7.5	Heinemann & Howard (1964)
ECB, *Escherichia coli* H540, DNA repair induction	+	NT	2500	Thielmann & Gersbach (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	(+)	50	Dunkel *et al*. (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	20	Gervasi *et al*. (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	–	38	Zeiger & Pagano (1989)
SA0, *Salmonella typhimurium*, TA100, reverse mutation	+	+	26	Adler *et al*. (1997)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	25	McCann *et al*. (1975)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	5	Rosenkranz & Poirier (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	5	Dunkel *et al*. (1984)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	2.5	Zeiger & Pagano (1989)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	4.3	Thier *et al*. (1995)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	8.6	Thier *et al*. (1996)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	167	Dunkel *et al*. (1984)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	50	Rosenkranz & Poirier (1979)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	167	Dunkel *et al*. (1984)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	167	Dunkel *et al*. (1984)

Table 28 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	60	Gervasi *et al*. (1985)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	(+)	(+)	167	Dunkel *et al*. (1984)
ECR, *Escherichia coli* B, reverse mutation	+	NT	1720	Glover (1956)
ECR, *Escherichia coli* B/r, reverse mutation	+	NT	860	Glover (1956)
KPF, *Klebsiella pneumonia*, fluctuation test	+	NT	4	Voogd *et al*. (1981)
SCH, *Saccharomyces cerevisiae* D4, mitotic gene conversion	+	NT	430	Zimmermann (1971)
SCH, *Saccharomyces cerevisiae* D81, mitotic crossing-over	+	NT	2000	Zimmermann & Vig (1975)
SCH, *Saccharomyces cerevisiae* D3, mitotic recombination	+	+	400	Simmon (1979)
SCH, *Saccharomyces cerevisiae* D7, gene conversion	+	+	130	Sandhu *et al.* (1984)
SCH, *Saccharomyces cerevisiae* D7, mitotic crossing-over	+	+	130	Sandhu *et al.* (1984)
SCF, *Saccharomyces cerevisiae*, mitochondrial mutation	+	NT	4000	Polakowska & Putrament (1979)
SCF, *Saccharomyces cerevisiae*, cytoplasmic petite mutation	–	NT	4000	Polakowska & Putrament (1979)
SCR, *Saccharomyces cerevisiae*, reverse mutation	+	NT	4000	Polakowska & Putrament (1979)
SCR, *Saccharomyces cerevisiae* D7, reverse mutation	+	+	130	Sandhu *et al.* (1984)
NCR, *Neurospora crassa*, reverse mutation	+	NT	1720	Pope *et al*. (1984)
NCR, *Neurospora crassa*, reverse mutation	+	NT	4300	Kolmark & Westergaard (1953)
DMM, *Drosophila melanogaster*, somatic mutation	+		430 feed	Olsen & Green (1982)
DMM, *Drosophila melanogaster*, somatic mutation	+		1000 feed	Graf *et al*. (1983)

Table 28 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+		100 inj	Bird & Fahmy (1953)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+		1000 inj	Fahmy & Fahmy (1970)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	+		175 feed	Sankaranarayanan *et al*. (1983)
DMC, *Drosophila melanogaster*, chromosome aberrations	+		1000 inj	Fahmy & Fahmy (1970)
DMH, *Drosophila melanogaster*, heritable translocations	+		1000 inj	Denell *et al*. (1978)
DIA, DNA–DNA cross-links, B6C3F_1 mouse liver DNA *in vitro*	+	NT	4	Ristau *et al*. (1990)
DIA, DNA single-strand breaks, male CD rat and male CD-1 mouse splenocytes *in vitro*	–	NT	13.7	Kligerman *et al*. (1996)
URP, Unscheduled DNA synthesis, male Sprague-Dawley rat primary hepatocytes *in vitro*	–	NT	8.6	Kornbrust & Barfknecht (1984)
UIA, Unscheduled DNA synthesis, Syrian hamster hepatocytes *in vitro*	+	NT	0.86	Kornbrust & Barfknecht (1984)
GCO, Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	+	NT	2.15	Zhu & Zeiger (1993)
G9H, Gene mutation, Chinese hamster V79 cells, *hprt* locus *in vitro*	(+)	NT	2	Nishi *et al*. (1984)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	0.3	McGregor *et al*. (1988)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	NT	0.025	Perry & Evans (1975)
SIC, Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	+	NT	0.1	Nishi *et al*. (1984)

Table 28 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	0.01	Sasiadek *et al*. (1991a)
SIM, Sister chromatid exchange, CD-1 mouse splenocytes *in vitro*	+	NT	0.43	Kligerman *et al*. (1996)
SIR, Sister chromatid exchange, CD rat splenocytes *in vitro*	+	NT	0.86	Kligerman *et al*. (1996)
MIA, Micronucleus test, rat spermatids (spermatocytes treated) *in vitro*	+	NT	0.43	Sjoblom & Lahdetie (1996)
CIM, Chromosomal aberrations, CD-1 mouse splenocytes *in vitro*	+	NT	3.44	Kligerman *et al*. (1996)
CIR, Chromosomal aberrations, Carworth Farm E rat liver epithelial (RL_1) cells *in vitro*	+	NT	0.1	Dean & Hodson-Walker (1979)
CIR, Chromosomal aberrations, CD rat splenocytes *in vitro*	+	NT	6.88	Kligerman *et al*. (1996)
TCM, Cell transformation, C3H 10T1/2 mouse cells *in vitro*	+	NT	0.0001	Nelson & Garry (1983)
TCL, Cell transformation, Syrian hamster lung epithelial M3E3/C3 cells *in vitro*	+	NT	0.009	Lichtenberg *et al*. (1995)
GIH, Gene mutation, human TK6 cells, *tk* locus *in vitro*	+	NT	0.2	Cochrane & Skopek (1993)
GIH, Gene mutation, human TK6 cells, *hprt* locus *in vitro*	+	NT	0.3	Cochrane & Skopek (1993)
GIH, Gene mutation, human TK6 cells, *hprt* locus *in vitro*	+	NT	0.34	Steen *et al*. (1997a)

Table 28 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.125	Wiencke *et al*. (1982)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.01	Porfirio *et al*. (1983)
SHL, Sister chromatid exchange, human lymphocytes[c] *in vitro*	–	NT	0.01	Porfirio *et al*. (1983)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.04	Sasiadek *et al*. (1991b)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+[d]	NT	0.13	Wiencke *et al*. (1991)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+[e]	NT	0.17	Landi *et al*. (1995)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+[e]	NT	0.17	Norppa *et al*. (1995)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+[e]	NT	0.5	Wiencke *et al*. (1995)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.17	Landi *et al*. (1996a,b)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.172	Pelin *et al*. (1996)
MIH, Micronucleus test, human blood lymphotyctes *in vitro*	+	NT	172	Vlachadimitropoulos *et al*. (1997)
CHF, Chromosome aberrations, human skin fibroblasts[f] *in vitro*	+	NT	0.01	Auerbach & Wolman (1976)
CHF, Chromosome aberrations, human skin fibroblasts *in vitro*	–	NT	0.01	Auerbach & Wolman (1976)
CHL, Chromosome aberrations, human lymphocytes[g] *in vitro*	+	NT	0.01	Cohen *et al*. (1982)
CHL, Chromosome aberrations, human lymphocytes *in vitro*	(+)	NT	0.1	Marx *et al*. (1983)

Table 28 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
CHL, Chromosome aberrations, human lymphocytes[c] *in vitro*	+	NT	0.1	Marx *et al*. (1983)
CHL, Chromosome aberrations, human lymphocytes *in vitro*	–	NT	0.01	Porfirio *et al*. (1983)
CHL, Chromosome aberrations, human lymphocytes[c] *in vitro*	+	NT	0.01	Porfirio *et al*. (1983)
CHL, Chromosome aberrations, human lymphocytes *in vitro*	+	NT	0.5	Wiencke *et al*. (1991)
CIH, Chromosome aberrations, human bone-marrow cells *in vitro*	(+)	NT	0.1	Marx *et al*. (1983)
CIH, Chromosome aberrations, human bone-marrow cells[c] *in vitro*	(+)	NT	0.1	Marx *et al*. (1983)
HMM, Host-mediated assay, reverse mutation in *Salmonella typhimurium* TA1530 in Swiss-Webster mice	+		444 im	Simmon *et al*. (1979)
HMM, Host-mediated assay, mitotic recombination in *Saccharomyces cerevisiae* D3 in Swiss-Webster mice	–		56 po	Simmon *et al*. (1979)
DVA, DNA single-strand breaks, male CD-1 mouse bone marrow and estis *in vivo*	+		15 ip × 1	Anderson *et al*. (1997)
DVA, DNA single-strand breaks, male Sprague-Dawley rat bone marrow *in vivo*	(+)		50 ip × 1	Anderson *et al*. (1997)
GVA, Gene mutation, B6C3F_1 mice, splenic T cells, *hprt* locus *in vivo*	+		21 ip × 3	Cochrane & Skopek (1993)
GVA, Gene mutation, B6C3F_1 mice, splenic T cells, *hprt* locus *in vivo*	+		7 ip × 3	Cochrane & Skopek (1994)
GVA, Gene mutation, male Lewis rats *in vivo* (*hprt* locus)	–		40 ip × 1	Tates *et al*. (1998)
GVA, Gene mutation, male (102/El × C3H/El)F_1 mice *in vivo* (*hprt* locus)	–		40 ip × 1	Tates *et al*. (1998)
GVA, Gene mutation, C57BL mice *in vivo* (*hprt* locus)	–		14 ip × 3	Tates *et al*. (1998)

Table 28 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SVA, Sister chromatid exchange, Swiss-Webster mouse bone marrow *in vivo*	+		1 ip × 1	Conner *et al*. (1983)
SVA, Sister chromatid exchange, Swiss-Webster mouse alveolar macrophages *in vivo*	+		1 ip × 1	Conner *et al*. (1983)
SVA, Sister chromatid exchange, Swiss-Webster mouse regenerating liver cells *in vivo*	+		1 ip × 1	Conner *et al*. (1983)
SVA, Sister chromatid exchange, NMRI mouse bone-marrow cells *in vivo*	+		22 inh 2 h	Walk *et al*. (1987)
SVA, Sister chromatid exchange, NMRI mouse bone-marrow cells *in vivo*	+		29 ip × 1	Walk *et al*. (1987)
SVA, Sister chromatid exchange, Chinese hamster bone-marrow cells *in vivo*	+		34 inh 2 h	Walk *et al*. (1987)
SVA, Sister chromatid exchange, Chinese hamster bone-marrow cells *in vivo*	+		32 ip × 1	Walk *et al*. (1987)
MVM, Micronucleus test, (102 × C3H)F_1 mouse splenocytes *in vivo*	+		15 ip × 1	Xiao & Tates (1995)
MVM, Micronucleus test, (102 × C3H)F_1 mouse spermatids *in vivo*	(+)		30 ip × 1	Xiao & Tates (1995)
MVM, Micronucleus test, (102/El × C3H/El)F_1 mouse bone marrow *in vivo*	+		9 ip × 1	Adler *et al*. (1995b)
MVM, Micronucleus test, male CD-1 mouse bone marrow *in vivo*	+		30 ip × 1	Anderson *et al*. (1997)
MVM, Micronucleus test, mouse spermatids and peripheral blood lymphocytes *in vivo*	+		15 ip × 1	Russo *et al*. (1997)
MVM, Micronucleus test, male (102/El × C3H/El)F_1 mice *in vivo*	+		30 ip × 1	Tates *et al*. (1998)

Table 28 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
MVR, Micronucleus test, Lewis rat spermatids *in vivo*	+		20 ip × 1	Xiao & Tates (1995)
MVR, Micronucleus test, Lewis rat splenocytes *in vivo*	+		40 ip × 1	Xiao & Tates (1995)
MVR, Micronucleus test, male Sprague-Dawley rat bone marrow *in vivo*	+		25 ip × 1	Anderson *et al.* (1997)
MVR, Micronucleus test, Sprague-Dawley rat bone-marrow *in vivo*	+		17 ip × 1	Lahdetie & Grawe (1997)
MVR, Micronucleus test, Sprague-Dawley rat spermatids *in vivo*	+		16.7 ip × 1	Lahdetie *et al.* (1997)
CBA, Chromosomal aberrations, NMRI mouse bone marrow *in vivo*	+		22 inh 2 h	Walk *et al.* (1987)
CBA, Chromosomal aberrations, NMRI mouse bone marrow *in vivo*	+		29 ip × 1	Walk *et al.* (1987)
CBA, Chromosomal aberrations, Chinese hamster bone marrow *in vivo*	+		34 inh 2 h	Walk *et al.* (1987)
CBA, Chromosomal aberrations, Chinese hamster bone marrow *in vivo*	+		32 ip × 1	Walk *et al.* (1987)
COE, Chromosomal aberrations, zygotes of (102/El × C3H/El)F_1 mice *in vivo*	+		17 ip × 1	Adler *et al.* (1995)
DLM, Dominant lethal test, (102/El × C3H/El)F_1 mice *in vivo*	+		18 ip × 1	Adler *et al.* (1995b)
BID, Binding (covalent) to DNA, Chinese hamster ovary AA8 cells *in vitro*	+	NT	43	Leuratti *et al.* (1993)
BID, Binding (covalent) to DNA, CHO AA8 cells *in vitro* (adenine adduct N6)	+	NT	43	Leuratti *et al.* (1994)

Table 28 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
BID, Binding (covalent) to calf thymus DNA *in vitro*	+	NT	3400	Mabon *et al*. (1996)
BVD, Binding (covalent) to DNA, female ICR mouse skin *in vivo*	+		6.5 skin paint	Mabon *et al*. (1996)
BVD, Binding (covalent) to DNA, female ICR mouse skin *in vivo*	+		60 skin paint	Mabon & Randerath (1996)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw /day; inj, injection; im, intramuscular; po, oral; ip, intraperitoneal; inh, inhalation
[c] Fanconi's anaemia (homozygotes and heterozygotes)
[d] Bimodal response, 24% positive, 76% negative; no correlation to GSTM1 deficiency
[e] Positive response correlates with GSTT1 deficiency
[f] Fanconi's anaemia (heterozygotes)
[g] Fanconi's anaemia (homozygotes and heterozygotes), ataxia telangiectasia, xeroderma pigmentosum, normal

aberrations in bone-marrow cultures from Fanconi's anaemia patients and normal individuals.

In the mouse host-mediated assay, diepoxybutane induced mutation in *S. typhimurium* TA1530 but did not induce mitotic recombination in *Saccharomyces cerevisiae* D3.

In a single study, gene mutations were induced at the *hprt* locus in splenic T cells of mice following intraperitoneal injection with diepoxybutane. Micronucleus frequencies were increased in splenocytes as well as spermatids of both mice and rats treated with a single intraperitoneal injection of diepoxybutane. Dose-related increases in the frequency of sister chromatid exchanges were observed in bone marrow and in alveolar macrophages from both intact and partially hepatectomized mice and in the regenerating livers of hepatectomized mice. Diepoxybutane also induced sister chromatid exchanges and chromosomal aberrations in Chinese hamster and NMRI mouse bone-marrow following exposure by inhalation or intraperitoneal injection. In one study, chromosomal aberrations were observed in zygotes from matings of untreated female mice with male mice exposed to diepoxybutane seven days earlier. Negative results were reported for the 14–28-day mating periods, indicating that only early spermatozoa were affected. Dominant lethal mutations were also induced in this study. The dominant lethal effect was restricted to spermatozoa for the two lower doses and only late spermatids could be evaluated at the highest dose because the number of pregnancies during the first eight mating days following treatment was greatly reduced.

Mechanism of mutation induction

The mechanisms by which epoxybutene and diepoxybutane induce mutagenicity have been examined using human and *lacI* transgenic cells in culture. By determining the spectra of mutations induced, the contribution of each metabolite to the genotoxic properties of the parent butadiene can be assessed.

Steen *et al.* (1997a,b) assessed the mutagenicity of epoxybutene and diepoxybutane at *hprt* in human TK6 lymphoblastoid cells exposed to 400 μM (epoxybutene) or 4 μM (diepoxybutane) for 24 h. These concentrations of epoxybutene and diepoxybutane resulted in approximately 10% survival relative to media controls and induced a five-fold increase in *hprt* mutant frequency. Molecular analysis of epoxybutene-induced *hprt* mutants revealed an increase (39/50, 78%) in single-base substitution mutations compared with media controls (22/43, 51%), and a shift in the spectrum of base substitution mutations at A:T base pairs (21/50, 42%) compared with media controls (8/43, 18%). The most significant change was a five-fold increase in A:T→T:A transversions among the epoxybutene-induced mutants. The DNA sequence context of the mutations at A:T base pairs among the epoxybutene-induced mutants showed a strand bias; in 19/21 (90%) of these mutants, the A was located in the non-transcribed DNA strand. All of the epoxybutene-induced A:T→T:A transversions displayed this strand bias. Molecular analysis of diepoxybutane-induced *hprt* mutants revealed an increase in 5′ partial deletion of the *hprt* gene (7/51, 14%) compared with media controls (1/43, 2%). Diepoxybutane-induced mutants also had an increased frequency of A:T→T:A transversions (9/51, 18%) compared with media controls

(2/43, 5%), but with the opposite strand bias compared with epoxybutene; in 8/11 (78%) of these mutants the T was located in the non-transcribed strand.

Saranko and Recio (1998) examined the mutagenicity of diepoxybutane at the *lacI* gene in Rat2 *lacI* transgenic fibroblasts exposed to 0, 2, 5 or 10 μM for 24 h. These concentrations of diepoxybutane resulted in approximately 100, 30 and 10% survival respectively, compared with media controls. There was no significant increase in *lacI* mutant frequency following exposure to 2, 5 or 10 μM diepoxybutane. However, all three of these exposure concentrations resulted in significant increases in the formation of micronuclei (2-, 2.5-, and 3.5-fold, respectively) in Rat2 cells. These experiments demonstrated the insensitivity of the lambda shuttle vector-based *lacI* transgenic system to the clastogenic effects of diepoxybutane. The inability of diepoxybutane to induce a mutational response in the Rat 2 *lacI* transgenic fibroblasts is probably due to the poor recovery of deletions by the *lacI* transgenic assay.

The results of these in-vitro studies demonstrate that epoxybutene and diepoxybutane differ in their mutagenic potency and mechanism of action. Epoxybutene is effective at producing base substitutions as well as large deletions and micronuclei.

DNA adducts

Butadiene

After exposure of $B6C3F_1$ mice and Wistar rats for up to 6.6 h to [1,4-^{14}C]butadiene (uptake of 0.24 mmol/kg), Laib *et al.* (1990) found alkylation of DNA to be similar in both species and a level of alkylation of nuclear proteins which was about twice as high in mice as in rats.

Acid hydrolysis of hepatic DNA isolated from mice exposed to [^{14}C]butadiene yielded two alkylation products: 7-*N*-(hydroxy-3-buten-2-yl)guanine and 7-*N*-(2,3,4-trihydroxybutyl)guanine. These were not found in similarly exposed rats (Jelitto *et al.*, 1989; Bolt & Jelitto, 1996). DNA adducts at the N7 position of guanine were also detected using ^{32}P-postlabelling in $B6C3F_1$ mouse lung following inhalation exposure to 200 ppm [440 mg/m^3] butadiene for 6 h per day for five days and in lung and liver of Sprague-Dawley rats treated under the same exposure conditions (Koivisto *et al*., 1996, 1997).

CB6 F_1 mice [sex not indicated] were exposed (6 h per day for five days) to 0, 50, 200, 500 and 1300 ppm [0, 110, 440, 1100 and 2870 mg/m^3] butadiene. In addition, Wistar rats [sex not indicated] were exposed up to 500 ppm butadiene. Using a post-labelling assay, dose-dependent formation of epoxybutene adducts at N^6 of adenine was found in lung DNA of both species at the higher concentrations. The mean adduct levels (fmol adducts/100 nmol 3′-dAMP) were similar in mouse lung (up to about 2.6 at 500 ppm) and rat lung (up to about 2.3 at 500 ppm), mean background levels being 0.5 in mice and 0.7 in rats (Koivisto *et al*., 1996; Sorsa *et al*., 1996). Corresponding mean adduct levels in the liver DNA of the rats exposed to 500 ppm were about 30, whereas background levels were about 2 (Sorsa *et al*., 1996).

Enantio- and regioisomeric formation of the epoxybutene adduct at guanine N7 of liver DNA (7.2 fmol/10 μg DNA = 2.4 adducts/10^7 nucleotides) was determined in male

Sprague-Dawley rats exposed for five days (6 h per day) to 200 ppm [440 mg/m^3] butadiene. The relative formation of the different isomers were 47, 22, 18 and 14%, corresponding to the adducts derived from *R*-epoxybutene (C-2″, C-1″) and from *S*-epoxybutene (C-2″, C-1″), respectively (Koivisto *et al.*, 1997).

After single exposures (7 h) of male B6C3F$_1$ mice to butadiene (100–2000 ppm [220–4400 mg/m^3]), dose-dependent DNA–DNA and DNA–protein cross-link formation was suggested from alkaline-elution profiles, the effect being stronger in lung DNA than in liver DNA. No cross-linking activity was found in Sprague-Dawley rats similarly exposed to butadiene (Vangala *et al.*, 1993).

Metabolites

Epoxybutene reacts with free DNA bases, nucleosides and DNA to form covalent adducts. Citti *et al*. (1984) characterized adducts formed *in vitro* between epoxybutene and deoxyguanosine or DNA (pH 7.2). They found *N*7-(2′-hydroxy-3′-buten-1′-yl)-guanine and *N*7-(1′-hydroxy-3′-buten-2′-yl)guanine in ratios of 59:4 using the nucleosides and of 54:46 using DNA. These results together with those of later investigations are summarized in Table 29. Selzer and Elfarra (1996a,b, 1997a,b) determined the pseudo-first-order rate constants from the in-vitro reactions between epoxybutene and guanosine, adenosine, deoxycytidine and thymidine at pH 7.4 and 37°C; they ranged from 2.67×10^{-4} to 2.63×10^{-2} per hour. Comparison of these rate constants indicates that the order of adduct formation at the various sites on the bases is likely to be as follows: α and β *N*7-guanosine > β *N*^{6}-adenosine, β *N*3-deoxyuridine, β *N*3-deoxycytidine > α *N*^{1}- and α *N*^{2}-guanosine, α *N*^{6}-adenosine, α *N*^{1}-inosine > β *N*3-thymidine, β *O*^{2}-deoxycytidine, α *N*3-deoxyuridine > α *N*3-thymidine. Thus, the pseudo-first-order constants suggest that the *N*3-thymidine adducts are among the least abundant under these in-vitro conditions. This order of formation may or may not be replicated in reactions of epoxybutene with DNA, where the molecular structure and hydrogen bonding at various sites may modify reactivity. The finding that thymidine adducts are likely to be less abundant than other adducts does not necessarily exclude them as mutagenic precursors, since analysis of *lacI* mutants from the bone marrow of B6C3F$_1$ mice exposed to butadiene showed an increase in mutations at A:T base pairs, with A:T→T:A transversions apparently occurring only in exposed mice (Sisk *et al.*, 1994).

Diepoxybutane also reacts with nucleosides, nucleotides and DNA. Adducts at N^6 of adenine were identified in incubations (pH 7) containing deoxyadenosine, deoxyadenosine monophosphate or poly(dA-dT)(dA-dT), as determined by mass spectrometry, or calf thymus DNA as determined by a high-performance liquid chromatography/^{32}P-postlabelling method (Leuratti *et al*., 1994). By the latter method, the authors demonstrated adduct formation to N^6 of adenine in DNA from Chinese hamster ovary cells incubated with diepoxybutane at 37°C.

In calf thymus DNA incubated with diepoxybutane, *N*7-(2′-hydroxy-3′,4′-epoxybut-1′-yl)guanine (Tretyakova *et al*., 1997b) and *N*7-(2′,3′,4′-trihydroxybut-1′-yl)guanine (Tretyakova *et al*., 1996, 1997b) [enantiomers not resolved] were formed, as characterized

Table 29. Reactivity of epoxybutene with DNA bases *in vitro*

Targets	Adducts formed	Kinetics	Comments (References)
Deoxyguanosine; Salmon testis DNA type III	*N*7-(2-Hydroxy-3-buten-1-yl)guanosine (I) *N*7-(1-Hydroxy-3-buten-2-yl)guanosine (II)	Half-lives of spontaneous depurination of I and II in DNA 50 h (pH 7.2; 37 °C).	NMR, MS HPLC, UV (Citti *et al.*, 1984)
Guanosine; Deoxyguanosine; Calf thymus DNA	Diastereomeric pairs of *N*7-(2-Hydroxy-3-buten-1-yl)guanosine (I) *N*7-(1-Hydroxy-3-buten-2-yl)guanosine (II)	Half-lives of spontaneous depurination of I and II in DNA 48 h (pH 7.4).	HPLC, UV, ECD, NMR, MS (Neagu *et al.*, 1995)
Deoxyadenosine; Deoxyadenosine monophosphate; Calf thymus DNA	Diastereomeric pairs of N^6-(1-Hydroxy-3-buten-2-yl)adenosine (III) N^6-(2-Hydroxy-3-buten-1-yl)adenosine (IV)		HPLC/^{32}P-post-labelling, MS/MS, CD, NMR (Koivisto *et al.*, 1995, 1996)
Guanosine	Diastereomeric pairs of *N*7-(2-Hydroxy-3-buten-1-yl)guanosine (I) *N*7-(1-Hydroxy-3-buten-2-yl)guanosine (II) N^2-(1-Hydroxy-3-buten-2-yl)guanosine (III) N^1-(1-Hydroxy-3-buten-2-yl)guanosine (IV)	Pseudo-first-order formation rate constant (pH 7.4; 37°C) at N7 about 10-fold higher than at N2 or N1. Half-lives of decomposition (pH 7.4; 37°C) of I 50 h, of II 90 h. III and IV stable up to 192 h (pH 7.4; 37°C).	HPLC, UV, NMR, FAB-MS (Selzer & Elfarra, 1996a)
Deoxyguanosine monophosphate; Salmon testis DNA	Diastereomeric pairs of *N*7-(2-Hydroxy-3-buten-1-yl)dGMP (I) *N*7-(1-Hydroxy-3-buten-2-yl)dGMP (II)	Half-lives of decomposition (pH 9.6; 37°C) of I 4.5 h, of II 5 h.	HPLC/^{32}P-postlabelling (Kumar *et al.*, 1996)
Adenine; Adenosine; Calf thymus DNA	N^1-(2-Hydroxy-3-buten-1-yl)adenine (I) N^1-(1-Hydroxy-3-buten-2-yl)adenine (II) *N*3-(2-Hydroxy-3-buten-1-yl)adenine (III) *N*3-(1-Hydroxy-3-buten-2-yl)adenine (IV)	Formation in DNA (pH 7.2; 37°C); V and VI 8-fold > IV; IV 2-fold > III; III 3-fold > I and II.	HPLC, UV, NMR, ESI^+-MS (Tretyakova *et al.*, 1997a)
Guanosine; Calf thymus DNA	*N*7-(2-Hydroxy-3-buten-1-yl)guanine (V) *N*7-(1-Hydroxy-3-buten-2-yl)guanine (VI)		

Table 29 (contd)

Targets	Adducts formed	Kinetics	Comments (References)
Adenosine	Diastereomeric pairs of N^1-(1-Hydroxy-3-buten-2-yl)adenosine (I) N^1-(2-Hydroxy-3-buten-1-yl)adenosine (II) N^6-(1-Hydroxy-3-buten-2-yl)adenosine (III) N^6-(2-Hydroxy-3-buten-1-yl)adenosine (IV) N^1-(1-Hydroxy-3-buten-2-yl)inosine (V)	Pseudo-first-order formation rate constants (pH 7.4; 37°C) of the sum of III and IV about 3-fold higher than of V. Half-lives of decomposition (pH 7.4; 37°C) of I 7 h, of II 9.5 h. III, IV, V stable up to 7 days (pH 7.4; 37°C). Dimroth rearrangement of I and II to III and IV (pH 7.4; 37°C). Deamination of I and II to V (pH 7.4; 80°C).	HPLC, UV, NMR, FAB-MS (Selzer & Elfarra, 1996b)
Thymidine	Diastereomeric pairs of *N*3-(2-Hydroxy-3-buten-1-yl)thymidine (I) *N*3-(1-Hydroxy-3-buten-2-yl)thymidine (II)	Pseudo-first-order formation rate constant (pH 7.4; 37°C) of I about 5- to 6-fold higher than of II. I and II stable up to 7 days (pH 7.4; 37°C).	HPLC, UV, NMR, FAB-MS (Selzer & Elfarra, 1997a)
Deoxycytidine	Diastereomeric pairs of *N*3-(2-Hydroxy-3-buten-1-yl)deoxycytidine (I) *N*3-(2-Hydroxy-3-buten-1-yl)deoxyuridine (II) *N*3-(1-Hydroxy-3-buten-2-yl)deoxyuridine (III) O^2-(2-Hydroxy-3-buten-1-yl)deoxycytidine (IV)	Pseudo-first-order formation rate constant (pH 7.4; 37°C) of I about 5- to 6-fold higher than of III and IV. Deamination of I to II (pH 7.4; 37°C). Half-lives of decomposition (pH 7.4; 37°C) of I c. 2.4 h, of IV 11 h; II and III stable up to 168 h (pH 7.4; 37°C).	HPLC, UV, ^{1}H-NMR, FAB-MS (Selzer & Elfarra, 1997b)

dGMP, deoxyguanosine monophosphate; NMR, nuclear magnetic resonance; MS, mass spectrometry; HPLC, high-performance liquid chromatography; ECD, electrochemical detection; FAB, fast atom bombardment; ESI^+, electron spray ionization; CD, circular dichroism

by UV spectrophotometry, electron spray ionization mass spectrometry and nuclear magnetic resonance. Incubation of diepoxybutane (methanol/Tris-HCl buffer 1:1; pH 7.2) with adenine yielded *N*3-, *N*7- and *N*9-(2′-hydroxy-3′,4′-epoxybut-1′-yl)adenine, which hydrolysed to the corresponding trihydroxybutyl adducts [enantiomers not resolved]. 2′-Deoxyadenosine reacted in aqueous solution with diepoxybutane, probably forming an N1 adduct, which after acid hydrolysis and heating yielded trihydroxybutyl adducts at N^6 through Dimroth rearrangement. Trihydroxybutyl adducts were also found at N3- and N^6 of adenine in calf thymus DNA following acidic hydrolysis (Tretyakova *et al*., 1997c). The molar ratios of adduct formation at N7 of guanine to N3 of adenine in calf thymus DNA were similar for epoxybutene (Tretyakova *et al*., 1997a) and diepoxybutane (Tretyakova *et al*., 1997b,c).

Skin application of diepoxybutane for three days to female ICR mice with a daily dose of 1.9–153 μmol per mouse led to the formation of three adenine adducts in skin DNA, as determined by ^{32}P-postlabelling. The relative adduct labelling values correlated linearly with dose, reaching a mean maximum value of 185.6 total adducts per 10^8 DNA nucleotides after application of 153 μmol [13–17 mg] diepoxybutane per mouse per day (Mabon *et al*., 1996; Mabon & Randerath, 1996).

Alterations of oncogenes and suppressor genes in tumours

Mouse tumours from the study of Melnick *et al*. (1990) were evaluated for the presence of oncogenes. Activated K-*ras* oncogenes were detected in 6/9 lung adenocarcinomas, 3/12 hepatocellular carcinomas and 2/11 lymphomas obtained from $B6C3F_1$ mice exposed to butadiene. A specific codon 13 mutation (guanine to cytosine transversion) was found in most of the activated K-*ras* genes (Goodrow *et al*., 1994). Activated K-*ras* genes have not been found in spontaneously occurring liver tumours or lymphomas from $B6C3F_1$ mice (Reynolds *et al*., 1987; Goodrow *et al*., 1994) and were observed in only 1/10 spontaneous lung tumours in this strain of mice (Goodrow *et al*., 1994).

Mutations of the *p53* and *ras* genes were also detected in lymphomas from butadiene-treated mice by Zhuang *et al*. (1997). Most of the lymphomas with *ras* mutations at codon 13 (CGC) were from the low-dose group (< 200 ppm [440 mg/m^3]) or from the high-dose group with shortened treatment time (26 weeks), while those with *p53* mutations were from the high-dose (625 ppm [1380 mg/m^3]) continuous-exposure group. These results suggest that the *ras* genes may be involved in the early stages of butadiene-induced lymphomagenesis, while the *p53* gene appears to be more involved with the late-stage progression of these tumours.

4.5 Mechanistic considerations

Mechanistic studies conducted in whole animals and in rodent and human tissues using biochemical and molecular biological approaches have provided important insights into the likely critical steps in the initiation of butadiene carcinogenicity and the identity of the most likely chemical species responsible for the development of tumours.

The initial step is metabolic activation of butadiene to its reactive epoxide metabolites by multiple cytochrome P450 enzymes, including cytochrome P450 2E1 (CYP2E1). Butadiene is bioactivated to at least two genotoxic metabolites, epoxybutene and diepoxybutane. These two metabolites have been studied in detail by numerous laboratories. A third genotoxic epoxide metabolite of butadiene, epoxybutanediol, has not been quantified in animals but adducts to haemoglobin that are presumed to be derived from this epoxide have been detected in rats and humans exposed to butadiene.

Following inhalation exposure to butadiene, blood concentrations of epoxybutene were up to eight-fold higher in mice than in rats and blood concentrations of diepoxybutane were 40-fold higher in mice than in rats. Further, tissue concentrations of epoxybutene were 3–10 times higher in mice than in rats and tissue concentrations of diepoxybutane were up to 100 times higher in mice than in rats. Mice are much more susceptible to the carcinogenic effects of butadiene than are rats, with female $B6C3F_1$ mice developing tumours at butadiene concentrations as low as 6.25 ppm [13.8 mg/m^3]. Rats, in contrast, developed tumours after exposure to butadiene at concentrations of 1000 and 8000 ppm [2200 and 17 700 mg/m^3]. Considering the higher mutagenic potency of diepoxybutane as compared with epoxybutene and epoxybutanediol, the correlation between the measured circulating blood and tissue levels of the epoxides, especially diepoxybutane, and the observed development of tumours is suggestive of the role of diepoxybutane in the initiation of cancers in rodents exposed to butadiene.

Data on the metabolism of butadiene *in vitro*, including activation and detoxication, indicate significant species differences, and suggest that levels of epoxides *in vivo* should be higher in mice than in rats. The data on metabolism and tissue concentrations of epoxybutene and diepoxybutane in mice and rats *in vivo* following inhalation exposures to butadiene are consistent with results *in vitro*. The substantial variation in enzymatic activity between tissues from humans for the conversion of epoxybutene to diepoxybutane suggests the potential for large interindividual variation among humans in susceptibility to the potential genotoxic effects of butadiene. Bioactivation of butadiene at low concentrations to epoxybutene and diepoxybutane is mediated primarily by CYP2E1, so that this isoenzyme may play a key role in mediating differences between species in response to butadiene.

Studies on the induction of mutations by epoxybutene and diepoxybutane and the resulting mutational spectra have demonstrated clear mechanistic differences between epoxybutene- and diepoxybutane-induced mutational events. The concentrations of diepoxybutane that are genotoxic *in vitro* are within the range of concentrations measured in the blood and tissues of mice exposed to butadiene by inhalation, while the concentrations of epoxybutene that are genotoxic *in vitro* are 10- to 100-fold greater than concentrations observed in blood of mice exposed to butadiene. The characterization of molecular events induced by epoxybutene and diepoxybutane indicates that epoxybutene-induced genotoxicity is primarily due to point mutations and small deletion events. Diepoxybutane induces not only point mutations and small deletions, but also large-scale deletions involving hundreds or thousands of base pairs at an equal frequency.

The molecular biology data suggest involvement of at least diepoxybutane in the development of cancer in rodents following butadiene exposure. However, the additive or possible synergistic involvement of one or both of the other butadiene epoxides cannot be discounted.

Haemoglobin binding indices of epoxides which are formed as metabolic intermediates in the butadiene pathway can be regarded as dose surrogates of the internal body burden of these compounds. The haemoglobin binding index of *N*-(2-hydroxy-3-butenyl)-valine, the adduct with epoxybutene, was about 1.5–5 times higher in butadiene-exposed mice than in rats. In exposed humans, the corresponding binding index was between 25 and 250 times lower than in rats. There are only two preliminary reports on the formation in butadiene-exposed rats and humans of haemoglobin adduct of epoxybutanediol which can arise from the oxidation of dihydroxybutene and/or the hydrolysis of diepoxybutane. Based on these data, binding indices can be estimated to be more than one order of magnitude lower in exposed humans than in exposed rats. Together with model predictions which are based on in-vitro data obtained with tissues of mouse, rat and human, the available in-vivo data indicate a considerably lower body burden of butadiene-derived epoxides in butadiene-exposed humans than in rats and mice.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

1,3-Butadiene is a monomer used in high volume in the manufacture of a wide range of polymers, including styrene–butadiene rubber, polybutadiene, nitrile rubber, acrylonitrile–butadiene–styrene resins and styrene–butadiene latexes. It is also an intermediate in the production of various other chemicals.

Occupational exposure to 1,3-butadiene occurs in the production of monomeric 1,3-butadiene and of 1,3-butadiene-based polymers and 1,3-butadiene-derived products. The mean full-shift, time-weighted average exposure levels measured for workers in these industries have usually been below 10 ppm [22 mg/m^3], although that level may be exceeded during some short-term activities. Recent data from monomer extraction and styrene–butadiene rubber plants showed lower average concentrations (< 5 ppm [< 11 mg/m^3]). 1,3-Butadiene is not usually found at detectable levels in workplace air during manufacture of finished rubber and plastic products.

The general population may be exposed to very low levels of 1,3-butadiene due to its occurrence in engine exhausts and cigarette smoke.

5.2 Human carcinogenicity data

One cohort study of workers in the United States who manufactured 1,3-butadiene monomer showed a moderate and significant excess of lymphohaematopoietic cancers based on 42 deaths. Persons employed before 1950 were especially at increased risk, but there was no convincing association with a cumulative exposure score. A total of

13 leukaemia cases only slightly and insignificantly contributed to the excess of the lymphohaematopoietic cancers.

A small cohort study of 1,3-butadiene production workers showed a significant excess of lymphosarcoma and reticulosarcoma, based on four cases. There was also an excess of stomach cancer, although represented by only five cases. Two leukaemia cases were found: this was slightly more than expected.

Several reports have been published on follow-up of styrene–butadiene rubber workers at eight plants in the United States and Canada. The most recent follow-up showed a consistent excess of leukaemia and a significant dose–response relationship with cumulative exposure to 1,3-butadiene, which remained after adjustment for exposure to styrene.

Evaluation of the human carcinogenicity of 1,3-butadiene hinges on evidence regarding leukaemia risks from one large and well conducted study and two smaller studies. The smaller studies neither support nor contradict the evidence from the larger study. The larger, United States–Canada study shows that workers in the styrene–butadiene rubber industry experienced an excess of leukaemia and that those with apparently high 1,3-butadiene exposure had higher risk than those with lower exposure. The evidence from this study strongly suggests a hazard, but the body of evidence does not provide an opportunity to assess the consistency of results among two or more studies of adequate statistical power. Further, while 1,3-butadiene was a major exposure in this cohort, there were others, and it remains possible that even if there is an increased risk of cancer in the styrene–butadiene rubber industry, it may be due to occupational exposures other than 1,3-butadiene.

5.3 Animal carcinogenicity data

1,3-Butadiene was tested for carcinogenicity by inhalation exposure in four experiments in mice and one experiment in rats.

In the studies in mice, tumours were induced in multiple organs at all exposure concentrations studied, ranging from 6.25 to 1250 ppm [13.8–2760 mg/m^3]. The tumours induced included malignant lymphomas and heart haemangiosarcomas. Neoplasms at multiple organ sites were induced in mice after as little as 13 weeks of exposure at exposure levels of 625 ppm.

In one inhalation study in rats, 1,3-butadiene increased the incidence of tumours at several sites. The tumour increases were mainly in organs in which tumours develop spontaneously. The response was seen mainly at 8000 ppm [17 700 mg/m^3].

The initial metabolite of 1,3-butadiene, 1,2-epoxy-3-butene, yielded equivocal results in carcinogenicity tests, whereas the subsequent metabolite, 1,2:3,4-diepoxybutane, was carcinogenic to mice and rats when administered by skin application or by subcutaneous injection.

5.4 Other relevant data

1,3-Butadiene is metabolized in experimental animals and human liver microsomes to epoxide metabolites, initially 1,2-epoxy-3-butene and subsequently 1,2:3,4-

diepoxybutane, by cytochrome P450. The epoxides can be inactivated by epoxide hydrolase and glutathione *S*-transferases. Adducts formed by reaction of 1,2-epoxy-3-butene and 3,4-epoxy-1,2-butanediol with haemoglobin and urinary mercapturic acids derived from 1,2-epoxy-3-butene have been detected in 1,3-butadiene-exposed workers. There are significant species differences in the metabolism of 1,3-butadiene both *in vitro* and *in vivo*. The in-vitro data are consistent with modelled and measured concentrations of 1,2-epoxy-3-butene and 1,2:3,4-diepoxybutane in 1,3-butadiene-exposed mice and rats. In these animals, blood and tissue levels of 1,2-epoxy-3-butene are several times higher in mice than in rats and those of 1,2:3,4-diepoxybutane up to 100 times higher in mice than in rats. There is considerable interindividual variability in the ability of human liver microsomes to metabolize 1,3-butadiene and 1,2-epoxy-3-butene *in vitro*. Mechanistic data suggest that the much higher carcinogenic potency of 1,3-butadiene in mice than in rats results predominantly from the high burden of 1,2:3,4-diepoxybutane.

The haemoglobin-binding index of 1,2-epoxy-3-butene can be considered as a dose surrogate for this metabolite; corresponding haemoglobin-binding indices have been published for mouse and rat. Haemoglobin-binding indices in occupationally exposed humans have also been estimated. In agreement with model predictions, these data demonstrate binding indices for 1,3-butadiene-exposed humans more than one order of magnitude lower than those in exposed rats.

There are conflicting results on whether 1,3-butadiene increases *hprt* mutations in lymphocytes from 1,3-butadiene-exposed humans compared with non-exposed controls. Sister chromatid exchanges, micronuclei, chromosomal aberrations and DNA strand breaks were not significantly elevated above control levels in peripheral blood lymphocytes of occupationally exposed workers. 1,3-Butadiene induced DNA adducts and damage in both mice and rats *in vivo*, although the damage was significantly greater in mice than in rats. 1,3-Butadiene is mutagenic in virtually all test systems both *in vitro* and *in vivo*. Where a direct comparison between rats and mice could be made for the same end-point, positive effects were observed primarily in mice.

Activated K-*ras* oncogenes have been detected in lymphomas and in liver and lung tumours induced in mice by 1,3-butadiene. Mutations in the *p53* tumour-suppressor gene have been detected in mouse lymphomas.

1,2-Epoxy-3-butene was directly mutagenic in bacteria and induced gene mutations, chromosomal aberrations and sister chromatid exchanges *in vivo* in rodents. Micronuclei were induced in both somatic and germ cells of mice and rats *in vivo*. It induced gene mutations and sister chromatid exchanges in cultured human lymphocytes but did not induce unscheduled DNA synthesis, micronuclei or chromosomal aberrations in mouse or rat cells *in vitro*.

1,2:3,4-Diepoxybutane is a potent bifunctional alkylating agent which reacts with DNA *in vitro* and *in vivo*. As a result, it is mutagenic in virtually all test systems including effects in somatic and germ cells of mammals exposed *in vivo*. *In vivo*, it induced DNA adducts, dominant lethal mutations and gene mutations in mice; chromosomal aberrations

and sister chromatid exchanges in Chinese hamsters and mice; and micronuclei in splenocytes and spermatids of rats and mice. It induced gene mutations, chromosomal aberrations and sister chromatid exchanges in human and mammalian cell cultures. In one study, 1,2:3,4-diepoxybutane induced DNA–DNA cross-links in murine hepatocytes *in vitro*. It induced somatic and sex-linked recessive lethal mutations, chromosomal deletions and heritable translocations in *Drosophila.* Gene mutations were induced in bacteria in the mouse host-mediated assay and *in vitro*. 1,2:3,4-Diepoxybutane also induced bacterial prophage and DNA repair.

5.5 Evaluation

There is *limited evidence* in humans for the carcinogenicity of 1,3-butadiene.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,3-butadiene.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,2:3,4-diepoxybutane.

Overall evaluation

1,3-Butadiene is *probably carcinogenic to humans (Group 2A)*.

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CHLOROPRENE

This substance was considered by previous Working Groups, in February 1978 (IARC, 1979) and March 1987 (IARC, 1987a). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

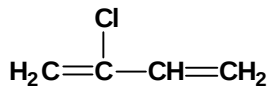
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 126-99-8
Chem. Abstr. Name: 2-Chloro-1,3-butadiene
Synonyms: 2-Chlorobutadiene; β-chloroprene

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_4H_5Cl Relative molecular mass: 88.54

1.1.3 *Chemical and physical properties of the pure substance*

From Lide (1995), unless otherwise specified

(*a*) *Description*: Colourless, flammable liquid (Lewis, 1993)
(*b*) *Boiling-point*: 59.4°C
(*c*) *Melting-point*: –130°C
(*d*) *Density*: d_4^{20} 0.956
(*e*) *Solubility*: Slightly soluble in water; miscible in acetone, benzene and diethyl ether
(*f*) *Volatility*: Vapour pressure, 26.6 kPa at 20°C; relative vapour density (air = 1), 3.06 (Verschueren, 1996)
(*g*) *Stability*: Flash-point, –20°C (Lewis, 1993)
(*h*) *Reactivity*: Readily forms dimers and oxidizes at room temperature (American Conference of Governmental Industrial Hygienists, 1991)
(*i*) *Octanol/water partition coefficient (P):* log *P*, 2.06 (United States National Library of Medicine, 1997a)

(*j*) *Conversion factor*: mg/m^3 = 3.62 × ppm[1]

1.1.4 *Technical products and impurities*

Chloroprene is available commercially on a restricted basis in the United States as crude β-chloroprene with a minimum purity of 95% (Lewis, 1993; DuPont Dow Elastomers, 1997). The principal impurities are dichlorobutene and solvents, with smaller amounts of 1-chlorobutadiene (α-chloroprene), chlorobutenes and dimers of both chloroprene and butadiene. Due to its reactivity, chloroprene is stored at 0°C or below under nitrogen and contains significant quantities of inhibitors, such as phenothiazine, *tert*-butylcatechol, picric acid and the ammonium salt of *N*-nitroso-*N*-phenylhydroxylamine, to prevent degradation and polymerization (Stewart, 1993). Generally within six weeks of manufacture, crude chloroprene is distilled to produce polymerization grade, which is used within approximately 24 h of distillation.

1.1.5 *Analysis*

The United States National Institute for Occupational Safety and Health has approved a method for the analysis of chloroprene in workplace air. The method [Method 1002] involves passing the sample through a solid sorbent tube of coconut shell charcoal, desorbing with carbon disulfide, and analysis by gas chromatography with flame ionization detection. The estimated limit of detection for this method is 0.03 mg per sample or 3.8 mg/m^3 assuming a maximum air sample of 8 L (Eller, 1994).

Several methods have been described for the determination of chloroprene in water. Huang *et al*. (1996) described a method for wastewater and underground water using gas extraction, thermal desorption and gas chromatography/mass spectrometry; the detection limit for this method was 0.02 µg/L. Kessels *et al*. (1992) described a purge-and-trap method for drinking-water using capillary gas chromatography with electron capture or flame ionization detection; the detection limit for this method was 0.01–0.09 µg/L.

Gas chromatography has been used to determine chloroprene as a residual monomer in polychloroprene latexes, with a sensitivity of less than 0.002 wt % (Bunyatyants *et al.*, 1976).

1.2 Production and use

1.2.1 *Production*

Chloroprene was first obtained as a by-product from the synthesis of divinylacetylene. When a rubbery polymer was found to form spontaneously, investigations were begun that defined the two methods of synthesis of chloroprene that have since been the basis of commercial production, and the first successful synthetic elastomer, Neoprene, or DuPrene as it was first called, was introduced in 1932 (Kleinschmidt, 1986; Stewart, 1993).

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[1]Calculated from: mg/m^3 = (relative molecular mass/24.47) × ppm, assuming a temperature of 25°C and a pressure of 101 kPa

Production of chloroprene today is completely determined by demand for the polymer. The only other use accounting for a significant volume is the synthesis of 2,3-dichloro-1,3-butadiene, which is used as a monomer in selected copolymerizations with chloroprene. The original commercial production was from acetylene through monovinylacetylene. Since the 1960s, because of the increasing price of acetylene and decreasing price of butadiene, the latter has displaced acetylene as the feedstock in most countries (Kleinschmidt, 1986; Stewart, 1993).

In the production of chloroprene from butadiene, there are three essential steps: liquid- or vapour-phase chlorination of butadiene to a mixture of 3,4-dichloro-1-butene and 1,4-dichloro-2-butene; catalytic isomerization of 1,4-dichloro-2-butene to 3,4-dichloro-1-butene; and caustic dehydrochlorination of the 3,4-dichloro-1-butene to chloroprene. By-products in the first step include hydrochloric acid, 1-chloro-1,3-butadiene, trichlorobutenes and tetrachlorobutanes, butadiene dimer and higher-boiling products. In the second step, the mixture of 1,4-dichloro-2-butene and 3,4-dichloro-1-butene isolated by distillation is isomerized to pure 3,4-dichloro-1-butene by heating to temperatures of 60–120°C in the presence of a catalyst. Finally, dehydrochlorination of 3,4-dichloro-1-butene with dilute sodium hydroxide in the presence of inhibitors gives crude chloroprene (Kleinschmidt, 1986; Stewart, 1993; DuPont Dow Elastomers, 1997).

Chloroprene production can be equated approximately to the amount of polymer produced. World production of dry polychloroprene was 135 thousand tonnes in 1960, 254 thousand tonnes in 1970, 314 thousand tonnes in 1980 and 321 thousand tonnes in 1989 (Stewart, 1993). World polychloroprene capacity in 1983 was reported to be (thousand tonnes): United States, 213; Germany, 60; France, 40; United Kingdom, 30; Japan, 85; and centrally planned economy countries, 220 (Kleinschmidt, 1986). Current capacities are reported to be (thousand tonnes): United States, 163; Germany, 60; France, 40; United Kingdom, 33; Japan, 88; central Europe and the Commonwealth of Independent States, 40; and People's Republic of China, 20 (International Institute of Synthetic Rubber Producers, 1997).

1.2.2 *Use*

Chloroprene is used almost exclusively in the production of the specialized elastomer known as polychloroprene. In the United States, more than 90% of the chloroprene produced annually is converted to the solid dry polychloroprene. Most of the remainder is used to produce polychloroprene latex, a colloidal suspension of polychloroprene in water. A small fraction is converted to the co-monomer, 2,3-dichloro-1,3-butadiene, for use in specialized copolymers (DuPont Dow Elastomers, 1997). The vulcanized products of polychloroprene have favourable physical properties and excellent resistance to weathering and ozone. Articles made with this rubber include electrical insulating and sheathing materials, hoses, conveyor belts, flexible bellows, transmission belts, sealing materials, diving suits and other protective suits. Adhesive grades of polychloroprene are used mainly in the footwear industry. Polychloroprene latexes have been used for dipped goods (balloons, gloves), latex foam, fibre binders, adhesives and rug backing (Kleinschmidt, 1986; Stewart, 1993).

1.3 Occurrence

1.3.1 *Natural occurrence*

Chloroprene is not known to occur as a natural product.

1.3.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), as many as 18 000 workers in the United States were potentially exposed to chloroprene (see General Remarks). National estimates of workers potentially exposed were not available from other countries. Occupational exposures to chloroprene have been measured mainly in polymer production.

During 1973, at a chloroprene polymerization plant in the United States, airborne concentrations of chloroprene were found to range from 14 to 1420 ppm [50–5140 mg/m^3] in the make-up area, from 130 to 6760 ppm [470–24 470 mg/m^3] in the reactor area, from 6 to 440 ppm [22–1660 mg/m^3] in the monomer recovery area and from 113 to 252 ppm [409–912 mg/m^3] in the latex area (Infante *et al*., 1977). Concentrations in the air inside a Russian polychloroprene rubber plant were 14.5–53.4 mg/m^3 (Mnatsakayan *et al*., 1972). In a Russian chloroprene latex manufacturing facility, chloroprene concentrations varied from 1 to 8 mg/m^3 (Volkova *et al*., 1976).

Recent data from two chloroprene polymerization plants in the United States with 650–800 exposed workers show relatively little decline in average exposure concentrations from the late 1970s through 1996 (Table 1). This is partly due to the fact that workers with jobs having low potential for exposure are no longer routinely monitored, resulting in upwardly biased time-weighted average exposures. At present, however, average exposures of process operators and mechanics are typically below 5 ppm, while other workers in these facilities are exposed to concentrations below 2 ppm (DuPont Dow Elastomers, 1997).

Exposure to residual chloroprene monomer in polychloroprene latex and polymer has also been described. In 1977, mean airborne concentrations of chloroprene of up to 0.2 ppm [0.72 mg/m^3] were reported in a roll building area at a metal fabricating plant in the United States where polychloroprene was applied extensively to metal cylinders before vulcanization (Infante, 1977). Workers in a Russian shoe factory were reportedly often exposed to chloroprene concentrations of 20–25 mg/m^3 (Buyanov & Svishchev, 1973).

1.3.3 *Environmental occurrence*

Industrial chloroprene emissions to the atmosphere reported to the United States Environmental Protection Agency for the Toxic Chemical Release Inventory totalled about 838 tonnes in 1987, 667 tonnes in 1991 and 446 tonnes in 1995 (United States National Library of Medicine, 1997b).

Chloroprene has been detected in industrial wastewater and nearby groundwater in the People's Republic of China (Huang *et al*., 1996), in wastewaters from polychloroprene and dichlorobutadiene production plants in Russia (Avetisyan *et al*., 1981; Geodakyan *et al*., 1981) and in waste gas from a chloroprene plant in Japan (Kawata *et al*., 1982).

Table 1. Average chloroprene concentrations in two polymerization plants in the United States, 1976–96

Year	Plant 1		Plant 2	
	No. of samples	Mean TWA[a] (mg/m^3)	No. of samples	Mean TWA[a] (mg/m^3)
1976	2331	19.0		
1977	2691	17.4		
1978	3676	10.2		
1979	1739	7.2		
1980	1520	4.9		
1981	1288	3.3		
1982	678	3.9		
1983	594	2.6		
1984	574	4.1		
1985	568	2.8		
1986	245	5.8		
1987	73	8.8		
1988	78	9.0		
1989	137	9.0		
1990	9	1.2		
1991	13	3.9		
1992	26	7.5		
1993	15	4.5	58	7.2
1994	8	22.3	72	7.3
1995	35	5.0	35	6.8
1996	14	0.6	35	2.3

From DuPont Dow Elastomers (1997)
[Means for 1993–96 were calculated by the Working Group from the raw data.]
[a] Time-weighted average

1.3.4 *Other*

It is reported that dry polychloroprene no longer contains detectable chloroprene (detection limit, 0.5 ppm). In polychloroprene latexes, residual chloroprene is less than 1%, varying with the manufacturing process and intended use (DuPont Dow Elastomers, 1998).

Chloroprene has been detected as an impurity at levels of several parts per million in commercial vinyl chloride in Italy (Sassu *et al*., 1968) and in Japan (Kurosaki *et al*., 1968), and in acrylonitrile in the USSR (Panina & Fain, 1968).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for chloroprene in several countries are given in Table 2.

Table 2. Occupational exposure limits and guidelines for chloroprene[a]

Country	Year	Concentration (mg/m^3)	Interpretation[b]
Australia	1993	36 (sk)	TWA
Belgium	1993	36 (sk)	TWA
Czechoslovakia	1991	50	TWA
		100	STEL
Denmark	1993	3.6 (sk)	TWA
Finland	1998	36 (sk)	TWA
		72	STEL (15-min)
France	1993	36	TWA
Germany	1998	None*	MAK
Hungary	1993	10	TWA
		30	STEL
Netherlands	1992	36	TWA
Poland	1993	2	TWA
Russia	1993	0.05	STEL
Sweden	1993	36 (sk)	TWA
		60	STEL
Switzerland	1993	36 (sk)	TWA
		72	STEL
United Kingdom	1993	30 (sk)	TWA
United States			
ACGIH (TLV)[b,c]	1997	36 (sk)	TWA
NIOSH (REL)	1997	3.6 (Ca)	Ceiling (15-min)
OSHA (PEL)	1996	90 (sk)	TWA

[a] From International Labour Office (1991); United States Occupational Safety and Health Administration (OSHA) (1996); American Conference of Governmental Industrial Hygienists (ACGIH) (1997a,b); United States National Library of Medicine (1997b); Deutsche Forschungsgemeinschaft (1998); Ministry of Social Affairs and Health (1998)

[b] TWA, time-weighted average; STEL, short-term exposure limit; MAK, maximum workplace concentration; TLV, threshold limit value; REL, recommended exposure limit; PEL, permissible exposure limit; Ca, potential occupational carcinogen; sk, skin notation

[c] Countries that follow the ACGIH recommendations for threshold limit values include Bulgaria, Colombia, Jordan, Korea, New Zealand, Singapore and Viet Nam

* Considered to be carcinogenic to man

2. Studies of Cancer in Humans

2.1 Case report and case series

One case has been reported of liver angiosarcoma (pathologically confirmed) in a worker exposed to polychloroprene who had no known occupational exposure to vinyl chloride (IARC, 1987b) or medical exposure to thorotrast (Infante, 1977). [It is unclear whether and how much this worker was exposed to chloroprene monomer.]

Khachatryan (1972a,b) reported on patients with skin and lung cancer who attended an oncology department in an industrial area of Armenia with a high prevalence of workers employed in the chemical industry. These included 18 cases of lung cancer and 21 of skin cancer among workers with high exposure to chloroprene.

2.2 Cohort studies

A historical prospective study of workers employed in the production of neoprene in two plants in the United States was conducted by Pell (1978). The cohort at the first plant comprised 234 male workers first employed at any time between 1931 and 1948. These men were followed from 1957 or 15 years after first exposure, whichever was later, until 1974. During this period, 39 deaths occurred in operation workers, giving a standardized mortality ratio (SMR) of 0.8 in comparison with United States mortality rates and 1.0 in comparison with death rates in the company as a whole. Twelve deaths were from cancer (9.7 expected from national rates) and five from cancer of the urinary organs (0.5 expected from national rates). Three of these deaths were from bladder cancer in men who had worked with β-naphthylamine (IARC, 1987c) and two were from cancer of the kidney. The cohort from the second plant comprised 1576 men identified from a wage roll of employees dated 30 June 1957. During follow-up to 1974 (99% successful), 193 deaths were observed [SMR, 0.7 based on national rates; 0.99 based on company rates], including 51 from cancer [SMR, 0.97 based on national rates]. There were 19 deaths from cancer of the digestive organs [SMR, 1.3 based on national rates] and two from cancer of the urinary organs [SMR, 0.7 based on national rates]. [The numbers of deaths from specific digestive tract cancers were not specified.]

Shouqi *et al*. (1989) investigated mortality from cancer at a plant in the People's Republic of China producing chloroprene monomer and neoprene. The cohort comprised 1258 employees identified from personnel records who could be assigned to certain exposure categories. In follow-up to 30 June 1983, 96.4% of the cohort were traced. Overall 16 cancer deaths were recorded among workers with a history of exposure to chloroprene, giving an SMR of 2.4 in comparison with mortality rates in the local area during 1973–75. A significant excess of liver cancer was reported among workers in the monomer workshop (4 observed versus 0.83 expected; SMR, 4.8). [The selection criteria for the cohort were not entirely clear and the use of reference rates from only a three-year period may have led to bias.]

Bulbulyan *et al*. (1998) conducted a cohort study among 5185 shoe-manufacturing workers, of whom 4569 were women, employed for at least two years during 1940–76 in

a factory in Moscow, Russia. The follow-up for mortality was between 1979 and 1993. A total of 131 workers (2.5%) were lost to follow-up. Chloroprene was the main solvent used in the glue and gluers were considered to be subject to high exposure. Workers employed in the same departments as gluers but indirectly exposed to chloroprene were considered to have medium exposure and workers only employed in other departments were considered to be unexposed to chloroprene. In the 1970s, chloroprene exposure for gluers was of the order of 20 mg/m^3. Other solvents to which gluers were exposed were benzene (IARC, 1987d), until the 1950s, and ethyl acetate. Other workers were exposed to leather dust and formaldehyde (IARC, 1995). The authors used Moscow mortality as a reference and conducted additional analyses based on internal comparisons. The overall SMR was 1.03 (95% CI, 0.97–1.1; 900 deaths) and there was an excess of cancer mortality (SMR, 1.2; 95% CI, 1.0–1.3; 265 deaths). The whole cohort experienced excess mortality from liver cancer (SMR, 2.4; 95% CI, 1.1–4.3; 10 deaths) and leukaemia (SMR, 1.9; 95% CI, 1.0–3.3; 13 deaths). When workers exposed to chloroprene were compared with unexposed workers, the relative risks were 4.2 (95% CI, 0.5–33; 9 deaths) for liver cancer, 3.8 (95% CI, 0.5–31; 9 deaths) for kidney cancer and 1.1 (95% CI, 0.3–3.7; 9 deaths) for leukaemia. Liver cancer mortality increased with duration of employment as a gluer ($p = 0.02$) and with cumulative exposure index ($p = 0.07$). [This trend may have included the unexposed group, in which case it would not provide evidence independent of the overall elevated relative risk for liver cancer.] No such trend was present for any other neoplasm. No information was available on the histology of the cases of liver cancer.

3. Studies of Cancer in Experimental Animals

Studies of the carcinogenicity of chloroprene by the oral route or inhalation, intratracheal administration, subcutaneous or intramuscular injection or skin application were reviewed by IARC (1979) and found inadequate for evaluation. These studies are not considered further.

3.1 Oral administration

3.1.1 *Rat*

Groups of 17 female BDIV rats were administered a single oral dose of 100 mg/kg bw chloroprene (99% pure, containing 0.8% 1-chlorobutadiene) in olive oil by gavage on day 17 of pregnancy; a control group of 14 females received olive oil alone. Progeny were treated once per week by gavage for up to 120 weeks with 50 mg/kg bw chloroprene in olive oil or olive oil alone. Litter sizes and preweanling body weights were not affected by exposure of the dams to chloroprene. The numbers of offspring receiving chloroprene were 81 males and 64 females. Fifty-three male and 53 female offspring served as controls. Survival of dams to 120 weeks was 13/17 exposed and 11/14 control rats. Survival of exposed offspring was 40/81 males and 43/64 females. Survival of control offspring was 27/53 males and 26/53 females. Body weights of exposed offspring did not

differ from those of controls. No increase was reported in site-specific or total tumours in the dams or offspring receiving chloroprene (Ponomarkov & Tomatis, 1980). [The Working Group noted that the animals may have tolerated higher doses.]

3.2 Inhalation exposure

3.2.1 *Mouse*

Groups of Kunming albino mice [age, initial numbers and sex unspecified] were exposed to chloroprene (99.8% pure) by whole-body inhalation at concentrations of 0, 2.9, 19 or 189 mg/m^3 in static inhalation chambers for 4 h per day on six days per week for seven months. [Details of the method of generation, exposure conditions and chamber analyses were lacking.] Survivors were killed when moribund or at the end of eight months. The first lung tumour was observed at six months and the effective numbers of mice were 77 control, 111 low-dose, 106 mid-dose and 132 high-dose. The incidences of mice with lung adenomas (predominantly papillary) were 1/77 (1.3%) control, 9/111 (8.1%) low-dose, 10/106 (9.4%) mid-dose and 26/132 (19.7%) high-dose. No other organs were examined (Dong *et al.*, 1989).

Groups of 50 male and 50 female B6C3F$_1$ mice, six weeks of age, were exposed to chloroprene for two years by whole-body inhalation at concentrations of 0, 12.8, 32 and 80 ppm [0, 46, 116 and 290 mg/m^3]. The chloroprene was > 99% pure, with seven more volatile components comprising 0.13% and less volatile components including chlorobutene (0.52%) and 1-chlorobutadiene (0.15%). The chloroprene vapour was generated at approximately 65°C and the vapour concentration in the chamber was regularly monitored and revealed no decomposition or degradation products exceeding 0.5%. No chloroprene dimers were found. All mice were killed and evaluated. Survival of exposed mice was reduced in males exposed to 32 and 80 ppm (27/50 controls, 27/50 low-dose, 14/50 mid-dose, 13/50 high-dose) and in all exposed females (35/50 controls, 16/50 low-dose, 1/50 mid-dose and 3/50 high-dose), with poor survival attributed to high rates of neoplasia. Body weight gain was similar in the exposed and control groups. Neoplasms of the lung, circulatory system, Harderian gland and mammary gland (females only) occurred with significantly increased incidence compared with controls (Table 3), and tumours of the forestomach, liver (females), kidney (males), skin and mesentery (females) and Zymbal gland (females) were also increased by chloroprene exposure (United States National Toxicology Program, 1998). [The Working Group noted that the livers of most control and exposed males, but not females, contained a spectrum of lesions consistent with *Helicobacter hepaticus* infection which may have compromised the detection of neoplastic effects in this organ.]

3.2.2 *Rat*

Groups of 50 male and 50 female Fischer 344/N rats, six weeks of age, were exposed to chloroprene for two years by whole-body inhalation at concentrations of 0, 12.8, 32 and 80 ppm [0, 46, 116 and 290 mg/m^3]. The chloroprene was > 99% pure, with seven more volatile components comprising 0.13%, and less volatile components including

Table 3. Incidence of neoplasms in chloroprene-treated B6C3F$_1$ mice

Sex	Male				Female			
Number	50	50	50	50	50	50	50	50
Exposure concentration (ppm)	0	12.8	32	80	0	12.8	32	80
Lung, alveolar bronchiolar								
Adenoma	8	18*	22*	28*	2	16[a]*	29*	26*
Carcinoma	6	12	23*	28*	2	14[a]*	16*	28*
Combined	13	28*	36*	43*	4	28[a]*	34*	42*
Circulatory system								
Haemangioma/haemangiosarcoma[b]	1	12*	18*	17*	4	6	18*	8
Harderian gland								
Adenoma/carcinoma	2	5	10*	12*	2	5	3	9*
Forestomach								
Papilloma	1	0	2	4	0	0	0	4
Kidney								
Tubular-cell adenoma	0	2[a]	3*	9*				
Liver, hepatocellular								
Adenoma/carcinoma					20	26[a]	20	30*
Zymbal gland								
Carcinoma					0	0	0	3
Mammary gland								
Carcinoma					3	4	7	12*
Skin								
Sarcoma					0	11*	11*	18*
Mesentery								
Sarcoma					0	4	8*	3

[a] $n = 49$

[b] Excludes haemangiomas/haemangiosarcomas of the liver in males, because these tumours have been associated with *Helicobacter hepaticus* infection which was present in these mice.

* $p < 0.05$ pairwise logistic regression

chlorobutene (0.52%) and 1-chlorobutadiene (0.15%); chloroprene dimers were not detected. The generation of the chloroprene vapour was the same as that described for the mouse study by the same investigators. The rats were then killed and evaluated. Survival of exposed rats was reduced in males exposed to 32 and 80 ppm (13/50 controls, 9/50 low-dose, 5/50 mid-dose, 4/50 high-dose), but not females (29/50 controls, 28/50 low-dose, 26/50 mid-dose and 21/50 high-dose). Body weight gain was similar in the exposed and control groups. The incidences of neoplasms of the oral cavity, thyroid gland, kidney, lung (males) and mammary gland (females) were increased by chloroprene exposure (see Table 4) (United States National Toxicology Program, 1998).

Table 4. Incidence of neoplasms in chloroprene-treated Fischer 344/N rats

Sex	Male				Female			
Number	50	50	50	50	50	50	50	50
Exposure concentration (ppm)	0	12.8	32	80	0	12.8	32	80
Oral cavity, squamous-cell Papilloma/carcinoma	0	2	5*	12*	1[a]	3	5	11*
Kidney, tubular-cell Adenoma/carcinoma	1	9*	6*	8*	0[a]	1	0	4
Thyroid gland, follicular-cell Adenoma/carcinoma	0	2	4[a]*	5*	1[a]	1	1	5**
Mammary gland Fibroadenoma					24[a]	32	36*	36*
Lung, alveolar/bronchiolar Adenoma/carcinoma	2	2	4[a]	6**				

[a] $n = 49$
* $p < 0.05$ pairwise logistic regression
** $p < 0.05$ trend logistic regression

Three groups of 100 male and 100 female Wistar rats, five weeks of age, were exposed by whole-body inhalation to 0 (control), 10 or 50 ppm [0, 36 or 180 mg/m^3] chloroprene for 6 h per day on five days per week for up to 24 months. Chloroprene vapour was generated from freshly distilled chloroprene (99.6% chloroprene, 0.3% α-chloroprene and < 50 ppm chloroprene dimers) by passing nitrogen through liquid chloroprene at 0°C. After 72 weeks on test, a technical fault in chamber operation procedures resulted in the accidental death of 87 male and 73 female low-dose rats. A slight but consistent growth retardation was found in males (approximately 10%) and females (approximately 5%) of the high-dose groups. Survival of high-dose rats (70–80%) was similar to that of the controls. Histological examinations were performed on 97 control, 13 low-dose and 100 high-dose males and 99 control, 24 low-dose and 100 high-dose females. The number of exposed females with mammary tumours was significantly increased compared with controls ($p < 0.05$). The incidence of adenomas was 3/99 in controls and 7/100 in high-dose females; that of fibroadenomas was 24/99 and 36/100; and that of adenocarcinomas was 5/99 and 3/100, respectively. The increases in individual incidences were not significant. In the region of the nose, squamous-cell carcinomas of uncertain origin were found in 3/100 high-dose males and 1/99 control females. Neither macroscopic nor microscopic examination clarified the exact origin of these tumours. If the tumours in males originated from the epidermis, the total number of squamous-cell carcinomas of the skin would be 5/100 in high-dose males, which would be significantly different ($p < 0.05$) from the incidence in the control group (0/97).

All other tumours were similarly distributed in test and control animals (Trochimowicz *et al*., 1998).

3.2.3 *Hamster*

Groups of 100 male and 100 female Syrian golden hamsters, six weeks of age, were exposed to chloroprene (99.6% pure) by whole-body inhalation at concentrations of 0 (control), 10 or 50 ppm [0, 36 or 180 mg/m^3] for 6 h per day on five days per week for 18 months. The chloroprene was generated in the same manner as that described for the rat study by the same investigators. Surviving animals were killed and all animals evaluated. Survival was 88% in control, 92% in 10-ppm and 93% in 50-ppm males and 63%, 75% and 72% for females. No increase in tumour incidence was observed in exposed animals (Trochimowicz *et al*., 1998).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Mouse liver microsomal preparations metabolized chloroprene *in vitro* into a volatile alkylating metabolite (Barbin *et al*., 1977; Bartsch *et al*., 1978).

Haley (1978) reviewed data on chloroprene metabolism. The most probable route is epoxidation and subsequent glutathione conjugation and mercapturic acid formation. [The Working Group noted that there is no information on the contribution of epoxide hydrolase to chloroprene epoxide metabolism.] Summer and Greim (1980) demonstrated the formation of glutathione conjugate, depletion of hepatic glutathione and increased excretion of urinary thioethers in the rat *in vivo* after oral administration of chloroprene. In isolated rat hepatocytes, the rate of glutathione depletion was dependent on cytochrome P450 activity, as deduced from an increased rate of depletion in cells from phenobarbital- or clophen-pretreated animals.

4.2 Toxic effects

4.2.1 *Humans*

The primary symptoms of acute exposure to high concentrations of chloroprene by inhalation in the chloroprene-rubber industry include nervous system depression, injury to the lungs, liver and kidney, irritation of the skin and mucous membrane, and respiratory difficulties (Nyström, 1948). Arevshatyan (1972) also reported pathomorphological changes in the peridontium (periodontitis, gingivitis, erosion of teeth and caries)

after inhalation of chloroprene. Dermal exposure to chloroprene and its polymers may result in dermatitis and hair loss (Schwartz, 1945; Nyström, 1948; Ritter & Carter, 1948).

Chronic exposure to chloroprene may result in symptoms such as headache, irritability, dizziness, insomnia, fatigue, respiratory irritation, cardiac palpitations, chest pain, gastrointestinal disorders, dermatitis, temporary loss of hair, conjunctivitis and corneal necrosis (Schwartz, 1945; Nyström, 1948; Barskii *et al*., 1972; Lloyd *et al*., 1975). According to Khachatryan and Oganesyan (1974), 44% of patients with chronic chloroprene poisoning had pathological changes in the cardiovascular and nervous systems. Reports have also been published of hepatomegaly, with a decrease in liver function tests, toxic hepatitis, dystrophy of the myocardium and changes in the nervous system (Orlova & Solov'ena, 1962), circulatory changes (Khachatryan & Oganesyan, 1974), anaemia (Nyström, 1948), hypoglycaemia (Mkhitaryan, 1960), and dysfunction of the central and peripheral nervous systems, particularly the cholinergic branch, as well as decreased blood cholinesterase activity (Gasparyan, 1965).

Bascom *et al*. (1988) reported a spectrum of respiratory illnesses which occurred in a group of workers who were exposed to heated chloroprene-based rubber. These included an acute sensitizing illness with dyspnoea and wheezing in some workers and pulmonary infiltrates with eosinophilia in others. One worker developed chronic obstruction of the airways with recurrent bronchitic illnesses.

Gooch and Hawn (1981) found no clinically significant or biochemical alterations in 563 workers occupationally exposed to chloroprene, as compared with workers never exposed to the chemical.

4.2.2 *Experimental systems*

The oral LD_{50} values for chloroprene in rats and mice are 251 and 260 mg/kg bw, respectively (Asmangulyan & Badalyan, 1971). The approximate LC_{50} for a 4-h inhalation exposure in Charles River male rats is 2300 ppm [8330 mg/m^3] (Clary *et al*., 1978).

von Oettingen *et al*. (1936) reported that the dose that killed 100% of the animals administered chloroprene by inhalation for 8 h was 2000 ppm [7240 mg/m^3] in rabbits and 4000–5000 ppm [14 500–18 100 mg/m^3] in rats. Death resulted from respiratory failure following symptoms that included inflammation of the mucous membranes of the eyes and nose, and depression of the central nervous system. The same study reported toxic effects in mice after an 8-h exposure to chloroprene levels of 12–130 ppm [43–470 mg/m^3]; the minimum lethal dose was 170 ppm [615 mg/m^3].

Plugge and Jaeger (1979) exposed fasted adult male Sprague-Dawley rats to 100, 150, 225 or 300 ppm [360, 540, 810 or 1090 mg/m^3] chloroprene by inhalation for 4 h and killed the animals 24 h after exposure ended. Liver non-protein sulfhydryl (NPSH) concentrations were increased 24 h after all exposures. Liver injury, as evidenced by increased serum sorbitol dehydrogenase activity, was observed in animals exposed to 225 and 300 ppm chloroprene. Lung NPSH concentrations were decreased significantly 24 h after the 100 and 300 ppm exposures. No other evidence of lung injury was

observed. A polychlorinated biphenyl mixture given orally before dosing with chloroprene prevented the liver damage and the reduction in lung NPSH.

Jaeger *et al*. (1975) found that chloroprene is markedly more hepatotoxic to fasted adult male Holtzman rats (250–350 g bw) than to rats fed *ad libitum*, following inhalation exposure to 500, 1000 or 2000 ppm [1810, 3620 or 7240 mg/m^3] chloroprene. These concentrations produced increases in serum alanine α-ketoglutarate transaminase activity and caused death in the fasted rats, while producing no effect in the fed rats. At 10 000 ppm [36 200 mg/m^3], the fed–fasted difference following chloroprene exposure disappeared.

Clary *et al*. (1978) conducted four-week inhalation studies (6 h per day, five days per week) in adult male and female Wistar rats, as well as Syrian hamsters. The animals were exposed to 39 [141 mg/m^3], 161 [582 mg/m^3] or 625–630 ppm [approximately 2260 mg/m^3]. Exposure to 39 ppm caused skin and eye irritation in both species and significant growth retardation. In the rats, repeated exposure to 625 ppm resulted in growth retardation and mortality. Mid-zonal liver degeneration and necrosis as well as increased liver and kidney weights were noted at the 625 ppm exposure level. Eye irritation, restlessness, lethargy, nasal discharge and discoloured urine were also observed. Gross pathological examination showed dark, swollen livers and greyish lungs containing haemorrhagic areas in most of the animals that died during exposure. Renal tubular epithelial degeneration in rats was also noted. Hair loss was observed primarily in the female rats at the highest and middle exposure levels. In hamsters, a single exposure to 630 ppm was lethal. Mid-zonal liver degeneration and necrosis and increased liver and kidney weights were found in most of the survivors of the middle exposure group. Some irritation of the nasal mucous membrane of the hamsters, evident as a slight flattening and thinning of the olfactory epithelium, was observed at both the 39 and 161 ppm exposure levels. Haematology and urinalysis were not affected significantly by exposure to chloroprene at any dose in either species.

Melnick *et al*. (1996) conducted 13-week inhalation studies in 4–5-week-old male and female Fischer 344/N rats and B6C3F$_1$ mice. The chloroprene vapour was generated at 65°C. All animals were exposed to chloroprene concentrations of 0, 5, 12, 32 or 80 ppm [0, 18, 43, 116 or 240 mg/m^3] for 6 h per day on five days per week. Rats were also exposed to 200 ppm [720 mg/m^3]; a pilot study had shown that this concentration was lethal to mice. In mice exposed to 80 ppm, there was a marginal decrease in body weight gain in males and epithelial hyperplasia of the forestomach in both sexes. No exposure-related effects were observed in organ weights, haematology or blood chemistry. In rats, exposure to 80 or 200 ppm chloroprene resulted in degeneration and metaplasia of the olfactory epithelium. Moreover, at the 200 ppm level, anaemia, hepatocellular necrosis and reduced sperm motility were also observed. Neurobehavioural assessment showed no effect on motor activity, startle response or forelimb/hindlimb grip strength of the rats.

No lethal effects were observed by Asmangulyan and Badalyan (1971) after repeated oral exposure of rats to 15 mg/kg bw chloroprene daily for five months, but renal and splenic damage was reported.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

Data from a single study of the effects of chloroprene on male reproductive function, reported in a review article (Sanotskii, 1976), were inadequate for evaluation by the Working Group.

4.3.2 *Experimental systems*

Salnikova and Fomenko (1973, 1975) exposed pregnant rats by inhalation to concentrations of chloroprene between 0.056 and 13 mg/m^3. Embryotoxic effects were observed at concentrations higher than 0.13 mg/m^3. The highest embryotoxic effect was seen when the dams inhaled 4.0 mg/m^3 chloroprene during the entire pregnancy, or intermittently on days 1–2, 3–4 or 11–12, or were given an oral dose of 0.5 mg/kg bw daily for 14 days or on days 3–4 or 11–12. Meningoencephaloceles, a teratogenic effect, was observed following chloroprene administration on days 5–6, 9–10, 11–12, 13–14 and 15–16 of gestation (Salnikova & Fomenko, 1975).

Davtyan *et al*. (1973) reported a significant increase in embryotoxicity when female white rats were fertilized by males that had been exposed to 3.8 mg/m^3 chloroprene for 4 h per day for 48 days. This report is not supported by a later study.

Culik *et al*. (1978) exposed male ChR-CD rats of reproductive age to 25 ppm [90 mg/m^3] chloroprene vapour for 4 h per day for 22 days and mated them weekly with untreated females for eight consecutive weeks. The reproductive capability of the males was not impaired.

Culik *et al*. (1978) also exposed pregnant ChR-CD rats to 0, 1, 10 and 25 ppm [0, 3.6, 36 and 90 mg/m^3] chloroprene vapour for 4 h per day during gestation days 3–20. They observed slight increases in resorptions and decreases in fetal body length in litters of dams exposed to 10 ppm and in fetal body weights and lengths in litters of dams exposed to 25 ppm. No other maternal, embryonal or fetal toxicity was observed.

As part of the investigation (Melnick *et al.*, 1996) with Fischer 344/N and B6C3F$_1$ mice described above (Section 4.2.2), reproductive tissue evaluations and oestrus cycle characterizations were made. Sperm mobility was reduced in rats from 87% in controls to 80% ($p < 0.01$) in the 200-ppm group, but there was no significant alteration in mice. Oestrus cycle length was unchanged in rats and mice.

4.4 Genetic and related effects

4.4.1 *Humans*

One study of chromosomal aberrations in chloroprene-exposed workers (Katosova & Pavlenko, 1985) was inadequately described for evaluation by the Working Group.

Cytogenetic examination was made of peripheral blood lymphocytes from women whose work involved exposure to chloroprene latex (Fomenko *et al.*, 1973). Twenty women, aged 19–23 years and employed for 1–4 years, were exposed to 3–7 mg/m^3; eight women, aged 19–50 years and employed for 1–20 years were exposed to 1–4 mg/m^3. These two groups were compared with a control group of 181 compiled

separately (Bochov *et al.*, 1972). The percentages of aberrant cells in the three groups were: controls, 1.19 ± 0.06; 1–4 mg/m^3 group, 2.5 ± 0.49 ($p < 0.05$); 3–7 mg/m^3 group, 3.49 ± 0.51 ($p < 0.001$).

4.4.2 *Experimental systems* (see Table 5 for references)

In one study involving vapour-phase exposure, chloroprene was mutagenic in *Salmonella typhimurium*, whereas a later investigation, also involving vapour-phase exposure, showed that freshly distilled chloroprene was not directly mutagenic, but direct mutagenic activity appeared upon storage for one or more day at –20°C (Westphal *et al.*, 1994). This study did not investigate the effect of metabolic activation; however, the most recent study (which did not involve vapour-phase exposure) did test chloroprene in the presence and absence of an exogenous metabolic system up to toxic doses and found no induction of mutation in *S. typhimurium*.

Chloroprene induced a small increase in sex-linked recessive lethal mutations in *Drosophila melanogaster* without a dose–response relationship in one study, but had no effect in another study. Chloroprene was not mutagenic to V79 Chinese hamster lung cells in the presence of S15 liver supernatants from phenobarbital-treated mice and rats.

Hamster lung cells exposed *in vitro* were transformed to phenotypes that could form tumours upon transplantation either subcutaneously into newborn hamsters or intraocularly into adults.

In one in-vivo study, induction of chromosomal aberrations in mouse bone-marrow cells and dominant lethal effects in male mice and rats were reported, following exposures to atmospheres containing any low concentration of chloroprene. Another in-vivo study, in which mice were exposed to atmospheres ranging up to 200 ppm [720 mg/m^3] chloroprene (a lethal concentration), found no induction of sister chromatid exchanges or chromosomal aberrations in bone-marrow cells or micronuclei in circulating blood cells.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Chloroprene is a monomer used almost exclusively for the production of polychloroprene elastomers and latexes. It readily forms dimers and oxidizes at room temperature. Occupational exposures occur in the polymerization of chloroprene and possibly in the manufacture of products from polychloroprene latexes.

Although few data are available on environmental occurrence, general population exposures are expected to be very low or negligible.

5.2 Human carcinogenicity data

The risk of cancer associated with occupational exposure to chloroprene has been examined in two well conducted studies, one in the United States and one in Russia. These investigations do not indicate a consistent excess of cancer at any site.

Table 5. Genetic and related effects of chloroprene

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	18	Bartsch *et al*. (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–[c]	–	177	Westphal *et al*. (1994)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	128	Zeiger *et al*. (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	128	Zeiger *et al*. (1987)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	128	Zeiger *et al*. (1987)
SA8, *Salmonella typhimurium* TA98, reverse mutation	–	–	128	Zeiger *et al*. (1987)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	(+)		3000 feed × 72h	Vogel (1979)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		1820 feed	Foureman *et al*. (1994)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		1820 inj	Foureman *et al*. (1994))
G9O, Gene mutation, Chinese hamster lung V79 cells, ouabain resistance *in vitro*	–	–	0.2% in air	Drevon & Kuroki (1979)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	–	0.2% in air	Drevon & Kuroki (1979)
TCL, Cell transformation, normal hamster lung cells, transplantability *in vitro*	+	NT	1	Menezes *et al.* (1979)
SVA, Sister chromatid exchange, male B6C3F$_1$ mouse bone-marrow cells *in vivo*	–		290 mg/m^3, 6 h/d × 12	Tice *et al*. (1988)
MVM, Micronucleus test, male B6C3F$_1$ mouse peripheral blood *in vivo*	–		290 mg/m^3, 6 h/d × 12	Tice *et al*. (1988)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CBA, Chromosomal aberrations, male B6C3F$_1$ mouse bone-marrow cells *in vivo*	+		1.85 mg/m^3, 2 mo	Sanotskii (1976)
CBA, Chromosomal aberrations, male B6C3F$_1$ mouse bone-marrow cells *in vivo*	–		290 mg/m^3, 6 h/d × 12	Tice *et al*. (1988)
DLM, Dominant lethal test, male C57BL/6 mice	(+)		1.85 mg/m^3, 2 mo	Sanotskii (1976)
DLR, Dominant lethal test, male white rats	+		0.14 mg/m^3, 4.5 mo	Sanotskii (1976)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; inj, injection

[c] Negative when freshly distilled, positive when aged

5.3 Animal carcinogenicity data

Chloroprene was tested for carcinogenicity in two studies in mice, in two studies in rats and in one study in hamsters, all by inhalation with samples of purity > 99%. Exposure of mice to chloroprene produced lung tumours in one study in which the lung was the only organ examined. In another study in mice, chloroprene produced neoplasia in the lung, circulatory system, Harderian gland, mammary gland, liver, kidney, skin, mesentery, forestomach and Zymbal gland. In one study in rats, chloroprene caused increased incidences of tumours of the oral cavity, thyroid gland, lung, mammary gland and kidney. In another study in a different strain of rats, the incidence of mammary tumours was increased in high-dose females only when mammary tumours of all types were combined. No increase in neoplasia was seen in hamsters.

5.4 Other relevant data

The observation of excretion of mercapturates of chloroprene indicates that glutathione conjugation occurs in rats.

Genetic toxicity assays with chloroprene may often have been complicated by impurities derived either from added stabilizers or from degradation and polymerization products. Consequently, positive and negative results have been reported for most assays, and it is notable that, often, the negative results were obtained using the higher dose levels of chloroprene.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of chloroprene.

There is *sufficient evidence* in experimental animals for the carcinogenicity of chloroprene.

Overall evaluation

Chloroprene is *possibly carcinogenic to humans (Group 2B)*.

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DICHLOROMETHANE

This substance was considered by previous Working Groups, in June 1978 (IARC, 1979), February 1982 (IARC, 1982), February 1986 (IARC, 1986), and March 1987 (IARC, 1987a). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

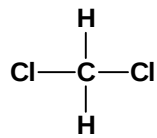
Chem. Abstr. Serv. Reg. No.: 75-09-2

Chem. Abstr. Name: Dichloromethane

IUPAC Systematic Name: Dichloromethane

Synonyms: Methane dichloride; methylene bichloride; methylene chloride; methylene dichloride

1.1.2 *Structural and molecular formulae and relative molecular mass*



CH_2Cl_2 Relative molecular mass: 84.93

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid with penetrating ether-like odour (Lewis, 1993; Budavari, 1996; Verschueren, 1996)

(*b*) *Boiling-point*: 40°C (Lide, 1995)

(*c*) *Melting-point*: –95.1°C (Lide, 1995)

(*d*) *Density*: d_4^{20} 1.3266 (Lide, 1995)

(*e*) *Spectroscopy data*: Ultraviolet (Grasselli & Ritchey, 1975), infrared (Sadtler Research Laboratories, 1995; prism [6620 (gas), 1011], grating [28523]), nuclear magnetic resonance (Sadtler Research Laboratories, 1995; proton [6401], ^{13}C [167]) and mass spectral data (Grasselli & Ritchey, 1975) have been reported.

(*f*) *Solubility*: Slightly soluble (1.38 g/100 mL) in water at 20°C; soluble in carbon tetrachloride; miscible in ethanol, diethyl ether and dimethylformamide (Lide, 1995; Budavari, 1996)

(*g*) *Volatility*: Vapour pressure, 58 kPa at 25°C (Lide, 1995); relative vapour density (air = 1), 2.93 (Verschueren, 1996)
(*h*) *Stability*: Vapour is nonflammable and is not explosive when mixed with air (Budavari, 1996) but may form explosive mixtures in atmospheres with higher oxygen content (Sax, 1984)
(*i*) *Reactivity*: Reacts vigorously with active metals (lithium, sodium, potassium) and with strong bases (potassium *tert*-butoxide) (Sax, 1984)
(*j*) *Octanol/water partition coefficient (P)*: log *P*, 1.25 (Hansch *et al*., 1995)
(*k*) *Conversion factor*: $mg/m^3 = 3.47 \times ppm$[1]

1.1.4 *Technical products and impurities*

Dichloromethane is available in several grades based on its intended end use: technical; aerosol; vapour degreasing; special; urethane; and decaffeination or Food Chemicals Codex/National Formula (food and pharmaceutical applications). Purity, when reported, ranges from 99 to 99.99%. Acidity (as hydrochloric acid) may be up to 5 mg/kg. The maximum concentration of water in these grades of dichloromethane is 100 mg/kg (Rossberg *et al*., 1986; Holbrook, 1993; Dow Chemical Co., 1995; Vulcan Chemicals, 1995, 1996a,b,c,d).

Small amounts of stabilizers are often added to dichloromethane at the time of manufacture to protect against degradation by air and moisture. The following substances in the listed concentration ranges are the preferred additives (wt %): ethanol, 0.1–0.2; methanol, 0.1–0.2; cyclohexane, 0.01–0.03; and amylene (2-methyl-2-butene), 0.001–0.01. Other substances have also been described as being effective stabilizers, including phenols (phenol, hydroquinone, *para*-cresol, resorcinol, thymol, 1-naphthol), amines, nitroalkanes (nitromethane), aliphatic and cyclic ethers, epoxides, esters and nitriles (Rossberg *et al*., 1986; Holbrook, 1993).

Trade names for dichloromethane include Aerothene MM, Narkotil, R30, Solaesthin, and Solmethine.

1.1.5 *Analysis*

Analytical methods are available for determination of dichloromethane in biological media and environmental samples. All methods involve gas chromatography in combination with a suitable detector. Very low detection limits have been achieved for most media (e.g., in food, 7 ng/sample; water, 0.01 µg/L; air, 1.76 $\mu g/m^3$; and blood, 0.022 mg/L) (WHO, 1996).

Selected methods for the analysis of dichloromethane in various matrices are identified in Table 1. The United States Environmental Protection Agency methods for analysing water (Methods 8010 and 8240) have also been applied to liquid and solid wastes (United States Environmental Protection Agency, 1982a,b). Volatile components of solid-

[1]Calculated from: mg/m^3 = (relative molecular mass/24.47) × ppm, assuming a temperature of 25°C and a pressure of 101 kPa

Table 1. Methods for analysis of dichloromethane

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Adsorb on activated charcoal; desorb with carbon disulfide	GC/FID	10 µg/sample	Eller (1994) [Method 1005]
	Adsorb on charcoal; desorb with carbon disulfide	GC/FID	94 µg/m^3	US Occupational Safety and Health Administration (1990) [Methods 59 & 80]
	Adsorb on carbon-based molecular sieve; desorb with 99:1 mixture of carbon disulfide/dimethylformamide in anhydrous sodium sulfate	GC/FID	697 µg/m^3	
Water	Purge (inert gas); trap (OV-1 on Chromosorb-W/Tenax/silica gel); desorb as vapour (heat to 180°C, backflush with inert gas) onto GC column	GC/ECD	0.25 µg/L	US Environmental Protection Agency (1996a)
		GC/ MS	2.8 µg/L	US Environmental Protection Agency (1996b)
	Add internal standard (isotope-labelled dichloromethane); purge; trap and desorb as above	GC/ MS	10 µg/L	US Environmental Protection Agency (1996c)
	Purge (80°C, nitrogen); trap (Tenax-GC); desorb (flash-heat) and trap in 'mini-trap'(Tenax-GC, –30°C); desorb (flash-heat) onto GC column	GC/EC	0.1 µg/L (tap-water)	Piet *et al*. (1985a)
		GC/MS	< 0.05 µg/L (tap-water) 0.1 µg/L (surface water)	
	Equilibrate sealed water sample at 30°C; inject aliquot of head-space vapour	GC/EC	3–15 µg/L	Piet *et al*. (1985b)
	Inject aqueous sample directly onto calcium carbide precolumn (to remove water)	GC/EC	400 µg/L	Boos *et al*. (1985)
Food	Dissolve (toluene); distil under vacuum	GC/EC GC/ECD	0.35 mg/kg 0.5 mg/kg	US Food and Drug Administration (1983)

[a] Abbreviations: GC/EC, gas chromatography/electron capture detection; GC/ECD, gas chromatography/electrolytic conductivity detection; GC/FID, gas chromatography/flame ionization detection; GC/MS, gas chromatography/mass spectrometry

waste samples are first extracted with polyethylene glycol or methanol before purge/trap concentration and analysis (United States Environmental Protection Agency, 1982b).

Exposures to dichloromethane can also be monitored in air using a direct-reading infrared analyser, with minimum concentrations of 0.7 mg/m^3 (0.2 ppm) (Goelzer & O'Neill, 1985).

1.2 Production and use

1.2.1 *Production*

Dichloromethane was first prepared by Regnault in 1840 by the chlorination of methyl chloride in sunlight. It became an industrial chemical of importance during the Second World War. Two commercial processes are currently used for the production of dichloromethane—hydrochlorination of methanol and direct chlorination of methane (Rossberg *et al*., 1986; Holbrook, 1993).

The predominant method of manufacturing dichloromethane uses as a first step the reaction of hydrogen chloride and methanol to give methyl chloride. Excess methyl chloride is then mixed with chlorine and reacts to give dichloromethane, with chloroform and carbon tetrachloride as co-products. This reaction is usually carried out in the gas phase thermally but can also be performed catalytically or photolytically. At low temperature and high pressure, the liquid-phase process is capable of giving high selectivity for dichloromethane (Rossberg *et al*., 1986; Holbrook, 1993).

The older and currently less used production method for dichloromethane involves direct reaction of excess methane with chlorine at high temperatures (400–500°C), or at somewhat lower temperatures either catalytically or photolytically. Methyl chloride, chloroform and carbon tetrachloride are also produced as co-products (Rossberg *et al*., 1986; Holbrook, 1993).

World production of dichloromethane increased from 93 thousand tonnes in 1960 to an estimated 570 thousand tonnes in 1980 (Edwards *et al*., 1982) and is believed to be still several hundred thousand tonnes. Production in the United States has shown a steady decline from 1981 to 1993, as shown by the following figures (thousand tonnes): 1981, 404; 1984, 275; 1987, 234; 1990, 209; 1993, 160 (Anon., 1994, 1997). The total amount produced in western Europe ranged from 331 500 tonnes in 1986 to 254 200 tonnes in 1991 (WHO, 1996).

1.2.2 *Use*

Most of the current applications of dichloromethane are based on its solvent properties. For use in paint strippers, one of its first applications, dichloromethane is blended with other chemical components to maximize its effectiveness against specific coatings. Typical additives include alcohols, acids, amines or ammonium hydroxide, detergents and paraffin wax (Rossberg *et al*., 1986; Holbrook, 1993; WHO, 1996).

Dichloromethane has been used as an extraction solvent for spices and beer hops and for decaffeination of coffee. It has also found use as a carrier solvent in the textile industry, in the manufacture of photographic film and as a blowing agent for polymer

foams. Dichloromethane is used as a solvent for vapour degreasing of metal parts and may also be blended with petroleum distillates and other chlorinated hydrocarbons for use as a dip-type cleaner in the metal-working industry, although consumption by this industry is declining because of recycling and recovery efforts on the part of end users. The reduction in use of 1,1,1-trichloroethane because of the Montreal Protocol and clean air legislation may increase the use of dichloromethane. It is also used as a component of low-pressure refrigerants, in air-conditioning installations, and as a low-temperature heat-transfer medium (Rossberg *et al*., 1986; Holbrook, 1993; WHO, 1996).

In chemical processing, dichloromethane is used in the manufacture of polycarbonate plastic from bisphenol and phosgene, the manufacture of photoresist coatings, and as a solvent carrier for the manufacture of insecticide and herbicide chemicals. It is used by the pharmaceutical industry as a process solvent in the manufacture of steroids, antibiotics, vitamins and, to a lesser extent, as a solvent in the coating of tablets. Other uses include grain fumigation, oil dewaxing, in inks and adhesives and in plastics manufacture (Rossberg *et al*., 1986; Holbrook, 1993).

The use of dichloromethane in western Europe has shown a decrease from 200 thousand tonnes in 1975–85 to 175 thousand tonnes per year in 1989 and to 150 thousand tonnes per year in 1992 (WHO, 1996). Estimated use patterns for dichloromethane in the United States are presented in Table 2.

Table 2. Estimated use patterns (%) for dichloromethane in the United States[a]

Use	1986	1989	1992	1995
Paint removers/strippers	23	28	31	40
Aerosols	20	18	8	–
Chemical processing	20	11	16	10
Exports	10	15	–	–
Pharmaceuticals	–	–	11	6
Metal degreasing/cleaning	8	8	11	13
Electronics	7	7	4	3
Urethane blowing agent	5	9	14	6
Miscellaneous[b]	7	4	5	22

[a] From Anon. (1986, 1989, 1992, 1995)

[b] Includes pesticides, food processing, synthetic fibres, paints and coatings, aerosols (for 1995) and film processing

1.3 Occurrence

1.3.1 *Natural occurrence*

Dichloromethane is not known to occur as a natural product.

1.3.2 *Occupational exposure*

The uses of dichloromethane reviewed in Section 1.2 can lead to human exposure.

According to the 1990–93 CAREX database for 15 countries of the European Union (Kauppinen *et al.*, 1998) and the 1981–83 United States National Occupational Exposure Survey (NOES, 1997), approximately 250 000 workers in Europe and as many as 1.4 million workers in the United States were potentially exposed to dichloromethane (see General Remarks).

Information on numbers of workers potentially exposed in other countries was not available to the Working Group.

Concentrations of dichloromethane measured in 1968–73 in a plant producing plastic films in the United States were 458–2060 mg/m^3 in the casting area, 583–3350 mg/m^3 in the filtration area, 625–659 mg/m^3 in the winding area and 160–1130 mg/m^3 in offices (United States National Institute for Occupational Safety and Health, 1976).

Table 3 summarizes personal occupational exposures measured in various industries using dichloromethane. The levels vary widely by operation and within operations. Concentrations exceeding 1000 mg/m^3 have been measured, e.g., in paint stripping, in the printing industry and in the manufacture of plastics and synthetic fibres. Full-shift exposures to levels above 100 mg/m^3 of dichloromethane are possible, e.g., in furniture-stripping shops and in certain jobs in aeronautical, pharmaceutical, plastic and footwear industries.

Workers exposed to dichloromethane may be exposed also to various other agents, depending on their specific tasks and working environments.

1.3.3 *Air*

The principal route of exposure to dichloromethane for the general population is inhalation of ambient air. Average daily intake of dichloromethane from urban air has been estimated to range from about 33 to 309 μg. Exposure to dichloromethane in indoor air may be much higher, especially from spray painting or other aerosol uses and from paint removal and metal degreasing. Dichloromethane is degraded in the atmosphere by reaction with hydroxyl radicals, with an atmospheric lifetime of less than one year. The compound is highly mobile in soil and volatilizes rapidly from surface water to the atmosphere (Agency for Toxic Substances and Disease Registry, 1993).

Because dichloromethane is highly volatile, most environmental releases are into the atmosphere. It is released to the atmosphere during its production, storage and transport, but most (more than 99%) of the atmospheric releases result from industrial and consumer uses. Estimates of annual global emissions of 500 thousand tonnes have been reported for dichloromethane. It has been estimated that 85% of the total amount of dichloromethane produced in the United States is released to the environment, mostly to the atmosphere. Industrial dichloromethane emissions to the atmosphere in the United States fell from about 58 thousand tonnes in 1988 to approximately 25 thousand tonnes in 1995 (United States National Library of Medicine, 1997a). The total emission into the air in western Europe was estimated to be 173 thousand tonnes for 1989 and

Table 3. Personal occupational exposures to dichloromethane

Location	Job classification	Concentration (mg/m^3 air)	Reference
Plastics industry (six plants, 1971–81)	Mixing, moulding, preforming, pressing	18–35 17.5–130	Cohen & Vandervort (1972) Wagner (1974)
	Foam gun operator, waxer	690–1600	Burroughs & Moody (1982)
	Fabrication, assembly, finishing	< 0.4–40 < 0.3–35 59 16–325	Cohen & Vandervort (1972) Hollett (1977) Markel & Jannerfeldt (1981) Markel & Slovin (1981)
Polyester industry (one plant, 1991)	Preparation, five jobs laboratory	396–742[a] 161[a]	Post *et al*. (1991)
Flexible polyurethane manufacturing (one plant, 1991)	Pouring line	120–260	Boeninger (1991)
Synthetic fibres industry (two plants, 1977–79)	Pressman Extrusion area Bobbin stores Textile department	916–1300 239–1950 264–729 10.6–967	Cohen *et al*. (1980)
	Extrusion and preparation	486–1648	Ott *et al*. (1983a)
Footwear manufacture (four plants, 1975–82)	Four-part machine Crimping	118–597[a] 31[a]	Tharr *et al*. (1982)
	Assembly, moulding	< 5–104 2–319	Gunter (1975) Hervin & Watanabe (1981)
Pharmaceutical industry (one plant, 1993)	Washing of gelatine capsules	3–201[a]	Ghittori *et al*. (1993)
Photographic film (cellulose triacetate) industry (one plant, 1975)	Dichloromethane area	33[a]	Friedlander *et al*. (1978)

Table 3 (contd)

Location	Job classification	Concentration (mg/m^3 air)	Reference
General manufacturing (air-conditioning and refrigeration equipment, vending machines, pipes, welding wire, toys, fibreglass boats, sporting goods, paints and coatings, drugs, medical equipment) (14 plants, 1972–81)	Degreasing, stripping, flushing, cleaning	7–1930	Burton & Shmunes (1973)
		14–101	Markel & Shama (1974)
		< 3.5–403	Hervin *et al*. (1974)
		180–2190	Lee (1980)
		27	Ruhe (1981)
		52–141	Ruhe *et al*. (1981)
		1.3–467	Ruhe *et al*. (1982)
	Production, operations (fabrication, moulding, waxing, laminating)	4–38[a]	Rosensteel & Meyer (1977)
		22–85	Markel (1980)
	Solvent control	507	Ruhe *et al*. (1982)
Laboratories (three laboratories, 1978–89)	Laboratory technician	23–172	Ruhe (1978)
		236–455	Salisbury (1981)
	Preparation of samples	3–29[a]	McCammon (1990)
Aeronautical industry (one workshop, 1994)	Paint stripping of aircraft	86–1240[a]	Vincent *et al*. (1994)
Furniture stripping shops (five shops, 1991)	Strippers	663[a]	McCammon *et al*. (1991)
	Washers	503[a]	
	Refinishers	108[a]	
Maintenance/repair (automotive, aircraft, furniture, general contracting, miscellaneous) (10 plants, 1976–81)	Paint stripping, sanding	38–2820	Okawa & Keith (1977)
		94–4882	Chrostek (1980)
		45–698	Hartle (1980)
	Painting	25	Gunter (1976)
		30–503	Ruhe & Anderson (1977)
		22–233	Chrostek & Levine (1981)

Table 3 (contd)

Location	Job classification	Concentration (mg/m^3 air)	Reference
Maintenance/repair (contd)	Other	2	Gunter (1976)
		30–412	Ruhe & Anderson (1977)
		2.61	White & Wegman (1978)
		3.4 –31.1	Albrecht (1982)
Printing (five plants, 1975–81)	Printing operation	17	Ahrenholz (1980)
		24–410	Lewis & Thoburn (1981)
		5–560	Quinn (1981)
		8.2–37	Gorman (1982)
	Press checking and cleaning	360–1550	Quinn (1981)
	Tank cleaning	84–17 890	Rivera (1975)
	Darkroom, drafting, folding, collating, office work	< 6–248	Quinn (1981)
Coffee decaffeination (one plant, 1978)	Processing/extracting/evaporating	1.4 –115	Cohen *et al*. (1980)
	Coffee handling, drying	1–86	

[a] Eight-hour time-weighted average. For data not so indicated, the basis of measurement was not reported.

180 thousand tonnes in 1991 (Agency for Toxic Substances and Disease Registry, 1993; WHO, 1996).

Dichloromethane has been detected in ambient air samples taken around the world, with background levels usually at about 0.17 μg/m^3. Concentrations in urban areas and in the vicinity of hazardous waste sites may be one to two orders of magnitude higher (up to 43 μg/m^3). Even higher levels (mean, 670 μg/m^3; peak level, 5000 μg/m^3) have been found in the indoor air of residences (WHO, 1996).

A large do-it-yourself consumer population uses paint strippers containing dichloromethane on furniture and woodwork. Formulations are available mainly in liquid form, but also, occasionally, as an aerosol. Exposures have been estimated on the basis of investigations of the use of household liquid products in the United States. The estimated levels ranged from less than 35 mg/m^3 to a few short-term exposures of 14 100–21 200 mg/m^3. The majority of the concentration estimates were below 1770 mg/m^3 (WHO, 1996).

Dichloromethane is also formed during water chlorination and is emitted into the air from wastewater in treatment plants (Agency for Toxic Substances and Disease Registry, 1993).

1.3.4 *Water*

About 2% of environmental releases of dichloromethane are to water. Industrial releases of dichloromethane to surface water and underground injection (potential groundwater release) reported to the United States Toxic Chemical Release Inventory in 1988 totalled 158 tonnes. Dichloromethane has been identified in industrial and municipal wastewaters from several sources at concentrations ranging from 0.08 μg/L to 3.4 g/L (Agency for Toxic Substances and Disease Registry, 1993; WHO, 1996).

Dichloromethane has been detected in surface water, groundwater and finished drinking-water throughout the United States. It was detected in 30% of 8917 surface water samples recorded in the STORET database of the United States Environmental Protection Agency, at a median concentration of 0.1 μg/L. In a New Jersey survey, dichloromethane was found in 45% of 605 surface water samples, with a maximum concentration of 743 μg/L. Dichloromethane has also been identified in surface waters in Maryland, in Lakes Erie and Michigan, and at hazardous waste sites (Agency for Toxic Substances and Disease Registry, 1993; WHO, 1996).

Dichloromethane is also present in small amounts in seawater. It has been found at up to 2.6 μg/L in coastal waters of the Baltic Sea. Levels up to 0.20 μg/L have been found in North Sea coastal waters. Dichloromethane is generally not detected in open ocean; a mean concentration of 2.2 ng/L has been reported in the southern Pacific Ocean (WHO, 1996).

Since volatilization is restricted in groundwater, concentrations of dichloromethane are often higher there than in surface water. Occurrence of dichloromethane in groundwater has been reported in several surveys across the United States, with concentrations ranging from 0 to 3600 μg/L (Agency for Toxic Substances and Disease Registry, 1993).

Dichloromethane has been detected in drinking-water supplies in numerous cities in the United States, with reported mean concentrations generally below 1 µg/L. It has also been identified in commercially bottled artesian water. Water chlorination in treatment plants appears to increase both the concentration and the frequency of occurrence of dichloromethane in drinking water supplies (Agency for Toxic Substances and Disease Registry, 1993; WHO, 1996).

Samples from 128 drinking-water wells in the United States showed that 3.1% of them had dichloromethane levels of 1–5 µg/L. Dichloromethane was detected in 98.4% of drinking-water samples from Santiago de Compostela, Spain, in 1987; the average concentration was 14.1 µg/L, with a range of 1.2–93.2 µg/L. A sampling of 630 public community water supplies (serving 690 million people in New Jersey, United States) in 1984 and 1985 detected dichloromethane in 2.6–7.1% of the samples; the median concentration ranged from 1.1 to 2.0 µg/L and the range for the whole sampling period was 0.5–39.6 µg/L (WHO, 1996).

Dichloromethane has been detected in both surface water and groundwater samples taken at hazardous waste sites. Data from the Contract Laboratory Program Statistical Database of the United States Environmental Protection Agency indicate that dichloromethane was present at geometric mean concentrations of 68 and 98 µg/L in surface water and groundwater samples, respectively, at about 30% of the sites sampled (Agency for Toxic Substances and Disease Registry, 1993).

Wastewater from certain industries has been reported to contain dichloromethane at average concentrations in excess of 1000 µg/L. Such industries include coal mining, aluminium forming, photographic equipment and supplies, pharmaceutical manufacture, organic chemical/plastics manufacture, paint and ink formulation, rubber processing, foundries and laundries. The maximal concentration measured was 210 mg/L in wastewater from the paint and ink industry and the aluminium-forming industry. In leachate from industrial and municipal landfills, dichloromethane concentrations were reported to range up to 184 mg/L (WHO, 1996).

1.3.5 *Soil/sediment*

The principal sources of dichloromethane releases to land are disposal of dichloromethane products and containers to landfills. Industrial releases of dichloromethane to land and off-site transfers to landfills reported to the Toxic Chemical Release Inventory in 1988 totalled about 71 tonnes. It is estimated that about 12% of dichloromethane releases to the environment are to land (Agency for Toxic Substances and Disease Registry, 1993).

Dichloromethane has been detected in soil and sediment samples taken at 36% of the hazardous waste sites included in the Contract Laboratory Program Statistical Database at a geometric mean concentration of 104 µg/kg (Agency for Toxic Substances and Disease Registry, 1993).

The levels of dichloromethane found in samples of sediment from Lake Pontchartrain, Louisiana, United States, ranged from not detectable to 3.2 µg/kg wet weight. In

Germany, levels found in sediments from the River Rhine in 1987–88 varied from not detectable to 30–40 μg/kg. At one site, concentrations of 220–2200 μg/kg were measured (WHO, 1996).

1.3.6 *Aquatic organisms*

Concentrations of dichloromethane in freshwater organisms have been reported for oysters and clams from Lake Ponchartrain, Louisiana, United States; levels ranging from 4.5 to 27 μg/kg wet weight were detected. Levels up to 700 μg/kg wet weight were found in marine bottom fish taken from Commencement Bay, Washington, United States. Data on biota collected in the STORET database of the United States Environmental Protection Agency showed an average level of 660 μg/kg in the 28% of the samples in which dichloromethane was detected (WHO, 1996).

1.3.7 *Foodstuffs*

Although dichloromethane has been used as a grain fumigant and in processing certain raw food commodities, there is little information on residual levels in food. At a large decaffeinating plant in the United States in 1978, monthly average residues in dichloromethane-decaffeinated coffee beans ranged from 0.32 to 0.42 mg/kg dichloromethane (115–295 samples analysed per month) (Cohen *et al.*, 1980). In seven types of decaffeinated ground coffee, the dichloromethane content ranged from < 0.05 to 4.04 mg/kg; in eight instant coffee samples, it ranged from < 0.05 to 0.91 mg/kg; and in 10 decaffeinated tea samples, it ranged from < 0.05 to 15.9 mg/kg (Page & Charbonneau, 1984). Dichloromethane apparently is no longer used for decaffeination in the United States (Agency for Toxic Substances and Disease Registry, 1993).

In an investigation of several halocarbons in table-ready foods, eight of the 19 foods examined contained dichloromethane levels above the quantification limit (not given). The following ranges were reported (μg/kg): butter, 1.1–280; margarine, 1.2–81; ready-to-eat cereal, 1.6–300; cheese, 3.9–98; peanut butter, 26–49; and highly processed foods (frozen chicken dinner, fish sticks, pot pie), 5–310 (Heikes, 1987).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for dichloromethane in several countries are given in Table 4.

In the United States, dichloromethane may be present as an extractant or process solvent residue in spice oleoresins at a level not to exceed 30 mg/kg [ppm] (including all chlorinated solvents), in hops extract at less than or equal to 2.2% and in coffee at a level not to exceed 10 mg/kg [ppm] (United States Food and Drug Administration, 1996).

The Joint FAO/WHO Expert Committee on Food Additives (WHO, 1983) withdrew the previously allocated temporary allowable daily intake (ADI) of 0–0.5 mg/kg body weight and recommended that the use of dichloromethane as an extraction solvent be limited, in order to ensure that its residues in food are as low as practicable.

Table 4. Occupational exposure limits and guidelines for dichloromethane[a]

Country	Year	Concentration (mg/m^3)	Interpretation[b]
Australia	1991	350 (C3)	TWA
Austria	1993	360	TWA
Belgium	1991	174 (C2)	TWA
Czechoslovakia	1991	500	TWA
		2500	STEL
Denmark	1991	175 (C, sk)	TWA
Finland	1998	350	TWA
		870	STEL
France	1991	360	TWA
		1800	STEL
Germany	1998	350 (C3)	TWA
Hungary	1993	10 (Ca)	STEL
Italy	1991	10	STEL
Japan	1991	350	TWA
The Netherlands	1993	350	TWA
		1740	STEL
The Philippines	1993	1740	TWA
Poland	1991	50	TWA
Russia	1991	350	TWA
		50	STEL
Sweden	1991	120 (sk)	TWA
		250	STEL
		500	STEL
Switzerland	1991	360	TWA
		1800	STEL
Thailand	1993	500	TWA
		1000	STEL
Turkey	1993	1740	TWA
United Kingdom	1991	350	TWA
		870 (MEL)	STEL
United States			
ACGIH (TLV)[c]	1997	174 (A3)	TWA
NIOSH (REL)	1997	(Ca, lfc)	
OSHA (PEL)	1997	87 (Ca)	TWA
		435	STEL

[a] From International Labour Office (1991); American Conference of Governmental Industrial Hygienists (ACGIH) (1997a,b); United States National Library of Medicine (1997b); United States Occupational Safety and Health Administration (OSHA) (1997); Deutsche Forschungsgemeinschaft (1998); Ministry of Social Affairs and Health (1998)

[b] TWA, time-weighted average; STEL, short-term exposure limit; MEL, maximum exposure limit; TLV, threshold limit value; REL, recommended exposure limit; PEL, permissible exposure limit; A3, animal carcinogen; C, suspected of being a carcinogen; C2, probable human carcinogen; C3, suspected of having a carcinogenic potential; Ca, potential occupational carcinogen; lfc, lowest feasible concentration; sk, skin notation

[c] Countries that follow the ACGIH recommendations for threshold limit values include Bulgaria, Colombia, Jordan, Korea, New Zealand, Singapore and Viet Nam.

The use of dichloromethane in hair sprays was banned in the United States by the Food and Drug Administration in 1989 (Agency for Toxic Substances and Disease Registry, 1993; WHO, 1996).

2. Studies of Cancer in Humans

2.1 Industry-based studies (Table 5)

Hearne *et al*. (1990) reported on mortality among a cohort of 1013 photographic film (cellulose triacetate) workers in the United States who were chronically exposed to dichloromethane. This subsumed earlier analyses by Friedlander *et al*. (1978), Hearne and Friedlander (1981) and Hearne *et al*. (1987). The cohort consisted of male workers employed between 1964 and 1970 who had worked for at least one year. Exposure was classified on the basis of work history abstracted from company records and industrial hygiene measurements. The 1975 mean personal time-weighted average (TWA) exposure was 33 ppm [115 mg/m^3], while the range of exposures based on area samples collected during 1959 to 1975 was 0 to 350 ppm [0–1215 mg/m^3] (Friedlander *et al.*, 1978). Four categories of cumulative exposure were used (< 400, 400–799, 800–1199, > 1200 ppm-years). Follow-up was from 1964 through 1988 and was reported to be more than 99% complete. There were 238 deaths in total [standardized mortality ratio (SMR), 0.7 using state rates; SMR, 0.8 using company rates] and 55 cancer deaths [SMR, 0.7 using both rates]. The numbers of observed cancers were less than or similar to the number expected for all sites except pancreas [SMR, 1.9, based on 8 deaths versus 4.2 expected from both state and company rates], but relative risk was not related to estimated cumulative exposure. No other significant association was reported.

Lanes *et al*. (1993) conducted a cohort mortality study of workers employed in the United States in the production of cellulose triacetate fibres, who were potentially exposed to dichloromethane. This extended earlier analyses by Ott *et al*. (1983a,b) and Lanes *et al*. (1990). The cohort consisted of 1271 workers who were employed for at least three months between 1954 and 1976. Based on a combination of personal and area samples, median TWA exposures in 1977 were reported to range from 140 to 745 ppm [486–2590 mg/m^3] in the exposed groups. Follow-up was from 1954 through 1990. Completeness of follow-up was not reported and those not known to have died were assumed to be alive at the end of follow-up. SMRs were calculated using county rates. In total, 172 deaths (SMR, 0.9) including 39 cancer deaths (SMR, 0.8; 95% confidence interval (CI), 0.6–1.5) were observed. The numbers of observed cancers were less than or similar to the number expected for all sites. The highest SMR was observed for cancer of the liver and biliary tract (SMR, 3.0; 95% CI, 0.8–7.6). Three deaths out of the four attributed to cancer of the liver and biliary tract were cancer of the biliary tract. Each of these deaths occurred among employees with longer than 10 years of employment and more than 20 years since first employment (SMR, 5.8; 95% CI, 1.6–14.9).

Table 5. Epidemiological results from industry-based studies relevant to the evaluation of dichloromethane carcinogenicity

Reference	Country	Cohort size/ no. of deaths	Cancer site[a]	Observed	RR	95% CI	Comment
Hearne *et al*. (1990)	United States	1013/238	All cancers	55	[0.7]	[0.6–1.0]	Expected numbers based on company-wide rates
			Lung	18	[0.8]	[0.5–1.2]	
			Liver	0	[0.0]	[0.0–6.2]	
			Pancreas	8	[1.9]	[0.8–3.8]	
			Brain	2	[1.0]	[0.1–3.6]	
Lanes *et al*. (1993)	United States	1271/172	All cancers	39	0.8	0.6–1.5	
			Lung	13	0.8	0.4–1.4	
			Liver/biliary	4	3.0	0.8–7.6	
			Pancreas	2	0.8	0.1–3.0	
			Breast	3	0.5	0.1–1.6	
Gibbs *et al*. (1996)	United States	Males: 1931/500[b]	All cancers	163	[0.8]	[0.7–1.0]	
			Lung	46	[0.7]	[0.5–1.0]	
			Liver/biliary	2	[0.5]	[0.1–2.0]	
			Pancreas	4	[0.6]	[0.2–1.5]	
			Prostate	22	[1.6]	[0.9–2.4]	
		Females: 978/124[b]	Cervix	6	[3.2]	[1.2–6.9]	
Tomenson *et al*. (1997)	United Kingdom	1473/287[b]	All cancers	68	0.7	0.5–0.8	
			Lung	19	0.5	0.3–0.8	
			Liver/biliary	0	0.0	[0.0–2.5]	
			Pancreas	3	0.7	0.1–2.0	
			Prostate	4	0.6	0.2–1.6	
			Brain	4	1.5	0.4–3.7	

Table 5 (contd)

Reference	Country	Cohort size/ no. of deaths	Cancer site[a]	Observed	RR	95% CI	Comment
Ott *et al*. (1985)	United States	226/42	All cancers	9	0.7	0.3–1.3	Expected from US rates
					[0.8]	[0.4–1.5]	Expected from company rates
			Respiratory	3	[0.7]	[0.1–2.0]	
			Digestive	6	[1.8]	[0.7–4.0]	
			Pancreas	3	[3.3]	[0.7–9.7]	
Blair *et al*. (1998)	United States	14 457/5 727	All cancers	1048	0.96	0.90–1.02	SMR, full cohort
			Non-Hodgkin lymphoma				
			men	6 exposed	3.0	0.9–10.0	Incident cancer,
			women	0 exposed	0.0	–	RR from Poisson
			Multiple myeloma				regression
			men	5 exposed	3.4	0.9–13.2	
			women	0 exposed	0.0	–	
			Breast, women	4 exposed	3.0	1.0–8.8	
Shannon *et al*. (1988)	Canada	Females: 203/19 incident cancers	All cancers	19	[1.6]	[0.9–2.4]	Incident cancers
			Breast	8	2.0	0.88–4.0	
			Cervix	1	1.1	0.03–5.9	

[a] Results are presented for all cancers, lung, liver/biliary, pancreas, prostate, breast, cervix and brain when reported.
[b] Results are presented only for the exposed portion of the cohort.

Gibbs *et al*. (1996) conducted a cohort mortality study of cellulose triacetate fibre workers exposed to dichloromethane at a facility in the United States similar to that reported by Lanes *et al*. (1993). The cohort consisted of 3211 workers who had been employed between 1970 and 1981 and had worked in the plant for three or more months. Follow-up was from 1970 through 1989 and comparisons were made with county rates. Completeness of follow-up was not reported and those not known to have died were assumed to be alive at the end of follow-up. In the areas of highest exposure, levels were reported to range from 300 to 1250 ppm [1040–4340 mg/m^3]. The cohort was divided into three exposure groups; none, low (50 to 100 ppm [174–347 mg/m^3]) and high (350 to 700 ppm [1215–2430 mg/m^3]) based on the area worked in and exposure levels reported by Ott *et al*. (1983). There were 302 workers classified as having no exposure. Among the 1931 dichloromethane-exposed male workers, there were 500 deaths [SMR, 0.8] and 163 cancer deaths [SMR, 0.8] observed. Results were reported for the following sites: trachea, bronchus and lung; liver and biliary tract; pancreas; prostate; and cervix. Only the SMR for prostate cancer [1.6; 95% CI, 0.9–2.4] was elevated and appeared to increase with level of exposure (SMR, 1.8; 95% CI, 1.0–3.0 for high exposure). There was also an excess of prostate cancer among workers who had been employed at the facility for 20 or more years (SMR, 2.9; $p < 0.05$). Among the 978 exposed women there was an excess of cervical cancer [SMR, 3.2; 95% CI, 1.2–6.9, based on 6 cases], which did not appear to be related to duration of employment or level of dichloromethane exposure. [The Working Group noted that the interpretation of the exposure measurements is not clear, since the units are in ppm and not ppm-years and it is not clear whether all jobs held at the facility were considered.]

Tomenson *et al*. (1997) performed a cohort mortality study of workers at a plant producing cellulose triacetate film base in the United Kingdom. The cohort comprised 1785 male workers who had been employed at the site at any time between 1946 and 1988, among whom 1473 had been employed in jobs with potential exposure to dichloromethane. Exposure assessment was based on time period and work group, and exposure levels were estimated from area samples. TWA exposures were estimated to range from 2 to 20 ppm [7–69 mg/m^3] before 1960, 6 to 127 ppm [21–441 mg/m^3] during the 1960s, 10 to 165 ppm [35–573 mg/m^3] during the 1970s and 7 to 88 ppm [24–305 mg/m^3] during the 1980s. Four exposure categories were established based on cumulative exposure to dichloromethane (never, < 400 ppm-years, 400–799 ppm-years, > 800 ppm-years). However, 30% of exposed workers could not be classified. Follow-up was from 1946 through 1994 and was reported to be > 99% complete. SMRs were calculated using rates for England and Wales. During the follow-up period there were 287 deaths (SMR, 0.7) and 68 cancer deaths (SMR, 0.7; 95% CI, 0.5–0.8) observed among the exposed workers. Only brain and central nervous system cancer (SMR, 1.5; 95% CI, 0.4–3.7; based on 4 cases) had appreciably more observed than expected cases and the excess was not limited to highly exposed workers. [The Working Group noted that 31 of the 68 cancer deaths among exposed workers were not assigned an exposure level, limiting the utility of the cumulative exposure analyses. The all-cancer SMR was unusually low.]

Ott *et al*. (1985) conducted a cohort mortality study of 1919 men employed for one year or longer between 1940 and 1969 at a chemical manufacturing facility in the United States. This cohort included 226 workers assigned to a unit which produced chlorinated methanes (methyl chloride (see this volume), dichloromethane, chloroform (IARC, 1987b) and carbon tetrachloride (see this volume)) and, recently, tetrachloroethylene (IARC, 1995a). Exposure levels were not reported. The follow-up period was from 1940 to 1979 and follow-up was 94% complete. Expected numbers were based on rates among white males in the United States for the full cohort and on the full cohort for sub-cohort analyses. Among the 226 workers producing chlorinated methanes, there were 42 deaths observed (SMR, 0.6, based on national rates) [SMR, 0.8, based on company rates], including nine from cancers [SMR, 0.8; 95% CI, 0.4–1.5, based on company rates] and three from pancreatic cancer [SMR, 3.3; 95% CI, 0.7–9.7, based on company rates]. Two of these three cases had been employed for less than five years, all three were first assigned to the chlorinated methane unit between 1942 and 1946, and the interval between first assignment to that unit and death was between 20 and 31 years. [The Working Group noted that the mix of exposures and the lack of information regarding exposure levels limits the ability to draw conclusions regarding the carcinogenicity of dichloromethane]

Blair *et al*. (1998) performed a retrospective cohort mortality study of 14 457 workers at a military aircraft maintenance facility in the United States, among whom 1222 were exposed to dichloromethane (Stewart *et al*., 1991). This was an update of an earlier study (Spirtas *et al*., 1991). The cohort consisted of civilian employees employed at a military air force base for at least one year between 1952 and 1956. Follow-up of the cohort was from 1952 through 1990 and comparisons were made with state rates and between exposed and unexposed cohort members. Mortality follow-up was 97% complete. In addition, incident cancers were identified using a statewide tumour registry with follow-up from 1973 to 1990. There were 5727 deaths (SMR, 0.97) and 1048 cancer deaths (SMR, 0.96; 95% CI, 0.90–1.02) identified in the full cohort. An extensive exposure assessment was performed to quantitatively classify exposure to trichloroethylene (IARC, 1995b) and to qualititatively classify exposure (ever/never) to other chemicals including various solvents (Stewart *et al*., 1991). Relative risks (RR) from chemicals other than trichloroethylene were examined by Poisson regression analyses of the cancer incidence data. Exposure to dichloromethane was associated with an increased risk of non-Hodgkin lymphoma (RR, 3.0; 95% CI, 0.9–10.0; 6 exposed cases) and multiple myeloma (RR, 3.4; 95% CI, 0.9–13.2; 5 exposed cases) among men, but not among women (0 exposed cases for both). Among women, exposure to dichloromethane was associated with the risk of breast cancer (RR, 3.0; 95% CI, 1.0–8.8; 4 exposed cases). Results for other cancer sites in relation to dichloromethane exposure were not reported. [The Working Group noted that overlapping exposures to solvents, some of which also showed associations with the cancers evaluated, and the lack of information about exposure levels limit the ability to draw conclusions regarding dichloromethane].

Shannon *et al*. (1988) reported the results of a study of cancer morbidity among Canadian lamp-manufacturing workers. The study had been initiated because of a reported cluster of five cancers among workers in the coiling and wire drawing (CWD) area. Although the study focused on exposure to dichloromethane, potential exposure to other solvents (e.g., trichloroethylene (IARC, 1995b)), strong acids (e.g., sulfuric or nitric acids (IARC, 1992)) and metals (e.g., arsenic (IARC, 1987c), chromium (IARC, 1990)) was also reported in the CWD area. The study population included 203 women and 46 men who had been employed for six or more months at some time between 1960 and 1975 in the CWD department. Incident cancers from 1964 to 1982 were ascertained using a population-based tumour registry and follow-up was stated to be over 90% complete. Among the 203 women who had been employed in the CWD department, the standardized incidence ratio (SIR) for all cancers was [1.6] [95% CI, 0.9–2.4 (n = 19)]. The SIR for breast cancer was 2.0 (95% CI, 0.9–4.0 (n = 8)) and was significantly elevated among those with ≥ 5 years employment and ≥ 15 years since first employment (SIR, 3.2; 95% CI, 1.1–7.5 (n = 5)). Only three cancers were observed among the 46 men who had been employed in the CWD department and their results were not reported. [The Working Group noted that one breast cancer was reported to be part of the initial cluster that led to the study and that the potential for exposure to multiple chemicals limits the ability to draw conclusions regarding dichloromethane.]

2.2 Community-based studies

Heineman *et al*. (1994) performed a case–control study to examine the relationship between occupational exposure to six chlorinated aliphatic hydrocarbons and risk of astrocytic brain cancer. Cases were 741 white men who died from central nervous system tumours in three areas of the United States (southern Louisiana, northern New Jersey, and Philadelphia) over a three-year period. Controls were 741 randomly selected men matched on age, year of death and study area, who died from other causes. The next-of-kin of 654 cases (88%) and 612 controls (83%) were located and 483 cases (74% of traced) and 386 of controls (63% of traced) were interviewed to ascertain detailed work history and other possible risk factors. The final data-set consisted of 300 cases and 320 controls, after exclusion of cases without a hospital diagnosis of astrocytic brain tumour and controls whose death might be linked to occupational exposure to chlorinated aliphatic hydrocarbons. Exposure was assessed using a semi-quantitative job–exposure matrix developed for the study (Gomez *et al*., 1994), and probability of exposure (unexposed, low, medium and high), duration of exposure (2–20 years and > 21 years), average intensity (low-medium, high) and cumulative exposure (low, medium and high) were examined. One hundred and nineteen cases and 108 controls were classified as having ever been exposed to dichloromethane. After adjustment for age and study area, there was a trend of increasing risk with increasing probability, duration and average intensity of exposure ($p < 0.05$), but not with cumulative exposure. The odds ratios for the highest categories were 2.4 (95% CI, 1.0–5.9, 19 exposed cases) for high probability, 1.7 (95% CI, 0.9–3.6, 24 exposed cases) for > 21 years, 2.2 (95% CI, 1.1–4.4, 28

exposed cases) for high average intensity, and 1.2 (95% CI, 0.6–2.5, 19 exposed cases) for high cumulative exposure. No association was found with any of the other five chlorinated hydrocarbons examined.

Cantor *et al*. (1995) performed a case–control study to examine the relationship between occupational exposures and female breast cancer mortality in the United States. Cases and controls were identified from a 24-state death certificate surveillance system. The case group comprised all deaths from female breast cancer between 1984 and 1989 (n = 59 515). For each case, four controls were selected from non-cancer deaths. Usual occupation and industry were coded from death certificates for cases and controls. After excluding those coded as homemakers, there were 29 397 white female cases and 4112 black female cases matched to 102 955 white and 14 839 black controls. Probability and level of workplace exposure to 31 chemical and physical agents were estimated using a job–exposure matrix and results for white women and black women were reported separately. No association was observed with probability of exposure to dichloromethane. However, a small elevated risk was observed for the highest exposure level among both white women (odds ratio, 1.2; 95% CI, 1.1–1.3) and black women (odds ratio, 1.5; 95% CI, 1.2–1.7) after adjustment for age and socioeconomic status. [The Working Group noted that usual occupation as recorded on death certificates may be a poor indicator of exposure to dichloromethane].

A population-based case–control study of cancer among male residents of Montreal, Canada, aged 35–70 years, included histologically confirmed cases of several types of cancer, newly diagnosed between 1979 and 1985 in 20 major hospitals (Siemiatycki, 1991). Interviews were carried out with 3730 cancer patients (response rate, 82%) and 533 age-stratified controls from the general population (response rate, 72%). The main cancer sites included were: oesophagus (99), stomach (251), colon (497), rectum (257), pancreas (116), lung (857), prostate (449), bladder (484), kidney (177), skin melanoma (103) and non-Hodgkin lymphoma (215). For each site of cancer analysed, two control groups were available: population controls and a cancer control group selected from among cases of cancer at the other sites studied. The interview was designed to obtain detailed lifetime job histories and information on potential confounders. Each job was reviewed by a team of chemists and industrial hygienists who translated jobs into occupational exposures using a checklist of 293 occupational substances. Analyses were carried out to estimate the odds ratio between cancer at each site and exposure to each substance. For each association, eight separate estimates were made: two control groups (population and cancer), two target populations (the entire population of Montreal and the 65% subset of the population who were French Canadian and who had a much more homogeneous genetic, social and environmental exposure profile than the population as a whole) and two exposure levels (any exposure and ‘substantial exposure’, defined on the basis of duration, intensity and frequency of purported exposure). The publication did not present all the odds ratios computed; while a set of results in the entire population and using cancer controls was presented for a subset of possible associations, for others, only the most significant results have been published. Dichloromethane was one of the

substances. About 2% of the study subjects had ever been exposed to dichloromethane. Among the main occupations to which dichloromethane exposure was attributed in this study were construction painters, paint mixers and cabinet makers. For most types of cancer examined (oesophagus, stomach, colon, pancreas, prostate, bladder, kidney, skin melanoma, lymphoma), there was no indication of an excess risk due to dichloromethane. However, for rectal cancer, based on five cases exposed at the 'substantial level', the odds ratio was 4.8 (90% CI, 1.7–13.8). For lung cancer, based on seven cases exposed at the 'substantial level', the odds ratio was 3.8 (90% CI, 1.2–12.0). [The interpretation of null results has to take into account the small numbers and low power. Workers typically had multiple exposures. This is particularly true among workers exposed to dichloromethane.]

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of male and female B6C3F_1 mice, seven weeks of age, were administered dichloromethane (containing < 300 mg/kg cyclohexane, < 20 mg/kg *trans*-1,2-dichloroethylene, < 10 mg/kg chloroform, < 2 mg/kg vinyl chloride and < 1 mg/kg each methyl chloride, ethyl chloride, vinylidene chloride, carbon tetrachloride and trichloroethylene) in the drinking-water for 104 weeks according to the study design shown in Table 6. No significant exposure-related trend in survival was found in males; in females, a significant trend towards longer survival in exposed groups was reported. In male mice, the incidences of hepatocellular adenoma were: 6/60 (10%), 4/65 (8%), 20/200 (10%), 14/100 (14%), 14/99 (14%) and 15/125 (12%); and the incidences of hepatocellular carcinomas were: 5/60 (8%), 9/65 (14%), 33/200 (17%), 18/100 (18%), 17/99 (17%) and 23/125 (18%) in control 1, control 2, low-dose, mid-dose 1, mid-dose 2 and high-dose groups, respectively. A slight but significant [$p = 0.035$] dose-related increase in the incidence of hepatocellular adenomas and/or carcinomas (combined) was observed in male mice: 11/60 (18%), 13/65 (20%), 51/200 (25%), 30/100 (30%), 31/99 (31%) and 35/125 (28%). However, the authors noted that tumour incidences in exposed groups were similar to those reported in historical controls (mean, 32.1%; range, 7–58%) (Serota *et al*., 1986a).

Groups of 50 male and 50 female Swiss mice, nine weeks of age, were administered 100 (low-dose) or 500 (high-dose) mg/kg bw dichloromethane (purity, > 99.9%) in olive oil by gavage once per day on four to five days per week for 64 weeks. Groups of 60 mice of each sex were given olive oil (vehicle-control). Animals were then kept under observation for their lifespan. Excess mortality was observed in male and female mice exposed to the high dose ($p < 0.01$). An increase in mortality appeared after 36 weeks of treatment and led to withdrawal of the treatment at 64 weeks. In mice that died by 78 weeks, the incidence of lung tumours in males was 1/14 control, 4/21 low-dose and 7/24

Table 6. Design of studies of dichloromethane in drinking-water

Group	No. of animals		Dose (mg/kg bw/day)
	Males	Females	
Mice[a]			
Control 1	60	50	0
Control 2	65	50	0
Low-dose	200	100	60
Mid-dose 1	100	50	125
Mid-dose 2	100	50	185
High-dose	125	50	250
Rats[b]			
Control 1	85	85	0
Control 2	50	50	0
Low-dose	85	85	5
Mid-dose 1	85	85	50
Mid-dose 2	85	85	125
High-dose	85	85	250
High-dose (78 weeks)	25	25	250

[a] From Serota *et al*. (1986a)
[b] From Serota *et al*. (1986b)

high-dose ($p < 0.05$) mice, respectively. At the end of the experiment, the cumulative incidence of lung tumours in males was 5/47, 5/28 and 9/36. No treatment-related increase in the incidence of any tumour in females or other type of tumour in males was reported (Maltoni *et al*., 1988). [The Working Group noted the short period of exposure and the high numbers of animals lost for examination.]

3.1.2 *Rat*

Groups of male and female Fischer 344 rats, seven weeks of age, were administered dichloromethane (containing < 300 mg/kg cyclohexane, < 20 mg/kg *trans*-1,2-dichloroethylene, 26 mg/kg chloroform and < 1 mg/kg each methyl chloride, vinyl chloride, ethyl chloride, vinylidene chloride and trichloroethylene) in the drinking-water for 104 weeks according to the study design shown in Table 6. Interim terminations were carried out at 26, 52 and 78 weeks in control group 1 and in the low-, mid-1 and -2 and high-dose groups, such that 50 males and 50 females per group received the treatment for 104 weeks. There was no significant difference in survival between the exposed and control groups. In females, the incidences of hepatocellular carcinomas after 104 weeks were: 0/85, 0/50, 0/85, 2/83, 0/85 and 2/85; those of neoplastic nodules [now classified as hepatocellular adenomas] were: 0/85, 0/50, 1/85, 2/83, 1/85 and 4/85; and those of

neoplastic nodules and/or hepatocellular carcinomas (combined) were: 0/85, 0/50, 1/85, 4/83, 1/85 and 6/85 in the six groups, respectively. This increasing trend was significant [$p < 0.01$]; however, tumour incidences in exposed groups were similar to those reported in historical controls in this laboratory (mean, 8%; range, 0–16%). In male rats, no increased incidence of liver tumours was observed at 104 weeks (neoplastic nodules: 4/85, 5/50, 2/85, 3/84, 3/85 and 1/85; carcinomas: 2/85, 2/50, 0/85, 0/84, 0/85 and 1/85; neoplastic nodules and/or carcinomas combined: 6/85, 7/50, 2/85, 3/84, 3/85 and 2/85). No other significant increase in tumour incidence was found (Serota *et al*., 1986b).

Groups of 50 male and 50 female Sprague-Dawley rats, 12 weeks of age, were administered 100 (low-dose) or 500 (high-dose) mg/kg bw dichloromethane (purity, > 99.9%) in olive oil by gavage once per day on four or five days per week for 64 weeks. A group of 50 rats of each sex was given olive oil (vehicle controls) and an additional group of 20 males and 26 females was kept untreated (untreated controls). Animals were then kept under observation for their lifespan. Excess mortality was observed in male and female rats administered dichloromethane at the high dose. An increase in mortality started to appear after 36 weeks of treatment and led to cessation of exposure after 64 weeks [details on mortality not reported]. There was no significant increase in tumour incidence associated with exposure (Maltoni *et al*., 1988). [The Working Group noted the short period of treatment and the inadequate reporting of the data.]

3.2 Inhalation exposure

3.2.1 *Mouse*

Groups of 50 male and 50 female B6C3F$_1$ mice, eight to nine weeks of age, were exposed to 0, 2000 or 4000 ppm [0, 6940 or 13 900 mg/m^3] dichloromethane (> 99% pure) by whole-body inhalation for 6 h per day on five days per week for 102 weeks and were killed after 104 weeks on study. The mean body weight of the high-dose male mice was generally comparable to that of controls until week 90, and that of high-dose females was somewhat lower from weeks 51 to 95. Survival to the end of the study period in males was: control, 39/50; low-dose, 24/50; and high-dose, 11/50; and that in females was: 25/50, 25/49 and 8/49. Significant dose-related increases in the incidence of lung and liver tumours were observed in exposed mice. The incidences of alveolar/bronchiolar adenomas were: males—3/50, 19/50 and 24/50 ($p < 0.001$); and females—2/50, 23/48 and 28/48 ($p < 0.001$). Those of alveolar/bronchiolar carcinomas were: males—2/50, 10/50 and 28/50 ($p < 0.001$); and females—1/50, 13/48 and 29/48 ($p < 0.001$). The incidences of hepatocellular adenomas were: males—10/50, 14/49 and 14/49 ($p = 0.075$); and females — 2/50, 6/48 and 22/48 ($p < 0.001$). The incidences of hepatocellular carcinomas were: males—13/50, 15/49 and 26/49 ($p = 0.016$); and females—1/50, 11/48 and 32/48 ($p < 0.001$) (United States National Toxicology Program, 1986).

Groups of 68 female B6C3F$_1$ mice, eight to nine weeks of age, were administered dichloromethane (> 99% pure) by whole-body inhalation at concentrations of 0 ppm (control) or 2000 ppm [6940 mg/m^3] for various lengths of time over a 104-week period (Table 7). Lung and liver were evaluated histopathologically. Survival was reduced

Table 7. Effect of various dichloromethane inhalation exposure regimens on survival and pulmonary and liver tumours in female B6C3F$_1$ mice

	Dichloromethane treatment							
	0 ppm 104 weeks	2000 ppm 26 weeks/ 0 ppm 78 weeks	0 ppm 78 weeks/ 2000 ppm 26 weeks	2000 ppm 52 weeks/ 0 ppm 52 weeks	0 ppm 52 weeks/ 2000 ppm 52 weeks	2000 ppm 78 weeks/ 0 ppm 26 weeks	0 ppm 26 weeks/ 2000 ppm 78 weeks	2000 ppm 104 weeks
Survival (%) (Kaplan–Meier)	58.8	47.1	54.1	34.4**	58.8	35.3**	47.1	40.4
Lung								
Adenomas								
Incidence	1/67	8/68	0/67	12/63	5/67	19/68	7/67	18/67
Carcinomas								
Incidence	4/67	17/68	3/67	36/63	6/67	25/68	7/67	31/67
Combined								
No. animals with adenomas or carcinomas/no. animals at risk	5/67	21/26**	3/67	40/63**	10/67	38/68**	13/67*	42/67**
Liver								
Adenomas								
Incidence	8/67	16/67	16/67	14/64	9/67	28/68	17/67	24/68
Carcinomas								
Incidence	11/67	14/67	13/67	18/64	12/67	25/68	20/67	35/68
Combined								
No. animals with adenomas or carcinomas/no. animals at risk	18/67	27/67	23/67	28/64*	21/67	42/68**	32/67*	47/68**

Incidence = No. of animals with tumour/No. of animals at risk
*p < 0.05; **p < 0.01 against control group
From Kari *et al.* (1993)

compared with controls in groups exposed to dichloromethane for the first 52, 78 or the complete 104 weeks of the study. The incidences of mice with lung adenomas, carcinomas or adenomas and carcinomas combined and the incidences of mice with hepatocellular adenomas, carcinomas or adenomas and carcinomas combined were increased in all groups in which exposure was begun during the first 26 weeks of the study [statistical analyses were reported only for the combined tumour incidences] (Table 7) (Kari *et al.*, 1993).

3.2.2 *Rat*

Groups of approximately 95 male and 95 female Sprague-Dawley rats, eight weeks of age, were administered dichloromethane by whole-body inhalation at concentrations of 0, 500, 1500 or 3500 ppm [1740, 5200 or 12 100 mg/m^3] for 6 h per day on five days per week for two years. The dichloromethane was > 99% pure, with ≤ 706 mg/kg (ppm) *trans*-1,2-dichloroethylene, ≤ 467 mg/kg cyclohexane, ≤ 576 mg/kg chloroform, ≤ 90 mg/kg vinylidene chloride, ≤ 20 mg/kg carbon tetrachloride, ≤ 23 mg/kg methyl bromide, ≤ 11 mg/kg ethyl chloride, ≤ 4.5 mg/kg methyl chloride and ≤ 1 mg/kg vinyl chloride. The numbers of animals still alive at the end of the study were 14, 14, 6 and 7 control, low-, mid- and high-dose males and 21, 24, 13 and 4 females, respectively. Mortality among high-dose females was significantly increased from the 18th month onwards. Non-neoplastic pathological changes in the liver and kidney were more frequently observed in treated animals. There was no significant increase in the incidence of benign or malignant mammary tumours; however, the total number of benign mammary tumours [type not specified] showed a slight dose-related increase in males (control, 8/95; low-dose, 6/95; mid-dose, 11/95; and high-dose, 17/97; $p = 0.046$), and a dose-related increase [$p < 0.001$] in the total number of benign mammary tumours [type not specified] was observed in females (165/96, 218/95, 245/95 and 287/97). The incidence of sarcomas located around the salivary glands was increased in mid- and high-dose males (1/93, 0/94, 5/91 and 11/88; $p = 0.002$ [$p < 0.001$, trend test]) (Burek *et al.*, 1984; United States Environmental Protection Agency, 1985). [The Working Group noted the reported occurrence of salivary gland sialodacryoadenitis early in the study.]

Groups of 50 male and 50 female Fischer 344/N rats, seven to eight weeks of age, were administered dichloromethane (> 99% pure) by whole-body inhalation at concentrations of 0, 1000, 2000 or 4000 ppm [0, 3470, 6940 or 13 900 mg/m^3] for 6 h per day on five days per week for 102 weeks and were killed after 104 weeks on study. Mean body weights of control and dosed rats of both sexes were comparable throughout the study. Survival of treated males was similar to that of controls. Survival at termination of the study was reduced in high-dose females compared with controls: control, 30/50; low-dose, 22/50; mid-dose, 22/50; and high-dose, 15/50. Increased incidences of benign mammary gland tumours (all fibroadenomas, except for one adenoma in the high-dose group) were observed in treated females (5/50, 11/50, 13/50 and 23/50; $p < 0.001$). There was a positive trend in the incidence of mammary gland adenoma or fibroadenoma

combined in males (0/50, 0/50, 2/50 and 5/50; $p < 0.01$). There was no difference in the distribution of other types of tumour between the control and treated groups (United States National Toxicology Program, 1986).

Groups of 54–70 male and female Sprague-Dawley rats, 13 weeks old, were administered 100 ppm [347 mg/m^3] dichloromethane (purity, > 99.9%) by whole-body inhalation for 7 h per day on five days per week. The exposure was started on breeders, and male and female offspring (12-day embryos). The breeders and a group of offspring were exposed for 104 weeks, another group of offspring was exposed for 15 weeks only. Control groups were composed of 60 female rats (breeder controls) and 158 males and 149 females (untreated controls). Animals were observed for their lifespan. No excess in mortality was found in the exposed groups. No significant increase in the incidence of any tumour type was noted (Maltoni *et al.*, 1988). [The Working Group noted the low exposure concentration.]

Groups of 90 male and 108 female Sprague-Dawley rats [age unspecified] were administered 0, 50, 200 or 500 ppm [0, 174, 694 or 1740 mg/m^3] dichloromethane (technical-grade; purity, > 99.5%) by whole-body inhalation for 6 h per day on five days per week for 20 (males) or 24 (females) months. A further group of 30 female rats was exposed to 500 ppm dichloromethane for the first 12 months and to room air for the last 12 months of the study (denoted 500/air). An additional group of 30 female rats was exposed to room air for the first 12 months followed by 500 ppm dichloromethane for the last 12 months of the study (denoted air/500). Subgroups of five rats per sex per exposure level were scheduled for interim terminations after 6, 12, 15 and 18 months of exposure to dichloromethane. No exposure-related adverse effect on body weight or mortality was observed. In females, the incidence of benign mammary tumours (adenomas and fibroadenomas combined) was 52/70, 58/70, 61/70 ($p < 0.05$, Fisher's exact test) and 55/70 in control, low-, mid- and high-dose groups, respectively. The multiplicity of benign mammary tumours was 1.8, 2.1, 2.0 and 2.2 ($p < 0.05$) in the control, low-, mid- and high-dose groups, respectively, and 2.3 ($p < 0.05$) and 2.7 ($p < 0.05$) in the air/500 and 500/air groups. No significant increase in the incidence of any other tumour type was seen in the exposed groups (Nitschke *et al.*, 1988).

3.2.3 *Hamster*

Groups of 95 male and 95 female Syrian golden hamsters, eight weeks of age, were administered dichloromethane by whole-body inhalation at concentrations of 0, 500, 1500 or 3500 ppm [0, 1740, 5200 or 12 100 mg/m^3] for 6 h per day on five days per week for two years. The dichloromethane was > 99% pure, with ≤ 706 mg/kg *trans*-1,2-dichloroethylene, ≤ 467 mg/kg cyclohexane, ≤ 576 mg/kg chloroform, ≤ 90 mg/kg vinylidene chloride, ≤ 20 mg/kg carbon tetrachloride, ≤ 23 mg/kg methyl bromide, ≤ 11 mg/kg ethyl chloride, ≤ 4.5 mg/kg methyl chloride and ≤ 1 mg/kg vinyl chloride. The numbers of animals surviving to the end of the study were 16, 20, 11 and 14 in males and 0, 4, 10 and 9 in females. The incidence of lymphosarcomas was slightly higher in exposed females than in controls: control, 1/91; low-dose, 6/92; mid-dose, 3/91; and high-dose, 7/91

($p = 0.032$) (Burek *et al.*, 1984; United States Environmental Protection Agency, 1985). [The Working Group noted that the higher survival in treated animals may have contributed to this non-dose-dependent result for which historical control data were not available.]

3.4 Intraperitoneal administration

Mouse: In a screening assay based on the production of lung adenomas in strain A mice, groups of 20 male mice, six to eight weeks of age, were administered thrice-weekly intraperitoneal injections of 0, 160, 400 or 800 mg/kg bw reagent-grade dichloromethane (purity, > 95%; impurities unspecified) in tricaprylin for a total of 16–17 injections (total doses: 2720, 6800 and 12 800 mg/kg bw in the treated groups, respectively). After 24 weeks, 18, 5 and 12 animals were still alive in the three treated groups, respectively; these and 15/20 surviving vehicle controls were killed and their lungs examined for tumours. No significant increase was found in the multiplicity of lung adenomas: vehicle-control, 0.27; low-dose, 0.94; mid-dose, 0.80; and high-dose, 0.50 (Theiss *et al.*, 1977).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

The metabolism of dichloromethane has been extensively reviewed (WHO, 1984; United States Environmental Protection Agency, 1985).

4.1.1 *Humans*

Liquid dichloromethane is absorbed through human skin, with maximum concentrations in expired air being reached 30 min after exposure (Stewart & Dodd, 1964). After 0.5–8 h inhalation exposure, concentrations of dichloromethane in the blood and expired air were directly proportional to dose, over the concentration range 173–1740 mg/m^3 (DiVincenzo *et al.*, 1972). It is distributed principally to adipose tissue. In male volunteers exposed to 2600 mg/m^3 for 1 h at a work intensity of 50 W, mean adipose tissue concentrations after 1, 4 and 22 h were 10.2, 8.4 and 1.7 mg/kg, respectively (Engström & Bjurström, 1977). Elevated carboxyhaemoglobin saturation and increased urinary formic acid concentrations have been found in exposed workers (Ku elová & Vlasák, 1966; DiVincenzo & Kaplan, 1981), but inhaled dichloromethane is excreted principally unchanged in expired air (Riley *et al.*, 1966).

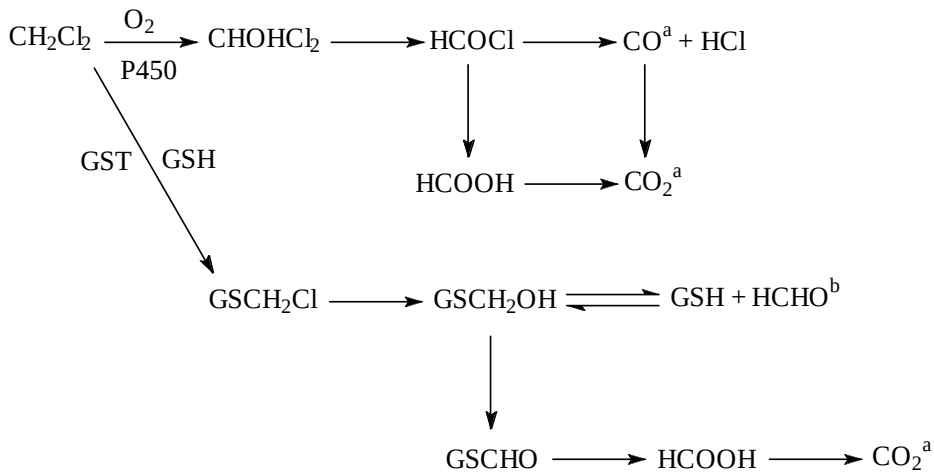
Dichloromethane can be conjugated with glutathione by human θ-class glutathione *S*-transferase (GST) T1-1, which is expressed in many human organs. However, the tissue-specific expression pattern differs from that of the α-, μ- and π-forms of GST, the θ-class being expressed only at very low levels in a small number of Clara cells and ciliated cells at the alveolar/bronchiolar junctions in human lung (Mainwaring *et al.*, 1996a; Sherratt *et al.*, 1997).

4.1.2 *Experimental systems*

Dichloromethane is rapidly absorbed through the lungs and distributed throughout the body reaching all organs, including the brain. Dichloromethane has a particular affinity for fat; concentrations in adipose tissues may be seven- to eight-fold higher than in other tissues (Savolainen *et al*., 1977). After inhalation or oral exposure of rats, the majority of the dose is exhaled unchanged (McKenna & Zempel, 1981; McKenna *et al*., 1982). Dichloromethane metabolites are also largely excreted via the lungs as carbon monoxide and carbon dioxide (DiVincenzo & Hamilton, 1975).

Two pathways for the metabolism of dichloromethane are known, one catalysed by a cytochrome P450 and the other by a GST (Kubic & Anders, 1975; Ahmed & Anders, 1976, 1978; Kubic & Anders, 1978; Gargas *et al.*, 1986). The P450-mediated oxidative pathway produces carbon monoxide and inorganic chloride, presumably via the highly unstable intermediate, formyl chloride. The glutathione pathway produces carbon dioxide after the formation of a postulated glutathione conjugate and formaldehyde (Figure 1).

Figure 1. Metabolic pathways for dichloromethane



[a] Metabolites identified *in vivo*
[b] Metabolite identified *in vitro*
All other metabolites are postulated intermediates
GSH, glutathione; GST, glutathione *S*-transferase

More recently the specific isoenzymes involved in the metabolism of dichloromethane have been identified as the cytochrome CYP2E1 (Kim & Kim, 1996) and the θ-class GST, GSTT1-1 (Meyer *et al*., 1991; Mainwaring *et al*., 1996b).

Early studies suggested that the relative activities of each pathway could be determined by measuring exhaled carbon monoxide (or carboxyhaemoglobin in blood) and carbon dioxide, resulting from the P450 and glutathione pathways respectively. How-

ever, it is now known that this is an unreliable method, since significant quantities of carbon dioxide are also derived from the oxidative pathway (Gargas *et al*., 1986).

The metabolism of dichloromethane is a saturable process. For example, 48 h after inhalation exposure of rats to 50 ppm, 500 ppm or 1500 ppm [74, 1740 or 5200 mg/m^3] dichloromethane, 5, 30 and 55% respectively of the chemical was expired unchanged (McKenna *et al*., 1982). Detailed investigations, in both rats and mice, have indicated that the cytochrome P450-mediated metabolism is a saturable high-affinity/low-capacity pathway. Saturation occurs at relatively low dose levels (< 500 ppm) in both rats and mice and results in similar levels of carboxyhaemoglobin in the blood (12–15%). Conversely, the GST-mediated pathway is low-affinity/high-capacity and exhibits dose-dependent linear kinetics (Gargas *et al*., 1986; ECETOC, 1987). This pathway is particularly active in mice; indeed it is the major pathway at the dose levels (2000 and 4000 ppm [6940 and 13 900 mg/m^3]) used in the carcinogenicity bioassays. *In vitro*, the relative rates for this pathway (as indicated by the yield of RNA–formaldehyde adducts) are 1, 2, 4 and 14 in Syrian hamster hepatocytes, in human hepatocytes with functional *GSTT1* genes and in rat and mouse hepatocytes, respectively (Casanova *et al*., 1997). Marked human inter-individual differences have been described for dichloromethane metabolism via the GST pathway. In 39 human samples, this activity varied between 0 and 3 nmol/min/mg cytosolic protein (Green, 1989; Reitz *et al*., 1989; Bogaards *et al*., 1993; Graves *et al*., 1995). Indeed a genetic polymorphism has been described for a θ-class GST found in erythrocytes (Bogaards *et al*., 1993; Hallier *et al*., 1994; Schroder *et al*., 1996). Some 10–40% of human samples studied appear to be deficient in this transferase activity.

The distribution of GSTT1-1 has been examined in mouse, rat and human liver and lung (Pemble *et al*., 1994; Mainwaring *et al*., 1996a; Sherratt *et al*., 1997; Mainwaring *et al*., 1998). GSTT1-1 mRNA and protein were visualized by in-situ hybridization and immunocytochemistry, respectively. High levels of both GSTT1-1 protein and mRNA were observed in mouse hepatocytes and mouse Clara cells, while much less was seen in rat or human liver and lung (Mainwaring *et al*., 1996a, 1998). Additionally, GSTT1-1 mRNA and protein were concentrated preferentially in certain cell types (mouse lung Clara cells, limiting plate hepatocytes) and, particularly in the nuclei of these cells in mice, whereas in rat and man, the distribution was more generalized.

A number of physiological toxicokinetic models have been developed to describe the metabolism of dichloromethane in mouse, rat, Syrian hamster and man following exposure either by inhalation or in drinking-water (Andersen *et al*., 1987; ECETOC, 1988; Reitz *et al*., 1989; Andersen *et al*., 1991).

4.2 Toxic effects

The toxicity of dichloromethane has been reviewed (Dhillon & Von Burg, 1995; WHO, 1996; Green, 1997).

4.2.1 *Humans*

The odour threshold of dichloromethane is about 200 ppm [694 mg/m^3] (Stahl, 1973). One of the products of dichloromethane metabolism is carbon monoxide, and the acute effects of dichloromethane poisoning are mainly due to carbon monoxide, which binds to haemoglobin and thus decreases the oxygen-transporting capacity of blood, and simultaneously increases the affinity of haemoglobin toward oxygen, thereby decreasing the liberation of oxygen to tissues (Shusterman *et al*., 1990; Dhillon & Von Burg, 1995).

Fatalities have been associated with acute or prolonged exposure to dichloromethane (Moskowitz & Shapiro, 1952; Ku elová *et al*., 1975; Stewart & Hake, 1976; Bonventre *et al*., 1977; Bakinson & Jones, 1985; Manno *et al*., 1989). Temporary neurobehavioural effects have been reported after exposure to doses as low as 200 ppm [694 mg/m^3] by some (Winnekke, 1974; Putz *et al*., 1976) but not by others (Gamberale *et al*., 1975).

An exposure-related increase in serum bilirubin was observed in workers exposed to dichloromethane, but no other sign of liver injury or haemolysis was reported (Ott *et al*., 1983a). A cross-sectional study of 24 employees at a fibre production plant showed no excess of electrocardiographic abnormalities among those exposed to 60–475 ppm [208–1650 mg/m^3] (time-weighted average) dichloromethane and monitored for 24 h (Ott *et al*., 1983b).

The following mortality studies are described in more detail in Section 2.1. No significant increase in overall mortality or deaths due to ischaemic heart disease was found among 1271 male and female employees exposed to 140–475 ppm [486–1650 mg/m^3] dichloromethane compared to the mortality of the general United States population (Ott *et al*., 1983c). An increased risk of ischaemic heart disease was found in comparison with an internal reference group. In another study in the United States of America on cellulose triacetate fibre workers (Gibbs *et al*., 1996), mortality from cardiovascular disease as a whole was not elevated, and in addition showed an inverse relationship with duration of exposure. In a further study on cellulose triacetate fibre workers in the United Kingdom (Tomenson *et al*., 1997), mortality from ischaemic heart disease was similarly less than expected from national rates. However, it was higher among the exposed than in the non-exposed cohort, and showed an association with the estimated lifetime exposure to dichloromethane. There was a statistically significant deficit in the mortality from non-malignant lung disease and cerebrovascular disease. In a third cohort study on dichloromethane-exposed workers (Hearne *et al*., 1990), which had a 90% probability of detecting an 1.3-fold risk of ischaemic heart disease, no elevated risk was observed. The risk of non-malignant pulmonary disease was not elevated either, and there was a (non-significant) deficit of cerebrovascular disease.

4.2.2 *Experimental systems*

Intraperitoneal LD_{50} values for dichloromethane are approximately 1.5 mL/kg bw (2000 mg/kg bw) in mice (Klaassen & Plaa, 1966) and 0.95 mL/kg bw (1300 mg/kg bw) in dogs (Klaassen & Plaa, 1967); the oral LD_{50} in rats ranges from 1.6 to 2.3 mL/kg bw (2100–3000 mg/kg bw) (Kimura *et al*., 1971); and the subcutaneous LD_{50} in mice is

approximately 76 mmol/kg bw (6400 mg/kg bw) (Kutob & Plaa, 1962). The LC_{50} values in mice, rats and guinea-pigs are 16 000 ppm [55 500 mg/m^3] (7-h exposure plus 1-h observation), 5.7% [200 000 mg/m^3] (15-min exposure) and 11 600 ppm [40 300 mg/m^3] (6-h exposure plus 18-h observation), respectively (Sviberly *et al*., 1947; Balmer *et al*., 1976; Clark & Tinston, 1982).

The acute toxicity of dichloromethane is expressed mainly as disturbances of the central nervous system, involving sleep disturbance and reductions in spontaneous activity (Heppel & Neal, 1944; Schumacher & Grandjean, 1960; Fodor & Winneke, 1971; United States National Institute for Occupational Safety and Health, 1976).

Hepatotoxic effects are seen after exposure to near-lethal concentrations of dichloromethane (Gehring, 1968). Inhalation exposure of guinea-pigs to 5200 ppm [18 000 mg/m^3] dichloromethane for 6 h increased hepatic triglyceride concentrations (Morris *et al*., 1979). Exposure of guinea-pigs to approximately 11 000 ppm [38 200 mg/m^3] dichloromethane for 6 h also increased hepatic triglyceride concentrations, but concomitant exposure to 21 400–24 100 ppm [40 200–45 300 mg/m^3] ethanol blocked this effect (Balmer *et al*., 1976). Continuous exposure of mice by inhalation to 5000 ppm [17 400 mg/m^3] dichloromethane caused swelling of the rough endoplasmic reticulum, fatty changes in the liver and necrosis of individual hepatocytes (Weinstein *et al*., 1972). Slight liver damage was also observed following administration of dichloromethane by gavage (133–665 mg/kg bw) to mice (Condie *et al*., 1983). In Sprague-Dawley rats, two doses of 1250 mg/kg dichloromethane by gavage 21 and 4 h before killing the animals did not affect serum alanine aminotransferase, or hepatic glutathione or cytochrome P450 content, but increased the hepatic ornithine decarboxylase activity in 3/15 animals (Kitchin & Brown, 1989). In a long-term bioassay of dichloromethane, increased incidences of haemosiderosis, cytomegaly, cytoplasmic vacuolation, necrosis, granulomatous inflammation and bile-duct fibrosis were observed in the livers of treated male and female Fischer 344/N rats (United States National Toxicology Program, 1986). Increased liver weight associated with glycogen accumulation in the hepatocytes, but no hepatotoxicity, was observed in another carcinogenicity study in mice, in which an elevated frequency of hepatic tumours was observed (Kari *et al*., 1993). The proportion of S-phase cells was frequently higher in altered foci than in cells from the areas of the liver with normal architecture, but similar to that of the altered foci from non-treated animals (Foley *et al*., 1993). Administration of dichloromethane to $B6C3F_1$ mice by gavage (1000 mg/kg, once) or inhalation (4000 ppm [13 900 mg/m^3] dichloromethane for 2 h) did not induce DNA synthesis, as measured by the number of cells in S-phase ([^{3}H]thymidine incorporation) (Lefevre & Ashby, 1989) and, when female $B6C3F_1$ mice were exposed to 1000, 2000, 4000 or 8000 ppm [3470, 6940, 13 900 or 27 800 mg/m^3] dichloromethane for 6 h per day on five days per week for up to four weeks, followed by a recovery period of 1–2 weeks (Foley *et al*., 1993), the hepatocyte labelling index was mostly decreased. There were, however, transient increases in the labelling index in the 4000- and 8000-ppm groups at two weeks and in the 1000-ppm group at one week. The labelling index of bronchiolar epithelium (in two branches proximal to the terminal

bronchiole and in the terminal bronchioles themselves) of female B6C3F$_1$ mice exposed to 2000 ppm dichloromethane for 2–26 weeks decreased to 40–60% of the value found in unexposed control mice. Exposure to 8000 ppm dichloromethane led to a smaller decrease in labelling index. No pathological change was found in the exposed lungs (Kanno *et al.*, 1993).

Inhalation exposure of male B6C3F$_1$ mice to dichloromethane (6 h, once) led to vacuolation of bronchiolar cells at exposure levels ≥ 2000 ppm [6940 mg/m^3], while no effect was observed at levels ≤ 1000 ppm [3470 mg/m^3] (Foster *et al*., 1994). Pretreatment with the cytochrome P450 inhibitor piperonyl butoxide (300 mg/kg intraperitoneally) 1 h before the exposure practically abolished the toxic effect upon bronchiolar cells, while buthionine sulfoximine (1 g/kg intraperitoneally), which decreased the pulmonary glutathione content by 50%, had no such protective effect. In Clara cells isolated after exposure to dichloromethane exposure (≥ 1000 ppm), the proportion of cells in the S-phase was increased.

Following intraperitoneal administration of dichloromethane at near-lethal doses, hydropic degeneration was observed in the kidneys of mice (Klaassen & Plaa, 1966), while no kidney damage was observed following administration of dichloromethane by gavage at dose levels of 133–665 mg/kg bw (Condie *et al*., 1983). Slight calcification of the renal tubules in dogs was seen after intraperitoneal administration of dichloromethane at near-lethal doses (Klaassen & Plaa, 1967). In rats, intraperitoneal administration of 1330 mg/kg bw dichloromethane produced renal proximal tubular swelling (Kluwe *et al*., 1982). After a similar dose by gavage, a transient elevation of blood urea nitrogen and decreased urine output, coinciding with cloudy swelling of tubular cells, were observed (Marzotko & Pankow, 1988). Urinary flow was already decreased at the lowest dose tested, 3.1 mmol/kg bw (263 mg/kg bw).

In gerbils exposed continuously by inhalation to 350 ppm [1210 mg/m^3], but not in those exposed to 210 ppm [730 mg/m^3], dichloromethane for up to three months, increased brain concentrations of two astroglial proteins (S-100 and GFA) and decreased cerebellar DNA concentrations were observed. Decreased hippocampal DNA concentrations were observed at both exposure levels (Rosengren *et al*., 1986).

Dichloromethane (≥ 6.3 mmol/kg bw) administered to rats by gavage induced increased urinary excretion of catecholamines in the urine in rats; cytomorphological changes and a decrease in chromaffin reaction were observed in the adrenal medulla at a dose level of 15.6 mmol/kg bw (1330 mg/kg) (Marzotko & Pankow, 1987).

In a two-year carcinogenicity test of inhaled dichloromethane, an increased incidence of testicular atrophy was observed in B6C3F$_1$ mice exposed to 4000 ppm [13 900 mg/m^3] for 6 h per day on five days a week (United States National Toxicology Program, 1986).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

A case–control study on 44 women who had had a spontaneous abortion was performed within a cohort of female workers employed in Finnish pharmaceutical

factories during 1973 or 1975–80. Three controls matched for age at conception within 2.5 years were chosen for each case (except two). Information about pregnancy outcome was collected from hospital data, and data on exposures from health personnel at the factories. The odds ratio for dichloromethane exposure, based on 11 exposed cases, was 2.3 (95% CI, 1.0–5.7); the odds ratio was also increased for exposure to many other solvents. The odds ratio for those exposed once a week or more during the first trimester of pregnancy was 2.8, and that for those exposed less often was 2.0 (Taskinen *et al*., 1986).

4.3.2 *Experimental systems*

In a teratology study, groups of Swiss Webster mice and Sprague-Dawley rats were exposed by inhalation to 0 or 1225 ppm [0 or 4250 mg/m^3] dichloromethane (purity, 97.9%) for 7 h per day on gestation days 6–15. Exposure of female mice resulted in a significant increase in body weight during and after exposure, while absolute but not relative liver weights were increased in both species. There was no significant increase in visceral anomalies in the fetuses of either species, but skeletal anomalies included decreased incidence of lumbar spurs and delayed ossification of the sternebrae in rats and increased incidence of a single extra sternal ossification centre in mice (Schwetz *et al*., 1975).

Female Long-Evans rats were exposed to 0 or 4500 ppm [0 or 15 600 mg/m^3] dichloromethane (> 97% pure) during either a three-week pregestational period or during the first 17 days of gestation or both. Ten females per group were allowed to give birth, and the offspring were examined for abnormal growth and behaviour. Dams exposed to dichloromethane during gestation had increased absolute and relative liver weights. There was no effect on litter size or viability, but fetal weight was reduced in both groups exposed during gestation. No treatment-related visceral or skeletal abnormality was detected in the fetuses of any exposure group, but a greater proportion of litters exposed during both the pregestational and gestational periods had fetuses with rudimentary lumbar ribs (Hardin & Manson, 1980). No difference in pup birth weight, viability or growth rate was observed, but alterations in spontaneous locomotor activities were seen in all exposure groups. No change was observed in running-wheel activity or acquisition of an avoidance response (Bornschein *et al*., 1980).

In a two-generation reproduction study (Nitschke *et al*., 1988), male and female Fischer 344 rats were exposed to 0, 100, 500 or 1500 ppm [0, 347, 1740 or 5200 mg/m^3] dichloromethane for 6 h per day on five days per week for 14 weeks and then mated to produce F_1 litters. After weaning, 30 randomly selected pups of each sex and dosage were exposed to dichloromethane for 17 weeks and subsequently mated to produce F_2 litters. Reproductive parameters examined included fertility, litter size, neonatal growth and survival. All adults and selected (10 per group) weanlings were examined for grossly visible lesions. Tissues from the selected weanlings were examined histopathologically. No adverse effects were observed on reproductive parameters, neonatal survival or neonatal growth; there were no gross or histopathological lesions.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 8 for references)

Gene mutations were induced in *Salmonella typhimurium* strains TA100, TA1535 and TA98 exposed to dichloromethane vapour in a closed chamber with or without the addition of exogenous metabolic activation. Glutathione-deficient strains of TA100 (NG 11 and NG 54) were less responsive to the effects of dichloromethane than were the parent strains. Studies using the liquid plate incorporation assay were negative, with the exception of one study which reported positive results in strain TA1535 transfected with rat θ-class GST 5-5+. Dichloromethane also induced mutation in *Escherichia coli* and gene conversion and mutation in *Saccharomyces cerevisiae*. In *Drosophila melanogaster* it did not induce sex-linked recessive lethal mutations.

Dichloromethane induced DNA–protein cross-links *in vitro* in hepatocytes of male B6C3F_1 mice but not in hepatocytes of Fischer 344 rats, Syrian hamsters or in human hepatocytes with functional *GSTT1* genes. DNA–protein cross-links were also induced in Chinese hamster ovary CHO cells exposed to dichloromethane with or without exogenous metabolic activation. DNA damage was greater, however, in the presence of metabolic activation.

Dichloromethane induced DNA single-strand breaks in AP rat primary hepatocytes and B6C3F_1 mouse hepatocytes and Clara cells, but not in Syrian hamster hepatocytes *in vitro.* DNA damage was reduced in Clara cells co-treated with buthionine sulfoximine, a glutathione-depleting agent. In one study, DNA single-strand breaks were increased in CHO cells cultured with dichloromethane in the presence, but not in the absence, of an exogenous metabolic activation system.

When tested in Chinese hamster lung V79 cells in the absence of exogenous metabolic activation, dichloromethane did not induce unscheduled DNA synthesis or *hprt* locus gene mutations but did induce a slight increase in sister chromatid exchange frequencies. It was mutagenic in CHO cells at the *hprt* locus in one study, in the presence of exogenous metabolic activation, and gave equivocal results in the mouse lymphoma $tk^{+/-}$ assay in another study. DNA sequence analysis of the *hprt* mutants of CHO cells treated with dichloromethane indicated that most were GC→AT transitions (4/8), with two GC→CG transversions and two AT→TA transversions. This pattern was more similar to that of 1,2-dibromoethane (ethylene dibromide) (see this volume) (7/9 being GC→AT transitions) than that of formaldehyde, a metabolite of dichloromethane that has been identified *in vitro* (see Section 4.1), for which all mutations were single-base transversions and 5/6 arose from AT base pairs (Graves *et al.*, 1996). Dichloromethane induced chromosomal aberrations in CHO cells in the presence and absence of an exogenous metabolic system in one of two studies, but did not increase sister chromatid exchange frequencies. Virus-infected Fischer rat and Syrian hamster embryo cells were transformed after treatment with dichloromethane *in vitro*. Neither DNA single-strand

Table 8. Genetic and related effects of dichloromethane

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SAF, *Salmonella typhimurium* BA/3, forward mutation, Ara resistance	+	(+)	325	Roldan-Arjona & Pueyo (1993)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	14	Simmon *et al.* (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	19	Jongen *et al.* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	18	Gocke *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	23	Jongen *et al.* (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	95	Green (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	NT	6800	Osterman-Golkar *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)[c]	NT	3700	Hughes *et al.* (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+[c]	+	150	Zeiger (1990)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	8.5	Dillon *et al.* (1992)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	17667	Graves *et al.* (1994)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	34	JETOC (1997)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	300	McGregor (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–[d]	NT	170	Thier *et al.* (1993)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	170	JETOC (1997)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	340	JETOC (1997)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	19	Jongen *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	72	Gocke *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	?[c]	?	1500	Zeiger (1990)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	34	JETOC (1997)

Table 8 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
ECF, *Escherichia coli* NR3835, forward mutation	+	NT	26500	Zielenska *et al*. (1993)
ECK, *Escherichia coli* K12, forward mutation, Rif resistance	–	(+)[e]	5100	Graves *et al*. (1994a)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	+	+	170	JETOC (1997)
ECR, *Escherichia coli* WP2 *uvrA*/pKM101, reverse mutation	+	+	21	Dillon *et al*. (1992)
ECR, *Escherichia coli* WP2 *uvrA*/pKM101, reverse mutation	+	+	170	JETOC (1997)
SCG, *Saccharomyces cerevisiae*, gene conversion	+	NT	13300	Callen *et al*. (1980)
SCH, *Saccharomyces cerevisiae*, homozygosis	+	NT	13300	Callen *et al*. (1980)
SCR, *Saccharomyces cerevisiae*, reverse mutation	+	NT	13300	Callen *et al*. (1980)
TSM, *Tradescantia* species, gene mutation	+	NT	100	Schairer & Sautkulis (1982)
DMX, *Drosophila melanogaster*, sex-linked mutation	–		52600	Gocke *et al*. (1981)
DMX, *Drosophila melanogaster*, sex-linked mutation	–		19.2	Kramers *et al*. (1991)
DIA, DNA–protein cross-links, B6C3F$_1$ mouse hepatocytes *in vitro*	+	NT	43	Casanova *et al*. (1997)
DIA, DNA–protein cross-links, Fischer 344 rat hepatocytes *in vitro*	–	NT	425	Casanova *et al*. (1997)
DIA, DNA–protein cross-links, Syrian hamster hepatocytes *in vitro*	–	NT	425	Casanova *et al*. (1997)
DIA, DNA–protein cross-links, human hepatocytes (expressing *GSTT1-1) in vitro*	–	NT	425	Casanova *et al*. (1997)
DIA, DNA single-strand breaks, B6C3F$_1$ mouse hepatocytes *in vitro*	+	NT	34	Graves *et al*. (1994b)

Table 8 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
DIA, DNA single-strand breaks, AP rat hepatocytes *in vitro*	+	NT	2550	Graves *et al.* (1994b)
DIA, DNA single-strand breaks, Chinese hamster ovary cells *in vitro*	–	+	5100	Graves *et al.* (1994b)
DIA, DNA single-strand breaks, Syrian hamster hepatocytes *in vitro*	–	NT	5100	Graves *et al.* (1995)
DIA, DNA single-strand breaks, $B6C3F_1$ mouse lung Clara cells *in vitro*	+[f]	NT	425	Graves *et al.* (1995)
DIA, DNA single-strand breaks and DNA-protein cross-links, Chinese hamster ovary CHO cells *in vitro*	(+)	+	3975	Graves & Green (1996)
UIA, Unscheduled DNA synthesis, Chinese hamster lung V79 cells *in vitro*	–	NT	65000	Jongen *et al.* (1981)
GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus *in vitro*	–	NT	65000	Jongen *et al.* (1981)
GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus *in vitro*	–	+	3975	Graves & Green (1996)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	52000	Jongen *et al.* (1981)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	?	?	3300	Myhr *et al.* (1990)
SIC, Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	(+)	NT	13000	Jongen *et al.* (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	13000	Thilagar & Kumaroo (1983)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	5000	Anderson *et al.* (1990)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	+	6500	Thilagar & Kumaroo (1983)

Table 8 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	5000	Anderson *et al.* (1990)
TRR, Cell transformation, RLV/Fischer rat	+	NT	14	Price *et al*. (1978)
T7S, Cell transformation, SA7/Syrian hamster embryo cells *in vitro*	+	NT	73	Hatch *et al*. (1982)
DIH, Single-strand breaks, human primary hepatocytes *in vitro*	–	NT	5100	Graves *et al*. (1995)
UHF, Unscheduled DNA synthesis, human AH fibroblasts *in vitro*	–	NT	65000	Jongen *et al*. (1981)
SHL, Sister chromatid exchanges, human lymphocytes *in vitro*	+[g]	NT	290	Hallier *et al*. (1993)
MIH, Micronucleus test, human MCL-5 and h2E1 lymphoblastoid cells *in vitro*	+[h]	NT	200	Doherty *et al*. (1996)
MIH, Micronucleus test, human AHH-1 lymphoblastoid cells *in vitro*	–	NT	850	Doherty *et al*. (1996)
DVA, DNA–protein cross-links, B6C3F$_1$/CrlBR mouse liver *in vivo*	+[i]		4000 ppm inh 6 h/d, 3 d	Casanova *et al*. (1992)
DVA, DNA–protein cross-links, Syrian hamster liver and lung *in vivo*	–		4000 ppm inh 6 h/d 3 d	Casanova *et al*. (1992)
DVA, DNA single-strand breaks, B6C3F$_1$ mouse liver *in vivo*	+		4831 ppm inh 6 h	Graves *et al*. (1994b)
DVA, DNA single-strand breaks, AP rat liver *in vivo*	–		4727 ppm inh 6h	Graves *et al*. (1994b)
DVA, DNA single-strand breaks, CD rat liver *in vivo*	+		1275 po × 1	Kitchin & Brown (1994)
DVA, DNA single-strand breaks, B6C3F$_1$ mouse liver *in vivo*	+[f]		4000 ppm inh 6 h	Graves *et al.* (1995)
DVA, DNA single-strand breaks, B6C3F$_1$ mouse lung *in vivo*	+[f]		2000 ppm inh 3 h	Graves *et al*. (1995)
DVA, DNA single-strand breaks, AP rat lung *in vivo*	–		4000 ppm inh 3 h	Graves *et al*. (1995)

Table 8 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
DVA, DNA–protein cross-links, B6C3F$_1$/CrlBR mouse liver *in vivo*	+		498 ppm inh 6 h/d, 2 d	Casanova *et al*. (1996)
DVA, DNA–protein cross-links, Syrian golden hamster liver *in vivo*	–		3923 ppm inh 6 h/d, 2 d	Casanova *et al*. (1996)
UPR, Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	–		1000 po × 1	Trueman & Ashby (1987)
UPR, Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	–		4000 ppm inh 6 h	Trueman & Ashby (1987)
UVM, Unscheduled DNA synthesis, B6C3F$_1$ mouse liver *in vivo*	–		4000 ppm inh 6 h	Trueman & Ashby (1987)
SVA, Sister chromatid exchange, B6C3F$_1$ mouse lung cells *in vivo*	+[j]		2000 ppm inh 6 h/d, 5 d/wk 12wk	Allen *et al*. (1990)
SVA, Sister chromatid exchange, B6C3F$_1$ mouse bone marrow *in vivo*	–		5000 sc × 1	Allen *et al*. (1990)
SVA, Sister chromatid exchange, C57BL/6J mouse bone marrow *in vivo*	–		1500 ip × 1	Westbrook-Collins *et al*. (1990)
MVM, Micronucleus test, NMRI mouse bone marrow *in vivo*	–		1700 ip × 2	Gocke *et al*. (1981)
MVM, Micronucleus test, C57BL/6J/Alpk mouse bone marrow *in vivo*	–		4000 po × 1	Sheldon *et al*. (1987)
MVM, Micronucleus test, B6C3F$_1$ mouse erythrocytes *in vivo*	(+)[j]		2000 ppm inh 6h/d, 5 d/wk 12 wk	Allen *et al*. (1990)
MVM, Micronucleus test, CD-1 mouse bone marrow *in vivo*	–		1720 ip × 1	Morita *et al*. (1997)
CBA, Chromosomal aberrations, Sprague-Dawley rat bone marrow *in vivo*	–		3500 ppm inh 6 h/d, 5 d/wk, 2 y	Burek *et al*. (1984)

Table 8 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
CBA, Chromosomal aberrations, B6C3F$_1$ mouse bone marrow *in vivo*	(+)		8000 ppm inh 6 h/d, 5 d/wk, 2 wk	Allen *et al*. (1990)
CBA, Chromosomal aberrations, B6C3F$_1$ mouse bone marrow *in vivo*	–		5000 sc × 1	Allen *et al*. (1990)
CBA, Chromosomal aberrations, C57BL/6J mouse bone marrow *in vivo*	–		1500 ip × 1	Westbrook-Collins *et al*. (1990)
CVA, Chromosomal aberrations, B6C3F$_1$ mouse lung cells *in vivo*	(+)		8000 ppm inh 6 h/d, 5 d/wk, 2 wk	Allen *et al*. (1990)
BVD, DNA binding, rat or mouse liver, lung, or kidney *in vivo*	–		NG	Ottenwalder & Peter (1989)

[a] +, positive; (+), weakly positive; -, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL (in bacterial tests, cells were exposed to dichloromethane vapour, so dose = µg/mL in atmosphere); in-vivo tests, mg/kg bw /day; inh, inhalation; po, oral; sc, subcutaneous; ip, intraperitoneal; NG, not given

[c] Negative in liquid plate incorporation assay

[d] Liquid plate incorporation assay; cells transfected with rat GST 5-5+ were positive at 42 µg/mL

[e] Positive with mouse liver S9, negative with rat liver S9

[f] Pre- or co-treatment with buthionine sulfoximine, a GSH depletor, caused a decrease in DNA damage

[g] Positive results were reported in lymphocytes from donors lacking GST activity

[h] Induction of kinetochore-positive and -negative micronuclei

[i] Negative in mouse lung

[j] The highest dose tested (8000 ppm 6 h/d, 5 d/w × 2 wk) was positive in erythrocytes and lung cells but negative in bone marrow

[k] Negative in lung cells at this dose; positive in erythrocytes after exposure to 8000 ppm 6 h/d [10 000 mg/kg bw], 5d/wk × 2 wk

breaks nor unscheduled DNA synthesis were induced in human primary hepatocytes or AH fibroblasts, respectively, following dichloromethane treatment. Sister chromatid exchanges were induced in human peripheral blood lymphocyte cultures but only in those from donors lacking GST activity towards methyl bromide. In a single study, dichloromethane induced kinetochore-staining micronuclei (which are indicative of aneuploidy) and kinetochore-negative micronuclei in human MCL-5 cells that stably express cDNA encoding human CYP1A2, CYP2A6, CYP3A4, CYP2E1 and epoxide hydrolase and in h2E1 cells, which contains a cDNA for CYP2E1. AHH-1 cells constitutively expressing CYP1A1 showed neither an increase in total micronucleus frequencies nor kinetochore-staining micronuclei.

DNA–protein cross-links were induced in the liver but not the lung of $B6C3F_1$/CrlBR mice exposed to dichloromethane. No DNA–protein cross-links were detected in Syrian hamster liver or lung after inhalation. Inhalation exposure of $B6C3F_1$ mice to dichloromethane induced DNA single-strand breaks in both lung and liver. Prior treatment of the mice with buthionine sulfoximine immediately before dichloromethane exposure reduced the amount of DNA damage to control levels. DNA single-strand breaks were not induced in liver or lung of AP rats but were seen in liver of CD rats treated by gavage. Dichloromethane did not induce unscheduled DNA synthesis in Fischer 344 rat or $B6C3F_1$ mouse hepatocytes *in vivo*. In a single study, mice treated with 2000 ppm [6940 mg/m^3] dichloromethane for 6 h per day on five days per week for 12 weeks showed an increased sister chromatid exchange frequency in lung cells and an increased frequency of micronuclei in peripheral blood erythrocytes. Exposure to higher concentrations (8000 ppm [27 800 mg/m^3] for two weeks) also induced an increase in sister chromatid exchange frequency in peripheral blood erythrocytes. Dichloromethane did not induce sister chromatid exchanges, micronuclei or chromosomal aberrations in bone marrow of mice treated by gavage or intraperitoneal or subcutaneous injection. A small increase in chromosomal aberrations in mouse bone marrow and lung cells was reported in one study following inhalation exposure to 8000 ppm dichloromethane for 6 h per day on five days per week for two weeks. Dichloromethane gave negative results in the rat bone-marrow chromosomal aberration assay. Covalent binding of dichloromethane to DNA was not observed in liver, kidney or lung of rats or mice exposed by inhalation, although metabolic incorporation of ^{14}C was found in normal deoxyribonucleosides in both species.

4.5 Mechanistic considerations

The currently available data lead to the suggestion that it is metabolism via the glutathione pathway, and not the cytochrome P450 pathway, that is related to the liver and lung carcinogenicity of dichloromethane in mice. This hypothesis is supported by the observations that the major species differences correspond to those of GST distribution and activity, and that the dose-dependent behaviour of the two pathways is consistent with the results of the carcinogenicity bioassays. For example, at the two high dose levels (2000 and 4000 ppm [6940 and 13 900 mg/m^3]) used in the National Toxicology Program

(NTP) mouse bioassay, the GST pathway would predominate and liver and lung tumour incidence was increased. Conversely, in the mouse drinking-water study where, presumably, lower blood levels of dichloromethane would be reached than in the inhalation bioassay, the dichloromethane would have been metabolized primarily via cytochrome P450, while GST-mediated metabolism would have been minimal (predicted by pharmacokinetic modelling to be two orders of magnitude lower than at the high dose used in the NTP study; Andersen *et al*., 1987), and no increased incidence of tumours was observed.

A variety of in-vivo (at dose levels used in the NTP studies) and in-vitro experiments using mouse hepatocytes and Clara cells have revealed DNA damage when the animals or cells were exposed to dichloromethane (Graves *et al*., 1994b, 1995). Cells depleted of glutathione, either *in vitro* or *in vivo*, had decreased DNA damage, thus strengthening the link with the GST pathway. DNA damage was not observed in hamster or human hepatocytes exposed to dichloromethane. This is consistent with the observed species differences in the degree of expression and the pattern of distribution of GSTT1-1, the enzyme responsible for the metabolic activation of dichloromethane.

A link has also been established between metabolism of dichloromethane by GST and mutagenicity in bacteria and Chinese hamster ovary (CHO) cells. Depletion of glutathione (*Salmonella typhimurium* and CHO cells) or expression of a rat GST (*S. typhimurium*) decreased or increased their mutagenicity respectively (Thier *et al*., 1993; Graves *et al*., 1994b). When liver subcellular fractions were used in these assays, only cytosol (GST), and not microsomes, supported the bioactivation of dichloromethane. Generally, throughout the mutagenicity assays, a good correlation is evident between glutathione and/or GST activity and genotoxicity (Table 9). Studies with CHO cells, measuring strand breaks and mutations at the *hprt* gene, suggest that the DNA damage was caused by *S*-chloromethylglutathione (Graves & Green, 1996; Graves *et al*., 1996).

DNA–protein cross-links caused by formaldehyde, a metabolite from the GST pathway, have been demonstrated in mice but not hamsters exposed to dichloromethane (Casanova *et al*., 1992). Similarly, in-vitro studies have not demonstrated DNA–protein cross-links in rat, hamster or human hepatocytes exposed to concentrations of dichloromethane of up to 5 mM. This is equivalent to the time-weighted average concentration predicted to occur in mouse liver during a 6-h inhalation exposure to a dichloromethane concentration of > 10 000 ppm [34 700 mg/m^3] (Casanova *et al.*, 1997).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Dichloromethane is used principally as a solvent, in paint removers, degreasers and aerosol products, and in the manufacture of foam polymers. Widespread exposure occurs during the production and industrial use of dichloromethane and during the use of a variety of consumer products containing dichloromethane. Substantial losses to the environment lead to ubiquitous low-level exposures from ambient air and water.

Table 9. Relationship of glutathione *S*-transferase (GST) status and dichloromethane-mediated DNA damage

System	GST-mediated metabolism of dichloro-methane	DNA damage without exogenous metabolic activation	Accurate prediction of DNA damage from GST status	Comments	Reference
Salmonella typhimurium BA13	ND	+	?		Roldan-Arjona & Pueyo (1993)
Salmonella typhimurium TA100	+	+	Yes	TA100 metabolizes dichloromethane	Simmon *et al*. (1977)
Salmonella typhimurium TA100	+	+	Yes		Jongen *et al*. (1978)
Salmonella typhimurium TA100	+	+	Yes		Gocke *et al*. (1981)
Salmonella typhimurium TA100	+	+	Yes		Jongen *et al*. (1982)
Salmonella typhimurium TA100	+	+	Yes		Green (1983)
Salmonella typhimurium TA100	+	(+)	(Yes)		Osterman-Golkar *et al.* (1983)
Salmonella typhimurium TA100	+	(+)	(Yes)		Hughes *et al*. (1987)
Salmonella typhimurium TA100	+	+	Yes		Zeiger (1990)
Salmonella typhimurium TA100	+	+	Yes		Dillon *et al.* (1992)
Salmonella typhimurium TA100	+	+	Yes		Graves *et al.* (1994a)
Salmonella typhimurium TA100	+	+	Yes		JETOC (1997)
Salmonella typhimurium TA1535	ND	+	?		McGregor (1979)
Salmonella typhimurium TA1535	ND	–	?		Thier *et al*. (1993)
Salmonella typhimurium TA1535+ transfected GST5-5	+	+	Yes	Transfected GST5-5 increased response	Thier *et al*. (1993)
Salmonella typhimurium TA1535	ND	+	?		JETOC (1997)
Salmonella typhimurium TA1537	ND	–	?		JETOC (1997)
Salmonella typhimurium TA98	ND	+	?		Jongen *et al*. (1978)
Salmonella typhimurium TA98	ND	+	?		Gocke *et al*. (1981)

Table 9 (contd)

System	GST-mediated metabolism of dichloro-methane	DNA damage without exogenous metabolic activation	Accurate prediction of DNA damage from GST status	Comments	Reference
Salmonella typhimurium TA98	ND	?	?		Zeiger (1990)
Salmonella typhimurium TA98	ND	+	?		JETOC (1997)
Escherichia coli NR3835	ND	+	?		Zielenska *et al*. (1994)
Escherichia coli K12	ND	+	?		Graves *et al*. (1994a)
Escherichia coli WP2/pKM101	ND	+	?		Dillon *et al*. (1992)
Escherichia coli WP2	ND	+	?		JETOC (1997)
Escherichia coli WP2/pKM101	ND	+	?		JETOC (1997)
Saccharomyces cerevisiae D7	ND	+	?		Callen *et al*. (1980)
Tradescantia	ND	+	?		Schairer & Sauttkulis (1982)
Drosophila melanogaster	ND	–	?		Gocke *et al*. (1981)
Drosophila melanogaster	ND	–	?		Kramers *et al*. (1991)
Single strand breaks, B6C3F$_1$ mouse hepatocytes *in vitro*	+	+	Yes	Deplete GSH and DNA damage decreases	Graves *et al*. (1994b)
Single strand breaks, AP rat hepatocytes *in vitro*	–	+	No	Very high dose (> 30 mM)	Graves *et al*. (1994b)
Single strand breaks, Chinese hamster ovary CHO cells				Not tested	Graves *et al*. (1994b)
Single strand breaks, B6C3F$_1$ Clara cells *in vitro*	+	+	Yes	Buthionine sulfo-ximine decreased DNA damage	Graves *et al*. (1995)
Single strand breaks and DNA-protein cross-links, CHO cells	+	(+)	(Yes)		Graves & Green (1996)

Table 9 (contd)

System	GST-mediated metabolism of dichloro-methane	DNA damage without exogenous metabolic activation	Accurate prediction of DNA damage from GST status	Comments	Reference
Unscheduled DNA synthesis, Chinese hamster V79 cells *in vitro*	ND	–	?		Jongen *et al*. (1981)
Gene mutation, CHO cells *hprt* locus	–	–	Yes		Jongen *et al*. (1981)
Gene mutation, CHO cells *hprt* locus	–	–	Yes		Graves & Green (1996)
Gene mutation, Chinese hamster V79 cells *hprt* locus	ND	–	?		Jongen *et al*. (1981)
Gene mutation, mouse lymphoma L5178Y cells *tk* locus	ND	?	?		Myrh *et al*. (1990)
Sister chromatid exchange, Chinese hamster V79 cells	ND	–	?		Jongen *et al*. (1981)
Sister chromatid exchange, CHO cells	–	–	Yes		Thilagar & Kumaroo (1983)
Sister chromatid exchange, CHO cells	–	–	Yes		Anderson *et al.* (1990)
Chromosomal aberrations, CHO cells	–	+	No		Thilagar & Kumaroo (1983)
Chromosomal aberrations, CHO cells	–	–	Yes		Anderson *et al.* (1990)
Cell transformation, RLV/F344 cells	ND	+	?		Price *et al*. (1978)
Cell transformation, SA7/Syrian hamster cells	ND	+	?		Hatch *et al*. (1982)
Single strand breaks, human hepatocytes *in vitro*	–	–	Yes		Graves *et al*. (1995)
Unscheduled DNA synthesis, human AH fibroblasts	ND	–	?		Jongen *et al*. (1981)

Table 9 (contd)

System	GST-mediated metabolism of dichloro-methane	DNA damage without exogenous metabolic activation	Accurate prediction of DNA damage from GST status	Comments	Reference
Sister chromatid exchange, human lymphocytes *in vitro*	–	+	No		Hallier *et al*. (1993)
Micronucleus test, human MCL5 cells	ND	+	?		Doherty *et al*. (1996)
Micronucleus test, human AHH-1 cells	ND	–	?		Doherty *et al*. (1996)
DNA–protein cross-links, B6C3F$_1$ mouse liver *in vivo*	+	+	Yes		Casanova *et al*. (1992)
Single strand breaks, B6C3F$_1$ mouse liver *in vivo*	+	+	Yes		Graves *et al*. (1994b)
Single strand breaks, AP rat liver *in vivo*	–	–	Yes		Graves *et al*. (1994b)
Single strand breaks, AP rat liver *in vivo*	–	+	No	Large po dose, small response	Kitchin & Brown (1994)
Single strand breaks, B6C3F$_1$ mouse lung and liver *in vivo*	+	+	Yes	Buthionine sulfoximine decreased DNA damage	Graves *et al*. (1995)
Single strand breaks, AP rat lung *in vivo*	–	–	Yes		Graves *et al*. (1995)
Unscheduled DNA synthesis, F344 rat hepatocytes *in vivo*	ND	–			Trueman & Ashby (1987)
Unscheduled DNA synthesis, B6C3F$_1$ mouse liver *in vivo*	ND	–			Trueman & Ashby (1987)
Sister chromatid exchange, B6C3F$_1$ mouse lung *in vivo*	+	+	Yes		Allen *et al*. (1990)
Sister chromatid exchange, B6C3F$_1$ mouse bone marrow *in vivo*	ND	–	?		Allen *et al*. (1990)
Sister chromatid exchange, C57BL/6J mouse bone marrow	ND	–	?		Westbrook-Collins *et al*. (1990)

Table 9 (contd)

System	GST-mediated metabolism of dichloromethane	DNA damage without exogenous metabolic activation	Accurate prediction of DNA damage from GST status	Comments	Reference
Micronucleus test, NMRI mouse bone marrow	ND	–	?		Gocke *et al*. (1981)
Micronucleus test, C57BL/6J mouse bone marrow	ND	–	?		Sheldon *et al*. (1987)
Micronucleus test, $B6C3F_1$ mouse erythrocytes *in vivo*	ND	(+)	?		Allen *et al*. (1990)
Chromosomal aberrations, CD rat bone marrow	ND	–	?		Burek *et al*. (1984)
Chromosomal aberrations, $B6C3F_1$ mouse bone marrow	ND	(+)	?		Allen *et al*. (1990)
Chromosomal aberrations, $B6C3F_1$ mouse bone marrow	ND	–	?		Allen *et al*. (1990)
Chromosomal aberrations, C57BL/6J mouse bone marrow	ND	–	?		Westbrook Collins *et al*. (1990)
Chromosomal aberrations, $B6C3F_1$ mouse lung	+	(+)	(Yes)		Allen *et al*. (1990)

Additionally *Salmonella typhimurium* TA100 strains (NG11 and NG54) are deficient in glutathione and were less responsive than their parent strains to mutagenesis induced by dichloromethane.
Parentheses mean weak response/poor correlation.
ND, no data available; po, oral
–/+, in GST status column; –, absence/or presence of GST; ( ), –/+, in DNA damage column, as in Table 8

5.2 Human carcinogenicity data

Seven cohort studies have examined the risk of cancer among populations exposed to dichloromethane. Two studies observed an excess of pancreatic cancer, but the three others which reported on this tumour did not. One study observed an excess of liver and biliary tract cancers among longer-term employees. One study observed an excess of prostate cancer that appeared to increase with level of exposure. One study observed an excess of breast cancer and gynaecological cancers among women with the highest likelihood of exposure and another study observed an excess of cervical cancer. With the exception of the prostate cancer excess observed in one study, all the excesses were based on small numbers. No estimates of exposure levels were available for two of the six studies.

Three case–control studies have examined the risk of cancer associated with dichloromethane exposure and provided data adequate for evaluation. One observed an association between estimated intensity, probability and duration of exposure and the risk of astrocytic brain tumours. A second, which focused on female breast cancer, observed an elevated risk in the highest exposure category but no association with probability of exposure. The third indicated an increased risk of rectal cancer and possibly lung cancer.

For no type of cancer was there a sufficiently consistent elevation of risk across studies to make a causal interpretation credible.

5.3 Animal carcinogenicity data

Dichloromethane was tested by oral administration in the drinking-water in one study in mice and one study in rats, by inhalation exposure in two studies in mice, three studies in rats and one study in hamsters and by intraperitoneal injection in a lung adenoma assay in mice. In the study in mice by oral administration, no increase in tumour incidence was observed. The study in rats by oral administration gave inconclusive results. In the two inhalation studies in mice, increased incidences of benign and malignant lung and liver tumours were observed in both sexes. In the three inhalation studies in rats, the incidence of benign mammary tumours was increased in one study in females of a strain in which the incidence of spontaneous mammary tumours is low, and the multiplicity was increased in two studies in females of a high-incidence strain. In one study, in males, the incidence of mammary gland adenomas and fibroadenomas was increased. Negative results were obtained in the lung adenoma test in mice and in the inhalation study in hamsters.

5.4 Other relevant data

Two dose-dependent alternative pathways involving cytochrome P450 and glutathione *S*-transferases are responsible for the metabolism of dichloromethane in human and rodent cells.

Dichloromethane is consistently mutagenic in microorganisms. Weaker and less consistent responses are seen in mammalian systems, predominantly in mice, both *in vitro* and *in vivo*.

It induced sister chromatid exchanges, chromosome breakage and chromosome loss *in vitro* in human cells. In-vitro results in rodent cells were inconclusive or negative.

Dichloromethane induced DNA single-strand breaks in mammalian cell cultures, but inconclusive or negative effects were reported for induction of gene mutations. It did not induce unscheduled DNA synthesis either *in vivo* in rodents or in human fibroblast cultures. It was genotoxic in fungi but not in *Drosophila* in the sex-linked recessive lethal assay.

Mechanistic studies have established a link between glutathione *S*-transferase-mediated metabolism of dichloromethane and its genotoxicity and carcinogenicity in mice. The glutathione *S*-transferase responsible for the metabolism of dichloromethane is expressed to significantly greater extents in mouse tissues than in rat, hamster or human tissues.

The available data suggest a plausible mechanism for the development of liver and lung tumours which occur in mice but not in rats exposed to dichloromethane.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of dichloromethane.

There is *sufficient evidence* in experimental animals for the carcinogenicity of dichloromethane.

Overall evaluation

Dichloromethane is possibly carcinogenic to humans (Group 2B).

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Part Two
Other Compounds Reviewed in Plenary Sessions

ACETALDEHYDE

Data were last reviewed in IARC (1985) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

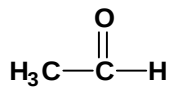
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 75-07-0
Chem. Abstr. Name: Acetaldehyde
IUPAC Systematic Name: Acetaldehyde
Synonyms: Acetic aldehyde; 'aldehyde'; ethanal; ethylaldehyde

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_2H_4O Relative molecular mass: 44.05

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid or gas with a characteristic pungent odour (Budavari, 1996; Verschueren, 1996)
(*b*) *Boiling-point*: 20.1°C (Lide, 1997)
(*c*) *Melting-point*: –123°C (Lide, 1997)
(*d*) *Solubility*: Miscible with water, benzene, diethyl ether and ethanol (Budavari, 1996; Lide, 1997)
(*e*) *Vapour pressure*: 98 kPa at 20°C; relative vapour density (air = 1), 1.52 (Verschueren, 1996)
(*f*) *Reactivity*: Flammable; polymerizes violently in the presence of trace amounts of metals or acids; can react violently with acid anhydrides, alcohols, ketones, phenols, ammonia, hydrocyanic acid, hydrogen sulfide, halogens, phosphorus, isocyanates, strong alkalis and amines (American Conference of Governmental Industrial Hygienists, 1991)
(*g*) *Flash-point*: –38°C, closed cup; –40°C, open cup (American Conference of Governmental Industrial Hygienists, 1991; Budavari, 1996)

(*h*) *Explosive limits*: Upper, 57%; lower, 4% by volume in air (American Conference of Governmental Industrial Hygienists, 1991)
(*i*) *Octanol/water partition coefficient (P)*: log *P*, 0.43 (Verschueren, 1996)
(*j*) *Conversion factor*: $mg/m^3 = 1.80 \times ppm$

1.2 Production and use

Production capacity for acetaldehyde in the United States in 1989 was 443 000 tonnes/year (Hagemeyer, 1991). Information available in 1995 indicated that it was produced in 16 countries (Chemical Information Services, 1995).

Acetaldehyde is used as an intermediate in the production of acetic acid, acetic anhydride, cellulose acetate, vinyl acetate resins, acetate esters, pentaerythritol, synthetic pyridine derivatives, terephthalic acid and peracetic acid. Synthetic pyridine derivatives, peracetic acid, acetate esters and pentaerythritol account for 40% of acetaldehyde demand (Hagemeyer, 1991). Other uses of acetaldehyde include: in the silvering of mirrors; in leather tanning; as a denaturant for alcohol; in fuel mixtures; as a hardener for gelatin fibres; in glue and casein products; as a preservative for fish and fruit; in the paper industry; as a synthetic flavouring agent; and in the manufacture of cosmetics, aniline dyes, plastics and synthetic rubber (American Conference of Governmental Industrial Hygienists, 1991; United States National Library of Medicine, 1998).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 220 000 workers in the United States were potentially exposed to acetaldehyde (see General Remarks). Occupational exposure to acetaldehyde may occur in its production, in the production of acetic acid, acetate esters and other chemicals and in other applications.

1.3.2 *Environmental occurrence*

Acetaldehyde is a natural product of combustion and photo-oxidation of hydrocarbons commonly found in the atmosphere. It is an important industrial chemical and may be released into the air or in wastewater during its production and use. It has been detected at low levels in drinking-water, surface water, rainwater, effluents, engine exhaust and ambient and indoor air samples. It is also photochemically produced in surface water. Acetaldehyde is an intermediate product in the metabolism of ethanol and sugars and therefore occurs in trace quantities in human blood. It is present in small amounts in all alcoholic beverages, such as beer, wine and spirits and in plant juices and essential oils, roasted coffee and tobacco smoke (Jira *et al*., 1985; Hagemeyer, 1991; United States National Library of Medicine, 1998).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not recommended an 8-h time-weighted average threshold limit value but has recommended 45 mg/m^3 as the ceiling value for occupational exposures to acetaldehyde in

workplace air. Values of 5–200 mg/m^3 for time-weighted averages have been used as standards or guidelines in other countries (International Labour Office, 1991).

No international guideline for acetaldehyde in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

2.1 Case series

In a survey of chemical plants (without prior hypothesis) in the German Democratic Republic, nine cancer cases were found in a factory where the main process was dimerization of acetaldehyde and where the main exposures were to acetaldol (3-hydroxybutanal), acetaldehyde, butyraldehyde, crotonaldehyde (IARC, 1995) and other higher, condensed aldehydes, as well as to traces of acrolein (IARC, 1985). Of the cancer cases, five were bronchial tumours and two were carcinomas of the oral cavity. All nine patients were smokers. The relative frequencies of these tumours were reported to be higher than those expected in the German Democratic Republic. [The Working Group noted the mixed exposure, the small number of cases and the poorly defined exposed population.]

2.2 Case–control studies

Acetaldehyde is the main metabolite of ethanol and this reaction is catalysed by alcohol dehydrogenases (ADH). Five ADHs have been characterized in humans, two of which (ADH2 and ADH3), are known to be polymorphic. In particular, polymorphism for ADH3 seems to strongly influence the metabolism of ethanol to acetaldehyde, with ADH_3^1 allele carriers being faster metabolizers than ADH_3^2 carriers. Acetaldehyde is metabolized by phase II enzymes, including aldehyde dehydrogenases (ALDH) and glutathione *S*-transferases (GST). ALDH2 is polymorphic; its mutant allele, $ALDH_2^2$, which leads to enzyme inactivity, is prevalent in Asian populations. GSTM1 is also polymorphic, with a null genotype $GSTM_1^0$ present mainly in European populations (Coutelle *et al.*, 1997). Therefore, carriers of ADH_3^2, $ALDH_2^2$ and $GSTM_1^0$ alleles are likely to be exposed to higher levels of acetaldehyde than are other people, following intake of a comparable amount of alcohol.

A Japanese case–control study (Yokoyama *et al*., 1996) of ALDH2-related risk for oesophageal squamous-cell carcinoma in alcoholics (40 cases and 55 controls) and non-alcoholic drinkers (29 cases and 28 controls) during 1991–95 showed a higher risk for oesophageal cancer in those with one $ALDH_2^2$ allele in both alcoholics (crude odds ratio, 7.6; 95% confidence interval (CI), 2.8–20.7) and non-alcoholic drinkers (odds ratio, 12.1; 95% CI, 3.4–42.8). Mantel–Haenszel adjustment for age and daily alcohol consumption had virtually no influence on the risk estimates [adjusted odds ratios not given]. As persons who have the mutant $ALDH_2^2$ allele have a high concentration of blood acetaldehyde after drinking alcohol, the results of this study were interpreted as strongly suggesting a carcinogenic role of acetaldehyde in humans.

As part of a population-based study of oral cancer (oral cavity and pharynx) in Puerto Rico in 1992–95, the alcohol dehydrogenase type 3 (ADH3) genotype was determined in 137 patients and 146 controls without cancer by molecular genetic analysis of oral epithelial cell samples (Harty *et al*., 1997). Participation rates were 48% among cases and 57% among controls. After adjustment for tobacco smoking, diet and alcohol drinking, the odds ratio for the ADH_3^{1-2} genotype was 0.7 (95% CI, 0.4–1.3) and that for the ADH_3^{2-2} genotype was 0.6 (95% CI, 0.3–1.6), using the ADH_3^{1-1} genotype as reference category. When non-drinkers with the ADH_3^{1-1} genotype were used as reference, the risk among drinkers of 57 or more drinks per week was modified by the ADH3 genotype: odds ratios were 40.1 (95% CI, 5.4–296), 7.0 (95% CI, 1.4–35.0) and 4.4 (95% CI, 0.7–33.3) for ADH_3^{1-1}, ADH_3^{1-2} and ADH_3^{2-2}, respectively. For lower alcohol consumption, the risks were not or only moderately elevated, without a clear pattern according to genotype. [The Working Group noted the low participation rate.]

Coutelle *et al*. (1997) conducted a case–control study in France among male heavy drinkers (more than 100 g of alcohol per day for more than 10 years). They included 21 cases of oral and pharyngeal cancer, 18 cases of laryngeal cancer and 37 heavy drinkers recruited in an alcoholism clinic. As compared to ADH_3^{1-1} or ADH_3^{2-2}, the ADH_3^{1-2} genotype was associated with an age-adjusted odds ratio of 2.6 (95% CI, 0.7–10.0) for oropharyngeal cancer and 6.1 (95% CI, 1.3–28.6) for laryngeal cancer. The GSTM1 null genotype had an odds ratio of 1.8 (95% CI, 0.5–6.2) for oropharyngeal cancer and 4.7 (95% CI, 1.0–21.8) for laryngeal cancer. The combination of ADH_3^{1-1} and GSTM1 null genotypes, as compared to the combination of ADH_3^{1-2} or ADH_3^{2-2} and GSTM1 non-null, gave an odds ratio of 4.3 (95% CI, 0.6–28.8) for oropharyngeal cancer and 12.9 (95% CI, 1.8–92.0) for laryngeal cancer.

In an abstract, Freudenheim *et al*. (1997) presented the results of a study conducted in western New York, United States, on 134 premenopausal and 181 postmenopausal cases of breast cancer and 356 population controls. Heavy alcohol intake was associated with an increased risk for premenopausal breast cancer (odds ratio, 3.5; 95% CI, 1.3–9.2) among ADH_3^{1-1} subjects but not among women with ADH_3^{1-2} or ADH_3^{2-2} genotypes. This association was not observed for postmenopausal breast cancer.

3. Studies of Cancer in Experimental Animals

Acetaldehyde was tested for carcinogenicity in rats by inhalation exposure and in hamsters by inhalation exposure and intratracheal instillation. Following inhalation exposure, an increased incidence of carcinomas was induced in the nasal mucosa of rats, and laryngeal carcinomas were induced in hamsters. In another inhalation study in hamsters, using a lower exposure level, and in an intratracheal instillation study, no increased incidence of tumours was observed. In hamsters, inhalation of acetaldehyde enhanced the incidence of respiratory-tract tumours produced by intratracheal instillation of benzo[*a*]-pyrene (IARC, 1985).

3.1 Inhalation exposure

Rat: In a study summarized from a preliminary report in the previous monograph, four groups of 105 male and 105 female Cpb:WU albino Wistar rats, six weeks of age, were exposed by whole-body inhalation to concentrations of 0, 750, 1500 or 3000 (reduced progressively over a period of 11 months to 1000 ppm due to toxicity) ppm [0, 1350, 2700 or 5400–1800 mg/m^3] acetaldehyde vapour [purity unspecified] for 6 h per day on five days per week for a maximum of 27 months. Each group comprised five subgroups, three of which were used for interim kills at weeks 13, 26 and 52, respectively. Of the animals killed at these intervals, only one had a tumour of the respiratory tract: a female in the high-dose group killed in week 53, bearing a nasal squamous-cell carcinoma. At day 468, the mortality rate in the high-dose group was 50% (28/55) for males and 42% (23/55) for females. By day 715, all high-dose rats had died and, at termination of the study at day 844, only a few animals were still alive in the mid-dose group. At the end of the study, the incidences of nasal carcinomas (carcinomas *in situ*, squamous-cell carcinomas and adenocarcinomas) were in males: 1/49, 17/52, 41/53 and 37/49 in the control, low-, mid- and high-dose groups, respectively; and in females: 0/50, 6/48, 34/53 and 43/53 in the control, low-, mid- and high-dose groups, respectively. One carcinoma *in situ* of the larynx was found in a female of the mid-dose group and one female of the low-dose group developed a poorly differentiated adenocarcinoma in the lung (Woutersen *et al*., 1986).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Human subjects retained 45–70% of acetaldehyde inhaled either orally or nasally.

N-Nitroso-2-methylthiazolidine 4-carboxylic acid (*cis*- and *trans*-isomers) was frequently detected in the urine of human subjects; a fraction of this may be formed as a two-step synthesis *in vivo* from acetaldehyde and L-cysteine to yield 2-methylthiazolidine 4-carboxylic acid, which is easily nitrosated (IARC, 1985).

4.1.2 *Experimental systems*

Acetaldehyde is oxidized to acetic acid by NAD^+-dependent aldehyde dehydrogenases (ALDH) in liver and nasal mucosal preparations. Its administration to rats causes an increase in urinary excretion of sulfur metabolites and it is known to react with cysteine to produce a thiazolidine 4-carboxylic acid derivative that can be *N*-nitrosated *in vivo* upon co-administration of nitrite (IARC, 1985). Many studies have been published subsequently, but these have been mainly in the context of ethanol metabolism.

Six dogs were each given a single 600 mg/kg bw dose of acetaldehyde by stomach tube. In two dogs, the maximum plasma concentration was reached after 15 min, while in

the others plasma acetaldehyde was either close to the limit of detection (2 ng/μL) or was not detectable. Urinary recovery of acetaldehyde was < 0.02% of the dose (Booze & Oehme, 1986).

The oxidation of acetaldehyde to acetic acid has been studied with NAD-linked ALDH purified from human, rat and Syrian hamster liver (Klyosov *et al*., 1996). The mitochondrial enzymes from these species have very similar kinetic properties, whereas human cytosolic ALDH1 has a K_m value of about 180 μM, compared with 15 μM and 12 μM for rats and hamsters, respectively. Apparently, in human liver, only mitochondrial ALDH oxidizes acetaldehyde at physiological concentrations, whereas both mitochondrial and cytosolic ALDHs of rodents can participate in acetaldehyde metabolism. The rodent cytosolic ALDHs are at least 10 times more sensitive that the human enzyme to inhibition by disulfiram.

In addition to forming adducts with cytosine and purine-containing nucleotides (IARC, 1985), acetaldehyde has been shown to form stable, cyclic imidazolidinones with the N-terminal valine of the α and β chains of haemoglobin (San George & Hoberman, 1986).

4.2 Toxic effects

4.2.1 *Humans*

The irritant effect of acetaldehyde vapour, which is reported to cause coughing and a burning sensation in the nose, throat and eyes, usually prevents exposure to a level sufficient to cause depression of the central nervous system. A splash of liquid acetaldehyde was reported to cause a burning sensation, lachrymation and blurred vision. Prolonged periods of contact with the skin result in erythema and burns; repeated contact may result in dermatitis, due either to primary irritation or to sensitization.

Intravenous infusion of 5% acetaldehyde [purity unspecified] at a rate of 20.6–82.4 mg/min for up to 36 min into normal human subjects caused an increase in heart rate, ventilation and dead space, and a decrease in alveolar carbon dioxide levels. These symptoms are qualitatively and quantitatively similar to those seen after ethanol intake in subjects previously treated with disulfiram (Antabuse), a known inhibitor of ALDH (IARC, 1985).

4.2.2 *Experimental systems*

Inhalation of acetaldehyde for four weeks by rats caused some degeneration of the nasal epithelium; a concentration of 400 ppm [720 mg/m^3] produced a slight degeneration of the olfactory epithelium. A similar concentration had no effect upon Syrian hamsters (IARC, 1985). The toxicology of acetaldehyde has been reviewed (Von Burg & Stout, 1991).

In the study by Booze and Oehme (1986) described above, all the dogs given a single 600 mg dose of acetaldehyde by stomach tube vomited and this condition lasted for several hours. The two dogs with the highest plasma levels of acetaldehyde developed slight tremors, but all dogs appeared to be normal 24 h after dosing.

Groups of weanling male and female Wistar rats were given acetaldehyde in the drinking-water to provide doses of 0, 25, 125 and 675 mg/kg bw per day for four weeks.

Food and water consumption was reduced and slight to moderate hyperkeratosis of the forestomach was observed in both sexes at the highest dose level (Til *et al*., 1988).

Male Wistar rats exposed to 243 ppm [437 mg/m^3] acetaldehyde atmospheres for 8 h per day on five days per week for five weeks showed increases in functional residual capacity, residual volume, total lung capacity and respiratory frequency. These changes were interpreted as being caused by damage to the peripheral regions of the lung parenchyma (Saldiva *et al*., 1985).

The progression and regression of nasal lesions were studied in groups of 30 male and 30 female Wistar rats exposed to acetaldehyde by inhalation for 6 h per day on five days per week at concentrations of 0, 750, 1500 and 3000 ppm [0, 1350, 2700 and 5400 mg/m^3] (the last dose was gradually reduced to 1500 ppm from week 20 to week 44) for 52 weeks. The animals were killed after recovery periods of 26 or 52 weeks. The main treatment-related effects included (1) focal basal cell hyperplasia of the olfactory epithelium in 750- and 1500-ppm group rats, (2) hyperplasia and metaplasia of the respiratory epithelium, often accompanied by keratinization and sometimes by proliferation of atypical basal cells, in 3000-/1500-ppm group rats and (3) rhinitis in some of 3000-/1500-ppm group rats. There was no restoration of the respiratory epithelium among 3000-/1500-ppm group rats, even after a recovery period of 52 weeks. Progression of the hyperplasia and metaplasia in the respiratory epithelium to squamous-cell carcinomas occurred during the first 26 weeks in 11 males and four females, but degeneration of the epithelium was less pronounced in the succeeding 26 weeks. Regeneration of the olfactory epithelium occurred in the 750- and 1500-ppm groups, but not in the 3000-/1500-ppm group (Woutersen & Feron, 1987).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

It is not known whether acetaldehyde, the primary metabolite of ethanol, is involved in the etiology of the human fetal alcohol syndrome (IARC, 1985).

4.3.2 *Experimental systems*

Fetal malformations were found in mice and rats treated with acetaldehyde *in vivo* and *in vitro*, and resorptions were observed in both species *in vivo* (IARC, 1985; WHO, 1995).

4.4 Genetic and related effects

The toxicity (including genotoxicity) of acetaldehyde has been reviewed (Dellarco, 1988; Feron *et al*., 1991; WHO, 1995).

4.4.1 *Humans*

Acetaldehyde–DNA adducts have been observed in granulocytes and lymphocytes of human alcohol abusers (Fang & Vaca, 1997).

4.4.2 *Experimental systems* (see Table 1 for references)

Acetaldehyde did not cause differential killing of repair-deficient *Escherichia coli* K-12 *uvrB*/*recA* cells and was not mutagenic to *Salmonella typhimurium* or *E. coli* WP2 *uvrA* after vapour exposure, with or without metabolic activation. It induced chromosome malsegregation in *Aspergillus nidulans* and was mutagenic in *Drosophila melanogaster* after injection but not after feeding.

In vitro and without exogenous metabolic activation, acetaldehyde induced gene mutations in mouse lymphoma L5178T cells, sister chromatid exchanges in Chinese hamster ovary cells and aneuploidy in embryonic Chinese hamster diploid fibroblasts. In human lymphocytes it also induced gene mutations and sister chromatid exchanges and, in addition, chromosomal aberrations and both positive- and negative-centromere-staining micronuclei. It did not cause morphological transformation in cultured mammalian cells when tested alone, but positive results were obtained when it was used in combination with the tumour promoter 12-*O*-tetradecanoylphorbol 13-acetate. It did not induce micronuclei in early spermatids of mice.

Acetaldehyde caused DNA strand breaks and cross-links in human lymphocytes *in vitro* without metabolic activation, but not in human bronchial epithelial cells and in human leukocytes. It has been shown to bind covalently to deoxynucleotides *in vitro* to form DNA–protein cross-links in rat nasal mucosa. Acetaldehyde–DNA adducts have been found *in vitro* in calf thymus DNA, in 2′-deoxyguanosine-3′-monophosphate and in liver from mice treated with ethanol (Fang & Vaca, 1995). Abnormal sperm morphology or spermocyte micronuclei were not observed in mice treated with an intraperitoneal injection of acetaldehyde.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to acetaldehyde may occur in its production, and in the production of acetic acid and various other chemical agents. It is a metabolite of sugars and ethanol in humans and has been detected in plant extracts, tobacco smoke, engine exhaust, ambient and indoor air, and in water.

5.2 Human carcinogenicity data

An increased relative frequency of bronchial and oral cavity tumours was found among nine cancer cases in one study of chemical workers exposed to various aldehydes. Oesophageal tumours have been associated with genetically determined, high metabolic levels of acetaldehyde after drinking alcohol.

Three case–control studies assessed the risk of oral, pharyngeal, laryngeal and oesophageal cancer following heavy alcohol intake, according to genetic polymorphism of enzymes involved in the metabolism of ethanol to acetaldehyde (alcohol dehydrogenase 3) and in the further metabolism of acetaldehyde (aldehyde dehydrogenase 2 and

Table 1. Genetic and related effects of acetaldehyde

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ECD, *Escherichia coli pol*A, differential toxicity (spot test)	–	NT	7800	Rosenkranz (1977)
ERD, *Escherichia coli* K-12 *uvrB/recA*, differential toxicity	–	NT	16317	Hellmér & Bolcsfoldi (1992)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	Mortelmas *et al*. (1986)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	0.5% in air	JETOC (1997)
SA4, *Salmonella typhimurium* TA104, reverse mutation	–	NT	2515	Marnett *et al*. (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	NT	7800	Rosenkranz (1977)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	Mortelmans *et al*. (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	0.5% in air	JETOC (1997)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Mortelmans *et al*. (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	0.5% in air	JETOC (1997)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	7800	Rosenkranz (1977)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Mortelmans *et al*. (1986)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1% in air	JETOC (1997)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	–	–	0.5% in air	JETOC (1997)
SCF, *Saccharomyces cerevisiae*, forward mutation	(+)	NT	23400	Bandas (19892)
ANN, *Aspergillus nidulans*, aneuploidy (chromosome malsegregation)	+	NT	200	Crebelli *et al*. (1989)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		22500 ppm inj × 1	Woodruff *et al*. (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		25000 ppm feed	Woodruff *et al*. (1985)
DIA, DNA–protein cross-links, Fischer 344 rat nasal mucosa cells *in vitro*	+	NT	4410	Lam *et al*. (1986)

Table 1 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	176	Wangenheim & Bolcsfoldi (1988)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	NT	3.9	Obe & Ristow (1977)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	NT	3.9	Obe *et al*. (1978)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	NT	1.9	Obe & Beek (1979)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	7.8	De Raat *et al*. (1983)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	NT	1.3	Brambilla *et al*. (1986)
MIA, Micronucleus test, Sprague-Dawley rat primary skin fibroblasts *in vitro*	+	NT	4.4	Bird *et al*. (1982)
CIR, Chromosomal aberrations, Sprague-Dawley rat primary skin fibroblasts *in vitro*	+	NT	44.1	Bird *et al*. (1982)
AIA, Aneuploidy, Chinese hamster embryonic diploid fibroblasts *in vitro*	+	NT	15.6	Dulout & Furnus (1988)
TCM, Cell transformation, C3H 10T½ mouse cells *in vitro*	–	NT	100	Abernathy *et al*. (1982)
TCL, Cell transformation, mammalian cells	–[c]	NT	0.44	Eker & Sanner (1986)
DIH. DNA strand breaks, human leukocytes *in vitro*	–	NT	441	Lambert *et al*. (1985)
DIH, DNA cross-links, human lymphocytes *in vitro*	+	NT	411	Lambert *et al*. (1985)
DIH, DNA strand breaks, human bronchial epithelial cells *in vitro*	–	NT	44	Saladino *et al*. (1985)
DIH, DNA–protein cross-links, human bronchial epithelial cells *in vitro*	–	NT	44	Saladino *et al*. (1985)
DIH, DNA strand breaks, human lymphocytes *in vitro*	+	NT	68.8	Singh & Khan (1995)
GIH, Gene mutation, human lymphocytes, *hprt* locus *in vtro*	+	NT	13	He & Lambert (1990)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	7.8	Obe *et al*. (1978)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	7.8	Ristow & Obe (1978)

Table 1 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	5.8	Jansson (1982)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	8	Bohlke *et al*. (1983)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	4.4	He & Lambert (1985)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	4.4	Knadle (1985)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	11	Norppa *et al*. (1985)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	15.6	Obe *et al*. (1986)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	4.4	Helander & Lindahl-Kiessling (1991)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	11	Sipi *et al*. (1992)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	20	Badr & Hussain (1977)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	(+)	NT	7.8	Obe *et al*. (1978)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	15.6	Obe *et al*. (1979)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	15.9	Böhlke *et al*. (1983)
CIH, Chromosomal aberrations, human Fanconi's anaemia lymphocytes *in vitro*	+	NT	7.8	Obe *et al*. (1979)
MIH, Micronucleus test, human lymphocytes *in vitro*	+[d]		26.5	Migliore *et al*. (1996)
DVA, DNA–protein cross-links, Fischer 344 rat nasal mucosa *in vivo*	+		1000 ppm inh 6 h/d × 5 d	Lam *et al*. (1986)
SVA, Sister chromatid exchange, male C3A mouse bone-marrow cells *in vivo*	+		0.4 µg/mouse ip × 1	Obe *et al*. (1979)
SVA, Sister chromatid exchange, Chinese hamster bone-marrow cells *in vivo*	+		0.5 ip × 1	Korte *et al.* (1981)
MVM, Micronucleus test, C57BL/6J × C3H/He mouse spermatocytes *in vivo*	–		375 ip × 1	Lähdetie (1988)

Table 1 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
COE, Chromosomal aberrations, rat embryos *in vivo*	+		7800 iam × 1	Bariliak & Kozachuk (1983)
BID, Binding (covalent) to calf thymus DNA *in vitro*	+	NT	44100	Ristow & Obe (1978)
BID, Binding (covalent) to calf thymus DNA *in vitro*	+	NT	78800	Fang & Vaca (1995)
BID, Binding (covalent) to deoxynucleosides *in vitro*	+	NT	7880	Vaca *et al*. (1995)
SPM, Sperm morphology, C57BL/6J × C3H/He mouse early spermatids *in vivo*	–		250 ip × 5	Lähdetie (1988)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; inj, injection; inh, inhalation; ip, intraperitoneal; iam, intra-amniotic

[c] Positive results when acetaldehyde treatment was followed by exposure of the cells to 12-*O*-tetradecanoylphorbol 13-acetate

[d] A dose-related increase in centromere-positive micronuclei was observed with fluorescence in-situ hybridization but it was not significantly different from the negative control

glutathione *S*-transferase M1). Despite limitations in the study design and the small size of most of the studies, these studies consistently showed an increased risk of alcohol-related cancers among subjects with the genetic polymorphisms leading to higher internal doses of acetaldehyde following heavy alcohol intake as compared to subjects with other genetic polymorphisms.

5.3 Animal carcinogenicity data

Acetaldehyde was tested for carcinogenicity in rats by inhalation exposure and in hamsters by inhalation exposure and by intratracheal instillation. It produced tumours of the respiratory tract following inhalation, particularly adenocarcinomas and squamous-cell carcinomas of the nasal mucosa in rats and laryngeal carcinomas in hamsters. In hamsters, it did not cause an increased incidence of tumours following intratracheal instillation. Inhalation of acetaldehyde enhanced the incidence of respiratory-tract tumours produced by intratracheal instillation of benzo[*a*]pyrene.

5.4 Other relevant data

Acetaldehyde is metabolized to acetic acid. During inhalation exposure of rats, degeneration of nasal epithelium occurs and leads to hyperplasia and proliferation.

Acetaldehyde causes gene mutations in bacteria and gene mutations, sister chromatid exchanges, micronuclei and aneuploidy in cultured mammalian cells, without metabolic activation. *In vivo*, it causes mutations in *Drosophila melanogaster* but not micronuclei in mouse germ cells. It causes DNA damage in cultured mammalian cells and in mice *in vivo*. Acetaldehyde–DNA adducts have been found in white blood cells from human alcohol abusers.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of acetaldehyde.

There is *sufficient evidence* in experimental animals for the carcinogenicity of acetaldehyde.

Overall evaluation

Acetaldehyde is *possibly carcinogenic to humans (Group 2B)*.

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AZIRIDINE

Data were last reviewed in IARC (1975) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

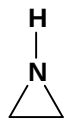
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 151-56-4
Chem. Abstr. Name: Aziridine
IUPAC Systematic Name: Ethylenimine
Synonyms: Azacyclopropane; dimethylenimine; ethyleneimine

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_2H_5N Relative molecular mass: 43.07

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Clear, colourless oily liquid with an intense odour of ammonia (American Conference of Governmental Industrial Hygienists, 1991; Budavari, 1996; Verschueren, 1996)
(*b*) *Boiling-point*: 56°C (Lide, 1997)
(*c*) *Melting-point*: –77.9°C (Lide, 1997)
(*d*) *Solubility*: Miscible with water; very soluble in diethyl ether; soluble in ethanol; and slightly soluble in chloroform (Budavari, 1996; Lide, 1997)
(*e*) *Vapour pressure*: 21 kPa at 20°C; relative vapour density (air = 1), 1.5 (Verschueren, 1996)
(*f*) *Flash point*: –11°C, closed cup (American Conference of Governmental Industrial Hygienists, 1991)
(*g*) *Reactivity*: Polymerizes explosively in contact with silver, aluminium or acid (American Conference of Governmental Industrial Hygienists, 1991; Budavari, 1996)

(*h*) *Explosive limits*: Upper, 46%; lower, 3.6% by volume in air (American Conference of Governmental Industrial Hygienists, 1991)
(*i*) *Conversion factor*: $mg/m^3 = 1.76 \times ppm$

1.2 Production and use

Global production capacity for aziridine is more than 12 000 tonnes per year (Scherr *et al.*, 1995). Information available in 1995 indicated that it was produced in Germany and Japan (Chemical Information Services, 1995).

Aziridine is an intermediate and monomer in the preparation of cationic polymers, such as polyaziridine (polyethyleneimine). These polymers are used to improve wet strength of paper, in fuel-oil and lubricant refining, as flocculating agents and in protective coatings, in textile finishing and for adhesives, polymer stabilizers, and surfactants (Lewis, 1993; Scherr *et al.*, 1995).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 1000 workers in the United States were potentially exposed to aziridine (see General Remarks). Occupational exposures to aziridine may occur in its production and in the preparation of polyaziridine polymers.

1.3.2 *Environmental occurrence*

No data on the environmental occurrence of aziridine were available to the Working Group.

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 0.88 mg/m^3 as the 8-h time-weighted average threshold limit value, with a skin notation, for occupational exposures to aziridine in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991). It is listed as an animal carcinogen in Germany (Deutsche Forschungsgemeinschaft, 1998).

No international guideline for aziridine in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Aziridine has been tested for carcinogenicity in two strains of mice by oral administration, producing an increased incidence of liver-cell and pulmonary tumours. Subcutaneous injection of single doses in suckling mice produced an increased incidence of lung tumours in males. In one experiment in rats, aziridine increased the incidence of tumours at the injection site following its subcutaneous injection in oil (IARC, 1975).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

[^{14}C]Aziridine injected intraperitoneally into rats is widely distributed, with some accumulation of radioactivity in liver, intestines, spleen and kidney. About half of the radioactivity was excreted in urine, 3–5% was expired as carbon dioxide and 1–3% was expired otherwise, probably as aziridine (IARC, 1975).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Degenerative changes occur in many organs of rats after administration of aziridine by various routes, including inhalation (IARC, 1975). Acute renal papillary necrosis is produced in rats and dogs administered aziridine. At low doses in rats, there was necrosis of interstitial cells, thin limbs of the loops of Henlé and vasa recta, while collecting ducts were spared. At higher doses, there was total papillary necrosis (Ellis *et al*., 1973; Ellis & Price, 1975; Axelsen, 1978).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

The mutagenicity of aziridine has been reviewed (Verschaeve & Kirsch-Volders, 1990). In particular, the many studies of the effects of aziridine on plants are referenced and discussed; these data are not addressed in the present review or in the accompanying table.

Aziridine induces gene mutations in *Salmonella typhimurium* and cultured Chinese hamster ovary CHO cells and sex-linked recessive lethal mutations in *Drosophila melanogaster*. It also induces gene conversion in *Saccharomyces cerevisiae* but not chromosomal loss in *D. melanogaster*. In cultured mammalian cell lines, it induces DNA strand breakage and chromosomal aberrations. Dominant lethal effects were induced in both *D. melanogaster* and mice. Adducts are formed between aziridine and [^{14}C]- or [^{3}H]-guanosine *in vitro* at pH 5–8, although the reaction rate was greater at pH values below 7. Two adducts were identified: imidazole-ring opened 7-alkylguanosine and 1-alkylguanosine, which accounted for 80% and 14% of all adduct radioactivity, respectively. At pH 6, intact 7-alkylation products were formed (Hemminki, 1984). The importance of ring-opening of the modified guanine (forming formamidopyrimidine residues) in mutagenesis has been investigated in CHO cells expressing the *E. coli fpg* gene, which encodes a DNA glycosylase that removes formamidopyrimidine residues (Cussac & Laval, 1996). At an aziridine concentration of 2 mM, the mutation frequency was reduced by at least 50% in the cells expressing *fpg*. In contrast, CHO cells transfected with rat *APDG* cDNA (encoding rat *N*3-methyladenine-DNA glycosylase, which removes both *N*3- and *N*7-alkylguanine residues) showed no reduction in mutation frequency when treated with aziridine. Thus, imidazole ring opening appears to be an important step in aziridine mutagenicity.

4.4.3 *Mechanistic consideration*

Based on the known chemical reactivity of aziridine and its ability to form adducts with DNA, sex-linked recessive lethal mutations in *Drosophila*, dominant lethal effects in *Drosophila* and mice, and gene mutation at the *hrpt* locus of CHO cells *in vitro*, it is probable that the biological effects of aziridine would be expressed in any mammalian species.

5. Summary of Data Reported and Evaluation[1]

5.1 Exposure data

Aziridine is a highly reactive and volatile chemical. Exposure to the compound may occur during its use as an intermediate and monomer in the production of cationic polymers.

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[1] Summary (but not the evaluation) prepared by the Secretariat after the meeting.

Table 1. Genetic and related effects of aziridine

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	5	McCann *et al*. (1975)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	5	McCann *et al*. (1975)
SCG, *Saccharomyces cerevisiae* D4, gene conversion	+	NT	860	Zimmerman (1971)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		NG	Shvartsman & Sharygina (1982)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		NG	Shvartsman *et al*. (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		43 feed	Zijlstra & Vogel (1988)
DMN, *Drosophila melanogaster*, ring-X chromosome loss	–		43 feed	Zijlstra & Vogel (1988)
DML, *Drosophila melanogaster*, dominant lethal test	+		NG	Shvartsman & Sharygina (1982)
DML, *Drosophila melanogaster*, dominant lethal test	+		430 inj	Šrám (1970)
DIH, DNA single-strand breaks, HeLa S3 cells *in vitro*	+	NT	21	Painter (1978)
GCO, Gene mutation, Chinese hamster ovary CHO cells, various loci *in vitro*	+	NT	2	Gupta & Singh (1982)
GCO, Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	+	NT	21	Cussac & Laval (1996)
CIH, Chromosomal aberrations, human WI-36 cells and leukocytes *in vitro*	+	NT	4	Chang & Elequin (1967)
DLM, Dominant lethal test, male C57BL/6 mice	+		5 ip × 1	Dean *et al*. (1981)

[a] +, positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; inj; injection; ip, intraperitoneal

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Aziridine was tested for carcinogenicity in mice by oral administration, producing an increased incidence of liver-cell and pulmonary tumours. Subcutaneous injection of single doses in suckling mice produced an increased incidence of lung tumours in males. In one experiment in rats it increased the incidence of tumours at the injection site following injection in oil.

5.4 Other relevant data

Aziridine produces genetic damage in bacteria, insects and mammalian cells in culture, as well as dominant lethal effects in mice. Opening of the aziridine ring appears to be an important metabolic step in its mutagenic action.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of aziridine were available.

There is *limited evidence* in experimental animals for the carcinogenicity of aziridine.

Overall evaluation

Aziridine is *possibly carcinogenic to humans (Group 2B)*.

In making the overall evaluation, the Working Group took into consideration that aziridine is a direct-acting alkylating agent which is mutagenic in a wide range of test systems and forms DNA adducts that are promutagenic.

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BENZOYL PEROXIDE

Data were last reviewed in IARC (1985) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

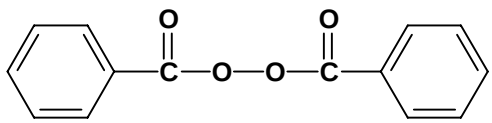
Chem. Abstr. Serv. Reg. No.: 94-36-0

Chem. Abstr. Name: Dibenzoyl peroxide

IUPAC Systematic Name: Benzoyl peroxide

Synonyms: Benzoic acid, peroxide; benzoperoxide; benzoyl superoxide; diphenylglyoxal peroxide

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{14}H_{10}O_4$ Relative molecular mass: 242.22

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: White granular crystalline solid with a faint odour of benzaldehyde (American Conference of Governmental Industrial Hygienists, 1991; Budavari, 1996)

(*b*) *Boiling-point*: May explode when heated (Budavari, 1996; Lide, 1997)

(*c*) *Melting-point*: 105°C (Lide, 1997)

(*d*) *Solubility*: Slightly soluble in water; soluble in acetone, diethyl ether, ethanol, and most other organic solvents (American Conference of Governmental Industrial Hygienists, 1991; Budavari, 1996; Lide, 1997)

(*e*) *Vapour pressure*: < 13 Pa at 20°C (American Conference of Governmental Industrial Hygienists, 1991)

(*f*) *Reactivity:* Highly flammable and explosive (American Conference of Governmental Industrial Hygienists, 1991)

(*g*) *Conversion factor:* $mg/m^3 = 9.91 \times ppm$

1.2 Production and use

Production of benzoyl peroxide in the United States in 1982 was 2300 tonnes. Information available in 1995 indicated that it was produced in 16 countries (Chemical Information Services, 1995).

Benzoyl peroxide is used as an initiator for polymerization of acrylates (including dental cements and restoratives) and other polymers; as a bleaching agent for flour, fats, oils, waxes and milk used in the preparation of certain cheeses; in pharmaceuticals for the topical treatment of acne; in rubber curing; and as a finishing agent for some acetate yarns (Anon., 1984; Lewis, 1993; Medical Economics Co., 1996; United States Food and Drug Administration, 1997).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 90 000 workers in the United States were potentially exposed to benzoyl peroxide (see General Remarks).

Occupational exposures to benzoyl peroxide may occur in its production and use in the plastics, rubber and pharmaceutical industries, and in food processing.

1.3.2 *Environmental occurrence*

No data on the environmental occurrence of benzoyl peroxide were available to the Working Group. General population exposures may occur as a result of its use in pharmaceutical and dental formulations.

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 5 mg/m^3 as the 8-h time-weighted average threshold limit value for occupational exposures to benzoyl peroxide in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for benzoyl peroxide in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

The potential carcinogenicity of exposure to benzoyl peroxide has been reviewed (Binder *et al*., 1995; Kraus *et al*., 1995).

Among a small factory population, two cases of lung cancer were found in men (one 40-year-old smoker and one 35-year-old nonsmoker) who were involved primarily in the production of benzoyl peroxide but were also exposed to benzoyl chloride (see this volume) and other chemicals (IARC, 1985).

In a study based on the Los Angeles County, United States, Cancer Surveillance Program, white male chemists with malignant melanoma and with other cancers (used as controls) were interviewed (Wright *et al.*, 1983). Four of the seven chemists with malignant melanoma gave a history of exposure to benzoyl peroxide (among many other chemicals) and none of the nine controls.

In a pilot case–control study of malignant melanoma in England (Cartwright *et al.*, 1988), 159 cases aged less than 45 years and seen between 1984 and 1986 were compared with 213 controls matched for general practitioner, sex and age. The risk ratio between past acne and malignant melanoma was 1.1 (95% confidence interval (CI), 0.7–1.9). The risk ratio between use of benzoyl peroxide and malignant melanoma was 0.5 (95% CI, 0.2–1.5).

A population-based case–control study of acne treatments as risk factors for skin cancer of the head and neck was carried out in the Province of Saskatchewan, Canada (Hogan *et al.*, 1991). The study was specifically designed to cover the age group who may have used benzoyl peroxide from the time it was marketed in Canada (1966). With interviews conducted in 1989, women aged 10–56 and men aged 10–51 years were included. Cases were identified from the files of the Saskatchewan cancer registry, and confirmed as being resident in Saskatchewan at the time of diagnosis and at the time of study. Four age- and sex-matched controls per case were identified from the files of the Saskatchewan Medicare Plan. All subjects were asked to complete a self-administered mailed questionnaire, that requested information on risk factors for skin cancer and all acne medications used. A list of 33 widely-used acne medications (including trade names) was supplied to facilitate recall of use at any time in the past. The response rate for the 964 cases was 91% and for the 3856 controls was 80%. Of the cases that responded, 92.3 (791) had basal-cell carcinoma, 4.8% (41) squamous-cell carcinoma and 2.9% (25) malignant melanoma. Nine per cent of the cases and 10.1% of the controls recalled use of preparations containing benzoyl peroxide, for average periods of 2.4 and 2.0 years, respectively. The odds ratio for use of benzoyl peroxide for all cases combined was 0.8 (95% CI, 0.5–1.3), and there was no association with the use of any single preparation containing benzoyl peroxide.

3. Studies of Cancer in Experimental Animals

Benzoyl peroxide was tested for carcinogenicity in mice and rats by oral administration in the diet and by subcutaneous administration, and in mice by skin application. In three studies by skin application in mice, benzoyl peroxide was tested for either initiating or promoting activity. All of the studies were inadequate for an evaluation of complete carcinogenicity; two studies indicated that benzoyl peroxide has promoting activity in mouse skin (IARC, 1985).

3.1 Skin application

Mouse: A group of 20 female SEN mice, four weeks of age, was treated twice weekly for 51 weeks with 0.2 mL of a 100 mg/mL solution of benzoyl peroxide in acetone applied to the skin shaved 48 h previously. A group of 15 mice receiving 0.2 mL acetone served as controls. At the termination of the experiment, there were no skin tumours among the control mice, compared with 8/20 in the benzoyl peroxide-treated mice ($p < 0.05$), of which 5/20 were squamous-cell carcinomas. The first tumour developed in week 24. Six of 20 mice showed epidermal hyperplasia (Kurokawa *et al*., 1984).

Groups of five male heterozygous TG.AC mice (carrying a v-Ha-*ras* gene) derived from the wild-type FVB/N strain were treated with 0, 1, 5 or 10 mg benzoyl peroxide in 0.2 mL acetone on the shaved dorsal skin twice a week for 20 weeks. Groups of five male FVB/N mice were similarly treated. No papillomas developed in the FVB/N mice. The incidences of papilloma-bearing mice in the four groups of TG:AC mice were 0/5, 0/5, 3/5 and 3/4, respectively (one papilloma-bearing mouse in the 10-mg group died before the end of the experiment) (Spalding *et al*., 1993).

3.2 Administration with known carcinogens

3.2.1 *Mouse*

Benzoyl peroxide was tested for promoting activity in groups of 20 and 15 female SEN mice receiving a single topical application of 20 nmol 7,12-dimethylbenz[*a*]anthracene (DMBA) followed by either 0.2 mL of a 100 mg/mL solution of benzoyl peroxide in acetone or acetone alone for 51 weeks. At the termination of the experiment, there were no skin tumours among the 15 control mice, compared with 20/20 in the benzoyl peroxide-treated mice ($p < 0.01$), of which 18/20 were squamous-cell carcinomas. The first tumour developed in week 8. All 20 treated mice showed epidermal hyperplasia (Kurokawa *et al*., 1984).

Groups of female SEN mice, five to seven weeks of age, were treated with a single topical application of 10 nmol DMBA on the shaved dorsal skin. Twice-weekly applications of 1 μg 12-*O*-tetradecanoylphorbol 13-acetate (TPA) were begun two weeks later and continued for 20 weeks. Beginning at week 21, one group of 21 papilloma-bearing mice continued to receive 1 μg TPA, while another group of 20 papilloma-bearing mice began twice-weekly treatments of 20 mg benzoyl peroxide. All solutions were applied in 0.2 mL acetone and treatments were ended at week 40. No new tumours appeared during weeks 21–40 in the benzoyl peroxide-treated group. At the end of the experiment, the proportion of mice with skin carcinomas was 70% in the benzoyl peroxide-treated group compared with 38% in the TPA-treated group and the cumulative number of carcinomas was 3.25-fold higher in the benzoyl peroxide-treated group. All skin tumours present at the end of the experiment were examined histologically. While no keratoacanthomas were identified in the TPA-treated group, 17 were found in the benzoyl peroxide-treated group. The authors concluded that benzoyl peroxide enhances the progression of benign to malignant tumours (O'Connell *et al*., 1986). However, in a similarly designed and

executed experiment, it was found that benzoyl peroxide did not enhance the progression of papillomas to squamous-cell carcinomas in SEN mice (Battalora *et al*., 1996).

Three groups of 16 male and 16 female *hr/hr* Oslo strain mice [age unspecified] were treated with a single topical application of 51.2 µg DMBA in 100 µL acetone. One group then received an application (rubbed into the skin [quantity not specified]) of Panoxyl, a gel containing 5% benzoyl peroxide used for the treatment of acne, twice each week for up to 60 or 61 weeks; a second group was similarly treated, but with gel not containing benzoyl peroxide and the third group was not treated further. A fourth group received Panoxyl treatment only. The total numbers of skin tumours in each group of mice were: DMBA alone, 22/32 (18 papillomas, 4 squamous-cell carcinomas); Panoxyl alone, 2/32 (2 squamous-cell carcinomas); DMBA + Panoxyl, 51/30 (49 papillomas, 3 squamous-cell carcinomas); DMBA + gel, 31/32 (31 papillomas). Both Panoxyl and the gel without benzoyl peroxide increased the multiplicity of papillomas induced by DMBA (Iversen, 1986).

The last experiment was repeated in part using *hr/hr* Oslo strain mice and extended by the use of SEN mice. The data were mainly presented as summary statistics. In contrast to the earlier results, the gel without benzoyl peroxide did not enhance DMBA carcinogenesis in *hr/hr* mice and Panoxyl did not enhance DMBA carcinogenesis in SEN mice. Groups of 32 *hr/hr* mice were also treated with ultraviolet radiation (UV) from new Phillips HP 3114 sunlamps, either alone twice a week or 5–30 min before treatment with Panoxyl or the gel without benzoyl peroxide. The numbers of mice with skin carcinomas (and the total numbers of skin tumours in each group of mice) were: UV alone, 26/32 (103 papillomas, 45 carcinomas); UV + Panoxyl, 22/32 (94 papillomas, 30 carcinomas); UV + gel, 23/32 (126 papillomas, 29 carcinomas). Thus, neither Panoxyl nor the gel without benzoyl peroxide had any significant effect upon the multiplicity of UV-induced skin tumours (Iversen, 1988).

One hundred and forty-eight Uscd (Hr) albino hairless mice [sex unspecified], three to four months of age, received 270 mJ/cm^2 UVB radiation to the posterior halves of their backs three times each week for eight weeks. The UVB source was an Hanovia air-cooled hot quartz contact lamp emitting 54 mJ/cm^2/s of UVB energy at a distance of 3.4 cm. Four weeks later the irradiated mice were divided into four groups that were treated in the irradiated area as follows: group 1 received 0.1 mL 0.1% croton oil in acetone five times per week for the duration of the study; group 2 received 0.1 mL acetone; group 3 received 0.1 mL benzoyl peroxide diluent; group 4 received 0.1 mL 5% benzoyl peroxide lotion [i.e., about 5 mg benzoyl peroxide]. At week 62, when the experiment was terminated, the tumour incidences in the irradiated areas of the skin were: group 1, 9/24; group 2, 1/20; group 3, 1/22; group 4, 2/26. Under the circumstances of the experiment, croton oil, but not benzoyl peroxide, enhanced the incidence of skin tumours induced by UVB radiation (Epstein, 1988).

A comparative initiation/promotion skin application study was conducted with B6C3F$_1$, CD-1 and SEN mice. In the portions of the study that are relevant to benzoyl peroxide, groups of 30 male and 30 female mice of each strain were treated on the shaved

skin as follows: A, acetone, the vehicle for all the substances (0.1 mL) alone, once per week; B, 2.5 μg DMBA, once, followed one week later with 0.1 mL acetone once per week for 51 weeks; C, 25 μg DMBA, once, followed one week later with 0.1 mL acetone once per week for 51 weeks; D, 20 mg benzoyl peroxide, followed one week later with 20 mg benzoyl peroxide in 0.2 mL acetone once per week for 51 weeks; E, as group B, followed one week later with 20 mg benzoyl peroxide in 0.2 mL acetone once per week for 51 weeks; F, as group C, followed one week later with 20 mg benzoyl peroxide in 0.2 mL acetone once per week for 51 weeks; G, 100 μg *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), once, followed one week later with 0.1 mL acetone once per week for 51 weeks; H, 500 μg MNNG, once, followed one week later with 0.1 mL acetone once per week for 51 weeks; I, as group D; J, as group G, followed one week later with 20 mg benzoyl peroxide in 0.2 mL acetone once per week for 51 weeks; and K, as group H, followed one week later with 20 mg benzoyl peroxide in 0.2 mL acetone once per week for 51 weeks. For each strain, survival of male and female mice in most groups was similar, but it was significantly reduced ($p < 0.01$) in male CD-1 and SEN mice of group H. Body weight gain also was similar in most groups, but was significantly reduced in female B6C3F_1 mice of group K ($p < 0.01$). The skin tumour responses are shown in Table 1. In neither sex of any strain did benzoyl peroxide act as a complete skin carcinogen, but it was active as a promoter in both sexes of SEN mice, following initiation with DMBA (at both dose levels) or MNNG (at both dose levels). The CD-1 strain and, in particular, the B6C3F_1 strain, were clearly less sensitive than the SEN strain (United States National Toxicology Program, 1996).

3.2.2 *Hamster*

Male Syrian hamsters [age unspecified] were randomized into five groups of 20 and treated as follows: group 1 received 1 mL acetone applied to the shaved dorsal area three times each week; group 2 received 10 mg/kg bw DMBA in sesame oil once by gavage; group 3 received 160 mg benzoyl peroxide in 1 mL acetone applied to the shaved dorsal skin three times per week; group 4 was treated with DMBA as group 2, followed one week later by 80 mg benzoyl peroxide in 1 mL acetone applied to the shaved dorsal skin three times per week; group 5 was treated with DMBA as group 2, followed one week later by treatment as group 3. After 16 months, all surviving hamsters were killed. The 25% survival in the groups was: group 1, 442 days; group 2, 376 days; group 3, 427 days; group 4, 342 days; group 5, 407 days. Histological assessment of the treated areas of skin indicated that the numbers of melanotic foci per hamster (arithmetic means and 95% CI) were: group 2, 6.3 (3.6–9.0); group 4, 17.4 (13.2–21.6); group 5, 26.9 (22.5–31.2); the corresponding numbers of melanotic tumours per hamster were: group 2, 0.6 (0.2–1.0); group 4, 2.2 (1.3–3.1); group 5, 2.9 (2.0–3.7). Benzoyl peroxide treatment enhanced the frequency of melanotic skin tumours in Syrian hamsters treated with DMBA (Schweizer *et al*., 1987).

Table 1. Skin tumour responses to treatment with carcinogens and/or benzyl peroxide in mice

Sex, group (treatment)	$B6C3F_1$	CD-1	SEN
Male A (acetone)	0/30	0/30	0/30
Male B (DMBA 2.5 µg)	0/30	0/30	0/29
Male C (DMBA 25 µg)	0/30	0/30	0/31
Male D (benzoyl peroxide/benzoyl peroxide)	0/30	0/30	0/30
Male E (DMBA 2.5 µg/benzoyl peroxide)	1/30	1/30	20/30
Male F (DMBA 25 µg/benzoyl peroxide)	1/30	6/30	22/30
Male G (MNNG 100 µg)	0/30	1/30	2/30
Male H (MNNG 500 µg)	1/30	7/30	19/30
Male I (benzoyl peroxide /benzoyl peroxide)	0/30	0/30	0/30
Male J (MNNG 100 µg/benzoyl peroxide)	0/30	1/30	9/30
Male K (MNNG 500 µg/benzoyl peroxide)	3/30	11/30	25/30
Female A (acetone)	0/30	0/30	0/29
Female B (DMBA 2.5 µg)	0/30	0/30	0/31
Female C (DMBA 25 µg)	0/30	0/30	0/29
Female D (benzoyl peroxide/benzoyl peroxide)	0/30	0/30	0/30
Female E (DMBA 2.5 µg/benzoyl peroxide)	4/30	1/30	22/30
Female F (DMBA 25 µg/benzoyl peroxide)	2/30	5/30	20/30
Female G (MNNG 100 µg)	0/30	0/30	0/30
Female H (MNNG 500 µg)	0/30	6/30	8/30
Female I (benzoyl peroxide/benzoyl peroxide)	0/30	0/30	1/30
Female J (MNNG 100 µg/benzoyl peroxide)	1/30	3/30	9/30
Female K (MNNG 500 µg/benzoyl peroxide)	3/30	13/30	16/30

From United States National Toxicology Program (1996)
DMBA, 7,12-dimethylbenz[*a*]anthracene; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine
All doses of benzoyl peroxide were 20 mg.

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Incubation of benzoyl peroxide with keratinocytes and the spin trap 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) results in the generation of an electron paramagnetic resonance spectrum characteristic of an alkyl radical adduct (Kensler *et al.*, 1988). Indeed, it has been known for a long time that benzoyl peroxide decomposes to benzoyloxyl and phenyl radicals in the presence of metals and heat. Electron paramagnetic resonance

spectroscopy and spin trapping in physiological media support the formation of benzoyloxyl and phenyl radicals, but not hydroxyl radicals (Hazlewood & Davies, 1996). The involvement of benzoyloxyl radicals in covalent binding to macromolecules is supported by the similar binding of both ring-^{14}C- and carbonyl-^{14}C-labelled benzoyl peroxide to protein. Binding to DNA was not observed in this study. Binding of labelled benzoic acid did not occur with either protein or DNA (Swauger *et al*., 1990). The production of free radicals continues at non-toxic concentrations of benzoyl peroxide in both freshly isolated and cultured human keratinocytes (Iannone *et al*., 1993).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

A single exposure to 10^{-10} mol/L benzoyl peroxide stimulated DNA synthesis in primary liver cells from four-day-old rats cultured in low-calcium medium. This effect was fully suppressed by simultaneous addition of α-tocopherol, selenous acid or superoxide dismutase (Romano *et al*., 1986). However, benzoyl peroxide was not mitogenic to human bronchial epithelial cells (Saladino *et al*., 1985).

Treatment of inbred SEN mouse skin with 20 mg benzoyl peroxide led to the transient induction of transforming growth factor β1 mRNA (Patamalai *et al*., 1994) and interleukin-1 (Lee *et al*., 1993). The number of mast cells also increased during benzoyl peroxide treatment, in a 30 μm-wide strip below the epidermis (de Rey *et al*., 1994).

A role for free radicals generated from benzoyl peroxide in tumour promotion is suggested by the general inhibitory (> 90%) effect of antioxidants, such as butylated hydroxytoluene, butylated hydroxyanisole, *para*-hydroxyanisole, disulfiram, α-tocopherol and ascorbic acid, the inhibitory effect of free radical scavengers, such as glutathione and *N*-acyl dihydroxylamines, and the enhancing effect of diethyl maleate, which reduces glutathione levels (Slaga, 1995).

4.3 Reproductive and developmental effects

The available data were inadequate to evaluate the teratogenic potential of benzoyl peroxide (IARC, 1985).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 2 for references)

Benzoyl peroxide was not mutagenic to bacteria, did not induce chromosomal aberrations in Chinese hamster lung cells and did not induce dominant lethal effects in mice. It has subsequently been shown to induce DNA single-strand breaks and DNA–protein

Table 2. Genetic and related effects of benzoyl peroxide

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500	Ishidate *et al*. (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500	Ishidate *et al*. (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500	Ishidate *et al*. (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	Ishidate *et al*. (1980)
SAS, *Salmonella typhimurium* TA92, reverse mutation	–	–	2500	Ishidate *et al*. (1980)
SAS, *Salmonella typhimurium* TA94, reverse mutation	–	–	2500	Ishidate *et al*. (1980)
CIC, Chromosomal aberrations, Chinese hamster lung CHL cells *in vitro*	–	NT	200	Ishidate *et al*. (1980)
AIA, Aneuploidy, Chinese hamster lung CHL cells *in vitro*	–	NT	200	Ishidate *et al*. (1980)
DIH, DNA single-strand breaks and DNA–protein cross-links, human bronchial epithelial cells *in vitro*	+	NT	242	Saladino *et al*. (1985)
DLM, Dominant lethal test, mice	–		62 ip × 1	Epstein *et al*. (1972)
ICR, Inhibition of gap-junctional intercellular communication, primary mouse keratinocytes *in vitro*	+	NT	40	Jansen *et al*. (1996)
ICR, Inhibition of gap-junctional intercellular communication, initiated primary mouse keratinocytes *in vitro*	+	NT	10	Jansen & Jongen (1996)
Increase in intercellular communication, Syrian hamster embryo cells *in vitro*	+	NT	242	Mikalsen & Sanner (1994)

[a] +, positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; ip, intraperitoneal

cross-links in cultured human bronchial epithelial cells. Benzoyl peroxide (10 μM, 1–2 h) produced a maximum three-fold increase in levels of 8-hydroxy-2′-deoxyguanosine in the DNA of cultured mouse keratinocytes, whereas the stable metabolic product, benzoic acid, did not produce this adduct (King *et al*., 1996). Results have been reported that are consistent with both the addition of benzoyloxyl and phenyl radicals to the C5–C6 double bond of pyrimidines and, to a lesser extent, hydrogen abstraction from sugar rings of RNA and DNA. The benzoyloxyl radical appears to be responsible for the majority of DNA strand breaks and high yields of altered bases through the formation of base adducts (Hazlewood & Davies, 1996).

Benzoyl peroxide generally inhibits gap-junctional intercellular communication in cultured cells. In contrast, an increase in gap-junctional intercellular communication was observed in a Syrian hamster embryo cell line. Changes in the expression of gap-junctional proteins (connexins) concomitant with inhibition of gap-junctional intercellular communication have been observed. In SEN mice treated with 83 μmol benzoyl peroxide, keratinocytes expressed the gap-junctional connexin 26 gene (not normally expressed in adult mouse skin), transiently increased the expression of connexin 43 and reduced the expression of connexin 31.1 (Budunova *et al*., 1995, 1996). In primary mouse keratinocyte cultures, benzoyl peroxide strongly decreased the amount of E-cadherin protein (Jansen *et al*., 1996).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to benzoyl peroxide may occur in its manufacture and use as an initiator in polymer production, food bleaching and rubber curing. Consumer exposure occurs from acne medications and dental products containing benzoyl peroxide.

5.2 Human carcinogenicity data

Two case–control studies have evaluated exposure to benzoyl peroxide among cases of malignant melanoma. One of these studies (the smallest) (among chemists) suggested a greater frequency of exposure among cases than controls. A third large population-based case–control study, designed specifically to evaluate the possible risk of benzoyl peroxide used as an acne medication among young persons, included largely cases of basal-cell carcinoma of the skin. There was no association with use of benzoyl peroxide in this study.

5.3 Animal carcinogenicity data

Benzoyl peroxide was tested in two studies by skin application in strains of mice susceptible to the development of skin papillomas and in several skin-painting studies in mice and in one study in hamsters in combination with known carcinogens. In one study by skin application in mice, it induced benign and malignant skin tumours and, in the

other study, benign skin tumours. Benzoyl peroxide was active as a skin tumour promoter in several strains of mice.

5.4 Other relevant data

Benzoyl peroxide forms radicals that are involved in its covalent binding to macromolecules. Its biological effects are inhibited by antioxidants.

Its genotoxic properties have received little attention. DNA damage has been observed in treated mammalian cells, but it is not mutagenic in bacteria and does not cause chromosomal damage in cultured mammalian cells or dominant lethal effects in mice.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of benzoyl peroxide.

There is *limited evidence* in experimental animals for the carcinogenicity of benzoyl peroxide.

Overall evaluation

Benzoyl peroxide is *not classifiable as to its carcinogenicity to humans (Group 3).*

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n-BUTYL ACRYLATE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

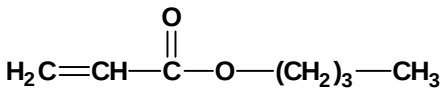
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 141-32-2
Chem. Abstr. Name: 2-Propenoic acid, butyl ester
IUPAC Systematic Name: Acrylic acid, n-butyl ester
Synonym: Butyl 2-propenoate

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_7H_{12}O_2$ Relative molecular mass: 128.17

1.1.3 *Chemical and physical properties of the pure substance*

From American Conference of Governmental Industrial Hygienists (1991) unless otherwise noted.

(*a*) *Description*: Colourless, flammable liquid
(*b*) *Boiling-point*: 145°C (Lide, 1997)
(*c*) *Melting-point*: –64.6°C (Lide, 1997)
(*d*) *Solubility*: Very slightly soluble in water (0.14% at 20°C); soluble in ethanol, diethyl ether and acetone
(*e*) *Vapour pressure*: 532 Pa at 20°C; relative vapour density (air = 1), 4.42
(*f*) *Flash-point*: 48.9°C, open cup
(*g*) *Conversion factor*: $mg/m^3 = 5.24 \times ppm$

1.2 Production and use

Production in the United States in 1993 was reported to be 340 035 tonnes (United States International Trade Commission, 1994). Information available in 1995 indicated that it was produced in nine countries (Chemical Information Services, 1995).

n-Butyl acrylate is used in the production of polymers and resins for textile and leather finishes, solvent coatings, adhesives, paints, binders and emulsifiers (Lewis, 1993; United States National Library of Medicine, 1997).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 40 000 workers in the United States were potentially exposed to *n*-butyl acrylate (see General Remarks). Occupational exposures may occur in its manufacture and use in the production of polymers and resins, including emulsion polymers for paints.

1.3.2 *Environmental occurrence*

n-Butyl acrylate may be released into the environment in fugitive and stack emissions or in wastewater during its production and use. It has been detected at low levels in ambient and urban air, groundwater and drinking-water samples (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 52 mg/m^3 as the 8-h time-weighted average threshold limit value for occupational exposures to *n*-butyl acrylate in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991). Germany reduced its 8-h time-weighted average MAK value to 11 mg/m^3 (Deutsche Forschungsgemeinschaft, 1998).

No international guideline for *n*-butyl acrylate in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

n-Butyl acrylate was tested for carcinogenicity by repeated skin applications in one experiment in male mice; no treatment-related tumour was observed. In a study reported as an abstract, in which male and female rats were exposed to *n*-butyl acrylate by inhalation for two years, no neoplastic effect was observed (IARC, 1986).

3.1 Inhalation exposure

Rat: In a study previously reported in an abstract, four groups of 86 male and 86 female Sprague-Dawley rats, five weeks of age, were administered *n*-butyl acrylate (purity, > 99.5%; main impurities, butyl propionate and isobutyl acrylate) by whole-body inhalation at concentrations of 0, 15, 45 and 135 ppm (0, 86, 258 and 773 mg/m^3) in air for 6 h per day on five days a week for 24 months. Interim kills were performed after 12 months (10 males and 10 females), 18 months (15 males and 15 females) and 24 months (10 males and 10 females). After a further six months, the study was terminated. No dose-related trend in mortality was observed. After 24 months of exposure, the mean cumulative mortality was approximately 20%. During the six-month post-exposure period, the cumulative mortality increased to approximately 45%. Exposure to *n*-butyl acrylate vapour did not lead to an increased frequency of any tumour type in any organ that could be related to the test substance (Reininghaus *et al*., 1991).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

In-vivo disposition in rats

Sanders *et al.* (1988) administered *n*-butyl [2,3-^{14}C]acrylate to rats orally at doses of 4, 40 and 400 mg/kg bw and intravenously at 40 mg/kg bw. After oral administration, *n*-butyl acrylate was very rapidly absorbed and hydrolysed to acrylic acid, with more than 75% of the dose eliminated as its metabolic end product $^{14}CO_2$. Some 10% of the dose was excreted in the urine, two metabolites being identified as the mercapturic acid *N*-acetyl-*S*-(2-carboxyethyl)cysteine and its sulfoxide. The elimination pattern of ^{14}C was essentially identical at all doses, but additional unidentified ^{14}C peaks were present in the urine at 400 mg/kg. Comparison of the data from the two routes of administration suggested that *n*-butyl acrylate exhibited a first-pass effect after oral dosing, but this was not investigated further. *n*-Butyl acrylate was rapidly and extensively excreted, the tissues being cleared of ^{14}C by 24–72 h. After an initial rapid reduction, a small amount of ^{14}C was retained in whole blood and adipose tissue, possibly by incorporation of ^{14}C via the one-carbon pool.

These findings were confirmed by Linhart *et al.* (1994a) using ^{13}C-labelled *n*-butyl acrylate with nuclear magnetic resonance analysis. These authors also found a significant enrichment of ^{13}C in 3-hydroxypropanoic acid in the urine of rats and, when esterase activity was inhibited with tri-*o*-tolyl phosphate, a third mercapturic acid, *N*-acetyl-*S*-

(butoxycarbonylethyl)cysteine, was found. This is derived from the reverse Michael addition of glutathione across the α,β-unsaturated bond of *n*-butyl acrylate. In further work, Linhart *et al.* (1994b) reported slight increases in the amounts of lactic and acetic acids in rat urine after administration of *n*-butyl acrylate.

In-vitro studies of hydrolysis

Miller *et al.* (1981) showed the rapid hydrolysis of *n*-butyl acrylate in whole homogenate of rat liver, the rate of ester disappearance being the same as that of appearance of acrylic acid. Among a series of acrylate esters, *n*-butyl acrylate was very rapidly hydrolysed by a 5000 × *g* supernatant of the nasal mucosa of mice (Stott & McKenna, 1985). This would lead to high local concentrations of the irritant acrylic acid, consistent with the nasal mucosa being a target organ for toxic effects of this ester when inhaled.

4.2 Toxic effects

4.2.1 *Humans*

The ability of *n*-butyl acrylate to cause allergic contact dermatitis was reported by Kanerva *et al*. (1988, 1996).

4.2.2 *Experimental systems*

No exposure-related clinical signs or lesions of systemic toxicity were observed in male and female Sprague-Dawley rats exposed by inhalation to *n*-butyl acrylate, at concentrations of 0, 15, 45 and 135 ppm [0, 86, 258 and 773 mg/m^3] over 24 months (Reininghaus *et al*., 1991). Atrophy of the neurogenic epithelial cells and hyperplasia of reserve cells were observed in the nasal mucosa of all *n*-butyl acrylate-treated animals. These changes were dose-related and mainly affected the anterior part of the olfactory epithelium. Opacity and neovascularization of the cornea were seen in the group exposed to 135 ppm *n*-butyl acrylate.

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

In a single study, *n*-butyl acrylate was not mutagenic to *Salmonella typhimurium* in the presence or absence of an exogenous metabolic activation system.

In Chinese hamsters and Sprague-Dawley rats exposed to 4300 mg/m^3 *n*-butyl acrylate by inhalation for 5–6 h per day for four days, no chromosomal damage was observed in single bone-marrow samples taken 5 h after cessation of exposure. [The

Table 1. Genetic and related effects of *n*-butyl acrylate

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1000	Waegemaekers & Bensink (1984)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1000	Waegemaekers & Bensink (1984)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1000	Waegemaekers & Bensink (1984)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1000	Waegemaekers & Bensink (1984)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	Waegemaekers & Bensink (1984)
MIA, Micronucleus test, Syrian hamster embryo cells *in vitro*	–	NT	10	Wiegand *et al.* (1989)
TCS, Cell transformation, Syrian hamster embryo cells	–	NT	10	Wiegand *et al.* (1989)
CBA, Chromosomal aberrations, Chinese hamster bone-marrow cells *in vivo*	–		820 ppm inh 5–6 h 4 d	Engelhardt & Klimisch (1983)
CBA, Chromosomal aberrations, Sprague-Dawley rat bone-marrow cells *in vivo*	–		820 ppm inh 5–6 h 4 d	Engelhardt & Klimisch (1983)
CBA, Chromosomal aberrations, rat bone-marrow cells *in vivo*	+		300 ip × 1	Fediukovich & Egorova (1991)

[a] +, positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; inh, inhalation; ip, intraperitoneal

Working Group noted that single samples were tested and the short period between cessation of exposure and sampling.] However, *n*-butyl acrylate induced chromosomal aberrations in the bone marrow of rats dosed by intraperitoneal injection.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to *n*-butyl acrylate may occur in its manufacture and its use in the production of polymers and other chemical products. It has been detected at low levels in ambient air and water.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

n-Butyl acrylate was tested in one study in mice by skin application and in one study in rats by inhalation exposure. No carcinogenic effect was observed.

5.4 Other relevant data

n-Butyl acrylate is rapidly absorbed and hydrolysed in experimental animals exposed orally. Exposure of rats to *n*-butyl acrylate vapours leads to hyperplasia of the nasal mucosa. In assays for genotoxicity/mutagenicity considered, results for *n*-butyl acrylate were generally negative.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of *n*-butyl acrylate were available.

There is *inadequate evidence* in experimental animals for the carcinogenicity of *n*-butyl acrylate.

Overall evaluation

n-Butyl acrylate is *not classifiable as to its carcinogenicity to humans (Group 3).*

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γ-BUTYROLACTONE

Data were last reviewed in IARC (1976) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

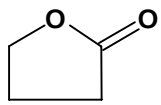
1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 96-48-0

Chem. Abstr. Name: Dihydro-2(3-H)-furanone

Synonyms: γ-BL; 1,4-butanolide; butyric acid lactone; 4-butyrolactone

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_4H_6O_2$ Relative molecular mass: 86.1

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid (Mercker & Kieczka, 1985; Budavari, 1996)

(*b*) *Boiling-point*: 204°C (Lide, 1997)

(*c*) *Melting-point*: –43.3°C (Lide, 1997)

(*d*) *Solubility*: Miscible with water, ethanol, diethyl ether, acetone and benzene (Lide, 1997)

(*e*) *Stability*: Stable at pH 7; rapidly hydrolysed by bases, slowly hydrolysed by acids (Weast, 1975)

(*f*) *Reactivity*: Reacts with inorganic acids and bases, alcohols and amines (Freifeld & Hort, 1967)

(*g*) *Conversion factor*: $mg/m^3 = 3.52 \times ppm$

1.2 Production and use

γ-Butyrolactone production in the United States in 1992 was estimated to be approximately 45 thousand tonnes per year (Datta, 1995). Information available in 1995 indicated that it was produced in six countries (Chemical Information Services, 1995).

γ-Butyrolactone is used principally as a chemical intermediate in the production of pyrrolidones, as an intermediate in organic synthesis, and as a solvent for many polymers (Mercker & Kieczka, 1985; Datta, 1995).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 27 000 workers in the United States were potentially exposed to γ-butyrolactone (see General Remarks). Occupational exposures may occur in its production, in the production of 2-pyrrolidone and related chemicals, and when it is used as a solvent.

1.3.2 *Environmental exposure*

γ-Butyrolactone has been found in alcoholic beverages, cooked meats, coffee, tomatoes and tobacco smoke (IARC, 1976; United States National Library of Medicine, 1998).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not recommended an 8-h time-weighted average threshold limit value for occupational exposures to γ-butyrolactone in workplace air.

No international guideline for γ-butyrolactone in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

γ-Butyrolactone was one of the several agents evaluated in the case–control studies of soft-tissue sarcoma and non-Hodgkin lymphoma nested within the IARC international cohort of pesticide production workers and sprayers (Kogevinas *et al.*, 1995), which are described in the monograph on polychlorophenols in this volume. One case of soft-tissue sarcoma and one control were classified as exposed (odds ratio, 5.0; 95% confidence interval (CI), 0.3–80). Two cases of non-Hodgkin lymphoma and three controls were classified as exposed (odds ratio, 3.0; 95% CI, 0.5–18).

3. Studies of Cancer in Experimental Animals

γ-Butyrolactone was tested for carcinogenicity in mice by oral administration, subcutaneous injection and skin application and in rats by oral and subcutaneous administration. No carcinogenic effects were observed (IARC, 1976).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F_1 mice, eight to nine weeks of age, received γ-butyrolactone (purity, > 97%) in corn oil by gavage on five days per week for two years. The doses administered were 0, 262, and 525 mg/kg bw for both male and female

mice. The mean body weights of dosed male mice were lower than those of the controls throughout the study, but the differences in mean body weights decreased when male mice were housed individually at week 67. The final mean body weights of dosed male mice were 6% lower than that of the controls. Mean body weights of dosed female mice were also lower than those of the controls throughout the study, and the final mean body weights were from 14% to 17% lower than that of the controls. The survival in high-dose male mice was significantly lower than that of the controls (35/50, 30/50, 12/50) due to bite wounds and fighting in high-dose males recovering from the sedative effects of γ-butyrolactone. The survival of female dosed mice was similar to that of the controls (38/50, 34/50, 38/50). Increased incidences of proliferative lesions of the adrenal medulla in low-dose male mice were associated with γ-butyrolactone administration (phaeochromocytoma, benign or malignant: 2/48, 6/50, 1/50; hyperplasia: 2/48, 9/50, 4/50). The incidence of hepatocellular neoplasms in both dose groups of male mice was lower than the incidence in the controls (hepatocellular adenoma or carcinoma: 24/50, 8/50, 9/50). No increase in the incidence of tumours at other sites was observed in either sex (United States National Toxicology Program, 1992).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344/N rats, eight to nine weeks of age, received γ-butyrolactone (purity, > 97%) in corn oil by gavage on five days per week for two years. The doses administered were 0, 112 and 225 mg/kg bw for male rats and 0, 225 and 450 mg/kg bw for female rats. The mean body weights of male rats given γ-butyrolactone were similar to those of the controls throughout the study. The mean body weight of high-dose females was 10–20% lower than that of the controls throughout the second year. The survival of high-dose male rats was slightly higher than that of the controls (control, 24/50; low-dose, 27/50; high-dose, 32/50) due primarily to a lower incidence of mononuclear cell leukaemia in the high-dose group (16/50, 15/50, 9/50). The survival of dosed females was similar to that of the controls (28/50, 27/50, 28/50). No increased incidence of neoplasms or non-neoplastic lesions in rats was reported (United States National Toxicology Program, 1992).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

γ-Butyrolactone rapidly hydrolyses in blood to γ-hydroxybutyric acid, which, when given to rats by inhalation, is mainly excreted as CO_2 (75–85% in 24 h) (IARC, 1976). The plasma half-life in rats of γ-butyrolactone after intravenous administration is less than one minute (Roth & Giarman, 1966, 1969).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

γ-Butyrolactone has relatively low toxicity, although sedative and hypnotic effects occur and bradycardia and coma can result from its ingestion (Higgins & Borron, 1996). These are likely to be due to its major metabolite, γ-hydroxybutyric acid, which is formed endogenously and found in low concentrations in the brain (Roth & Giarman, 1969; Borbély & Huston, 1972; Gold & Roth, 1977). γ-Butyrolactone did not sensitize guinea-pigs, following skin application (IARC, 1976).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Groups of 10 pregnant rats received up to 500 mg/kg bw per day γ-butyrolactone by gavage on gestation days 6–15. No embryotoxicity was observed on day 21 of gestation (Kronevi *et al*., 1988).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

A large proportion of the genetic toxicity data on γ-butyrolactone is derived from a collaborative study involving up to seventeen laboratories.

γ-Butyrolactone does not induce DNA damage or mutations in bacteria, gene conversion or aneuploidy in yeast. Sex-linked recessive lethal mutations were not induced in *Drosophila melanogaster*. In cultured human cells, there was no indication of induction of gene mutations in one study. In contrast, sister chromatid exchanges and chromosomal aberrations were increased in one study with Chinese hamster ovary cells in the presence of an exogenous metabolic activation system, while chromosomal aberrations were not increased in another study in rat liver cells. Micronuclei were not induced in the bone-marrow cells of exposed mice in two studies.

Table 1. Genetic and related effects of γ-butyrolactone

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage, induction, SOS repair test, DNA strand breaks or cross-links	–	–	12 500	Thomson (1981)
ECL, *Escherichia coli pol A*/W3110-P3478, differential toxicity (liquid suspension test)	(+)	NT	NG	Rosenkranz *et al*. (1981)
ERD, *Escherichia coli rec* strains, differential toxicity	–	–	500	Green (1981)
ERD, *Escherichia coli rec* strains, differential toxicity	–	–	500	Ichinotsubo *et al*. (1981a)
ERD, *Escherichia coli rec* strains, differential toxicity	–	–	1000	Tweats (1981)
BRD, *Bacillus subtilis rec* strains, differential toxicity	–	+[c]	22 400 µg/disk	Kada (1981)
SAF, *Salmonella typhimurium* TM677, forward mutation, 8-azaguanine resistance	–	–	1000	Skopeck *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Baker & Bonin (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation (fluctuation test)	–	–	500	Hubbard *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Ichinotsubo *et al*. (1981b)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500	MacDonald (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Nagao & Takahashi (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	NT	5000	Richold & Jones (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1000	Rowland & Severn (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Simmon & Shepherd (1981)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	–	1250	Trueman (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	250	Venitt & Crofton-Sleigh (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	Haworth *et al*. (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Baker & Bonin (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	Richold & Jones (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation (fluctuation test)	–	–	500	Gatehouse (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1000	Rowland & Severn (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	NG	Simmon & Shepherd (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	NT	–	1250	Trueman (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Baker & Bonin (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation (fluctuation test)	–	–	500	Gatehouse (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1000	MacDonald (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Nagao & Takahashi (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Richold & Jones (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1000	Rowland & Severn (1981)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Simmon & Shepherd (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	NT	–	1250	Trueman (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Haworth *et al*. (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	500	Baker & Bonin (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	5000	Richold & Jones (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1000	Rowland & Severn (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	NG	Simmon & Shepherd (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	NT	–	1250	Trueman (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Baker & Bonin (1981
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation (fluctuation test)	–	–	500	Gatehouse (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation (fluctuation test)	–	–	500	Hubbard *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Ichinotsubo *et al*. (1981b)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	MacDonald (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Nagao & Takahashi (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Richold & Jones (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	Rowland & Severn (1981)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Simmon & Shepherd (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	NT	–	1250	Trueman (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250	Venitt & Crofton-Sleigh (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Haworth *et al*. (1983)
SAS, *Salmonella typhimurium* TA92, reverse mutation	–	–	1000	Brooks & Dean (1981)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation (fluctuation test)	–	–	500	Gatehouse (1981)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	–	–	NG	Matsushima *et al*. (1981)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	–	250	Venitt & Crofton-Sleigh (1981)
EC2, *Escherichia coli* WP2, reverse mutation	–	–	250	Venitt & Crofton-Sleigh (1981)
ECR, *Escherichia coli* WP2 *uvrA*/pKM101, reverse mutation	–	–	NG	Matsushima *et al*. (1981)
SCG, *Saccharomyces cerevisiae* D4, gene conversion	–	–	166	Jagannath *et al*. (1981)
SCG, *Saccharomyces cerevisiae* JD1, gene conversion	?[d]	NT	500	Sharp & Parry (1981a)
SCG, *Saccharomyces cerevisiae* D7, gene conversion	–	–	2250	Zimmermann & Scheel (1981)
SCH, *Saccharomyces cerevisiae* 'race XII', homozygosis by mitotic recombination, ade2 locus	–	–	1000	Kassinova *et al*. (1981)
SCR, *Saccharomyces cerevisiae*, XV185-14C, reverse mutation	–	?	22	Mehta & von Borstel (1981)
SZF, *Schizosaccharomyces pombe*, forward mutation	–	–	20	Loprieno (1981)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SCN, *Saccharomyces cerevisiae* D6, aneuploidy	–	–	1000	Parry & Sharp (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		0.2% feed	Vogel *et al*. (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations (*y mei-9^{a} mei-41^{DS}*)	–		0.2% feed	Vogel *et al*. (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		28000 ppm feed	US National Toxicology Program (1992)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	1000	Perry & Thomson (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	+	3010	Loveday *et al*. (1989)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	+	2580	Loveday *et al*. (1989)
CIR, Chromosomal aberrations, rat liver RL_1 cells *in vitro*	–	NT	250	Dean (1981)
GIH, Gene mutation, human fibroblast HSC172 cell line, diphtheria toxin resistance *in vitro*	–	–	500	Gupta & Goldstein (1981)
MVM, Micronucleus test, $B6C3F_1$ mouse bone-marrow cells *in vivo*	–		984 ip × 2	Salamone *et al*. (1981)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVM, Micronucleus test, CD-1 mouse bone-marrow cells *in vivo*	–		495 ip × 2	Tsuchimoto & Matter (1981)
SPM, Sperm morphology, (CBA × BALB/c)F_1 mice *in vivo*	–		560 ip × 5	Topham (1981)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; ip, intraperitoneal
[c] S-9 from Japanese yellowtail fish
[d] Positive in dimethyl sulfoxide, negative in ethanol

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to γ-butyrolactone may occur in its production and use as an intermediate and as a solvent. It has been detected in alcoholic beverages, tobacco smoke, coffee and several foodstuffs.

5.2 Human carcinogenicity data

No adequate data were available to the Working Group.

5.3 Animal carcinogenicity data

γ-Butyrolactone was tested for carcinogenicity in two studies in mice and two studies in rats by oral administration. It was also tested in mice by skin application in two studies and by subcutaneous injection in mice and rats in single studies. No carcinogenic effect was observed.

5.4 Other relevant data

γ-Butyrolactone rapidly hydrolyses in blood to γ-hydroxybutyric acid. γ-Butyrolactone has been extensively studied in in-vitro genetic toxicity tests in which the overwhelming majority of results did not indicate activity. Positive results were obtained in one study for chromosomal aberrations and sister chromatid exchanges in a Chinese hamster cell line. No mutagenic activity was observed *in vivo* in *Drosophila* or in mouse bone marrow micronucleus tests.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of γ-butyrolactone.

There is *evidence suggesting lack of carcinogenicity* of γ-butyrolactone in experimental animals.

Overall evaluation

γ-Butyrolactone is *not classifiable as to its carcinogenicity to humans* (Group 3).

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CAPROLACTAM

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

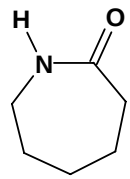
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 105-60-2
Chem. Abstr. Name: Hexahydro-2*H*-azepin-2-one
IUPAC Systematic Name: Hexahydro-2*H*-azepin-2-one
Synonyms: 2-Ketohexamethylenimine; 2-oxohexamethylenimine

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_6H_{11}NO$ Relative molecular mass: 113.16

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: White crystalline solid (American Conference of Governmental Industrial Hygienists, 1991)
(*b*) *Boiling-point*: 270°C (Lide, 1997)
(*c*) *Melting-point*: 69.3°C (Lide, 1997)
(*d*) *Solubility*: Very soluble in water, benzene, diethyl ether, and ethanol; soluble in methanol, tetrahydrofurfuryl alcohol, dimethylformamide, chlorinated hydrocarbons, and petroleum fractions (Budavari, 1996; Lide, 1997)
(*e*) *Vapour pressure*: 800 Pa at 120°C (American Conference of Governmental Industrial Hygienists, 1991)
(*f*) *Flash-point*: 125°C, open cup (Budavari, 1996)
(*g*) *Conversion factor*: $mg/m^3 = 4.6 \times ppm$

1.2 Production and use

Production in the United States in 1993 was reported to be 649 825 tonnes (United States International Trade Commission, 1994). Estimated production capacities of caprolactam in 1990 were reported as (thousand tonnes): United States, 640; western Europe, 860; eastern Europe, 895; Japan, 500; Latin America, 150; Asia, 290 (Fisher & Crescentini, 1992).

Caprolactam is used primarily in the manufacture of synthetic fibres and resins (especially nylon 6), bristles, film, coatings; synthetic leather, plasticizers and paint vehicles; as a cross-linking agent for polyurethanes; and in the synthesis of the amino acid lysine (Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 25 000 workers in the United States were potentially exposed to caprolactam (see General Remarks). Occupational exposures to caprolactam may occur in the manufacture of the chemical and of polycaprolactam (nylon 6) fibres and resins.

1.3.2 *Environmental occurrence*

Caprolactam may be released to the environment during its manufacture and use in the preparation of resins and plastics (United States National Library of Medicine, 1997). It has been detected in surface water, groundwater and drinking-water (IARC, 1986).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 1 mg/m^3 as the 8-h time-weighted average threshold limit value for occupational exposures to caprolactam dust in workplace air and 23 mg/m^3 for the vapour. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for caprolactam in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Caprolactam was tested for carcinogenicity in mice and rats by oral administration in the diet. No carcinogenic effect was observed (IARC, 1986).

3.1 Multistage protocols and preneoplastic lesions

Rat: A group of 15 male F344/DuCrj rats, six weeks of age, was administered a single intraperitoneal injection of 100 mg/kg bw *N*-nitrosodiethylamine (NDEA), followed by four twice weekly intraperitoneal injections of 20 mg/kg bw *N*-methyl-*N*-nitrosourea (MNU) during weeks 1 and 2 and administration of 0.1% *N*-bis(2-hydroxypropyl)nitrosamine in the drinking-water during weeks 3 and 4. The rats were then given 10 000 mg caprolactam [purity unspecified]/kg diet (ppm) for 16 weeks. A group of 30 rats was given basal diet after the first-step procedure and served as controls. In addition, five rats received vehicles without carcinogens during the first-step treatment period and were then given 10 000 mg caprolactam/kg diet (ppm) for 16 weeks. Animals were killed at week 20 and histological examination of most organs and any gross lesions and quantitation of glutathione *S*-transferase (placental form) (GST-P)-positive foci of the liver were performed. Caprolactam showed no modifying effect in any organ (Fukushima *et al*., 1991).

Two groups of 14 and 15 male Fischer 344 rats, six weeks of age, were administered a single intraperitoneal injection of 200 mg/kg bw NDEA in 9% (w/v) saline. After a two-week recovery period, rats were given either 10 000 mg caprolactam [purity unspecified]/kg diet (ppm) or basal diet for six weeks. At week 3, all rats were subjected to a two-thirds partial hepatectomy and killed at week 8. Quantitative analysis of GST-P-positive foci of the liver was performed. There were no significant differences in either the numbers or areas of GST-P-positive foci between the caprolactam-treated group and the controls (Hasegawa & Ito, 1992).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

The major urinary metabolites of caprolactam were identified in male Sprague-Dawley rats given 3% caprolactam in the diet for two to three weeks. Twenty-four-hour urine samples were collected during the final week and metabolites isolated by ion-exchange chromatography and characterized by infrared and nuclear magnetic resonance spectroscopy. The major metabolite (16% of the dose) was 4-hydroxycaprolactam or the corresponding free acid: this rearranges in acid to an equilibrium mixture of 6-amino-α-caprolactone and 6-amino-4-hydroxyhexanoic acid. A small amount of 6-aminohexanoic acid was also excreted (Kirk *et al*., 1987).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Oral treatment of adult female Sprague-Dawley rats with 425 mg/kg bw caprolactam 21 and 4 h before killing resulted in a significant increase in serum alanine aminotransferase activity (33%), while hepatic ornithine decarboxylase activity and cytochrome P450 content were not changed significantly (Kitchin & Brown, 1989).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Caprolactam was evaluated for developmental toxicity in both rats and rabbits (Gad *et al*., 1987). Rats were dosed by gavage on days 6–15 of gestation with 0, 100, 500 or 1000 mg/kg bw per day. No skeletal anomalies or major malformations were observed in the pups, while, in the high-dose group, maternal survival rate and fetal viability were decreased. Rabbits were dosed by gavage on days 6–28 of gestation with 0, 50, 150 or 250 mg/kg bw per day. No embryotoxicity or teratogenicity was observed. In the groups dosed with 150 and 250 mg/kg bw per day, fetal weights were decreased and in the groups given 250 mg/kg bw there was an increased incidence of thirteen ribs.

In a three-generation reproduction study, Fischer 344 rats were given 0, 1000, 5000 and 10 000 mg caprolactam/kg diet (ppm) (Serota *et al*., 1988). Each generation was treated over a 10-week period. In both the parental generations and the offspring, reduced body weights were found in the high-dose groups. Otherwise, no treatment-related effect on gross appearance, gross pathology, survival rate or number of pups was observed. In some instances, significantly reduced body weights were also observed in adult animals receiving 5000 mg caprolactam/kg diet.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

The genetic and related effects of caprolactam have been reviewed (Ashby & Shelby, 1989; Brady *et al*., 1989)

Caprolactam gave negative results across a wide range of in-vitro and in-vivo short-term tests. It did not induce mutation in *Salmonella typhimurium* or gene mutation or aneuploidy in *Aspergillus nidulans* in the presence or absence of an exogenous metabolic activation system. In *Saccharomyces cerevisiae*, no gene conversion was induced and

Table 1. Genetic and related effects of caprolactam

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SAF, *Salmonella typhimurium* TM677, forward mutation	–	–	500	Liber (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	25000	Greene *et al*. (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Baker & Bonin (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500	Matsushima *et al*. (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500	Rexroat & Probst (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	Zeiger & Haworth (1985)
SA2, *Salmonella typhimurium* TA102, reverse mutation	–	–	500	Baker & Bonin (1985)
SA2, *Salmonella typhimurium* TA102, reverse mutation	–	–	2500	Matsushima *et al*. (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	25000	Greene *et al*. (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500	Rexroat & Probst (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	Zeiger & Haworth (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	25000	Greene *et al*. (1979)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500	Rexroat & Probst (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	25000	Greene *et al*. (1979)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2500	Rexroat & Probst (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	25000	Greene *et al*. (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Baker & Bonin (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	Matsushima *et al*. (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	Rexroat & Probst (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Zeiger & Haworth (1985)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	500	Baker & Bonin (1985)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	2500	Matsushima *et al*. (1985)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	5000	Zeiger & Haworth (1985)
SCG, *Saccharomyces cerevisiae* D7, gene conversion	–	–	5000	Arni (1985)
SCG, *Saccharomyces cerevisiae* JD1, gene conversion	–	–	2000	Brooks *et al*. (1985)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SCG, *Saccharomyces cerevisiae* PV-2 and PV-3, gene conversion	–	–	1000	Inge-Vechtomov *et al*. (1985)
SCG, *Saccharomyces cerevisiae* D7-144, gene conversion	+	(+)	400	Mehta & von Borstel (1985)
SCG, *Saccharomyces cerevisiae* D7, gene conversion	–	–	2000	Parry & Eckardt (1985a)
SCH, *Saccharomyces cerevisiae* D7, homozygosis	–	–	5000	Arni (1985)
SCH, *Saccharomyces cerevisiae* PV4a and PV4b, homozygosis	–	–	1000	Inge-Vechtomov *et al*. (1985)
SCH, *Saccharomyces cerevisiae* D6 and D61-M, homozygosis	–	–	5000	Parry & Eckardt (1985b)
SCH, *Saccharomyces cerevisiae* D61-M, homozygosis	–	NT	15000	Zimmermann *et al*. (1985)
SCF, *Saccharomyces cerevisiae* D5, forward mutation	–	NT	2000	Ferguson (1985)
SCF, *Saccharomyces cerevisiae* PV-1, forward mutation	–	–	1000	Inge-Vechtomov *et al*. (1985)
SCR, *Saccharomyces cerevisiae* D7, reverse mutation	–	–	5000	Arni (1985)
SCR, *Saccharomyces cerevisiae* PV2 and PV3, reverse mutation	–	–	1000	Inge-Vechtomov *et al*. (1985)
SCR, *Saccharomyces cerevisiae* XV185-14C, reverse mutation	+	+	100	Mehta & von Borstel (1985)
SCR, *Saccharomyces cerevisiae* RM52, reverse mutation	–	–	800	Mehta & von Borstel (1985)
SCR, *Saccharomyces cerevisiae* D7, D6 and D61-M, reverse mutation	–	–	2000	Parry & Eckardt (1985a,b)
SZF, *Schizosaccharomyces pombe*, forward mutation	–	–	1900	Loprieno *et al*. (1985)
ANF, *Aspergillus nidulans*, forward mutation	–	NT	1000	Carere *et al*. (1985)
SCN, *Saccharomyces cerevisiae* D6 and D61-M, aneuploidy	–	–	5000	Parry & Eckardt (1985b)
SCN, *Saccharomyces cerevisiae* D61-M, aneuploidy	(+)	NT	7500	Zimmermann *et al*. (1985)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
ANN, *Aspergillus nidulans*, aneuploidy	–	NT	500	Carere *et al*. (1985)
DMG, *Drosophila melanogaster*, genetic crossing over/recombination	–		565 feed	Vogel (1985)
DMG, *Drosophila melanogaster*, genetic crossing over/recombination	–		5000 feed	Wurgler *et al*. (1985)
DMM, *Drosophila melanogaster*, somatic mutation	+		45000 feed	Fujikawa *et al*. (1985)
DMM, *Drosophila melanogaster*, somatic mutation	(+)		565 feed	Vogel (1985)
DMM, *Drosophila melanogaster*, somatic mutation	+		1000 feed	Wurgler *et al*. (1985)
DMM, *Drosophila melanogaster*, somatic mutation (mitotic recombination (SMART) test)	(+)		425 feed	Vogel (1989)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	(+)		1700 feed	Vogel (1989)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		15000 ppm inj	Foureman *et al*. (1994)
DIA, DNA single-strand breaks, Fischer 344 rat hepatocytes *in vitro*	–	NT	3390	Bradley (1985)
DIA, DNA single-strand breaks, Chinese hamster ovary CHO cells *in vitro*	–	–	11300	Douglas *et al*. (1985)
DIA, DNA single-strand breaks, Chinese hamster ovary CHO cells *in vitro*	–	–	NG	Lakhanisky & Hendrickx (1985)
URP, Unscheduled DNA synthesis, male Fischer 344 rat primary hepatocytes *in vitro*	–	NT	113	Probst & Hill (1985)
URP, Unscheduled DNA synthesis, male Fischer 344 rat primary hepatocytes *in vitro*	–	NT	1000	Williams *et al*. (1985)
GCO, Gene mutation, Chinese hamster ovary CHO cells *in vitro*	–	–	5000	Greene *et al*. (1979)
GCO, Gene mutation, Chinese hamster ovary CHO cells *in vitro*	–	–	2000	Zdzienicka & Simons (1985)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	–	3000	Fox & Delow (1985)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	–	1000	Kuroda *et al*. (1985)
G9O, Gene mutation, Chinese hamster lung V79 cells, ouabain resistance *in vitro*	NT	–	113	Kuroki & Munakata (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	–	11000	Amacher & Turner (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	–	15000	Knaap & Langebroek (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	–	5000	Myhr *et al*. (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	–	10000	Oberly *et al*. (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	NT	1000	Styles *et al*. (1985)
G51, Gene mutation, mouse lymphoma L5178Y cells, ouabain resistance *in vitro*	–	–	200	Garner & Campbell (1985)
G51, Gene mutation, mouse lymphoma L5178Y cells, *hprt* locus *in vitro*	–	–	200	Garner & Campbell (1985)
G51, Gene mutation, mouse lymphoma L5178Y cells, *hprt* locus *in vitro*	–	–	15000	Knaap & Langebroek (1985)
GIA, Gene mutation, mouse BALB/c–3T3 cells, ouabain resistance *in vitro*	NT	?	15000	Matthews *et al*. (1985)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	1130	Douglas *et al*. (1985)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	5000	Gulati *et al*. (1985)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	5000	Lane *et al*. (1985)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	17000	Natarajan *et al*. (1985)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	10600	Norppa & Järventaus (1989)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	–	–	5650	van Went (1985)
SIR, Sister chromatid exchange, Wistar rat liver cell line (RL_4) *in vitro*	–	NT	1000	Priston & Dean (1985)
MIA, Micronucleus test, Chinese hamster ovary CHO cells *in vitro*	–	–	113	Douglas *et al*. (1985)
CIC, Chromosomal aberrations, Chinese hamster lung CH1-L cells *in vitro*	–	NT	2000	Danford (1985)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	5000	Gulati *et al*. (1985)
CIC, Chromosomal aberrations, Chinese hamster lung CHL cells *in vitro*	–	?	10000	Ishidate & Sofuni (1985)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	17000	Natarajan *et al*. (1985)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	2500	Palitti *et al*. (1985)
CIR, Chromosomal aberrations, Wistar rat liver RL_4 cells *in vitro*	–	NT	1000	Priston & Dean (1985)
AIA, Aneuploidy, Chinese hamster lung CH1-L cells *in vitro*	–	NT	2000	Danford (1985)
TBM, Cell transformation, mouse BALB/c–3T3 cells	–	+	2500	Matthews *et al*. (1985)
TCM, Cell transformation, mouse C3H 10T½ cells	(+)	–	4570	Lawrence & McGregor (1985)
TCM, Cell transformation, mouse C3H 10T½ cells	–	NT	1000	Nesnow *et al*. (1985)
TCS, Cell transformation, Syrian hamster embryo, clonal assay	+	NT	10	Barrett & Lamb (1985)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
TCS, Cell transformation, Syrian hamster embryo, clonal assay	–	NT	1000	LeBoeuf *et al*. (1989)
TCS, Cell transformation, Syrian hamster embryo, clonal assay	?	NT	300	Sanner & Rivedal (1985)
TFS, Cell transformation, Syrian hamster embryo, focus assay	–	NT	6000	Greene *et al*. (1979)
TRR, Cell transformation, RLV/Fischer rat cells	–	NT	50	Suk & Humphreys (1985)
T7S, Cell transformation, SA7/Syrian hamster embryo cells	–	NT	7000	Greene *et al*. (1979)
T7S, Cell transformation, SA7/Syrian hamster embryo cells	–	NT	5000	Hatch & Anderson (1985)
GIH, Gene mutation, human lymphocytes *in vitro*	–	–	8000	Crespi *et al*. (1985)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	–	1000	Obe *et al*. (1985)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	+	270	Howard *et al*. (1985)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	?	NT	7500	Kristiansen & Scott (1989)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	+	4250	Norppa & Jarventaus (1989)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	(+)	(+)	5500	Sheldon (1989a)
AIH, Aneuploidy, human lymphocytes *in vitro*	+	+	2125	Norppa & Jarventaus (1989)
DVA, DNA single-strand breaks, male Fischer 344 rat hepatocytes *in vivo*	–		750 po × 1	Bermudez *et al*. (1989)
DVA, DNA single-strand breaks/alkaline-labile sites, Sprague-Dawley rat hepatocytes *in vivo*	–		425 po × 2	Kitchin & Brown (1989)
UPR, Unscheduled DNA synthesis, male Fischer 344 rat hepatocytes *in vivo*	–		750 po × 1	Bermudez *et al*. (1989)
UVR, Unscheduled DNA synthesis, Fischer 344 rat spermatocytes *in vivo*	–		750 po × 1	Working (1989)
MST, Mouse spot test, (C57BL × T)F_1 mice	?		500 ip × 1	Fahrig (1989)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
MST, Mouse spot test $(T \times HT)F_1$ mice	?		500 ip × 1	Neuhauser-Klaus & Lehmacher (1989)
SVA, Sister chromatid exchange, $B6C3F_1$ mouse bone marrow *in vivo*	–		700 ip × 1	McFee & Lowe (1989)
MVM, Micronucleus test, ICR/JCL mouse bone marrow *in vivo*	–		500 ip × 1	Ishidate & Odagiri (1989)
MVM, Micronucleus test, C57BL/6J mouse bone marrow *in vivo*	–		700 po × 1	Sheldon (1989b)
CBA, Chromosomal aberrations, $B6C3F_1$ mouse bone marrow *in vivo*	–		1000 po × 1	Adler & Ingwersen (1989)
CBA, Chromosomal aberrations, $B6C3F_1$ mouse bone marrow *in vivo*	–		700 ip × 1	McFee & Lowe (1989)
SPM, Sperm morphology, $B6C3F_1$ mice *in vivo*	–		1125 po × 5	Salamone (1989)
ICR, Inhibition of cell communication, Chinese hamster lung V79/4K-1 and V79-M13 cells *in vitro*	–	NT	400	Scott *et al*. (1985)
ICR, Inhibition of cell communication, Chinese hamster lung V79 cells *in vitro*	–	NT	2250	Umeda *et al*. (1985)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested; ?, inconclusive

[b] HID, highest ineffective dose; LED, lowest effective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; inj, injection; po, oral; ip, intraperitoneal

there was no induction of point mutations in three of four studies or aneuploidy in one of two studies. In *Drosophila melanogaster*, it induced somatic cell mutations in four studies and a marginal increase in sex-linked recessive lethal mutations in one of two studies.

Neither DNA single-strand breaks nor unscheduled DNA synthesis were induced in cultures of rat primary hepatocytes and DNA strand breaks were not induced in Chinese hamster ovary cells treated with caprolactam. Gene mutations were not induced in Chinese hamster ovary, lung V79 or mouse lymphoma L5178Y cells *in vitro*. Caprolactam did not increase the frequency of sister chromatid exchanges, micronuclei, chromosomal aberrations or aneuploidy in Chinese hamster cell cultures nor did it inhibit intercellular communication. Marginally positive results were reported in tests for morphological transformation using mouse BALB/c-3T3, C3H 10T½, and Syrian hamster embryo cells, while results from virally enhanced cell transformation tests were negative.

Caprolactam did not induce gene mutations in human lymphoblastoid AHH-1 cells or sister chromatid exchanges in human lymphocyte cultures, but it did increase the frequency of chromosomal aberrations in four studies and, in a single study, aneuploidy in human lymphocytes *in vitro*.

Caprolactam treatment *in vivo* did not increase DNA single-strand breaks in hepatocytes or unscheduled DNA synthesis in spermatocytes of rats, did not induce sister chromatid exchanges, micronuclei or chromosomal aberrations in mouse bone marrow and did not induce morphological abnormalities in mouse sperm. Inconclusive results were reported in two mouse spot test studies for gene mutations.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to caprolactam, a monomer used in high volume, can occur in its manufacture and the manufacture of nylon 6. It has been detected in surface water, groundwater and drinking-water.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Caprolactam was tested for carcinogenicity by oral administration in the diet of mice and rats. No increase in the incidence of tumours was observed. Caprolactam was also tested for promoting effects in two multistage studies in male rats. In one, oral administration of caprolactam in the diet after treatment with several carcinogens showed no modifying effect on carcinogenicity in any organ or on glutathione *S*-transferase (placental form) (GST-P)-positive foci of the liver. In the other study, oral administration of caprolactam in the diet with a two-thirds partial hepatectomy after treatment with *N*-nitrosodiethylamine did not increase the numbers or areas of GST-P-positive foci in the liver.

5.4 Other relevant data

Caprolactam is metabolized in rats to a number of metabolites including 4-hydroxycaprolactam. In rats, it exhibits some hepatotoxicity at high doses.

Caprolactam was not mutagenic to rodents *in vivo*. It induced chromosomal aberrations and aneuploidy in human lymphocytes *in vitro*, but no other evidence of mutagenicity has been found in a variety of tests with rodent cell cultures. Results for morphological transformation in mammalian cells were inconclusive. Caprolactam was mutagenic in somatic and to a lesser degree to germ cells in *Drosophila melanogaster*. Caprolactam was not genotoxic in bacteria.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of caprolactam were available.

There is *evidence suggesting a lack of carcinogenicity* of caprolactam in experimental animals.

Overall evaluation

Caprolactam is *probably not carcinogenic to humans (Group 4)*.

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CARBON TETRACHLORIDE

Data were last reviewed in IARC (1979) and the compound was classified in *IARC Monographs* Supplement 7 (1987a).

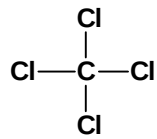
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 56-23-5
Chem. Abstr. Name: Tetrachloromethane
IUPAC Systematic Name: Carbon tetrachloride
Synonyms: Benzinoform; carbona

1.1.2 *Structural and molecular formulae and relative molecular mass*



CCl_4 Relative molecular mass: 153.82

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless, clear, nonflammable, liquid with a characteristic odour (Budavari, 1996)
(*b*) *Boiling-point*: 76.8°C (Lide, 1997)
(*c*) *Melting-point*: –23°C (Lide, 1997)
(*d*) *Solubility*: Very slightly soluble in water (0.05% by volume); miscible with benzene, chloroform, diethyl ether, carbon disulfide and ethanol (Budavari, 1996)
(*e*) *Vapour pressure*: 12 kPa at 20°C; relative vapour density (air = 1), 5.3 at the boiling-point (American Conference of Governmental Industrial Hygienists, 1991)
(*f*) *Conversion factor*: $mg/m^3 = 6.3 \times ppm$

1.2 Production and use

Production in the United States in 1991 was reported to be approximately 143 thousand tonnes (United States International Trade Commission, 1993). Information

available in 1995 indicated that carbon tetrachloride was produced in 24 countries (Chemical Information Services, 1995).

Carbon tetrachloride is used in the synthesis of chlorinated organic compounds, including chlorofluorocarbon refrigerants. It is also used as an agricultural fumigant and as a solvent in the production of semiconductors, in the processing of fats, oils and rubber and in laboratory applications (Lewis, 1993; Kauppinen *et al*., 1998).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1990–93 CAREX database for 15 countries of the European Union (Kauppinen *et al.*, 1998) and the 1981–83 United States National Occupational Exposure Survey (NOES, 1997), approximately 70 000 workers in Europe and as many as 100 000 workers in the United States were potentially exposed to carbon tetrachloride (see General Remarks). Occupational exposure to carbon tetrachloride may occur in the chemical industry, in laboratories, and during degreasing operations.

1.3.2 *Environmental occurrence*

The major source of carbon tetrachloride in air is industrial emissions. Carbon tetrachloride has been detected in surface water, groundwater and drinking-water as a result of industrial and agricultural activities. Carbon tetrachloride has also been found in wastewater from iron and steel manufacturing, foundries, metal finishing, paint and ink formulations, petroleum refining and nonferrous metal manufacturing industries (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 31 mg/m^3 as the 8-h time-weighted average threshold limit value, with a skin notation, for occupational exposures to carbon tetrachloride in workplace air. Values of 10–65 mg/m^3 have been used as standards or guidelines in other countries (International Labour Office, 1991).

The World Health Organization has established an international drinking-water guideline for carbon tetrachloride of 2 µg/L (WHO, 1993).

2. Studies of Cancer in Humans

2.1 Industry-based studies (Table 1)

Ott *et al*. (1985) conducted a cohort mortality study of 1919 men employed for one or more years between 1940 and 1969 at a chemical manufacturing facility in the United States. This cohort included 226 workers assigned to a unit which produced chlorinated methanes (methyl chloride (see this volume), dichloromethane (see this volume), chloroform (IARC, 1987b), and carbon tetrachloride) and, recently, tetrachloroethylene (IARC,

Table 1. Epidemiological results from industry-based studies relevant to the evaluation of carbon tetrachloride

Reference	Country	Cohort size/ no. of deaths	Cancer site[a]	Observed	RR	95% CI	Comment
Ott *et al*. (1985)	United States	226/42	All cancers	9	0.7	0.3–1.3	Expected from US rates
					[0.8]	[0.4–1.5]	Expected from company rates
			Respiratory	3	[0.7]	[0.1–2.0]	
			Digestive	6	[1.8]	[0.7–4.0]	
			Pancreas	3	[3.3]	[0.7–9.7]	
Blair *et al*. (1990)	United States	5365/1129	All cancers	294	[1.2]	1.0–1.3	
			Lung	47	1.3	0.9–1.7	
			Oesophagus	13	2.1	1.1–3.6	
			Pancreas	15	1.2	0.7–1.9	
			Lympho/reticulosarcoma	7	1.7	0.7–3.4	
			Hodgkin's disease	4	2.1	0.6–5.3	
			Leukaemia	7	0.9	0.4–1.8	
			Other lymphatic	4	0.7	0.2–1.8	
			Breast	36	1.0	0.7–1.4	
Blair *et al*. (1998)	United States	14475/3832	All cancers[b]	641	0.90	0.83–0.97	SMR, full cohort
			Non-Hodgkin lymphoma, women	8 exposed	3.3	0.9–12.7	Incident cancer, RR from Poisson regression
			Non-Hodgkin lymphoma, men	14 exposed	1.2	0.4–3.3	
			Multiple myeloma, women	4 exposed	2.0	0.4–9.1	
			Multiple myeloma, men	10 exposed	1.2	0.4–3.7	
			Breast, women	18 exposed	1.3	0.7–2.5	
Wilcosky *et al*. (1984)	United States	6678	Lymphocytic leukaemia (white men)	8 exposed	15.3	$p < 0.0001$	Odds ratios from nested case–control analysis
			Lymphosarcoma (white men)	6 exposed	4.2	$p < 0.05$	
Bond *et al*. (1986)	United States	19608	Lung cancer		0.8	0.6–1.1	Odds ratio from nested case–control analysis

[a] Results are presented for all cancers, lung, oesophagus, pancreas, lymphatic and haematopoietic cancers, and breast when reported.
[b] Includes entire cohort regardless of potential exposure to dichloromethane.

1995). Exposure levels were not reported. The follow-up period was from 1940 to 1979 and follow-up was 94% complete. Expected numbers were based on national rates for white males in the United States for the full cohort and on the rates for the full cohort for sub-cohort analyses. There were 42 deaths observed among the 226 workers (standardized mortality ratio (SMR), 0.6, based on national rates) [SMR, 0.8, based on company rates]. Nine cancers were observed [SMR, 0.8; 95% confidence interval (CI), 0.4–1.5, based on company rates], including three pancreatic cancers [SMR, 3.3; 95% CI, 0.7–9.7, based on company rates]. Two of the three workers who died of pancreatic cancer had been employed for less than five years. All three were first assigned to the chlorinated methane unit between 1942 and 1946, and the interval between first assignment to the unit and death was between 20 and 31 years. [The Working Group noted that the mix of exposures and the lack of information regarding exposure levels limits the ability to draw conclusions regarding the carcinogenicity of carbon tetrachloride.]

Blair *et al*. (1990) studied the risk of cancer and other causes of death among a cohort of 5365 members of a dry-cleaners union in the United States. The cohort consisted of persons who were union members for one year or more before 1978 and had been employed in dry-cleaning establishments. Carbon tetrachloride was used extensively in dry-cleaning between 1930 and 1960, although other solvents, such as Stoddard solvent, were also widely used. The mean year at entry into the cohort was 1956. Follow-up was from 1948 through 1978 and was 88% complete. For individuals lost to follow-up, person-years were counted only until last date known alive. The exposure assessment classified members by level of exposure to solvents, but not type of solvent. Three time-weighted average (TWA) exposure categories for solvents (none, medium, high) were assigned weights of 0, 7, 40 for cumulative exposure analysis. Expected deaths were calculated from national rates for the United States and the overall SMR (based on 1129 deaths) was 0.9. Cancer deaths amounted to 294 (SMR, 1.2). A significant excess of oesophageal cancer (SMR, 2.1; 95% CI, 1.1–3.6, based on 13 cases) and non-significant excesses of several other cancers were found. However, only the risk of lymphatic and haematopoietic cancers appeared to be related to level of solvent exposure (SMR, 4.0 for high exposure, based on five cases). The authors state that mortality patterns among those entering the union after 1960, when the use of tetrachloroethylene was predominant, were similar to those in people entering before 1960.

Blair *et al*. (1998) performed a retrospective cohort mortality study of 14 457 workers employed for at least one year between 1952 and 1956 at an aircraft maintenance facility in the United States. Among this cohort were 6737 workers who had been exposed to carbon tetrachloride (Stewart *et al*., 1991). The methods used for this study are described in greater detail in the monograph on dichloromethane. An extensive exposure assessment was performed to classify exposure to trichloroethylene quantitatively and to classify exposure (ever/never) to other chemicals qualitatively (Stewart *et al*., 1991). Risks from chemicals other than trichloroethylene were examined in a Poisson regression analysis of cancer incidence data. Among women, exposure to carbon tetrachloride was associated with an increased risk of non-Hodgkin lymphoma (relative risk (RR), 3.3; 95% CI,

0.9–12.7; 8 exposed cases) and multiple myeloma (RR, 2.0; 95% CI, 0.4–9.1; 4 exposed cases), but among men the corresponding risks were lower (non-Hodgkin lymphoma: RR, 1.2; 95% CI, 0.4–3.3; 14 exposed cases and multiple myeloma: RR, 1.2; 95% CI, 0.4–3.7; 10 exposed cases). No association was observed with breast cancer and no other site-specific results for carbon tetrachloride were presented. Exposure levels for carbon tetrachloride were not reported. [The Working Group noted that overlapping exposures limit the ability to draw conclusions regarding carbon tetrachloride.]

A nested case–control study within a cohort of rubber workers in the United States was performed to examine the relationship between exposure to solvents and the risk of cancer (Checkoway *et al*., 1984; Wilcosky *et al*., 1984). The cohort consisted of 6678 male rubber workers who either were active or retired between 1964 and 1973. The cases comprised all persons with fatal stomach cancer (n = 30), respiratory system cancer (n = 101), prostate cancer (n = 33), lymphosarcoma (n = 9) or lymphocytic leukaemia (n = 10). These sites were chosen because they were those at which cancers had been found to be in excess in an earlier cohort analysis (McMichael *et al*., 1976). The controls were a 20% age-stratified random sample of the cohort (n = 1350). Exposure was classified from a detailed work history and production records. An association was observed between exposure for one year or more to carbon tetrachloride and lymphocytic leukaemia (odds ratio (OR), 15.3; $p < 0.0001$, based on eight exposed cases) and lymphosarcoma (OR, 4.2; $p < 0.05$, based on six exposed cases) after adjusting for year of birth. The relative risk associated with 24 solvents was examined and levels of exposure were not reported. [The Working Group noted that overlapping exposures limit the ability to draw conclusions regarding carbon tetrachloride.]

Bond *et al*. (1986) conducted a nested case–control study of lung cancer among a large cohort of chemical workers in the United States. The cohort consisted of 19 608 white male workers employed for one year or more between 1940 and 1980 at a large facility which produced chlorinated solvents, plastics, chlorine, caustic soda, ethylene (IARC, 1994a), styrene (IARC, 1994b), epoxy latex, magnesium metal, chlor-nitrogen agricultural chemicals and glycols (Bond *et al*., 1985). The cases were 308 lung cancer deaths that occurred among cohort members between 1940 and 1981. Two control groups, one consisting of other deaths (n = 308) and the other a 'living' series (n = 97), were matched on race, year of birth, and year of hire. Occupational exposures were classified on the basis of work history records and information regarding exposure to chemical and physical agents collected for each work area [levels of exposure to carbon tetrachloride were not reported], while information on smoking and other potential confounders was collected by interview. No association was observed between having been exposed to carbon tetrachloride (ever versus never) and lung cancer (OR, 0.8; 95% CI, 0.6–1.1).

2.2 Community-based studies

Linet *et al*. (1987) performed an analysis to compare two different methods for determining occupational exposure in a population-based case–control study of chronic

lymphocytic leukaemia. Incident cancers were identified using hospital records, and controls matched on age, race and sex were selected from among patients with nonmalignant diseases from the same hospitals. The study included 342 cases and an equal number of controls [participation rates were not reported]. Relative risks derived from exposures classified on the basis of the job–exposure matrix developed by Hoar *et al.* (1980) were compared with those derived from a classification of exposure based on the National Occupational Hazard Survey (NOHS). The prevalence of exposure among cases and controls using the job–exposure matrix developed by Hoar *et al*. (1980) was 10.5% and 10.2%, respectively. The prevalence of exposure among cases and controls using the job–exposure matrix based on the NOHS was 3.8% and 5.2%, respectively. No association between chronic lymphocytic leukaemia and carbon tetrachloride exposure was observed in either set of analyses (odds ratio, 1.1; 95% CI, 0.6–2.0 for the Hoar method; and odds ratio, 0.8; 95% CI, 0.4–1.9 for the NOHS method). [The Working Group expressed concern regarding the sensitivity and specificity of the exposure assessment used.]

Heineman *et al*. (1994) performed a case–control study to examine the relationship between occupational exposure to six chlorinated aliphatic hydrocarbons and risk of astrocytic brain cancer. The study was conducted in three areas of the United States, and 300 cases and 320 controls were included in the analysis. The methods used for this study are described in greater detail in the monograph on dichloromethane. Exposure was assessed using a semi-quantitative job–exposure matrix developed for the study (Gomez *et al*., 1994), and probability of exposure, duration of exposure, average intensity and cumulative exposure were examined. There were 137 cases and 123 controls classified as ever exposed. The odds ratios for the highest-exposure categories were 0.8 (95% CI, 0.4–1.9; 13 exposed cases) for high probability of exposure, 1.6 (95% CI, 0.9–2.8; 36 exposed cases) for more than 21 years of exposure, 2.9 (95% CI, 1.2–7.1; 22 exposed cases) for high average intensity, and 1.6 (95% CI, 0.8–3.2; 24 exposed cases) for high cumulative exposure.

Cantor *et al*. (1995) performed a case–control study to examine the relationship between occupational exposures and female breast cancer mortality in 24 states of the United States. The methods used for this study are described in greater detail in the monograph on dichloromethane. Probability and level of workplace exposure to 31 chemical and physical agents were estimated using a job–exposure matrix. No association was found with probability of exposure to carbon tetrachloride. After adjustment for age and socioeconomic status, a slightly elevated risk was observed for the highest exposure level among white women (odds ratio, 1.2; 95% CI, 1.1–1.3) but not among black women. [The Working Group noted that the usual occupation from death certificate in combination with a job–exposure matrix may be a poor indicator of exposure to carbon tetrachloride.]

Holly *et al*. (1996) performed a case–control study of intraocular melanoma to examine the role of chemical exposures. Cases were white male patients referred to the Ocular Oncology Unit at the University of California San Francisco (United States) between 1978 and 1987. Two white males matched on age and geographical area were selected for each case using random-digit dialling. A total of 221 cases and 447 control

(93% and 85% participation rates, respectively) were interviewed for the study. An association with exposure (ever versus never) to 'carbon tetrachloride and other cleaning fluids' was observed (odds ratio, 2.3; 95% CI, 1.3–4.1). [The Working Group expressed concern regarding the potential for recall bias from exposures based on self-reporting. The broad category of 'carbon tetrachloride and other cleaning fluids' limits the ability to draw inferences regarding carbon tetrachloride alone.]

In the Montreal case–control study carried out by Siemiatycki *et al.* (1991) (see the monograph on dichloromethane in this volume), the investigators estimated the associations between 293 workplace substances and several types of cancer. Carbon tetrachloride was one of the substances. About 4% of the study subjects had ever been exposed to carbon tetrachloride. Among the main occupations to which carbon tetrachloride exposure was attributed were fire fighters, machinists and electricians. For most types of cancer examined (oesophagus, stomach, colon, pancreas, prostate, kidney, skin melanoma), there was no indication of an excess risk. For non-Hodgkin lymphoma, based on three cases exposed at any level, the odds ratio was 0.4 (90% CI, 0.1–1.0). For rectal cancer, based on 16 cases exposed at any level, the odds ratio was 2.0 (90% CI, 1.2–3.3). For bladder cancer, in the population subgroup of French Canadians (the majority ethnic group in this region), based on nine cases exposed at the 'substantial' level, the odds ratio was 2.5 (90% CI, 1.2–5.1). [The interpretation of null results has to take into account the small numbers and presumed low levels of exposure.]

3. Studies of Cancer in Experimental Animals

Carbon tetrachloride was tested for carcinogenicity in several experiments in mice by oral and intrarectal administration and in rats by oral and subcutaneous administration and by inhalation exposure; it was also tested in one experiment in hamsters and one experiment in trout by oral administration. In various strains of mice, it produced liver tumours, including hepatocellular carcinomas. In various strains of rats, it produced benign and malignant liver tumours; and in one experiment with subcutaneous injection, an increased incidence of mammary adenocarcinomas was observed. In hamsters and trout, increased incidences of liver tumours were observed; however, these studies were considered to be inadequate (IARC 1979).

3.1 Oral administration

Rat: A group of 20 female Sprague-Dawley rats, weighing 200 ± 20 g, was administered 0.08–1.6 mL/rat carbon tetrachloride [purity unspecified] by gavage once a week for 30 weeks. The initial dose was 0.08 mL/rat for six weeks followed by 1.1 mL/rat for four weeks and then increasing to 1.6 mL/rat. Animals were killed at the end of 30 weeks and the livers were examined histologically. Hepatocellular carcinomas occurred in 6/20 rats (Frezza *et al.*, 1994). [The Working Group noted that no controls were used in this study.]

3.2 Inhalation exposure

3.2.1 *Mouse*

Groups of 50 male and 50 female BDF_1 (C57BL/6 × DBA/2) mice, six weeks of age, were exposed by whole-body inhalation to 0, 5, 25 or 125 ppm [0, 32, 157 or 787 mg/m^3] carbon tetrachloride (purity, > 99%) for 6 h per day on five days a week for 104 weeks. The incidence of hepatocellular adenomas (9/50, 10/50, 27/50 and 16/50 males; 2/50, 8/49, 17/50 and 5/49 females) was significantly increased in mid- and high-dose males and in low-dose and mid-dose females. The incidence of hepatocellular carcinomas (17/50, 12/50, 44/50 and 47/50 males; 2/50, 1/49, 33/50 and 48/49 females) was increased in mid- and high-dose males and females. Incidence of phaeochromocytomas of the adrenal gland (0/50, 0/50, 16/50 and 31/50 males; 0/50, 0/49, 0/50 and 22/49 females) was increased in mid- and high-dose males and in high-dose females (Nagano *et al.*, 1998).

3.2.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were exposed by whole-body inhalation to 0, 5, 25 or 125 ppm [0, 32, 15 or 787 mg/m^3] carbon tetrachloride (purity, > 99.8%) for 6 h per day on five days per week for 104 weeks. The incidence of hepatocellular adenomas (0/50, 1/50, 1/50 and 21/50 males; 0/50, 0/50, 0/50 and 40/50 females) and of hepatocellular carcinomas (1/50, 0/50, 0/50 and 32/50 males; 0/50, 0/50, 3/50 and 15/50 females) was significantly increased in high-dose rats of each sex (Nagano *et al.*, 1998).

3.3 Multistage protocols and preneoplastic lesions

3.3.1 *Mouse*

Three groups of 30 male and 30 female C57BL/6 mice, six to eight weeks old, received a single-dose irradiation with 0, 170 or 330 rad of fast neutrons. Nine weeks later all mice received a single subcutaneous injection of 3 g/kg bw carbon tetrachloride [purity unspecified] dissolved in corn oil. Animals were observed for lifetime and were necropsied after death. Histological examinations were performed on the livers of all animals and on all other organs or tissues with macroscopic lesions. The incidence of liver carcinomas was increased in high-dose females (330 rad neutrons + carbon tetrachloride, 11/27; 330 rad neutrons + corn oil, 1/17; 330 rad neutrons alone, 2/14) [statistical significance unspecified]. No liver carcinomas were observed in females receiving carbon tetrachloride alone (0/30) (Habs *et al.*, 1983).

Groups of 8–12 female $B6C3F_1$ mice were administered 1.6 g/kg bw carbon tetrachloride [purity unspecified] dissolved in corn oil by gavage once every other week (four or eight times, starting at 4, 18 or 26 weeks of age) after a single dose of 15 mg/kg bw *N*-nitrosodiethylamine (NDEA) given at seven days of age. Gross and histological examinations were performed on the liver of all surviving mice killed at 36 weeks of age. An increased number and volume of the hepatocellular nodules [lesion histology not described] was observed compared with mice administered NDEA alone ($p < 0.01$ by

Scheffe's test). No hepatocellular nodules were observed in mice receiving carbon tetrachloride alone (Dragani *et al*., 1986).

3.3.2 *Rat*

Groups of 12 male Fischer rats, weighing approximately 150 g, were given 200 mg/kg of diet [ppm] 2-acetylaminofluorene for two weeks and received by gavage a single dose of 1.6 g/kg bw carbon tetrachloride dissolved in olive oil at the end of week 1. Subsequently, phenobarbital was added to the diet at a concentration of 500 mg/kg of diet for six weeks and a two-thirds partial hepatectomy was performed at the end of week 3. Animals were killed at the end of week 8. Quantitative analysis of hyperplastic nodules of the liver [lesion histology not described] was carried out. The number and area of hyperplastic nodules per cm^2 (1.44 ± 1.05 and 0.77 ± 0.71 mm^2, respectively) were significantly higher in animals receiving carbon tetrachloride than in animals that did not receive carbon tetrachloride treatment (0.30 ± 0.30 and 0.18 ± 0.17 mm^2, respectively) (number, $p < 0.01$; area, $p < 0.05$) [statistical method unspecified]. No hyperplastic nodules were observed in the group not given 2-acetylaminofluorene (Takano *et al*., 1980).

A group of 24 male and 21 female inbred ACI rats [age unspecified] was administered 0.5 mL/kg bw carbon tetrachloride [purity unspecified] by gavage followed 24 h later by intraperitoneal injections of 25 mg/kg bw methylazoxymethanol acetate once a week for four weeks and animals were observed until they were killed 30 weeks later. A group of 15 males and 15 females received the methylazoxymethanol acetate treatment alone. Organs [unspecified] were examined histologically. There was no significant difference in the number of animals bearing tumours of the whole intestine (males: carbon tetrachloride + methylazoxymethanol acetate, 18/19; methylazoxymethanol acetate alone, 10/15; females: 13/17 and 10/14, respectively). No intestinal tumours were observed in a group receiving carbon tetrachloride only (0/15 males). However, in males, the multiplicity of tumours in the small intestine (3.4; no. of tumours/no. of tumour-bearing rats) was significantly higher in the carbon tetrachloride + methylazoxymethanol acetate group than that in rats receiving methylazoxymethanol acetate alone (1.4; $p < 0.025$ by *t*-test) (Kazo *et al*., 1985).

A group of 17 male Fischer 344 rats, weighing 160–170 g, received thrice-weekly intraperitoneal injections of 10 mg/kg bw NDEA dissolved in 0.9% saline up to a total dose of 200 mg/kg bw (treatment lasted six weeks). Starting two weeks later, the rats were administered 0.2 mL/kg bw carbon tetrachloride [purity unspecified] dissolved in corn oil by gavage twice a week for three months. All animals were killed eight months after the start of the experiment and a complete necropsy was performed. The incidence of hepatocellular carcinomas in the group receiving NDEA + carbon tetrachloride (17/17) was significantly higher than in a group that received NDEA only (9/17) ($p < 0.005$, by chi-square test). No hepatocellular carcinomas were observed in a group of 15 rats receiving carbon tetrachloride only (Zalatnai *et al*., 1991).

Newborn Sprague-Dawley rats received a single intraperitoneal injection of 15 mg/kg bw NDEA dissolved in 0.1 mL normal saline one day after parturition. From

three weeks of age, female rats received twice-weekly intraperitoneal injections of a 33% solution of carbon tetrachloride [purity unspecified] in 0.25 mL mineral oil for nine weeks. Animals were killed at week 12 and the livers were examined histologically by staining with haematoxylin and eosin and by glutathione *S*-transferase placental form (GST-P) staining. The incidence of foci of cellular alterations and of neoplastic nodules was 15/20 and 13/20, respectively, in the NDEA + carbon tetrachloride group compared with 10/10 and 0/10 in the group not receiving carbon tetrachloride treatment (NDEA group). Most of the nodular lesions were GST-P-positive. The number and area of GST-P-positive neoplastic nodules and/or foci per cm^2 were significantly larger in the NDEA + carbon tetrachloride group (7.27 ± 3.18 and 4.34 ± 4.41 mm^2, respectively) than in the NDEA group (3.97 ± 1.86 and 0.29 ± 0.16 mm^2, respectively ($p < 0.001$, Student's *t*-test) (Cho & Jang, 1993).

3.3.3 *Hamster*

Groups of 11–15 male Syrian hamsters, six weeks of age, were administered carbon tetrachloride by gavage at a dose of 0 or 0.1 mL/animal every two weeks for 30 weeks alone or beginning one week after a single intraperitoneal injection of 6 mg/kg bw NDEA. At the end of the study at 30 weeks, carbon tetrachloride alone produced no liver tumours compared with 1/15 (7%) in hamsters given NDEA and 11/13 (85%) in hamsters given NDEA followed by carbon tetrachloride (Tanaka *et al*., 1987).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

The absorption, distribution, metabolism and excretion have previously been reviewed (IARC, 1979; McGregor & Lang, 1996).

Liquid carbon tetrachloride on intact mouse skin was absorbed at a rate of 8.3 µg/cm^2/minute (Tsuruta, 1975). Jakobson *et al.* (1982) examined the percutaneous uptake by guinea-pigs of liquid carbon tetrachloride (1 mL in a glass depot, covering 3.1 cm^2 of clipped skin). A peak blood level of about 1 mg carbon tetrachloride/L was reached within 1 h. Despite continuation of the exposure, the blood levels declined during the following hours, possibly due to local vasoconstriction, rapid transport from blood to adipose tissues or biotransformation processes. McCollister *et al.* (1951) exposed the clipped skin of one male and one female monkey to [^{14}C]carbon tetrachloride vapour (whole body exposure). After exposure to 3056 mg/m^3 for 3 h, the blood of the female ained radioactivity equivalent to a carbon tetrachloride level of 12 µg/100 g and the

expired air contained 0.8 µg/L. After exposure to 7230 mg/m^3 for 3.5 h, the blood of the male contained a carbon tetrachloride-equivalent level of 30 µg/100 g and the expired air contained 3 µg/L.

Many early studies examining hepatotoxicity of carbon tetrachloride used corn oil as a dosing vehicle for laboratory animals, but corn oil has been found to markedly delay the absorption of carbon tetrachloride from the gastrointestinal tract (Kim *et al.*, 1990). More recent studies have used Emulphor®, a polyethoxylated oil, in concentrations up to 10% in an aqueous vehicle for carbon tetrachloride. Aqueous solutions of carbon tetrachloride in Emulphor® were administered to Sprague-Dawley rats both as a bolus and during gastric infusion at a constant rate over a 2-h period (Sanzgiri *et al.*, 1997). Uptake and tissue levels of carbon tetrachloride after gastric infusion were less than after bolus dosing. When the concentration of Emulphor® was varied up to 10%, absorption (and distribution) of carbon tetrachloride was not affected (Sanzgiri & Bruckner, 1997).

Following inhalation exposure of rats to 406 ppm [2600 mg/m^3] carbon tetrachloride for 4 h, the blood level was 10.5 mg/L, but dropped to 50% of this value in less than 30 min (Frantik & Benes, 1984). Carbon tetrachloride, administered by inhalation to rats, mice or monkeys, is distributed to most tissues, including fat, liver, brain, bone marrow and kidney (McCollister *et al.*, 1951; Bergman, 1984; Paustenbach *et al.*, 1986). In mice exposed to [^{14}C]carbon tetrachloride, much of the radioactivity became non-volatile and a portion appeared to be non-extractable (Bergman, 1984).

The discrepancy between bolus oral administration of carbon tetrachloride (the route used for most toxicity and mechanistic studies) and inhalation exposure, the route most representative of human exposure, has been addressed by Sanzgiri *et al*. (1995), who studied the kinetics of carbon tetrachloride in rats at doses of (1) 100 and 1000 ppm [630 and 6300 mg/m^3] by inhalation for 2 h (equivalent to a systemically administered dose of 17.5 and 179 mg/kg bw), (2) as a gavage bolus emulsion of 17.5 and 179 mg/kg bw and (3) as a gastric infusion emulsion at these dose levels over a period of 2 h. The concentration of carbon tetrachloride in arterial blood were considerably higher in the bolus-administered groups. In the groups administered 17.5 and 179 mg/kg bw, respectively, C_{max} and AUC values were approximately six- and 16-fold higher in the bolus-administered groups than the inhalation-exposed groups. C_{max} and AUC values were slightly lower following gastric infusion than after inhalation, probably due to first-pass metabolism effects. A pharmacokinetic model has been developed for carbon tetrachloride in order to study its interaction with methanol (Evans & Simmons, 1996). The metabolic rate (V_{max}) for carbon tetrachloride was 0.11 mg/h, and increased about 4.5-fold 24 h after exposure to methanol (10 000 ppm, 6 h), but < 2-fold 48 h after methanol treatment. The K_m value was 1.3 mg/L.

Known metabolites of carbon tetrachloride include chloroform, carbon monoxide, carbon dioxide, hexachloroethane and phosgene (Poyer *et al.*, 1978; Shah *et al.*, 1979; Ahr *et al.*, 1980; Kubic & Anders, 1980; Nastaincyzyk *et al.,* 1991). Metabolism of carbon tetrachloride is initiated by cytochrome P450-mediated transfer of an electron to the C–Cl

bond, forming an anion radical that eliminates chloride, thus forming the trichloromethyl radical. The isoenzymes implicated in this process are CYP2E1 and CYP2B1/2B2 (Raucy *et al*., 1993; Gruebele *et al*., 1996).

4.2 Toxic effects

The toxicity of carbon tetrachloride has been reviewed (Recknagel *et al*., 1989; McGregor & Lang, 1996).

4.2.1 *Humans*

Numerous poisonings and fatalities have occurred due to ingestion or inhalation of carbon tetrachloride. The major pathological changes have been seen in the liver and kidney (IARC, 1979). Minor changes in enzyme levels reflecting hepatic effects were observed among workers exposed to carbon tetrachloride levels that were generally below 5 ppm [32 mg/m^3] (Tomenson *et al*., 1995). In a case series of carbon tetrachloride-exposed workers, fulminant hepatic damage was observed only in the two individuals who were heavy users of alcoholic beverages, suggesting a synergistic effect between ethanol and carbon tetrachloride (Manno *et al*., 1996).

4.2.2 *Experimental systems*

High doses of carbon tetrachloride kill animals within hours by central nervous system depression; smaller doses produce death by liver damage after several days. Repeated administration of carbon tetrachloride induces liver cirrhosis (IARC, 1979). This observation of liver damage was substantiated in a carcinogenicity study comparing responses in different strains of rats (Reuber & Glover, 1970). Severe cirrhosis was observed in all (16/16) Sprague-Dawley rats at 5–16 weeks (the time of death of the animals) and in 13/17 Black rats at 7–18 weeks. In Wistar rats, 6/12 rats developed moderate and 6/12 severe cirrhosis by 17–68 weeks, while the cirrhosis was mild in 2/13, moderate in 7/13 and severe in 4/13 Osborne-Mendel rats at 10–105 weeks; in Japanese rats, the cirrhosis was mild in 9/15, moderate in 5/15 and severe in 1/15 rats at 8–78 weeks. Lipid peroxidation, presumably initiated by a free-radical metabolite of carbon tetrachloride, seems to be the most important factor in carbon tetrachloride-induced liver toxicity. Similar events may be responsible for tissue damage in lung, kidney, testes, adrenals and placenta. Induction and inhibition of drug-metabolizing enzymes alters the hepatotoxicity of carbon tetrachloride (IARC, 1979).

A single oral bolus of carbon tetrachloride (17.5 or 179 mg/kg) to male Sprague-Dawley rats induced a dose-dependent increase in serum sorbitol dehydrogenase and alanine aminotransferase activities, and a decrease in the hepatic cytochrome P-450 content and glucose-6-phosphatase activity. When the same dose was given as a gastric infusion for 2 h, or by inhalation, the effects were much smaller (Sanzgiri *et al*., 1995). In contrast, continuous inhalation exposure (16 ppm [100 mg/m^3]) for four weeks was more hepatotoxic to rats than a fluctuating, but similar cumulative exposure (87 ppm [550 mg/m^3] 6 h per day, five days per week) (Plummer *et al*., 1990). No significant

difference was observed in the toxicity of carbon tetrachloride administered orally in either corn oil, Emulphor or Tween-85 (Raymond & Plaa, 1997).

Carbon tetrachloride induced hepatic cell proliferation, increasing the frequency of cells in S-phase from < 1% in control animals to about 10% in male and female $B6C3F_1$ mice 48 h after dosing with 100 mg/kg by gavage; in male Fischer 344 rats, a similar increase was observed after a dose of 400 mg/kg (Mirsalis *et al*., 1985) to about 30%. In CD-1 mice, an increase to about 30% was observed 48 h after a single oral dose of 50 mg/kg (Doolittle *et al*., 1987). In male Fischer 344 rats, the frequency of S-phase cells was elevated in one study to 30% 24 h after administration of 0.4 mL/rat, the only dose tested (Cunningham & Matthews, 1991). In male Fischer 344 rats administered 400 mg/kg carbon tetrachloride orally, it was increased to 3% in animals fed *ad libitum* and to 15% in fasting rats (Asakura *et al*., 1994). Twenty-four hours after an intraperitoneal dose of 400 mg/kg carbon tetrachloride to male Fischer 344 rats fed *ad libitum*, an increase to 5% was observed (Mirsalis *et al*., 1985). An even lower response, to approximately 2%, was observed in male Tif:RAIf rats 24 h or 48 h after treatment with 400 mg/kg by gavage (Puri & Müller, 1989). In Sprague-Dawley rats, an increase in DNA synthesis was observed 48 h after an intragastric dose (0.25 mL/100 g [4000 mg/kg bw]) of carbon tetrachloride, and the number of *ras* transcripts was elevated 36–48 h after dosing (Goyette *et al*., 1983). After a single intraperitoneal dose (1.25 mL/kg [2000 mg/kg] bw) of carbon tetrachloride to female Sprague-Dawley rats, sequential transient expression of c-*fos* (peak at 1 h in pericentral hepatocytes and at 1–12 h in mesenchymal cells), c-*jun* (1 h), c-*myc* (3–12 h), c-Ha-*ras* (12–24 h), and c-Ki-*ras* (12–24 h) RNA transcripts was observed; the pattern of proto-oncogene expression spread later to the peripheral parts of the hepatic lobulus (Herbst *et al*., 1991). A rapid transient increase of 8–10-fold in c-*fos* and c-*jun* mRNA (1–2 h after treatment) was also observed in the liver of male Sprague-Dawley rats after a single dose of 160 mg/kg carbon tetrachloride (Zawaski *et al*., 1993). An increase in c-*fos*, c-*jun* and c-*myc* mRNA was also observed in male Wistar rats after a single intragastric dose of carbon tetrachloride (2 mL/kg [3200 mg/kg] bw) (Coni *et al*., 1990, 1993). These authors also concluded that elevations in c-*fos* and c-*myc* RNA are not inevitably linked with liver hyperplasia. Concentrations of *ras* and *myc* proteins were assessed by immunohistochemical techniques in periportal areas of rat liver after a dose of 0.25 mL/100 g [4000 mg/kg] bw carbon tetrachloride; staining throughout the lobule was greatest 96 h after dosing (Richmond *et al*., 1992). The sequence of *fos*, *myc* and Ha-*ras* mRNA expression, followed by hepatocyte proliferation, was observed also in Fischer 344 rats after a single intraperitoneal dose of 2000 mg/kg carbon tetrachloride by gavage (Goldsworthy *et al*., 1994). Injection of a polyclonal antiserum to murine tumour necrosis factor α (TNF-α) 1 h before a challenge with carbon tetrachloride (0.1 mL/kg [0.15 mg/kg bw]) blocked the increase in c-*fos* and c-*jun* mRNA expression, DNA binding of the activator protein-1 (AP-1) nuclear transcription factor and the subsequent increase of S-phase cells, while at the same time delaying liver repair, as shown by the prolonged elevation of serum alanine and aspartate aminotransferases and sorbitol dehydrogenase in female $B6C3F_1$ mice. When recombinant TNF-α was injected into mice, rapid expression

of c-*jun* and c-*fos* proto-oncogene mRNA was observed (Bruccoleri *et al.*, 1997). This result supports the notion, formulated after the demonstration of increased expression of TNF-α after administration of a hepatotoxic dose of carbon tetrachloride, that TNF-α has a role in hepatocellular regeneration after carbon tetrachloride administration (Czaja *et al.*, 1989). It has also been demonstrated, however, that injection of a soluble TNF-α receptor preparation to rats had a protective effect against a single, 2.5 mL/kg [4000 mg/kg] bw dose of carbon tetrachloride by reducing serum aminotransferase levels and the extent of histological liver damage, as well as reducing mortality following a single 6000 mg/kg bw dose (Czaja *et al.*, 1995)

Like several naturally occurring tumour promoters, carbon tetrachloride (at millimolar concentrations) increased 43 kDa protein phosphorylation by rabbit platelets *in vitro*, and activated protein kinase C in a cell-free system (Roghani *et al.*, 1987). Carbon tetrachloride (≥ 15 mg/kg) greatly enhanced hepatic ornithine decarboxylase activity, even at dose levels that also decreased the hepatic total cytochrome P450 concentrations but did not induce elevated serum alanine aminotransferase levels (Kitchin & Brown, 1989). Electrical and dye coupling between hepatocytes *in vitro* was reversibly blocked by carbon tetrachloride (650 μmol/L); this activity was substantially reduced by the cytochrome P450 inhibitor SKF 525-A and by β-mercaptoethanol (Sáez *et al.*, 1987). Injection of carbon tetrachloride (1 mL/kg [1600 mg/kg] bw) to male Sprague-Dawley rats caused a transient decrease in hepatic connexin 32 content (Miyashita *et al.*, 1991). Repeated administration of carbon tetrachloride (0.5 mL/kg bw injections twice a week for 12 weeks), which led to liver cirrhosis, also decreased the connexin 32 content of the liver in male Sprague-Dawley rats (Nakata *et al.*, 1996).

Oral dosage of carbon tetrachloride (2.5 mL/kg [4000 mg/kg] bw) decreased ATP-dependent calcium uptake of liver microsomes within 30 min in Sprague-Dawley rats (Moore *et al.*, 1976). The cytosolic calcium concentration increased 100-fold in hepatocytes exposed to carbon tetrachloride (1 mmol/L [1500 μg/mL]), and this was paralleled by inhibition of the endoplasmic reticulum Ca-Mg ATPase (Long & Moore, 1986). The inhibition of the ATPase by carbon tetrachloride exposure has been confirmed (Srivastava *et al.*, 1990), and has led to the hypothesis that this is the specific mechanism by which radical intermediates from carbon tetrachloride cause cell death. The calcium-chelating agents, Calcion and alizarin sodium sulfonate, administered 6 or 10 h after a necrogenic intraperitoneal dose of carbon tetrachloride (1 mL/kg [1600 mg/kg] bw), markedly decreased the necrotizing effect of carbon tetrachloride on the liver, and decreased the hepatic calcium concentration, but did not affect carbon tetrachloride-induced lipid peroxidation *in vitro* or lipid accumulation in the liver (de Ferreyra *et al.*, 1989, 1992). Carbon tetrachloride (0.01–0.12 mmol/L) induced complete release of calcium from calcium-loaded microsomes in the presence of NADPH; this release was blocked by adding the spin-trapping agent, phenyl-*tert*-butylnitrone (PBN) after a lag period that was dependent on the concentration of carbon tetrachloride. The lag period was shortened in microsomes from pyrazole-treated rats, which showed elevated activity for *para*-nitrophenol oxidation, and was lengthened in the presence of the CYP2E1 inhibitor,

methylpyrazole, or an anti-CYP2E1 antibody. Calcium release was practically complete at concentrations of carbon tetrachloride that had no effect on the Ca-Mg ATPase activity. Ruthenium red, a specific ryanodine receptor inhibitor, completely blocked the carbon tetrachloride-induced calcium release at a concentration (0.02 mmol/L) which had no effect on *para*-nitrophenol hydroxylation or on formation of PBN–carbon tetrachloride adducts (Stoyanovsky & Cederbaum, 1996). These results support the notions that the hepatotoxicity of carbon tetrachloride requires metabolism to the trichloromethyl radical, and that it is mediated by calcium release from intracellular stores, most likely from the ryanodine-sensitive calcium store.

Several studies have demonstrated that ethanol, methanol and other alcohols potentiate the hepatic toxicity of carbon tetrachloride (Traiger & Plaa, 1971; Cantilena *et al*., 1979; Harris & Anders, 1980; Ray & Mehendale, 1990; Simko *et al*., 1992). Dietary ethanol (2 g/80 mL liquid diet for three weeks) potentiated the hepatoxicity of carbon tetrachloride (inhalation exposure to 10 ppm [63 mg/m^3] for 8 h), measured by serum aminotransferases and liver malonaldehyde concentrations, in male Wistar rats (Ikatsu *et al*., 1991; Ikatsu & Nakajima, 1992). Only a minor potentiating effect on weight gain, but no potentiating effect on carbon tetrachloride-induced hepatotoxicity was observed, when rats were treated simultaneously with ≤ 0.5 mL/kg ethanol and 20 mg/kg carbon tetrachloride by gavage for 14 days (Berman *et al*., 1992). Micronodular cirrhosis was observed in all treated male black-headed Wistar rats after 10 weeks of inhalation exposure to carbon tetrachloride (80 ppm [500 mg/m^3], 6 h per day, 5 days per week) when the animals were simultaneously given ethanol as a part of a liquid diet, whereas no animal treated with either ethanol or carbon tetrachloride alone developed cirrhosis (Hall *et al*., 1991). Similar cirrhosis was observed also in male Porton rats treated with carbon tetrachloride and ethanol (Hall *et al*., 1994). Inhalation exposure to methanol (10 000 ppm for 6 h) increased the hepatotoxicity of carbon tetrachloride (a single gavage dose of 0.075 mL/kg [120 mg/kg] bw after 24 h) (Simmons *et al*., 1995). Similar exposure to methanol also increased the toxicity of inhaled carbon tetrachloride (100, 250 or 1000 ppm [630, 1550, 6300 mg/m^3] for 6 h, 26–27 h after the beginning of the methanol exposure). This potentiation subsided when the interval between methanol and carbon tetrachloride exposures was increased by 24 h (Evans & Simmons, 1996). Malonaldehyde generation induced by carbon tetrachloride *in vitro* was enhanced by prior exposure of the rats to methanol (10 000 ppm for 6 h); this enhancement coincided with increased microsomal activity of *para*-nitrophenol hydroxylase, used as a marker of CYP2E1; inhibition of CYP2E1 by allyl sulfone abolished the carbon tetrachloride-induced lipid peroxidation (Allis *et al*., 1996). Malonaldehyde–DNA adducts have been detected in livers of rats and Syrian hamsters treated with carbon tetrachloride (Chaudhary *et al*., 1994; Wang & Liehr, 1995). Imidazole and pyrazole, inducers of CYP2E1, caused 3–25-fold enhanced rates of carbon tetrachloride-induced lipid peroxidation (and chloroform production from carbon tetrachloride); the increase was directly related to the microsomal concentration of CYP2E1 (Johansson & Ingelman-Sundberg, 1985).

Acetone, methyl ethyl ketone (2-butanone) and methyl isobutyl ketone (4-methylpentan-2-one) (6.8 mmol/kg bw for 3 days) increased the hepatotoxicity of carbon tetrachloride to Sprague-Dawley rats (Raymond & Plaa, 1995a); this enhancement of toxicity was coincident with increased microsomal aniline hydroxylase activity (Raymond & Plaa, 1995b). In addition to the effect on cytochrome P450, acetone, but not the other ketones, increased basal canalicular membrane fluidity, as measured by fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene or 1-[4-(trimethylammoniumphenyl)-6-phenyl]-1,3,5-hexatriene (Raymond & Plaa, 1996).

Treatment of male athymic nude rats, male and female Sprague-Dawley rats, and male Fischer 344 rats with vitamin A (75 mg/kg per day for seven days) greatly enhanced the hepatotoxicity of carbon tetrachloride (0.2 or 0.1 (Fischer 344 rats) mL/kg [320 or 160 mg/kg] bw intraperitoneally), while it protected BALB/c, C3H/HeJ, athymic nude and Swiss-Webster mice against carbon tetrachloride hepatotoxicity (0.0125, 0.015, 0.015 and 0.02 mL/kg [20, 24, 24 and 32 mg/kg] bw, respectively) (Hooser *et al*., 1994). In male Sprague-Dawley rats, vitamin A (≥ 100 000 IU/kg/day for three weeks or 250 000 IU/kg/day for ≥ 1 week) greatly increased the hepatotoxicity of carbon tetrachloride (0.15 mL/kg [240 mg/kg] intraperitoneally) (ElSisi *et al*., 1993c). There was a simultaneous six- to eight-fold increase in the amount of exhaled ethane and a less than two-fold increase in covalent binding to liver proteins in rats treated with vitamin A (250 000 IU [75 mg]/kg/day for one week) and [^{14}C]carbon tetrachloride (0.15 mL/kg [240 mg/kg bw]) in comparison with rats treated with carbon tetrachloride alone, but no increase in exhaled $^{14}CO_2$, exhaled organics or metabolites excreted in the urine, or in covalent binding to hepatic lipids (ElSisi *et al.*, 1993a). Aminobenzotriazole (50 mg/kg intraperitoneally, 2 h before carbon tetrachloride), an inhibitor of cytochrome P450, blocked the vitamin A-induced potentiation of the hepatotoxicity of carbon tetrachloride (ElSisi *et al*., 1993b). A single dose of vitamin A (75 mg/kg orally) 24 h before carbon tetrachloride also very significantly potentiated carbon tetrachloride hepatotoxicity. While the total cytochrome P450 content of the liver was not affected by retinol treatment, the concentration (Western blot analysis) and activity (aniline hydroxylase) of CYP2E1 were both elevated. Isolated hepatocytes from retinol-treated rats were more susceptible to carbon tetrachloride (Badger *et al*., 1996).

An intravenous injection of gadolinium chloride (10 mg/kg) 24 h before an intragastric dose of carbon tetrachloride (4000 mg/kg) nearly completely protected rats against hepatic necrosis, as measured by serum aspartate aminotransferase levels and trypan blue exclusion, without having any effect on CYP2E1 (Edwards *et al*., 1993). This was interpreted to indicate a role of Kupffer cells in carbon tetrachloride-induced hepatic damage, since gadolinium chloride at this concentration strongly inhibits Kupffer cell phagocytosis (Husztik *et al*., 1980). A similar dose of gadolinium chloride was, however, reported to decrease the total amount of hepatic cytochrome P450 in rats, as well as the activity of aniline *para*-hydroxylase (Badger *et al*., 1997). In support of the role of Kupffer cells in carbon tetrachloride-induced hepatic damage, it was reported that gadolinium chloride (10 mg/kg intravenously 24 h before carbon tetrachloride administration) prevented and

methyl palmitate (another Kupffer cell inhibitor) attenuated the periportal oedema observed using proton magnetic imaging 1–2 h after carbon tetrachloride administration (0.8 mL/kg [1280 mg/kg] intraperitoneally) (Towner *et al*., 1994). In-vivo spin trapping using PBN and subsequent electron paramagnetic resonance study of the liver indicated that gadolinium chloride did not affect the generation of trichloromethyl radical from carbon tetrachloride (Towner *et al*., 1994). Gadolinium chloride (10 mg/kg intravenously), methyl palmitate, polyethylene glycol-coupled superoxide dismutase and polyethylene glycol-coupled catalase protected Sprague-Dawley rats against vitamin A-induced potentiation of carbon tetrachloride hepatotoxicity, both after a single oral dose and after daily oral dosing for seven days with 75 mg/kg bw retinol (ElSisi *et al*., 1993a; Sauer & Sipes, 1995; Badger *et al*., 1996). Dietary α-tocopherol (250 mg/kg diet) partly protected male Wistar rats against hepatic damage induced by carbon tetrachloride (0.15 mL [240 mg] injected intraperitoneally three times per week for five weeks) (Parola *et al*., 1992). A single intraperitoneal dose of α-tocopheryl hemisuccinate (0.19 mmol, about 100 mg/kg) gave partial protection against the hepatotoxicity of carbon tetrachloride (1.0 g/kg bw by gavage) administered 18 h later (Tirmenstein *et al*., 1997). However, a much more pronounced protection, apparent as a decrease in mortality, less pronounced histological damage, and lower serum aminotransferase levels, resulted from intravenous administration of α-tocopherol as a suspension or in liposomes, which are accumulated in Kupffer cells (Yao *et al*., 1994; Liu *et al*., 1995). If incorporated into liposomes, other antioxidants, such as butylated hydroxytoluene and ascorbic acid palmitate, also protected mice against carbon tetrachloride toxicity (Yao *et al*., 1994).

Carbon tetrachloride (intraperitoneally, daily for seven days) affected both humoral and cell-mediated immune responses in female $B6C3F_1$ mice; the most sensitive parameters were the T-cell-dependent antibody-forming cell response to sheep red blood cells (effect observed at ≥ 500 mg/kg), mixed lymphocyte response (≥ 1000 mg/kg) and the proliferative response to concanavalin A and lipopolysaccharide (≥ 1000 mg/kg) (Kaminski *et al*., 1989). The effects were prevented by treatment of the animals with aminoacetonitrile, a competitive inhibitor of cytochrome P450, but enhanced by treatment with ethanol, an inducer of CYP2E1 (Kaminski *et al*., 1990). Incubation of serum from carbon tetrachloride-treated mice with neutralizing monoclonal antibodies towards transforming growth factor (TGF) β1 reversed the immunosuppression, indicating that TGF β1 at least in part mediates the immunosuppression induced by carbon tetrachloride (Delaney *et al*., 1994).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Carbon tetrachloride increased fetal mortality in mice after a single intraperitoneal or subcutaneous dose of 150 mg/kg late in gestation (IARC, 1979).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 2 for references)

Carbon tetrachloride was not mutagenic in bacteria. It induced intra-chromosomal and mitotic recombination but not aneuploidy in *Saccharomyces cerevisiae*; aneuploidy was detected in another single study in *Aspergillus nidulans*. *In vivo*, in a single study with *Drosophila melanogaster*, no sex-linked recessive mutations were observed.

In mammalian in-vitro systems, in single studies, carbon tetrachloride induced cell transformation in Syrian hamster cells and kinetochore-positive micronuclei (which are indicative of aneuploidy) and kinetochore-negative micronuclei in human MCL-5 cells that stably express cDNAs encoding human CYP1A2, CYP2A6, CYP3A4, CYP2E1 and epoxide hydrolase and in h2E1 cells, which contain a cDNA for CYP2E1. AHH-1 cells constitutively expressing CYP1A1 showed neither an increase in total micronucleus frequencies nor kinetochore-staining micronuclei.

Neither sister chromatid exchanges nor chromosomal aberrations were induced in cultured human lymphocytes.

In vivo in rat hepatocytes, unscheduled DNA synthesis was not induced, and no DNA repair intermediate products were found after exposure to carbon tetrachloride; neither micronuclei nor polyploidy were induced in a single study with the same experimental system. Carbon tetrachloride did not induce micronuclei in mouse bone-marrow cells or peripheral erythrocytes.

In vitro, carbon tetrachloride binds covalently to DNA. Inhibition of intercellular communication was observed *in vivo* in rats and induction of TNF-α expression *in vivo* in mice.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to carbon tetrachloride may occur in its production, in the production of refrigerants, in laboratories and during degreasing operations. It has been detected at low levels in ambient air and water.

5.2 Human carcinogenicity data

The risk of cancer from carbon tetrachloride has been examined in five occupational populations. In three of four studies that collected information on non-Hodgkin lymphoma (two cohort investigations and one independent nested case–control study), associations with exposure to carbon tetrachloride were suggested. However, not all of these studies distinguished exposure to carbon tetrachloride specifically, and the associations were not strong statistically. In the fourth study (another cohort investigation), few men were exposed to carbon tetrachloride and the risk of non-Hodgkin lymphoma was not reported.

Table 2. Genetic and related effects of carbon tetrachloride

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS response, *Salmonella typhimurium* TA1535/pSK1002, *umu* test	–	NT	5300	Nakamura *et al*. (1987)
SAF, *Salmonella typhimurium* BA13, Ara forward mutation	?	–	190	Roldán-Arjona & Pueyo (1993)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	McCann *et al*. (1975)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1400	Barber *et al*. (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	McCann *et al*. (1975)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1400	Barber *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1400	Barber *et al*. (1981)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	NT	(+)	160	Norpoth *et al*. (1980)
SCG, *Saccharomyces cerevisiae* D7, gene conversion	+	NT	5200	Callen *et al*. (1980)
SCH, *Saccharomyces cerevisiae* D7, homozygosis	+	NT	5200	Callen *et al*. (1980)
SCH, *Saccharomyces cerevisiae* RS112, intra-chromosomal recombination	+	NT	4000	Schiestl *et al*. (1989)
SCH, *Saccharomyces cerevisiae* AGY3, intra-chromosomal recombination	+	NT	2000	Galli & Schiestl (1996)
ANG, *Aspergillus nidulans*, crossing-over	(+)	NT	8000	Gualandi (1984)
SCR, *Saccharomyces cerevisiae*, reverse mutation	+	NT	5200	Callen *et al*. (1980)
ANF, *Aspergillus nidulans*, forward mutation	(+)	NT	8000	Gualandi (1984)
SCN, *Saccharomyces cerevisiae* D61-M, aneuploidy	–	NT	5000	Whittaker *et al*. (1989)
ANN, *Aspergillus nidulans*, aneuploidy	+	NT	0.02% (v:v)	Benigni *et al*. (1993)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		25000 ppm feed	Foureman *et al*. (1994)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		2000 ppm inj	Foureman *et al*. (1994)
DIA, DNA strand breaks/cross-links, rat hepatocytes *in vitro*	(+)	NT	462	Sina *et al*. (1983)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SIR, Sister chromatid exchange, rat epithelial-type RL1 cells *in vitro*	–	NT	0.02	Dean & Hodson-Walker (1979)
CIR, Chromosomal aberrations, rat epithelial-type RL1 cells *in vitro*	–	NT	0.02	Dean & Hodson-Walker (1979)
AIA, Aneuploidy, Chinese hamster ovary CHO cells *in vitro*	+	NT	8000	Coutino (1979)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	3	Amacher & Zelljadt (1983)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	–	48	Garry *et al.* (1990)
MIH, Micronucleus test, AHH-1 (CYP1A1 native) *in vitro*	–	NT	1540	Doherty *et al*. (1996)
MIH, Micronucleus test, MCL-5 (cDNAs for CYP1A2, 2A6, 3A4, 2E1 and epoxide hydrolase) *in vitro*	+[c]	NT	770	Doherty *et al*. (1996)
MIH, Micronucleus test, h2E1 (cDNA for CYP2E1) *in vitro*	+[c]	NT	308	Doherty *et al*. (1996)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	–	–	38	Garry *et al.* (1990)
DVA, DNA strand breaks/cross-links, NMRI mouse liver *in vivo*	–		4000 po × 1	Schwarz *et al*. (1979)
DVA, DNA strand breaks/cross-links, Fischer 344 rat liver *in vivo*	–		400 po × 1	Bermudez *et al*. (1982)
DVA, DNA strand breaks/cross-links, BD-VI rat liver *in vivo*	–		4000 ip × 1	Barbin *et al*. (1983)
DVA, DNA strand breaks/cross-links, Sprague-Dawley rat liver *in vivo*	–		200 ip × 1	Brambilla *et al*. (1983)
RVA, DNA repair intermediates, Wistar rat hepatocytes *in vivo*	–		800 ip × 1	Stewart (1981)
UPR, Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	–		100 po × 1	Mirsalis & Butterworth (1980)
UPR, Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	–		400 po × 1	Bermudez *et al*. (1982)
MVM, Micronucleus test, BDF_1 mouse bone marrow *in vivo*	–		2000 po × 1	Suzuki *et al.* (1997)
MVM, Micronucleus test, BDF_1 mouse peripheral erythrocytes *in vivo*	–		3000 ip × 1	Suzuki *et al.* (1997)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVM, Micronucleus test, CBA × C57BL/6 mouse hepatocytes *in vivo*	–		0.05–0.1 mL/5L inh	Uryvaeva & Delone (1995)
CBA, Chromosomal aberrations, 101/H and C57BL/6 mouse bone marrow *in vivo*	–		8000 im × 1	Lil'p (1983)
AVA, Aneuploidy, CBA × C57BL/6 mouse hepatocyte polyploidy *in vivo*	–		0.05–0.1 mL/5L inh	Uryvaeva & Delone (1995)
BVD, Binding (covalent) to DNA, A/J mouse liver *in vivo*	+		1.4 ip × 1	Diaz Gomez & Castro (1980)
BVD, Binding (covalent) to DNA, Sprague-Dawley rat liver *in vivo*	+		1.4 ip × 1	Diaz Gomez & Castro (1980)
BVD, Binding (covalent) to DNA, Syrian hamster liver *in vivo*	+		1200 ip × 1	Castro *et al*. (1989)
BVD, Binding (covalent) to DNA, C3H mouse liver *in vivo*	+		1200 ip × 1	Castro *et al*. (1989)
BVD, Binding (covalent) to DNA, Sprague-Dawley rat liver *in vivo*	+		1200 ip × 1	Castro *et al*. (1989)
Decreased connexin 32 expression, Sprague-Dawley rat liver *in vivo*	+		800 ip × 24	Nakata *et al*. (1996)
Induction of TNF-α expression, $B6C3F_1$ mouse liver *in vivo*	+		160 ip × 1	Bruccoleri *et al*. (1997)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; inh, inhalation; po, oral; ip, intraperitoneal; im, intramuscular

[c] Greater number of kinetochore-positive micronuclei than kinetochore-negative micronuclei

A nested case–control study of lung cancer in a cohort of chemical workers showed no association with exposure to carbon tetrachloride.

Four population-based case–control studies have examined associations of carbon tetrachloride with chronic lymphocytic leukaemia, brain cancer, female breast cancer and intraocular melanoma. Findings were generally unremarkable. In a fifth case–control study, which examined several cancers, no association was found with non-Hodgkin lymphoma, although the power to detect an increased risk was low.

5.3 Animal carcinogenicity data

Carbon tetrachloride was tested for carcinogenicity by various routes of administration. It produced liver neoplasms in mice and rats and mammary neoplasms in rats following subcutaneous injection. In one study in mice by inhalation, an increased incidence of phaeochromocytomas was reported. In experiments involving administration of carbon tetrachloride after known carcinogens, the occurrence of tumours and/or preneoplastic lesions of the liver in mice, rats and hamsters was enhanced.

5.4 Other relevant data

Carbon tetrachloride is metabolized by CYP2 enzymes; several reactive metabolites have been postulated, including radicals and phosgene. *In vitro*, DNA binding of carbon tetrachloride is observed in several cellular systems; no such binding *in vivo* has been reported.

Carbon tetrachloride induces hepatic cell proliferation and DNA synthesis.

Carbon tetrachloride has a mutagenic effect and induces aneuploidy in several in-vitro systems.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of carbon tetrachloride.

There is *sufficient evidence* in experimental animals for the carcinogenicity of carbon tetrachloride.

Overall evaluation

Carbon tetrachloride is *possibly carcinogenic to humans (Group 2B)*.

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CATECHOL

Data were last reviewed in IARC (1977) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

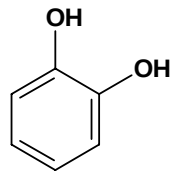
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 120-80-9
Chem. Abstr. Name: 1,2-Benzenediol
IUPAC Systematic Name: Pyrocatechol
Synonyms: Catechin; 1,2-dihydroxybenzene

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_6H_6O_2$ Relative molecular mass: 110.11

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless monoclinic crystals (Budavari, 1996)
(*b*) *Boiling-point*: 245°C (Lide, 1997)
(*c*) *Melting-point*: 105°C (Lide, 1997)
(*d*) *Solubility:* Very soluble in water, benzene, chloroform, diethyl ether, ethanol, pyridine and aqueous alkalis (Budavari, 1996; Lide, 1997)
(*e*) *Vapour pressure*: 4 Pa at 20°C; relative vapour density (air = 1), 3.79 (Verschueren, 1996; United States National Library of Medicine, 1997)
(*f*) *Flash-point:* 127.2°C, open cup (American Conference of Governmental Industrial Hygienists, 1991)
(*g*) *Conversion factor*: $mg/m^3 = 4.5 \times ppm$

1.2 Production and use

Worldwide consumption of catechol in 1980 was estimated to be about 20 thousand tonnes. Catechol is currently produced in France, Italy, Japan, the United Kingdom and the United States (Hamamoto & Umemura, 1991; Krumenacker *et al.*, 1995).

Approximately 50% is used as starting material for insecticides, 35–40% for perfumes and drugs and 10–15% for polymerization inhibitors and other chemicals. Catechol has also been used as an antiseptic, in photography, dyestuffs, electroplating, specialty inks, antioxidants and light stabilizers, and in organic synthesis (Hamamoto & Umemura, 1991; Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 14 000 workers in the United States were potentially exposed to catechol (see General Remarks). Occupational exposures to catechol may occur in its production, in the production of insecticides, perfumes and drugs, in metal-plating shops and in coal-processing.

1.3.2 *Environmental occurrence*

Catechol occurs naturally in fruits and vegetables such as onions, apples and crude beet sugar, and in trees such as pine, oak and willow. Catechol may be released to the environment during its manufacture and use. It has been detected at low levels in ambient and urban air, groundwater, drinking-water and soil samples. It has been found in wastewaters from coal conversion, coal-tar chemical production and bituminous shale (United States National Library of Medicine, 1997). It is present in cigarette smoke at 100–360 µg per cigarette (IARC, 1986).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 23 mg/m^3 as the 8-h time-weighted average threshold limit value, with a skin notation, for occupational exposures to catechol in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for catechol in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

In skin painting studies in mice, catechol increased the carcinogenic effects of benzo[*a*]pyrene on the skin (IARC, 1977).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 30 male and 30 female B6C3F$_1$ mice, six weeks of age, were administered catechol (> 99% pure) at 0 or 0.8% in the diet for 96 weeks. Catechol reduced the body weight gain of both males and females but did not affect survival. In exposed mice, the incidence of forestomach hyperplasia (16/30 males, 25/29 females) was increased. Forestomach papillomas occurred in one male and one female compared with none in controls. In the glandular stomach, 29/30 males and 21/29 females exhibited adenomatous hyperplasia, but no adenocarcinomas. No increase in the incidence of other neoplasms was observed (Hirose *et al*., 1990, 1993a).

3.1.2 *Rat*

Two groups of 30 male Fischer rats, eight weeks of age, were administered catechol (purity, > 99%) at 0 or 0.5% in the drinking-water for 78 weeks. Catechol alone did not increase the incidence of any tumour type (La Voie *et al*., 1985). [The Working Group noted the short duration of the study.]

Groups of 30 male MRC-Wistar rats, six weeks of age, were administered catechol (purity, > 99%) at concentrations of 0 or 2 mg/kg in the diet for up to 15 months. Catechol alone induced no increase in neoplasms (Mirvish *et al*., 1985). [The Working Group noted the short duration of the study.]

Groups of 30 male and 30 female Fischer 344 rats, six weeks of age, were administered catechol (> 99% pure) at 0 or 0.8% in the diet for 104 weeks. Catechol reduced the body weight gain of both males and females and increased the liver weight of males but did not affect survival. In exposed rats, forestomach hyperplasia was increased in both sexes (24/28 males, 23/28 females) and papillomas occurred in 2/24 (7%) males, compared with none in controls. In the glandular stomach, 100% of exposed males and females exhibited adenomatous hyperplasia and adenocarcinomas occurred in 15/28 males and 12/28 females ($p < 0.001$) compared with none in controls. No change in the incidence of other neoplasms was observed (Hirose *et al*., 1990, 1993a).

Groups of 20 or 30 male Wistar (Crj:Wistar), WKY (WKY/NCrj), Lewis (LEW/Crj) and SD (Crj:CD) rats, six weeks of age, were administered catechol (> 99% pure) in the diet at 0 or 0.8% for 104 weeks. Weight gain was reduced in all exposed groups but no effect on survival was observed. In the forestomach, the incidence of hyperplasia was significantly increased in exposed Wistar, WKY and SD rats compared with controls. Papillomas occurred in 6/30 SD rats ($p < 0.05$), 2/30 Wistar rats and 1/30 WKY rats and carcinomas in 1/30 SD and 1/30 Wistar rats compared with none in controls. In the

glandular stomach, all strains developed 97–100% incidence of adenomas compared with none in controls and adenocarcinomas occurred in 23/30 ($p < 0.01$) SD, 22/30 ($p < 0.01$) Lewis, 20/30 ($p < 0.01$) Wistar and 3/30 ($p > 0.05$) WKY rats compared with none in controls. No increase in any other tumour type was found in exposed WKY rats, while pituitary adenomas/carcinomas were decreased in exposed Wistar rats (4/30 versus 8/20 controls; $p < 0.05$) and SD rats (6/30 versus 11/21 controls; $p < 0.05$) and pituitary adenomas in Lewis rats (2/30 versus 14/20 controls; $p < 0.01$). In Wistar rats, islet-cell adenomas/carcinomas were also decreased (0/30 versus 5/20 controls; $p < 0.01$) (Tanaka *et al*., 1995).

3.2 Skin application

Mouse: Groups of 30 female SEN mice, six weeks of age, were administered catechol (purified by recrystallization) at 0 or 2000 μg/animal topically three times per week for 490–560 days. Catechol alone induced no skin tumours and none occurred in a total of 125 control mice (Van Duuren *et al*., 1986).

Groups of 30 female Crl:DC-1(1CR) BR mice, seven weeks of age, were administered catechol [purity unspecified] topically five times per week for 48 weeks at a dose of 0 or 250 μg per animal. Catechol alone induced no skin tumours. In a second experiment, groups of 30 mice were administered acetone or 500 μg catechol per animal 10 times every other day and, 10 days after the last exposure, 12-*O*-tetradecanoylphorbol 13-acetate (TPA) was applied as a promoter for 20 weeks. In mice given catechol before promotion, 5/29 developed skin tumours [unspecified] compared with 3/29 mice given acetone plus TPA (Melikian *et al*., 1989).

3.3 Administration with known carcinogens

3.3.1 *Rat*

Groups of 15 male Fischer 344 rats, six weeks of age, were administered 0 or 0.05% *N*-nitrosobutyl-*N*-(4-hydroxybutyl)amine in the drinking-water for two weeks followed by ureteric ligation one week later to initiate bladder carcinogenesis. Catechol [purity unspecified] was administered at concentrations of 0 or 0.8% in the diet for 22 weeks and all animals were killed at week 24. When catechol was administered after initiation, no increase in bladder tumours was produced (Miyata *et al*., 1985).

Groups of 10–20 male Fischer 344 rats, seven weeks of age, received catechol (> 99.8% pure) in the diet at concentrations of 0 or 1.5% for four weeks followed by 0.8% for 47 weeks either with no other exposure or one week after exposure to *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine to initiate stomach carcinogenesis. With catechol alone, the incidence of forestomach papillomas was 1/15 compared with 0/10 in untreated controls. Glandular stomach adenocarcinomas were found in 3/15 rats compared with 0/10 in controls. Catechol increased the incidence of squamous-cell carcinomas of the forestomach induced by the initiator from 5/19 to 19/19 ($p < 0.001$). In the glandular stomach, the incidence of adenocarcinomas in the pyloric region was 18/19 ($p < 0.001$) compared with none in rats given only the initiator (Hirose *et al*., 1987).

Groups of 7–10 male Sprague-Dawley rats, weighing 200 g, were administered catechol (purity, > 98%) at concentrations of 0 or 100 mg/kg in the diet for six weeks beginning one week after partial hepatectomy and intraperitoneal injection of 30 mg/kg bw *N*-nitrosodiethylamine to initiate liver carcinogenesis. Catechol after initiation did not increase the multiplicity of liver enzyme-altered (γ-glutamyltranspeptidase) foci (Stenius *et al*., 1989).

Groups of 11–14 male Fischer 344 rats, five weeks of age, were administered catechol (< 99% pure) at 0 or 0.8% alone for 52 weeks or after exposure to six intraperitoneal injections of 25 mg/kg bw *N*-nitrosomethyl-*n*-amylamine to initiate upper digestive tract carcinogenesis. Catechol given after carcinogen increased the incidence of papillomas of the tongue from 1/11 in rats given carcinogen alone to 8/14 ($p < 0.02$) and carcinomas of the oesophagus from 0/11 in controls to 9/14 ($p < 0.001$) (Yamaguchi *et al*., 1989).

Groups of 10 or 19 male Fischer 344 rats, six weeks of age, were administered catechol (purity, > 98%) in the diet at 0.8% for 36 weeks alone or after exposure to 0.05% *N*-nitrosobutyl-*N*-(4-hydroxybutyl)amine in the drinking-water for four weeks to initiate bladder carcinogenesis. Catechol did not affect body weight or bladder weight, but when given after initiator, it reduced final body weight, but did not affect bladder weight. Catechol did not induce bladder lesions. Feeding of catechol after the initiator did not increase the incidence or multiplicity of bladder neoplasms induced by initiation alone (Kurata *et al*., 1990).

Groups of 5–30 male Fischer 344/DuCrj rats, nine weeks of age, were administered catechol [purity unspecified] at a concentration of 0.8% in the diet for 16 weeks either alone or after a single intraperitoneal injection of 100 mg/kg bw *N*-nitrosodiethylamine, 20 mg/kg bw *N*-methyl-*N*-nitrosourea (four times) and 0.1% *N*-nitroso-*N*-bis(2-hydroxypropyl)amine in the drinking-water during weeks 3–4. Catechol alone induced low incidences of hyperplasia of the forestomach and hyperplasia and adenoma of the glandular stomach. The incidence of forestomach papillomas in rats given carcinogens was 0%, whereas in rats treated with carcinogens and catechol, the incidence of forestomach papillomas was 35% and forestomach carcinomas occurred in 5% [no numerical values given]. Catechol did not affect the incidence of oesophageal, thyroid or bladder tumours (Fukushima *et al*., 1991).

Groups of 15 or 20 male Fischer 344/DuCrj rats, six weeks of age, were given a single intraperitoneal injection of 100 mg/kg bw *N*-nitrosodiethylamine, followed by four injections of 20 mg/kg bw *N*-methyl-*N*-nitrosourea during weeks 1 and 2, then four subcutaneous injections of 40 mg/kg bw 1,2-dimethylhydrazine and 0.05% *N*-nitrosobutyl-*N*-(4-hydroxybutyl)amine and 0.1% *N*-nitroso-*N*-bis(2-hydroxypropyl)amine in the drinking-water during weeks 3 and 4, to initiate carcinogenesis in multiple organs. Rats were then fed with diet containing 0.8% catechol [purity unspecified] for the next 24 weeks or for 100 weeks. Rats given catechol for only 24 weeks were either killed at the end of this time or were maintained thereafter on basal diet. A control group was given the multiple initiation treatments only. Catechol given for 24 weeks after initiation

reduced body weight gain compared to initiation alone. After 24 weeks of exposure, catechol induced combined forestomach papillomas and carcinomas in 10/13 ($p < 0.01$) and glandular stomach adenomas in 11/13 ($p < 0.01$) compared with none in rats given initiators alone. In the group given catechol for 24 weeks after initiation and then maintained on basal diet, all rats were dead by 64 weeks versus 72 weeks for those given only initiation. In this group, no increase in cancer was observed. In the group given continuous catechol administration after initiation, all rats were dead at 56 weeks versus 72 weeks with only initiation. Catechol exposure increased the incidence of combined forestomach squamous-cell papilloma and carcinoma to 18/19 ($p < 0.01$) compared with 9/20 with initiation, and the incidence of glandular stomach adenoma to 9/19 ($p < 0.01$) compared with 1/20 (Hagiwara *et al*., 1993).

Groups of 10 or 15 male Fischer 344 rats, six weeks of age, were administered catechol (> 98% pure) in the diet at concentrations of 0 or 0.8% either alone or after exposure to a standard protocol of treatment with *N*-nitrosodiethylamine, *N*-methyl-*N*-nitrosourea, 1,2-dimethylhydrazine, *N*-nitrosobutyl-*N*-(4-hydroxybutyl)amine and *N*-nitroso-*N*-bis(2-hydroxypropyl)amine to initiate carcinogenesis in multiple organs. Catechol alone or after initiation reduced weight gain and induced mild hyperplasia in the forestomach and adenomas in the glandular stomach in 10/10 rats ($p < 0.001$) compared with 0/10 in unexposed controls. In initiated rats, catechol produced carcinoma *in situ* or squamous carcinoma in 6/15 rats ($p < 0.05$) compared with 0/14 rats given the initiators only. It also increased the incidence of glandular stomach adenomas and carcinomas to 4/15 ($p < 0.05$) versus 0/14 rats subjected to initiation only (Hirose *et al*., 1993b).

Groups of 20 male Wistar/Crj rats, six weeks of age, were administered catechol [purity unspecified] in the diet at a concentration of 0.8% for 36 weeks either alone or starting one week after exposure to 0.1% *N*-nitrosoethyl-*N*-(hydroxyethyl)amine in the drinking-water for three weeks to initiate liver and kidney carcinogenesis. The final body weights of rats given catechol were lower than those of rats given either basal diet or initiator. Catechol alone did not affect liver weights but increased relative kidney weights. When catechol was given after the initiator, there was no effect on liver or kidney weights. Catechol did not enhance the incidence of preneoplastic or neoplastic lesions in the liver or kidneys (Okazaki *et al*., 1993).

Groups of 20 male Fischer 344 rats, five weeks of age, were administered catechol (purity, > 99%) at concentrations 0 or 0.8% in the diet for 52 weeks alone or beginning one week after a single intragastric instillation of 150 mg/kg bw *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine to initiate stomach carcinogenesis. Catechol alone induced no neoplasms, but increased the incidence of forestomach hyperplasia compared with that in unexposed rats. In initiated rats, catechol exposure led to forestomach squamous-cell carcinoma in 17/20 ($p < 0.01$) compared with 6/18 in rats without catechol. In the glandular stomach, catechol after initiation induced adenocarcinomas in 15/20 ($p < 0.01$) rats compared with 0/18 rats receiving initiation treatment alone (Kawabe *et al*., 1994).

3.3.2 *Hamster*

Groups of Syrian golden hamsters, six weeks of age, were exposed to catechol (purity, > 98%) at concentrations of 0 or 1.5% in the diet for 16 weeks either alone (10 and 15 hamsters, respectively) or after two subcutaneous injections of 70 mg/kg bw *N*-nitroso-bis(2-oxopropyl)amine (20 hamsters) to initiate pancreatic carcinogenesis. Catechol alone did not affect body weights or pancreas weights compared with untreated controls, but reduced relative liver weight. Given after initiator, it did not affect body weight or pancreas weight, but reduced liver weight compared with hamsters given initiator. All animals were killed at 20 weeks. Catechol alone did not induce neoplastic lesions in pancreas or liver lesions. In hamsters given catechol after initiator, no increase in pancreatic lesions was found. In the forestomach and glandular stomach of hamsters given catechol, a higher frequency of epithelial hyperplasias was observed than in control groups [numerical data not provided] (Maruyama *et al*., 1991). Similarly, no enhancement of pancreatic carcinogenesis was observed in a later study using *N*-nitroso-*N*-bis(2-hydroxypropyl)amine as an initiator of pancreatic carcinogenesis (Maruyama *et al*., 1994).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, excretion and metabolism

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Proposed metabolic pathways of catechol are summarized in Figure 1. The major metabolic pathways in experimental animals are sulfation and glucuronidation.

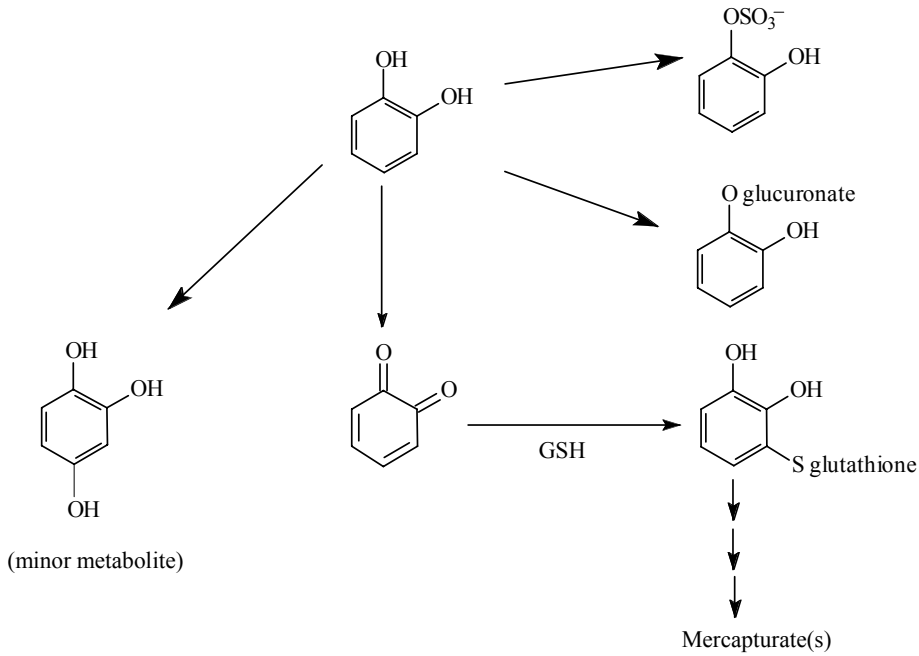
Catechol may be oxidized by peroxidases to the reactive intermediate benzo-1,2-quinone, which readily binds to proteins (Bhat *et al*., 1988); this process, catalysed by rat or human bone-marrow cells in the presence of H_2O_2 (0.1 mM), is stimulated by phenol (0.1–10 mM), and decreased by hydroquinone and by glutathione, which conjugates with benzo-1,2-quinone. These phenols (phenol, catechol and hydroquinone) may play a role in benzene toxicity to bone marrow: all three are formed as benzene metabolites (Smith *et al*., 1989) and they interact in several ways as far as their bioactivation by (myelo)peroxidases is concerned (Smith *et al*., 1989; Subrahmanyam *et al*., 1990).

4.2 Toxic effects

4.2.1 *Humans*

It was noted previously that skin contact with catechol causes dermatitis, and absorption through the skin may give rise to symptoms similar to those seen in phenol poisoning (IARC, 1977).

Figure 1. Metabolism of catechol



4.2.2 *Experimental systems*

Administration of catechol (1.5% in the diet) for 20 weeks induced mild to moderate hyperplasia but no papillomatous lesions in the forestomach in Syrian hamsters. Labelling index, after an intraperitoneal dose of [^{3}H]thymidine, was elevated in the pyloric region, but not in the forestomach or urinary bladder (Hirose *et al*., 1986).

In male Fischer rats, oral administration of catechol for four or eight weeks (0.8% in the diet) caused hyperplasia in the forestomach epithelium (4/5 rats) and increased DNA synthesis, as measured by a BrdU-labelling index, from 6.3% in controls to 16.8% ($p < 0.01$) after eight weeks (Shibata *et al*., 1990a,b). In pyloric mucosa of Fischer 344 rats given dietary catechol (0.8%) for four weeks, cell proliferation was observed (cells/pit column: control, 20.8; treated, 35.5; $p < 0.05$), accompanied by submucosal cell growth and an increase in DNA synthesis from 5.0% in controls to 10.3% ($p < 0.05$) (Ohgaki *et al*., 1989). The pyloric mucosa of Fischer 344 rats given dietary catechol (0.8%) for eight weeks also showed an increase in pepsinogen-altered preneoplastic foci from 0.2/100 pyloric glands in controls to 3.6/100 pyloric glands ($p < 0.05$) and an increased DNA labelling index from 12.4% in controls to 20.6% ($p < 0.01$) (Shibata *et al*., 1990a,b). After 60 weeks of dietary administration of 0.8% catechol to WKY/Ncrj rats, adenomatous hyperplasia and Pg1-altered foci were observed. The CCGG sites but not CGCG sites of the *Pg1* gene showed slightly increased methylation frequency in adenomatous tissues, while the methylation pattern of the *Pg1* gene was not significantly different from that of normal tissue in the Pg1-altered foci (Tatematsu *et al*., 1993). After

dietary administration of 0.8% catechol to Fischer 344 rats for 12, 24, 48 or 72 weeks and recovery on basal diet for 84, 72, 48 or 24 weeks, respectively, mucosal thickness and DNA labelling indices in the glandular stomach were significantly reduced in comparison with the values from catechol-fed rats that were not permitted a recovery period (Hirose *et al*., 1992).

Catechol (approx. 10^{-5} mol/L) inhibited the growth of bone-marrow cells from female C57BL/6 × DBA/2 mice (Seidel *et al*., 1991) and from male C57 and SW mice (Neun *et al*., 1992). Catechol (25, 50, 75 or 100 mg/kg bw, single intraperitoneal administration) decreased the incorporation of ^{59}Fe to erythrocytes in a dose-dependent fashion in female Swiss mice, when administered with phenol (50 mg/kg bw, single intraperitoneal administration) (Snyder *et al*., 1989). Catechol induced apoptosis in the human leukaemia cell line HL60 at concentrations (50 μmol/L) at which necrosis was not observed (Moran *et al*., 1996). On the other hand, catechol (≥ 0.5 μmol/L) prevented elimination by apoptosis of G418-resistant, transformed Swiss 3T3 M × Cl1 cells by co-cultured TGF-β-treated C3H 10T½ cells (Schaeffer *et al*., 1995).

A high concentration (0.5 mmol/L) of catechol induced a small-scale cytosol-to-membrane transport of protein kinase C, followed by inactivation of the enzyme activity, in cultured LL/2 lung carcinoma cells (Gopalakrishna *et al*., 1994).

In a study on the immunotoxic effects of cigarette tar components, it was shown that catechol at a concentration that did not affect the viability of the cells (50 μmol/L) decreased IL-2-dependent DNA synthesis and cell proliferation by > 90% in cultured human lymphoblasts (Li *et al*., 1997). Catechol did not inhibit Fc-receptor-mediated phagocytosis in mouse peritoneal macrophages at the highest concentrations tested (0.1 mmol/L) (Manning *et al*., 1994). Catechol (≤ 10 mmol/L) had no effect on the colony formation of granulocytes/macrophages induced by a recombinant granulocyte/macrophage colony-stimulating factor of murine bone-marrow cells (Irons *et al*., 1992).

Catechol (100 mg/kg bw, a single oral dose) given to male Sprague-Dawley rats did not affect the urinary excretion of malonaldehyde but did increase hepatic ornithine decarboxylase activity from a control level of 15.5 pmol/mg/h to 99.3 pmol/mg/h and, *in vitro*, 0.3 mmol/L induced rapid depletion of the glutathione content of isolated hepatocytes (Stenius *et al*., 1989). Addition of 0.25 mM catechol to HL-60 cells increased endogenous hydrogen peroxide levels three-fold, but 0.25 mM hydroquinone had no effect upon resting levels, whereas 0.25 mM catechol + 0.05 mM hydroquinone provoked a five-fold increase in endogenous hydrogen peroxide (Lévay & Bodell, 1996).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Catechol had no adverse effects upon cultured rat conceptuses at a concentration of 50 μmol/L, but killed all embryos at 100 μmol/L (Chapman *et al*., 1994).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Catechol did not induce gene mutations in *Salmonella typhimurium* or DNA repair in a mouse host-mediated assay in *Escherichia coli.*

In studies with eukaryotic cell in-vitro assays, most experiments were performed in the absence of an exogenous metabolic activation system, and almost all results indicated genetic toxicity. In a single study with the yeast *Saccharomyces cerevisiae*, catechol induced forward mutation but not gene conversion or homozygosis. When incubated with cultured non-human mammalian cells, catechol induced DNA strand breaks in two studies (which included one with rat primary hepatocytes), gene mutations (three studies) and sister chromatid exchanges, chromosomal aberrations, aneuploidy and cell transformation (all within the same study). Another cell transformation assay with BALB/3T3 cells showed no response at relative cloning efficiencies lower than 27%. Inhibition of gap-junctional intercellular communication was also demonstrated in one study. Mutagenic activity at the *tk* locus of mouse lymphoma cells was blocked by superoxide dismutase (McGregor *et al*., 1988). In cultured human lymphocytes, catechol induced DNA strand breaks (one study) and sister chromatid exchanges (three studies). Also in human lymphocytes, micronuclei and chromosome loss (as indicated by kinetochore staining) were induced by catechol co-incubated with hydroquinone, but not in the absence of hydroquinone. Two- to three-fold increases in total micronuclei were observed at doses down to 0.5 μM, but with no response increasing with dose (Yager *et al.*, 1990). Perhaps of relevance to some of these in-vitro effects, catechol (1 mM) did not inhibit topoisomerase I activity, whereas topoisomerase II was inhibited by the same concentration (but not by 0.5 mM) and even by 0.01 mM in the presence of horseradish peroxidase (Chen & Eastmond, 1995; Franz *et al*., 1996).

In single in-vivo studies, catechol did not induce DNA strand breaks or somatic cell mutations in the mouse spot test (one study). On the other hand, micronuclei were induced in mouse bone marrow (three of four studies). In one of these positive micronucleus test studies, the effect was greater after intraperitoneal injection than after gavage administration, while, in the other positive study, the effect of an intraperitoneal injection was enhanced by either phenol or hydroquinone.

Adducts

Catechol added to HL-60 cells or administered intraperitoneally at 75 mg/kg bw to $B6C3F_1$ mice, from which bone-marrow cells were sampled, did not induce formation of 8-hydroxydeoxyguanosine, as might be expected if there had been oxidative damage to DNA. When catechol was administered with hydroquinone, however, an increase in 8-hydroxydeoxyguanosine was observed (Kolachana *et al*., 1993). Leanderson and Tagesson (1990) found no covalent binding of catechol to DNA *in vitro*. Using a ^{32}P-postlabelling

Table 1. Genetic and related effects of catechol

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS induction, *Salmonella typhimurium*/pSK1002, *umu* test	–	–	3300	Nakamura *et al*. (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	NT	500	Nazar *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1667	Haworth *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	Yoshida & Fukuhara (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1667	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1667	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Yoshida & Fukuhara (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1667	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Yoshida & Fukuhara (1983)
SCG, *Saccharomyces cerevisiae* MP1, gene conversion	–	NT	2500	Fahrig (1984)
SCH, *Saccharomyces cerevisiae* MP1, homozygosis	–	NT	2500	Fahrig (1984)
SCF, *Saccharomyces cerevisiae* MP1, forward mutation	+	NT	2500	Fahrig (1984)
DIA, DNA strand breaks/alkali-labile sites, rat primary hepatocytes *in vitro*	(+)	NT	330	Solveig Walles (1992)
DIA, DNA strand breaks, mouse lymphoma L5178YS cells *in vitro*	–	NT	110	Pellack-Walker & Blumer (1986)
DIA, DNA strand breaks/cross-links, mouse lymphoma cells *in vitro*	+	+	55	Garberg *et al*. (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	2.5	McGregor *et al*. (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	1.14	Wangenheim & Bolcsfoldi (1988)
GIA, Gene mutation, Syrian hamster embryo cells, *hprt* locus *in vitro*	+	NT	0.33	Tsutsui *et al*. (1997)
GIA, Gene mutation, Syrian hamster embryo cells, Na^+/K^+ ATPase locus *in vitro*	+	NT	1.1	Tsutsui *et al*. (1997)
SIS, Sister chromatid exchange, Syrian hamster embryo cells *in vitro*	+	NT	1.1	Tsutsui *et al*. (1997)
CIS, Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	+	NT	0.33	Tsutsui *et al*. (1997)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
AIA, Aneuploidy, Syrian hamster embryo cells *in vitro*	+	NT	3.3	Tsutsui *et al*. (1997)
TBM, Cell transformation, BALB/3T3 mouse cells, focus assay	–	NT	2	Atchison *et al*. (1982)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	0.11	Tsutsui *et al*. (1997)
DIH, DNA strand breaks/alkali-labile sites, human lymphocytes, comet assay *in vitro*	(+)[c]	+	11	Anderson *et al*. (1995)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	4	Morimoto & Wolff (1980)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	33	Morimoto (1983)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	6	Erexson *et al*. (1985)
MIH, Micronucleus test, human lymphocytes *in vitro*	+[c]	NT	22	Yager *et al*. (1990)
MIH, Micronucleus test, human lymphocytes *in vitro*	–	NT	8.3	Robertson *et al.* (1991)
HMM, Host-mediated assay, *Escherichia coli* K-12 *uvr B/rec A* DNA repair in blood, liver, lungs, kidneys, testicles of male NMRI mice	–		200 po × 1	Hellmér & Bolcsfoldi (1992)
DVA, DNA strand breaks/cross-links, Fischer 344 rats *in vivo*	–		90 po × 1	Furihata *et al*. (1989)
MST, Mouse spot test, C579BL × T mouse embryos	–		22 ip × 1	Fahrig (1984)
MVM, Micronucleus test, male NMRI mouse bone marrow *in vivo*	–		42 sc × 6	Tunek *et al*. (1982)
MVM, Micronucleus test, male CD-1 mouse bone marrow *in vivo*	+		40 po × 1	Ciranni *et al*. (1988a)
MVM, Micronucleus test, pregnant female CD-1 mouse bone marrow and fetal liver *in vivo*	+		40 po × 1	Ciranni *et al*. (1988b)
MVM, Micronucleus test, CD-1 mouse bone marrow *in vivo*	+		10 ip × 1	Marrazzini *et al*. (1994)
ICR, Inhibition of cell communication, Chinese hamster lung V79 cells	+	NT	0.25	Bohrman *et al*. (1988b)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; po, oral; ip, intraperitoneal; sc, subcutaneous

[c] Higher percentage stained kinetochore-positive compared to controls

technique, Lévay and Bodell (1996) found that treatment of HL-60 cells with 0.5 mM catechol for 24 h resulted in a relative adduct level of 0.21×10^{-7}. Addition of 0.05–0.25 mM hydrogen peroxide increased the relative adduct level to $0.83–2.10 \times 10^{-7}$, whereas co-administration of hydrogen peroxide with 1,2,4-benzenetriol had no additional effect.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to catechol may occur in its production, in the production of insecticides, perfumes and drugs, in metal plating and in coal processing. Catechol occurs naturally in fruits and vegetables. It is present in cigarette smoke and has been detected at low levels in ambient air and water.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Catechol was tested for carcinogenicity by oral administration in one study in mice and in two studies in rats. No increase in the incidence of malignant tumours was found in mice. In rats, it induced adenocarcinomas in the glandular stomach in several strains. In one study in mice by skin application, no skin tumour was observed. In several experiments in rats involving administration with known carcinogens, catechol enhanced the incidence of papillomas of the tongue, carcinomas of the oesophagus, squamous-cell carcinomas of the forestomach and adenocarcinomas of the glandular stomach.

5.4 Other relevant data

Catechol is oxidized by peroxidases to the reactive intermediate benzo-1,2-quinone, which binds to protein. The acute toxicity of catechol is relatively low. In humans, the irritant action of catechol can lead to dermatitis and other dermal lesions. Chronic oral treatment of rodents causes hyperplasia of the forestomach and pyloric mucosa.

Catechol was shown to cause gene mutations in mammalian cells *in vitro*. Chromosomal aberrations and sister chromatid exchanges were reported in mammalian cells in culture. After application to mice, catechol was negative in one and positive in three studies of micronucleus formation in bone marrow.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of catechol were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of catechol.

Overall evaluation

Catechol is *possibly carcinogenic to humans (Group 2B)*.

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α-CHLORINATED TOLUENES AND BENZOYL CHLORIDE

Data were last reviewed in IARC (1982) and the compounds were classified in *IARC Monographs* Supplement 7 (1987a).

1. Exposure Data

Benzyl chloride

1.1 Chemical and physical data

1.1.1 *Nomenclature*

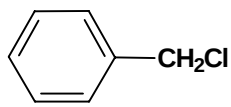
Chem. Abstr. Serv. Reg. No.: 100-44-7

Chem. Abstr. Name: (Chloromethyl)benzene

IUPAC Systematic Name: α-Chlorotoluene

Synonyms: Chloromethyl benzene; chlorophenylmethane; α-tolyl chloride

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_7H_7Cl Relative molecular mass: 126.6

1.1.3 *Chemical and physical properties of the pure substance*

From Lide (1997), unless otherwise specified

(*a*) *Description:* Colourless liquid with a pungent odour (Lewis, 1993)

(*b*) *Boiling-point:* 179°C

(*c*) *Melting-point:* –45°C

(*d*) *Density*: d_{10}^{20} 1.10

(*e*) *Solubility*: Insoluble in water; slightly soluble in carbon tetrachloride; miscible with chloroform, diethyl ether and ethanol (Budavari, 1996)

(*f*) *Vapour pressure*: 133 Pa at 22°C; relative vapour density (air = 1), 4.36 (Verschueren, 1996)

(*g*) *Stability*: Decomposes in hot water to benzyl alcohol (United States Environmental Protection Agency, 1980); decomposes rapidly when heated in the presence of iron (Budavari, 1996); combustible (Lewis, 1993)

(*h*) *Reactivity*: Undergoes reactions both at the side-chain containing the chlorine and at the aromatic ring (Gelfand, 1979)

(*i*) *Flash-point*: 67°C (closed cup); 74°C (open cup) (Lin & Bieron, 1993)
(*j*) *Explosive limit*: Lower, 1.1% by volume of air (Lin & Bieron, 1993)
(*k*) *Octanol/water partition coefficient (P)*: log *P*, 2.30 (Verschueren, 1996)
(*l*) *Conversion factor*: $mg/m^3 = 5.18 \times ppm$

1.2 Production and use

The chemical processes associated with the manufacture of chlorinated toluenes are summarized in Figure 1.

Plant capacities for the production of benzyl chloride in western countries totalled 144 thousand tonnes in 1989. Total production in these countries in 1988 was approximately 93 thousand tonnes, with production in the United States of 26 500 tonnes or 54% of capacity (Lin & Bieron, 1993). Information available in 1995 indicated that benzyl chloride was produced in 16 countries (Chemical Information Services, 1995).

More than two-thirds of the benzyl chloride produced is used in the manufacture of butyl benzyl phthalate, a plasticizer used extensively in vinyl flooring and other flexible poly(vinyl chloride) uses such as food packaging. Other significant uses are the manufacture of benzyl alcohol and benzyl chloride-derived quaternary ammonium compounds, each of which consumes more than 10% of the benzyl chloride produced. In the dye industry, benzyl chloride is used as an intermediate in the manufacture of triphenylmethane dyes. Derivatives of benzyl chloride are processed further to pharmaceutical, perfume and flavour products (Lin & Bieron, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 27 000 workers in the United States were potentially exposed to benzyl chloride (see General Remarks). Occupational exposures to benzyl chloride may occur in its production and use as a chemical intermediate.

1.3.2 *Environmental occurrence*

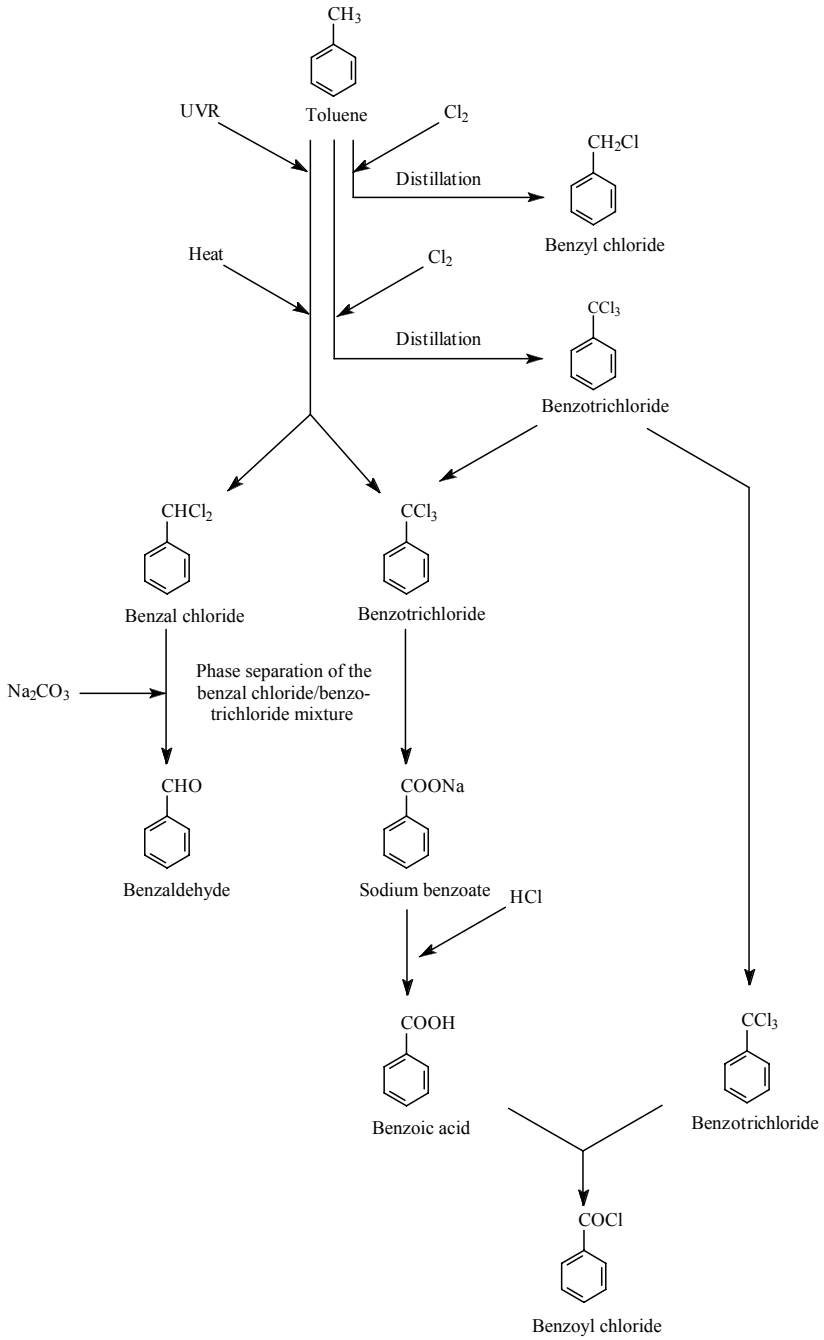
Benzyl chloride has been detected in surface water, industrial effluents and river water (Sheldon & Hites, 1978; Hushon *et al.*, 1980).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 5.2 mg/m^3 as the 8-h time-weighted average threshold limit value for occupational exposures to benzyl chloride in workplace air.

No international guideline for benzyl chloride in drinking-water has been established (WHO, 1993).

Figure 1. Chemical processes associated with the manufacture of chlorinated toluenes and benzoyl chloride



From Sorahan *et al.* (1983)

Benzal chloride

1.1 Chemical and physical data

1.1.1 *Nomenclature*

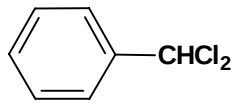
Chem. Abstr. Serv. Reg. No.: 98-87-3

Chem. Abstr. Name: (Dichloromethyl)benzene

IUPAC Systematic Name: α,α-Dichlorotoluene

Synonyms: Benzyl dichloride; benzylene chloride; benzylidine chloride; chlorobenzal; (dichloromethyl)benzene; dichlorophenylmethane; dichlorotoluene

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_7H_6Cl_2$ Relative molecular mass: 161.0

1.1.3 *Chemical and physical properties of the pure substance*

From Lide (1997), unless otherwise specified

(*a*) *Description*: Colourless liquid with a pungent odour (Lewis, 1993; Budavari, 1996)

(*b*) *Boiling*-point: 205°C

(*c*) *Melting*-point: –17°C

(*d*) *Density*: d_4^{14} 1.26

(*e*) *Solubility:* Insoluble in water; very soluble in diethyl ether and ethanol

(*f*) *Vapour pressure*: 133 Pa at 35.4°C (Lin & Bieron, 1993)

(*g*) *Stability*: Hydrolysed to benzaldehyde under both acid and alkaline conditions (Gelfand, 1979); fumes in air (Budavari, 1996)

(*h*) *Reactivity*: Undergoes reactions both at the side-chain containing the chlorine atoms and at the aromatic ring (Gelfand, 1979)

(*i*) *Conversion factor:* $mg/m^3 = 6.58 \times ppm$

1.2 Production and use

Information available in 1994 indicated that benzal chloride was produced in two countries (Belgium, Japan) (Chemical Information Services, 1994).

Benzal chloride is used almost exclusively for the manufacture of benzaldehyde and cinnamic acid (Lewis, 1993; Budavari, 1996).

1.3 Occurrence

1.3.1 *Occupational exposure*

No information on occupational exposure to benzal chloride was available to the Working Group.

1.3.2 *Environmental exposure*

Benzal chloride has been detected in surface waters (Hushon *et al.*, 1980).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not proposed any occupational exposure limit for benzal chloride in workplace air. Russia has a short-term exposure limit of 0.5 mg/m^3 for exposure in workplace air. Sweden lists benzal chloride as a probable human carcinogen and Finland and Germany list benzal chloride as suspected of having carcinogenic potential (International Labour Office, 1991).

No international guideline for benzal chloride in drinking-water has been established (WHO, 1993).

Benzotrichloride

1.1 Chemical and physical data

1.1.1 *Nomenclature*

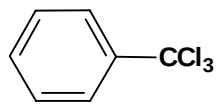
Chem. Abstr. Serv. Reg. No.: 98-07-7

Chem. Abstr. Name: (Trichloromethyl)benzene

IUPAC Systematic Name: α,α,α-Trichlorotoluene

Synonyms: Benzenyl chloride; benzenyl trichloride; benzylidyne chloride; benzyl trichloride; phenyl chloroform; phenyltrichloromethane; toluene trichloride; trichloromethylbenzene

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_7H_5Cl_3$ Relative molecular mass: 195.5

1.1.3 *Chemical and physical properties of the pure substance*

From Lide (1997), unless otherwise specified

(*a*) *Description*: Colourless to yellowish oily liquid with a pungent odour (Lewis, 1993; Budavari, 1996)

(*b*) *Boiling point*: 221°C

(*c*) *Melting-point*: –5°C

(*d*) *Density*: d_4^{20} 1.37

(*e*) *Solubility*: Insoluble in water; soluble in benzene, diethyl ether and ethanol

(*f*) *Vapour pressure*: 20 Pa at 20°C (Verschueren, 1996); relative vapour density (air = 1), 6.77 (Lin & Bieron, 1993)

(*g*) *Stability*: Unstable; hydrolyses in the presence of moisture; fumes in air (Budavari, 1996)

(*h*) *Reactivity*: Undergoes reactions both at the side-chain containing the chlorine atoms and at the aromatic ring (Gelfand, 1979)
(*i*) *Flash-point*: 127°C (open cup) (Budavari, 1996)
(*j*) *Octanol/water partition coefficient (P)*: log *P*, 4.1 (Verschueren, 1996)
(*k*) *Conversion factor*: $mg/m^3 = 8.00 \times ppm$

1.2 Production and use

Total production capacity in the western countries in 1988 for benzotrichloride was 68 thousand tonnes; production in 1988 was approximately 31 500 tonnes (Lin & Bieron, 1993). Information available in 1994 indicated that benzotrichloride was produced in eight countries (Chemical Information Services, 1994).

Benzotrichloride is mostly used as a chemical intermediate, primarily for benzoyl chloride. Lesser amounts are used in the manufacture of benzotrifluoride, as a dyestuff intermediate, and in producing hydroxybenzophenone ultraviolet light stabilizers (Lin & Bieron, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

No information on occupational exposure to benzotrichloride was available to the Working Group.

1.3.2 *Environmental occurrence*

Benzotrichloride has been detected in surface waters (Hushon *et al.*, 1980).

1.4 Regulations

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not recommended an 8-h time-weighted average threshold limit value but has recommended 0.8 mg/m^3 as the ceiling value for occupational exposures to benzotrichloride in workplace air. Russia has a short-term exposure limit of 0.2 mg/m^3 for exposure in workplace air. Sweden lists benzotrichloride as a probable human carcinogen and Finland and Germany list benzotrichloride as suspected of having carcinogenic potential (International Labour Office, 1991).

No international guideline for benzotrichloride in drinking-water has been established (WHO, 1993).

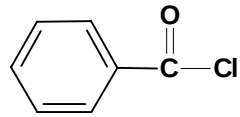
Benzoyl chloride

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 98-88-4
Chem. Abstr. Name: Benzoyl chloride
IUPAC Systematic Name: Benzoyl chloride
Synonym: Benzene carbonyl chloride

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_7H_5ClO Relative molecular mass: 140.6

1.1.3 *Chemical and physical properties of the pure substance*

From Lide (1997), unless otherwise specified

(*a*) *Description*: Colourless liquid with a pungent odour (Lewis, 1993)
(*b*) *Boiling point*: 197.2°C
(*c*) *Melting-point*: 0°C
(*d*) *Density*: d_4^{20} 1.21
(*e*) *Solubility*: Decomposes in water and ethanol; soluble in benzene, carbon disulfide and carbon tetrachloride; miscible with diethyl ether (Budavari, 1996)
(*f*) *Vapour pressure*: 53 Pa at 20°C; relative vapour density (air = 1), 4.88 (Verschueren, 1996)
(*g*) *Flash-point*: 72.2°C (Lewis, 1993)
(*h*) *Conversion factor*: $mg/m^3 = 5.75 \times ppm$

1.2 Production and use

Information available in 1995 indicated that benzoyl chloride was produced in 11 countries (Chemical Information Services, 1995).

Benzoyl chloride is used in the manufacture of benzoyl peroxide and dye intermediates, for acylation (introduction of the benzoyl group into alcohols, phenols and amines), and as an analytical reagent (Lewis, 1993; Budavari, 1996).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 6900 workers in the United States were potentially exposed to benzoyl chloride (see General Remarks). Occupational exposures to benzoyl chloride may occur in its production and use as a chemical intermediate.

1.3.2 *Environmental occurrence*

No information on environmental occurrence of benzoyl chloride was available to the Working Group.

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not recommended an 8-h time-weighted average threshold limit value but has

recommended 2.8 mg/m^3 as the ceiling value for occupational exposures to benzoyl chloride in workplace air. Hungary has an 8-h time-weighted average exposure limit of 5 mg/m^3 and Russia has a short-term exposure limit of 5 mg/m^3 for occupational exposure in workplace air (International Labour Office, 1991).

No international guideline for benzoyl chloride in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

Six cases of respiratory tract cancer were reported among benzoyl chloride production workers in two small plants in Japan. The cases occurred in people aged ≤ 44 years, three of whom were nonsmokers (IARC, 1982, 1987a).

A mortality study of 953 workers potentially exposed to various chlorinated toluenes and benzoyl chloride was conducted in a factory in England (Sorahan *et al*., 1983). Included were workers employed for six or more months between 1961 and 1970 and followed through 1976 for vital status. Standardized mortality ratios (SMR) were calculated using mortality rates from England and Wales as the referent. Although workers with exposures to specific chlorinated toluenes could not be evaluated, groups with low (n = 153) and high (n = 163) exposures to chlorinated toluenes were identified from job titles. Significant excesses occurred for all deaths combined for the high-exposure (SMR, 1.6; 25 obs./15.4 exp.) but not the low-exposure (SMR, 1.2; 66 obs./56.1 exp.) groups. The high-exposure group also had significant excesses for all cancers (SMR, 2.5; 10/4.0), cancer of the digestive system (SMR, 4.0; 5/1.2) and cancer of the respiratory system (SMR, 2.8; 5/1.8). Among the low-exposure group, a significant excess occurred for mouth and throat cancer (SMR, 5.7; 2/0.35).

Sorahan and Cathcart (1989) extended the follow-up of their cohort through 1984 and conducted a nested case–control study of lung cancer to obtain more detailed information on occupational risks and to control for possible confounding by smoking. Twenty-six lung cancers were each matched to three controls by age and year of starting employment. A significant excess for lung cancer occurred among the high-exposure group (SMR, 3.3; 10/3.0), but not among the low-exposure group (SMR, 1.4; 16/11.5). Conditional logistic regression of the case–control data revealed relative risks of 1.4 (95% confidence interval (CI), 0.4–4.2) for benzotrichloride, 1.1 (95% CI, 0.3–4.2) for other chlorinated toluenes and 3.0 (95% CI, 0.3–25.8) for smoking. The relative risks for chemicals are expressed per 10 years of exposed employment.

A mortality study was conducted among 697 male employees (610 whites and 11 assumed to be white) at a chlorination plant in Tennessee (United States) (Wong, 1988). The cohort consisted of all employees at the plant between 1943 and 1980. Almost all of the cohort held jobs with potential exposure to benzotrichloride, benzyl chloride or benzoyl chloride, there being substantial overlap between these groups. The mortality data were compared with the United States national age- and cause-specific rates for five-

year time periods from 1940 to 1982. Respiratory tract cancer mortality was elevated for the entire cohort (7, including 6 lung cancers observed, 2.8 expected; SMR, 2.5; 95% CI, 1.0–5.0) and the white employees alone (7 observed, 2.7 expected; SMR, 2.7; 95% CI, 1.1–5.5). The respiratory cancer mortality was similarly elevated for the three specific chemical exposure subgroups. The values were: benzotrichloride SMR, 2.6 ($p < 0.05$); benzyl chloride or benzoyl chloride SMR, 2.6 ($p < 0.05$). The cohort was also divided according to length of employment (< 15 years and 15+ years). The respiratory tract cancer SMRs were 1.3 and 3.8 ($p < 0.05$), respectively. The author concluded that the data suggest an association between the process of toluene chlorination at the plant and an increased risk of respiratory cancer. [The Working Group noted that more precise identification of a single causative exposure is not possible from this study.]

3. Studies of Cancer in Experimental Animals

Benzyl chloride was tested in mice by skin application and in rats by subcutaneous injection. Sarcomas at the injection site in rats were observed in 6/8 high-dose and 3/14 low-dose compared with none in controls. Skin carcinomas were observed in 3/20 exposed mice whereas none was observed in the vehicle (benzene) control mice. When benzyl chloride was administered to mice and rats in corn oil by gavage, increased incidences of papillomas and carcinomas of the forestomach were observed in mice of each sex, and the incidence of thyroid C-cell tumours was increased in female rats but decreased in male rats; a few neoplasms of the forestomach were observed in male rats (IARC, 1982, 1987a).

Benzal chloride was tested in two experiments in mice by skin application, the results of which were reported together. In the first experiment, the total dose of benzal chloride was about 289 mg per mouse during a 50-week dosing period, after which all mice were killed at week 82. No skin tumours developed in 20 controls, while, in the treated group of 19 (14 of which had died by the end of the experiment), nine mice had squamous cell carcinomas of the skin and two had skin fibrosarcomas. In the other experiment in which the total benzal chloride dose was about 1109 mg per mouse, but which was terminated after just 43 weeks, 2/10 mice developed skin papillomas compared with 0/10 in the controls (IARC, 1982).

Benzotrichloride was tested in three studies by skin application to female mice. It produced squamous cell carcinomas of the skin and lung tumours in all three experiments; upper digestive tract tumours were also observed in two of the three experiments. Increases in the incidence of tumours at other sites were reported. In a strain A mouse lung tumour bioassay, benzotrichloride increased the incidence of lung adenomas (IARC, 1982, 1987a).

Benzoyl chloride was tested in two experiments by skin application to female mice. A few skin carcinomas and lung adenomas were observed, but their incidence was not significant. However, no skin tumours occurred in controls of either experiment or lung tumours in controls of one of them (IARC, 1982).

3.1 Oral administration

Mouse: Groups of 40 female ICR mice, nine weeks of age, were administered benzotrichloride (reagent grade) by gavage at doses of 0, 0.0315, 0.125, 0.5 and 2 µL/mouse twice per week for 25 weeks and the experiment was terminated at 18 months. The mortality of exposed mice increased dose-dependently. The 0.5 and 2 µL doses induced forestomach papillomas and carcinomas in 23/40 ($p < 0.01$; Fisher's exact test) and 25/38 ($p < 0.01$) mice, respectively, compared with 0/39 controls. Higher incidences of lung adenomas and carcinomas were also found: 7/39 [not significant] at 0.0315 µL, 26/39 ($p < 0.01$) at 0.125 µL, 35/40 ($p < 0.01$) at 0.5 µL and 24/38 ($p < 0.01$) at 2 µL compared with 1/39 controls. The highest dose also induced thymic lymphomas in 8/38 ($p < 0.01$) mice compared with 1/39 controls (Fukuda *et al*., 1993).

3.2 Inhalation exposure

Mouse: Groups of female ICR-Jcl mice were exposed to air or benzotrichloride vaporized at either 50°C (6.7 ± 1.66 ppm [54 ± 13 mg/m^3]) for five months or 20 ± 5°C (1.62 ± 0.43 ppm [13 ± 3.4 mg/m^3]) for 12 months, for 30 min per day on two days per week. Afterwards, they were observed for a further five months (50°C vaporization) or three months (20°C vaporization). In the control, exposed (50°C) and exposed (20°C) groups, respectively, lung tumours were observed in 3/30 (3 adenomas), 17/32 (16 adenomas, 1 adenocarcinoma) and 30/37 (17 adenomas, 13 adenocarcinomas) mice; skin tumours were observed in 0/30, 8/32 (4 papillomas, 4 carcinomas; $p < 0.02$) and 10/37 (6 papillomas, 4 carcinomas) mice; and malignant lymphomas were observed in 0/30, 8/32 ($p < 0.02$) and 4/37 mice. The differences in incidence were significant (Yoshimura *et al*., 1986).

Groups of female ICR-Jc1 mice were exposed to air or to benzoyl chloride vaporized at 50°C [concentration not stated] for 30 min per day on two days per week for five months. They were then observed for a further seven to nine months (12–14 months total). In the control and exposed groups, respectively, lung tumours were observed in 3/30 (3 adenomas) and 3/28 (1 adenoma and 2 adenocarcinomas) mice and skin papillomas in 0/30 and 2/28 mice. The differences in incidence were not significant (Yoshimura *et al*., 1986). [The Working Group noted the short duration of exposure and observation time, allowing only comparison with mice simultaneously exposed to benzotrichloride.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Benzyl chloride is absorbed through lung and gastrointestinal tract. It can react with tissue proteins and is metabolized in rodents and rabbits to *N*-acetyl-*S*-benzylcysteine (benzyl mercapturic acid) through side-chain conjugation and to benzoic acid and the glycine conjugate of benzoic acid (hippuric acid). The percentages of the dose excreted in the urine as benzyl mercapturic acid in rats, guinea-pigs and rabbits, respectively, were 27%, 4% and 49%. Rabbits also excrete about 37% as benzoic acid (17% free acid and 20% conjugated). In rats, 30% of the dose was recovered as the hippuric acid derivative (IARC, 1982).

In rats receiving [^{14}C]benzyl chloride in corn oil by gavage, the peak plasma level was reached after 30 min. The distribution half-life was 1.3 h, while the elimination half-life was 58.5 h. After 48 h, the higher concentrations were found in the stomach, gastric contents, ileum and duodenum, followed by liver, adrenal, bone marrow and blood. After 72 h, approximately 76% was excreted in urine and, in expired air, 7% as $^{14}CO_2$ and less than 1.3% as benzyl chloride or its metabolites. Urinary metabolites were identified as *S*-benzyl-*N*-acetyl cysteine, benzyl alcohol and benzaldehyde (Saxena & Abdel-Rahman, 1989).

No data were available on the disposition of benzotrichloride, benzal chloride or benzoyl chloride.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Benzyl chloride, benzal chloride, benzotrichloride and benzoyl chloride are irritant to the eyes, skin and respiratory tract of mice exposed by skin application (IARC, 1982).

In rats and mice, benzyl chloride, benzotrichloride and benzal chloride produce signs of central nervous system toxicity and hyperaemia of the extremities (IARC, 1982).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Oral administration to rats of 0.006 mg/kg bw benzyl chloride per day on days 1–19 of gestation increased embryolethality, but doses of 0.0006 or 0.00006 mg/kg bw per day did not produce any malformations (IARC, 1982).

No data were available on the reproductive and developmental effects of benzotrichloride, benzal chloride or benzoyl chloride.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Benzyl chloride was the subject of a large, multilaboratory investigation, as a consequence of which there are numerous data that were tabulated in a previous volume (IARC, 1987b). A few additional data have become available (see Table 1). Benzyl chloride induced DNA damage and mutations in bacteria. It induced somatic and sex-linked recessive lethal mutations in *Drosophila melanogaster* and mitotic recombination, gene conversion, mutation and DNA damage in fungi. Benzyl chloride induced sister chromatid exchanges, chromosomal aberrations, mutations and DNA strand breaks in cultured rodent cells. In cultured human cells, it induced DNA strand breaks, but not chromosomal aberrations; conflicting results were obtained for induction of sister chromatid exchanges. Benzyl chloride did not induce micronuclei in mice *in vivo*. [^{14}C]Benzyl chloride injected intravenously into mice arylated DNA in various organs, the higher concentrations one hour after injection being found in brain and testis, followed by liver and lung. The principal adduct cochromatographed with *N7*-benzylguanine (Solveig Walles, 1981).

Benzal chloride induced DNA damage and mutations in bacteria.

Benzotrichloride induced DNA damage and mutations in bacteria.

No activity of benzoyl chloride was observed in single bacterial tests for either differential toxicity or mutation induction.

Lung adenomas derived from control and benzotrichloride-treated strain A/J mice (Stoner *et al*., 1986) were examined for the presence of activated K-*ras* proto-oncogenes. DNA segments were amplified using the polymerase chain reaction and sequenced to identify the mutations. An activated K-*ras* protooncogene was detected in all of the lung tumours tested. In the control mouse lung tumours (described in an earlier publication, You *et al*., 1989), activating mutations were in both codon 12 (6/10, 60%) and codon 61 (3/10, 30%) with several types of nucleotide substitution. In contrast, all of the activating mutations in tumours from benzotrichloride-treated mice (24/24) were in codon 12 and were exclusively GC→AT transitions, whereas only 27% of the K-*ras* mutations in spontaneous tumours were GC→AT transitions. The authors conclude that this result may indicate a direct genotoxic effect of benzotrichloride, although selective promotion of the GC→AT transition during tumorigenesis induced by benzotrichloride cannot be excluded (You *et al*., 1993).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Little information on occupational or environmental exposures to these chemicals was available to the Working Group.

5.2 Human carcinogenicity data

Small cohort studies of occupational exposures to α-chlorinated toluenes and benzoyl chloride in the United States and England each noted an approximately three-fold excess of lung cancer.

Table 1. Genetic and related effects of chlorinated toluenes and benzoyl chloride

	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Benzyl chloride				
ECD, *Escherichia coli pol* A, differential toxicity (spot test)	+	NT	275 000	Fluck *et al*. (1976)
ECD, *Escherichia coli pol* A, differential toxicity (spot test)	–	NT	10 000	Rosenkranz & Poirier (1979)
ECL, *Escherichia coli pol* A, differential toxicity (liquid suspension test)	+	+	10	Rosenkranz & Poirier (1979)
BSD, *Bacillus subtilis rec*, differential toxicity	(+)	NT	10 000 µg/disk	Yasuo *et al* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	500	Yasuo *et al*. (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	125	Simmon (1979a)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	–	31.5	Neudecker *et al*. (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	+	50	Ashby *et al*. (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	(+)	500	Brooks & Gonzalez (1982a)
SA0, *Salmonella typhimurium* TA100, reverse mutation (spot test)	?	?	2000 µg/disk	Hyldig-Nielsen & Hartley-Asp (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	125	Jones & Richold (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	25	Kirkland *et al*. (1982a)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	200	Ladner (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	112	Moore & Chatfield (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	NT	150	Pour *et al*. (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation (fluctuation test)	NT	–	250	Sargent & Regnier (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	NT	1250	Trueman & Callander (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	(+)	250	Varley (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	(+)	250	Venitt *et al*. (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	250	Watkins & Rickard (1982)

Table 1 (contd)

	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	5	Booth *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	40	Hemminki *et al*. (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	–	5000	Rosenkranz & Poirier (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	125	Simmon (1979a)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2000	Brooks & Gonzalez (1982a)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	125	Jones & Richold (1982)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	NT	500	Kirkland *et al*. (1982a)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	200	Ladner (1982)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	NT	1250	Trueman & Callander (1982)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	250	Varley (1982)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Watkins & Rickard (1982)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	250	Booth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	125	Simmon (1979a)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2000	Brooks & Gonzalez (1982a)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	125	Jones & Richold (1982)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	NT	500	Kirkland *et al*. (1982a)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	200	Ladner (1982)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	NT	1250	Trueman & Callander (1982)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	250	Varley (1982)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Watkins & Rickard (1982)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	250	Booth *et al*. (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	5000	Rosenkranz & Poirier (1979)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	125	Simmon (1979a)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2000	Brooks & Gonzalez (1982a)

Table 1 (contd)

	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA8, *Salmonella typhimurium* TA1538, reverse mutation (spot test)	–	–	2000 µg/disk	Hyldig-Nielsen & Hartley-Asp (1982)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	125	Jones & Richold (1982)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	500	Kirkland *et al.* (1982a)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	200	Ladner (1982)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	1250	Trueman & Callander (1982)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	250	Varley (1982)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	500	Watkins & Rickard (1982)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	250	Booth *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	125	Simmon (1979a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2000	Brooks & Gonzalez (1982a)
SA9, *Salmonella typhimurium* TA98, reverse mutation (spot test)	–	–	2000 µg/disk	Hyldig-Nielsen & Hartley-Asp (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	125	Jones & Richold (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	500	Kirkland *et al.* (1982a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	200	Ladner (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	250	Pour *et al.* (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250	Sargent & Regnier (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation (fluctuation test)	+	+	10	Styles & Pritchard (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	1250	Trueman & Callander (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250	Varley (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250	Venitt *et al.* (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Watkins & Rickard (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250	Booth *et al.* (1983)

Table 1 (contd)

	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	40	Hemminki *et al.* (1983)
SAS, *Salmonella typhimurium* TA1536, reverse mutation	–	–	125	Simmon (1979a)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	+	NT	250	Kirkland *et al.* (1982a)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	+	+	100	Venitt *et al.* (1982)
EC2, *Escherichia coli* WP2, reverse mutation	+	–	400	Yasuo *et al.* (1978)
EC2, *Escherichia coli* WP2, reverse mutation	+	NT	25	Kirkland *et al.* (1982a)
SSB, *Saccharomyces cerevisiae* D6, strand breaks, cross-links	+	NT	100	Tippins (1982)
SSD, *Saccharomyces cerevisiae rad* mutants, differential toxicity	+	NT	100	North & Parry (1982)
SCG, *Saccharomyces cerevisiae* JD1, gene conversion	+	+	250	Brooks & Gonzalez (1982b)
SCG, *Saccharomyces cerevisiae* D7, gene conversion	+	NT	125	Goodwin & Parry (1982)
SCG, *Saccharomyces cerevisiae* D4, gene conversion	+	NT	220	Mitchell & Gilbert (1982)
SCG, *Saccharomyces cerevisiae* JD1, gene conversion	+	+	0.5	Parry (1982a)
SCG, *Saccharomyces cerevisiae* JD1, gene conversion	+	+	125	Wilcox & Parry (1982)
SCH, *Saccharomyces cerevisiae* D3, homozygosis	(+)	(+)	400	Simmon (1979b)
SCH, *Saccharomyces cerevisiae* D7, homozygosis	+	NT	50	Kelly & Parry (1982)
SCH, *Saccharomyces cerevisiae* D6, homozygosis	+	+	25	Parry (1982b)
ANG, *Aspergillus nidulans*, genetic crossing-over	+	NT	100	Igwe & Cohn (1982)
ANG, *Aspergillus nidulans*, genetic crossing-over	+	+	500	Watkins (1982)
SCF, *Saccharomyces cerevisiae* D7, forward mutation	–	NT	500	Goodwin & Parry (1982)
SCF, *Saccharomyces cerevisiae* D4, forward mutation	+	NT	330	Mitchell & Gilbert (1982)
ANR, *Aspergillus nidulans*, reverse mutation	+	NT	100	Igwe & Cohn (1982)
NCR, *Neurospora crassa*, reverse mutation	+	NT	50	Luker (1982)
SCN, *Saccharomyces cerevisiae* D6, aneuploidy	–	–	200	Parry (1982b)
ANN, *Aspergillus nidulans*, aneuploidy	–	–	2500	Watkins (1982)

Table 1 (contd)

	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DMM, *Drosophila melanogaster*, somatic mutation and recombination	+		126 feed	Fahmy & Fahmy (1982)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	(+)		252 feed	Fahmy & Fahmy (1982)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		504 feed	MacDonald & Telford (1982)
DIA, DNA strand breaks, cross-links, Chinese hamster lung V79 cells *in vitro*	+	NT	126	Swenberg (1981)
GCO, Gene mutation, Chinese hamster ovary CHO cells *in vitro*	+	NT	18	Phillips & James (1982)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	NT	+	11	Lee & Webber (1982)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	+	NT	18	Mirzayans *et al*. (1982a)
G9O, Gene mutation, Chinese hamster lung V79 cells, ouabain resistance *in vitro*	–	NT	25	Mirzayans *et al*. (1982a)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	–	10	Ross & McGregor (1982)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	8	McGregor *et al*. (1988)
G51, Gene mutation, mouse lymphoma L5178Y cells, ouabain resistance *in vitro*	–	NT	NG	Booth *et al*. (1983)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	NT	10	Phillips & James (1982)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	NT	12.7	Hemminki *et al*. (1983)

Table 1 (contd)

	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	NT	14	Phillips & James (1982)
CIC, Chromosomal aberrations, Chinese hamster lung cells *in vitro*	(+)	+	30	JETOC (1997)
CIR, Chromosomal aberrations, rat cells *in vitro*	+	NT	15	Malallah *et al*. (1982)
TCM, Cell transformation, C3H 10T½ mouse cells *in vitro*	–	–	20	Poole & McGregor (1982)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay *in vitro*	+	NT	0.1	Pienta *et al* (1977)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay *in vitro*	–	NT	5	Poiley *et al*. (1980)
DIH, DNA strand breaks, cross-links, human alveolar tumour cells *in vitro*	+	NT	125	Mirzayans *et al*. (1982b)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	NT	10	Hartley-Asp (1982a)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	5	Kirkland *et al*. (1982b)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	10	Hartley-Asp (1982a)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	10	Kirkland *et al*. (1982b)
BFA, Urine of mice, *Salmonella typhimurium* TA100, TA98, TA1535, TA1537, TA1538 mutagenicity	–		550 ip × 2	Jones & Richold (1982)
HMM, Host-mediated assay, *Salmonella typhimurium* TA1530, TA1538 and *Saccharomyces cerevisiae* D3 in Swiss-Webster mice	–		4400 im × 1	Simmon *et al*. (1979)
MVM, Micronucleus test, male TuckTO mice *in vivo*	–		300 ip × 2	Danford & Parry (1982)
MVM, Micronucleus test, NMRI mice *in vivo*	–		400 po × 2	Hartley-Asp (1982b)
MVM, Micronucleus test, CD-1 mice *in vivo*	–		876 po × 2	Holmstrom *et al*. (1982)
MVM, Micronucleus test, CD-1 mice *in vivo*	–		550 ip × 2	Richardson *et al*. (1982)

Table 1 (contd)

	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVM, Micronucleus test, (CBA × BALB/c)F_1 mice *in vivo*	–		2000 sc × 1	Scott & Topham (1982)
Benzal chloride				
BSD, *Bacillus subtilis rec*, differential toxicity	+	NT	5000 µg/disk	Yasuo *et al*. (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	100	Yasuo *et al*. (1978)
EC2, *Escherichia coli* WP2, reverse mutation	–	+	100	Yasuo *et al*. (1978)
Benzotrichloride				
BSD, *Bacillus subtilis rec*, differential toxicity	+	NT	500 µg/disk	Yasuo *et al*. (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	195	Yasuo *et al*. (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	100	Yasuo *et al*. (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	100	Yasuo *et al*. (1978)
EC2, *Escherichia coli* WP2, reverse mutation	–	+	100	Yasuo *et al*. (1978)
Benzoyl chloride				
BSD, *Bacillus subtilis rec*, differential toxicity	+	NT	100 µg/disk	Yasuo *et al*. (1978)
EC2, *Escherichia coli* WP2, reverse mutation	–	+	500	Yasuo *et al*. (1978)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; ip, intraperitoneal; im, intramuscular; po, oral; sc, subcutaneous

5.3 Animal carcinogenicity data

Benzyl chloride, benzal chloride, benzotrichloride and benzoyl chloride have been studied by skin application to mice. Small numbers of skin tumours were produced by benzyl chloride and benzoyl chloride, while clear increases in skin tumours were produced by benzal chloride and benzotrichloride. Following subcutaneous injections to rats, benzyl chloride produced some injection site tumours. Administration by gavage of benzyl chloride to mice and rats produced forestomach tumours in mice and a few neoplasms of the forestomach were observed in male rats. Benzotrichloride administered by gavage to mice produced tumours of the forestomach and lungs. In addition, benzotrichloride and benzoyl chloride were administered by inhalation to mice: benzotrichloride produced increases in the incidences of tumours of the lung and skin, whereas no significant increase in tumour incidence was observed after benzoyl chloride administration.

5.4 Other relevant data

No studies were available on the disposition of benzotrichloride, benzal chloride or benzoyl chloride. Benzyl chloride is rapidly absorbed and distributed from the gastrointestinal tract. Excretion is mainly in urine as *S*-benzyl-*N*-acetylcysteine, benzyl alcohol and benzaldehyde.

All of the compounds are irritant to the skin and mucous membranes.

Benzyl chloride, benzal chloride and benzotrichloride, but not benzoyl chloride, are bacterial mutagens. Only benzyl chloride has been more extensively tested. It is genotoxic to fungi, *Drosophila melanogaster* and cultured mammalian cells, but did not increase the frequency of micronuclei in mice.

5.5 Evaluation

There is *limited evidence* in humans for the carcinogenicity of α-chlorinated toluenes and benzoyl chloride.

There is *sufficient evidence* in experimental animals for the carcinogenicity of benzyl chloride.

There is *limited evidence* in experimental animals for the carcinogenicity of benzal chloride.

There is *sufficient evidence* in experimental animals for the carcinogenicity of benzotrichloride.

There is *inadequate evidence* in experimental animals for the carcinogenicity of benzoyl chloride.

Overall evaluation

Combined exposures to α-chlorinated toluenes and benzoyl chloride are *probably carcinogenic to humans (Group 2A)*.

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1,2-DIBROMO-3-CHLOROPROPANE

Data were last reviewed in IARC (1979) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

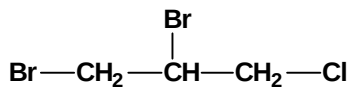
Chem. Abstr. Serv. Reg. No.: 96-12-8

Chem. Abstr. Name: 1,2-Dibromo-3-chloropropane

IUPAC Systematic Name: 1,2-Dibromo-3-chloropropane

Synonyms: DBCP; dibromochloropropane

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_3H_5Br_2Cl$ Relative molecular mass: 236.33

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Brown liquid with a pungent odour (Budavari, 1996)

(*b*) *Boiling-point*: 196°C (Lide, 1997)

(*c*) *Melting-point:* 6°C (Agency for Toxic Substances and Disease Registry, 1992)

(*d*) *Solubility*: Slightly soluble in water; miscible with oils, dichloropropane and isopropanol (Budavari, 1996)

(*e*) *Vapour pressure*: 106 Pa at 21°C; relative vapour density (air = 1), 2.09 at 14°C (Verschueren, 1996; United States National Library of Medicine, 1997)

(*f*) *Flash point*: 76.6°C, open cup (Agency for Toxic Substances and Disease Registry, 1992)

(*g*) *Conversion factor:* $mg/m^3 = 9.7 \times ppm$

1.2 Production and use

Estimates of annual production of 1,2-dibromo-3-chloropropane in the United States during 1974–75 ranged from eight to nine thousand tonnes. Commercial production is believed to have ceased worldwide (Agency for Toxic Substances and Disease Registry, 1992).

1,2-Dibromo-3-chloropropane has been used as a pesticide, nematocide and soil fumigant (Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

No current data on numbers of exposed workers were available to the Working Group. Occupational exposures to 1,2-dibromo-3-chloropropane have occurred during its production and use.

1.3.2 *Environmental occurrence*

Use of 1,2-dibromo-3-chloropropane as a pesticide, soil fumigant and a nematocide resulted in the direct release of this compound to the environment. Its production and use as an intermediate in organic synthesis also may have resulted in its release to the environment through various waste streams. It has been detected at low levels in ambient and urban air, groundwater, drinking-water and soil samples (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The United States Occupational Safety and Health Administration (OSHA) (1996) has established 0.0001 mg/m^3 as the permissible exposure limit for occupational exposures to 1,2-dibromo-3-chloropropane in workplace air.

The World Health Organization has established an international drinking-water guideline for 1,2-dibromo-3-chloropropane of 1 μg/L (WHO, 1993).

2. Studies of Cancer in Humans

2.1 Cohort studies

A group of some 3500 workers classified as having had exposure to several brominated chemicals, including 1,2-dibromo-3-chloropropane, was studied in four facilities in the United States. Among the 1034 workers ever exposed to 1,2-dibromo-3-chloropropane, nine respiratory cancers were observed compared with 5.0 expected; of these seven were due to lung cancer (4.8 expected) (IARC, 1987).

Olsen *et al*. (1995) studied mortality among a cohort of 548 male 1,2-dibromo-3-chloropropane production workers. This was an update of an earlier study performed by Hearn *et al*. (1984). The workers were identified on the basis of employment records or self-declaration of exposure to 1,2-dibromo-3-chloropropane and were followed from 1957 through 1989. A total of 68 deaths were identified (standardized mortality ratio (SMR), 0.8) and overall cancer mortality was similar to expected (SMR, 1.0; n = 19), based on mortality of white men in the United States. There were seven lung cancer deaths compared with 7.1 expected (SMR, 1.0; 95% confidence interval (CI), 0.4–2.0), but an excess of lung cancer (SMR, 3.4; 95% CI, 0.7–9.6), based on three cases, was

observed among the 81 workers categorized as having been directly exposed for one or more years. Exposure levels were not reported.

Brown (1992) conducted a cohort mortality study of workers employed at four pesticide manufacturing plants. The 1158 workers employed at Plant 3 of the study, which produced aldrin and dieldrin, were also potentially exposed to 1,2-dibromo-3-chloropropane produced at the plant between 1975 and 1976. The cohort included all white males employed for six or more months before 1964 with follow-up through 1987. Although overall cancer mortality at Plant 3 was not elevated (SMR, 0.9; 95% CI, 0.7–1.1; $n = 72$), an excess of liver and biliary tract cancer was observed (SMR, 3.9; 95% CI, 1.3–9.2; 5 observed). All of the deaths occurred at least 15 years after first employment (SMR, 4.9), but no association was observed with duration of employment. The SMR for lung cancer was 0.7 (95% CI, 0.4–1.0). Levels of exposure were not reported. Amoateng-Adjepong *et al*. (1995) reported the results of an update of the same cohort with three additional years of follow-up. No new association was reported.

Wesseling *et al*. (1996) reported the results of a retrospective cohort study of cancer incidence among banana plantation workers in Costa Rica where 1,2-dibromo-3-chloropropane was used as a soil fumigant. Other pesticides were also used. The cohort consisted of 29 565 men and 4892 women on the payrolls of banana companies, as reported to the social security system, at any time between 1972 and 1979. Follow-up was performed using national cancer registry records from 1981 to 1992. Duration of employment during the period 1972–79 period was also available. Overall cancer rates for both men and women were less than expected based on national rates. The standardized incidence ratio (SIR) for lung cancer among men was 1.1 (95% CI, 0.7–1.5; 30 cases). Excesses were observed for melanoma (SIR, 2.0; 95% CI, 0.9–3.6; 10 cases) and penile cancer (SIR, 1.5; 95% CI, 0.6–3.2; 6 cases) among men and for cervical cancer (SIR, 1.8; 95% CI, 1.2–2.4; 36 cases) and leukaemia (SIR, 2.7; 95% CI, 0.9–6.4; 5 cases) among women. Excesses, based on small numbers, were observed among men employed for three or more years for lung (SIR, 1.7; 95% CI, 0.9–2.9; 12 cases), melanoma (SIR, 3.2; 95% CI, 0.9–3.3; 4 cases), penile (SIR, 2.0; 95% CI, 0.3–1.4; 2 cases) and brain cancer (SIR, 2.3; 95% CI, 0.9–5.0; 6 cases), and among women for leukaemia (SIR, 5.6; 95% CI, 0.7–20.3; 2 cases). It was not possible to link cancer incidence results to specific exposures and exposure levels were not reported.

2.2 Case–control studies

Wong *et al*. (1989) performed both ecological analyses and case–control studies to examine the relationship between gastric cancer and leukaemia and 1,2-dibromo-3-chloropropane contamination of drinking-water in Fresno County, California (United States). The concentration of 1,2-dibromo-3-chloropropane was estimated based on water systems by census tract (Whorton *et al.*, 1987). The studies were precipitated by public concern over 1,2-dibromo-3-chloropropane contamination of drinking-water wells in various farming areas of the county, and an analysis by the Department of Health suggesting elevated stomach cancer and leukaemia mortality in the county. In the ecological

analyses, no correlation between gastric cancer and leukaemia rates from 1960 to 1983 and estimated 1,2-dibromo-3-chloropropane concentration in water based on census tracts and residence at time of death was observed after adjustment for age, sex and race. For the case–control analyses, fatal gastric cancer ($n = 263$) and leukaemia ($n = 259$) cases were identified and 203 and 225 were included in the study. Three or four controls for each case, matched on age, race and year, were randomly chosen from among other Fresno County deaths. Attempts were made through the use of mailed questionnaires and residential directories to identify the residence of cases and controls at time of death and at one year and ten years before death. No association was observed with estimated 1,2-dibromo-3-chloropropane levels based on census tract. Nonsignificant increased risks of both gastric cancer (odds ratio, 3.1; 95% CI, 1.0–9.8) and leukaemia (odds ratio, 3.9; 95% CI, 0.7–21.5) were associated with estimated 1,2-dibromo-3-chloropropane concentrations above 1.0 ppb (μg/L) based on the water system data alone, 10 years before death in multiple logistic regression analysis.

3. Studies of Cancer in Experimental Animals

1,2-Dibromo-3-chloropropane was tested in one experiment in mice and one in rats by oral administration. It produced squamous-cell carcinomas of the forestomach in animals of both species and adenocarcinomas of the mammary gland in female rats (IARC, 1979).

3.1 Inhalation exposure

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F_1 mice, four to five weeks of age, were administered 1,2-dibromo-3-chloropropane (96% pure), containing small amounts of epichlorohydrin (0.6%) and 1,2-dibromoethane (0.07%), by whole-body inhalation at concentrations of 0 (control), 0.6 or 3 ppm [0, 4 or 29 mg/m^3] for 6 h per day on five days per week for 76–103 weeks. Survival was significantly decreased in all treated groups. 1,2-Dibromo-3-chloropropane increased the incidence of lung and nasal tumours, as shown in Table 1 (United States National Toxicology Program, 1982).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, five to six weeks of age, were administered 1,2-dibromo-3-chloropropane (96% pure), containing small amounts of epichlorohydrin (0.6%) and 1,2-dibromoethane (0.07%), by whole-body inhalation at concentrations of 0 (control), 0.6 or 3 ppm [0, 4 or 29 mg/m^3] for 6 h per day on five days per week for 84–103 weeks. Survival of high-dose rats was reduced and all surviving rats were killed at week 84. Increased incidence of tumours of the nasal cavity and of the tongue in both sexes and of the pharynx in females was observed, as shown in Table 2 (United States National Toxicology Program, 1982).

Table 1. Primary tumour incidence in B6C3F$_1$ mice exposed by inhalation to 1,2-dibromo-3-chloropropane

Site/tumour	Animals with tumours					
	Males			Females		
	Chamber control	0.6 ppm	3.0 ppm	Chamber control	0.6 ppm	3.0 ppm
Lung/bronchus/bronchiole[a,b]	0/41	3/40	11/45[c]	4/49	12/48[d]	18/47[c]
Nasal cavity[a,e]	0/45	1/42	18/48[c]	0/50	11/50[c]	38/50[c]

From United States National Toxicology Program (1982)
[a] Dose-related trends ($p < 0.001$)
[b] Papillary adenoma or carcinoma, squamous-cell carcinoma, alveolar/bronchiolar adenoma or carcinoma
[c] Greater than controls ($p < 0.001$)
[d] Greater than controls ($p < 0.05$)
[e] Carcinoma, squamous-cell papilloma or carcinoma, adenocarcinoma, adenomatous polyp, unspecified malignant neoplasm

Table 2. Primary tumour incidence in Fischer 344 rats exposed by inhalation to 1,2-dibromo-3-chloropropane

Site/tumour	Animals with tumours					
	Males			Females		
	Chamber control	0.6 ppm	3.0 ppm	Chamber control	0.6 ppm	3.0 ppm
Adrenal gland cortical adenoma	1/49	6/49	3/48	0/50	7/50[a]	5/48[b]
Mammary gland fibroadenoma	0/50	0/50	0/49	4/50	13/50[b]	4/50
Nasal cavity and turbinates[c,d]	0/50	32/50[e]	39/49[e]	1/50	21/50[e]	42/50[e]
Pharynx squamous-cell papilloma or carcinoma[f]	0/50	3/50	1/49	0/50	0/50	6/50[b]
Tongue squamous-cell papilloma or carcinoma[c]	0/50	1/50	11/49[e]	0/50	4/50	9/50[e]

From United States National Toxicology Program (1982)
[a] Greater than controls ($p < 0.01$)
[b] Greater than controls ($p < 0.05$)
[c] Dose-related trends ($p < 0.001$)
[d] Carcinoma, squamous-cell papilloma or carcinoma, adenoma, adenocarcinoma, adenomatous polyp and carcinosarcoma
[e] Greater than controls ($p < 0.001$)
[f] Dose-related trend, females ($p < 0.001$)

3.2 Other systems

Fish: A group of 100 *Danio rerio* (H) fish of both sexes, 10–12 months old, were exposed to 20 µg/L water 1,2-dibromo-3-chloropropane (purity, > 95%) (equivalent to 0.2 $LD_{50/30}$) added to the water every two weeks for eight weeks, after which the fish were kept in fresh water without 1,2-dibromo-3-chloropropane for 12 more weeks. A negative control group of 40 fish received dimethyl sulfoxide (DMSO) at a concentration of 90 mg/L water [exact test regimen not reported]. A positive-control group of 100 fish received *N*-nitrosodimethylamine (NDMA) at a concentration of 50 mg/L water [exact test regimen not reported]. Twenty-one fish in the 1,2-dibromo-3-chloropropane group, 39 fish in the DMSO group and 51 in the NDMA group lived for 20 weeks, the minimum duration required for the appearance of the first liver tumour. The incidences of liver tumours were 9/21, 0/39 and 22/51 for the 1,2-dibromo-3-chloropropane, DMSO and NDMA groups, respectively. The nine liver tumours in the 1,2-dibromo-3-chloropropane group consisted of seven hepatocellular carcinomas and two cholangiocarcinomas (Belitsky *et al*., 1994).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Several purified human GST forms readily metabolized 1,2-dibromo-3-chloropropane in descending order of activity from GST A1-2 > A2-2 ≈ A1-1 > M1a-1a > M3-3 ≈ P1-1 (Søderlund *et al*., 1995).

The in-vitro metabolic activation of 1,2-dibromo-3-chloropropane, measured as radiolabel covalently bound to macromolecules, is three-fold faster in rat testicular cells than in human testicular cells (Bjørge *et al*., 1996a).

4.1.2 *Experimental systems*

In rats, 1,2-dibromo-3-chloropropane is rapidly absorbed after oral administration in water (T_{max}, 0.20 h after 1 mg/kg bw); corn oil as a vehicle delays absorption (T_{max}, 1.56 h), but does not affect bioavailability. 1,2-Dibromo-3-chloropropane is distributed and eliminated biexponentially, mainly as metabolites with a half-life of 2–3 h. There is no saturation of absorption, distribution or elimination up to 10 mg/kg bw (Gingell *et al*., 1987).

Metabolism of 1,2-dibromo-3-chloropropane proceeds via oxidation by cytochrome P450 enzyme(s) and conjugation with glutathione (Omichinski *et al*., 1987, 1988; Simula *et al*., 1993; Weber *et al*., 1995). The metabolism is measurable as formation of water-soluble metabolites (mainly several *N*-acetylcysteine conjugates in bile and urine) and metabolites covalently bound to macromolecules (Kato *et al*., 1979; Dohn *et al*., 1988; Pearson *et al*., 1990a,b; Weber *et al*., 1995). Metabolism to water-soluble products

occurs in isolated rat liver, kidney and testicular cells, the rate of formation decreasing in this order (Søderlund *et al*., 1995). Various testicular cell types isolated from rats show differences in their rates of activation of 1,2-dibromo-3-chloropropane to metabolites that bind to macromolecules (Bjørge *et al*., 1995). Rats and guinea-pigs seem to be more sensitive than Syrian hamsters and mice to testicular damage because of a higher ability to activate 1,2-dibromo-3-chloropropane to DNA-damaging species (Låg *et al*., 1989a).

1,2-Dibromo-3-chloropropane is metabolically activated into several products (see Figure 1; Pearson *et al*., 1990a,b). The principal adduct in rat and mouse tissues after in-vivo administration was *S*-[1-(hydroxymethyl)-2-(*N*7-guanyl)-ethyl]glutathione, which was also detected in several rat tissues, both target and non-target, after in-vivo administration of 1,2,3-trichloropropane, a structurally related chemical (La *et al*., 1995). Several studies suggest that cytochrome P450-mediated metabolism is of minor importance for organ toxicity (Omichinski *et al*., 1987; Låg *et al*., 1989b; Søderlund *et al*., 1995).

4.1.3 *Comparison of human and rodent data*

The rate of metabolic activation of 1,2-dibromo-3-chloropropane in human testicular cells is abour one-third that of rat cells. No other data are available for comparison. Nevertheless, since P450 isoenzymes and several GST enzymes are rather similar in terms of substrate selectivity between humans and rats, it is expected that human tissues should be capable of activating 1,2-dibromo-3-chloropropane via both P450- and GST-mediated pathways.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

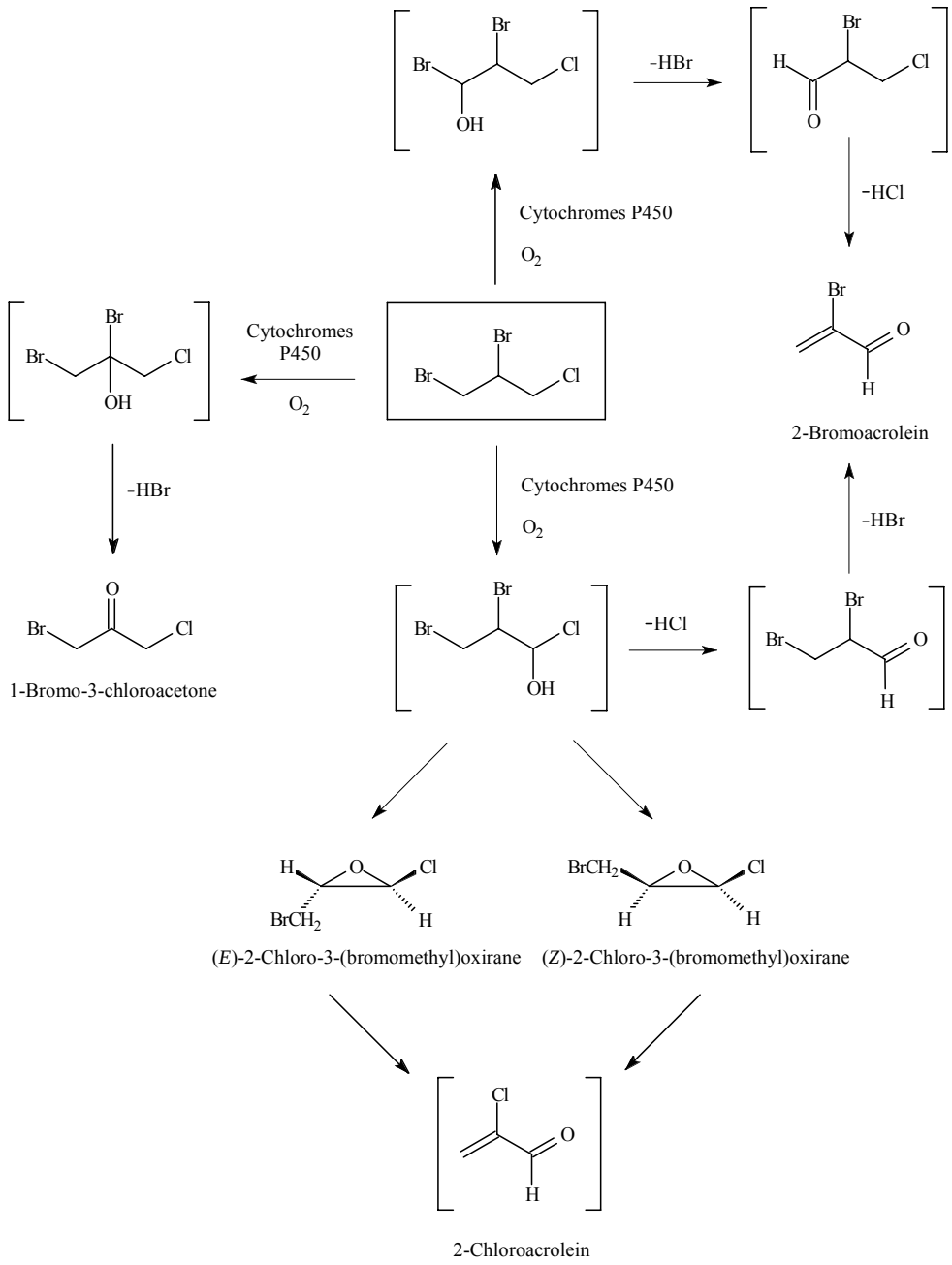
4.2.2 *Experimental systems*

Groups of rats were exposed by inhalation to 1,2-dibromo-3-chloropropane for 7 h per day on five days per week for 10 weeks. The testis was the primary target for toxicity, atrophy being observed at concentrations of 10 ppm [97 mg/m^3] and above (Torkelson *et al*., 1961).

Extensive renal necrosis and elevated plasma urea and creatinine levels were noted in male Mol:WIST rats 48 h after intraperitoneal administration of 170–340 mmol/kg bw [40.2–80.3 mg/kg bw] 1,2-dibromo-3-chloropropane (Søderlund *et al*., 1990). In the same study, significantly less damage was found in male Bom:NMRI mice and male Mol:DH guinea-pigs after higher doses (up to 680 mmol/kg bw [160.7 mg/kg bw]). No nephrotoxicity was detected in male Lak:LVG/SYR Syrian hamsters at doses of 170–680 mmol/kg bw. In guinea-pigs and mice, the high doses (> 510 mmol/kg bw) of 1,2-dibromo-3-chloropropane resulted in central nervous system depression and death in a number of animals.

Male Fischer 344 rats dosed by gavage with 29 mg/kg bw 1,2-dibromo-3-chloropropane on five days per week for two weeks developed hyperkeratosis and hyperplasia of

Figure 1. Proposed oxidative metabolism pathway for 1,2-dibromo-3-chloro-propane



From Pearson *et al.* (1990a)
Bracketed structures have not been isolated.

the forestomach. A lower dose 915 mg/kg bw had no significant effect (Ghanayem *et al*., 1986).

Necrosis and atrophy of the olfactory epithelium in the nasal cavity resulted from inhalation exposure to 5 and 25 ppm [50 and 240 mg/m^3] 1,2-dibromo-3-chloropropane for 6 h per day on five days per week for 13 weeks in both male and female Fischer 344 rats and B6C3F$_1$ mice. At 1 ppm, respiratory changes were observed that included cytomegaly, focal hyperplasia and, to lesser extents, squamous metaplasia and loss of cilia (Reznik *et al*., 1980).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

Several studies have found decreased sperm counts, altered sperm morphology and decreased spermatogenic activity in workers occupationally exposed to 1,2-dibromo-3-chloropropane, with the drop in sperm count correlating with the length of exposure, but other studies have failed to find any effect (reviewed by Whorton & Foliart, 1988). Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were increased from 7.9 and 14.0 ImU/mL to 29.0 and 21.7 ImU/mL, respectively, in highly exposed men (physicians' estimation of exposure) (Olsen *et al*., 1990). The hormonal effects of 1,2-dibromo-3-chloropropane appear to be reversible after 12–16 months of cessation of exposure in many cases of oligospermia and in some cases of azospermia (Whorton & Milby, 1981; Eaton *et al*., 1986; Olsen *et al*., 1990; Potashnik & Porath, 1995). It has been reported that the use of 1,2-dibromo-3-chloropropane in the 1970s caused the sterilization of approximately 1500 banana workers (approximately 20–25% of the workforce) in Costa Rica (Thrupp, 1991).

4.3.2 *Experimental systems*

Groups of male Sprague-Dawley rats were given subcutaneous injections of 1,2-dibromo-3-chloropropane at 7, 30 or 90 days of age either once (50 mg/kg bw) or repeatedly (20 mg/kg bw once a week for three weeks) (Sod-Moriah *et al*., 1990). Results were similar following either single or repeated injections. In the 7- and 90-day-old rats, the weights of the testes, epididymis, prostate and seminal glands were significantly reduced. Additionally, the plasma FSH and LH levels were significantly increased. Little change in reproductive organs or hormone levels was observed in the 30-day-old rats. No changes in the weights of non-reproductive organs were observed.

In a study on the effects of fetal exposure to 1,2-dibromo-3-chloropropane, pregnant Sprague-Dawley rats were treated with 25 mg/kg bw 1,2-dibromo-3-chloropropane for two, four or six days beginning on gestational days 18.5, 16.5 or 14.5, respectively (Warren *et al*., 1988). A decrease in testicular weights of 75% to > 90% was found in adult males treated *in utero* that related to the duration of treatment. Many of the adults treated with 1,2-dibromo-3-chloropropane on gestational days 16.5–18.5 lacked seminiferous tubules. In-utero treatment for six days reduced intratesticular testosterone level by 50%.

Adult males treated *in utero* on gestational days 16.5–18.5 also exhibited increased feminine behaviour and decreased masculine behaviour.

There are species differences in sensitivity to 1,2-dibromo-3-chloropropane-mediated testicular damage. Låg *et al*. (1989a) found marked necrosis and atrophy of seminiferous epithelium in male Mol:WIST rats and male Mol:DH guinea-pigs 10 days after a single injection of 340 mmol/kg bw [80 mg/kg bw] 1,2-dibromo-3-chloropropane and no significant difference in the seminiferous epithelium of male Bom:NMRI mice or Lak:LVG/SYR Syrian hamsters. Indicators of testicular DNA damage correlated with the relative susceptibilities of the different species to 1,2-dibromo-3-chloropropane-induced testicular damage.

In a continuous breeding study, exposure of Swiss CD-1 mice to 100 mg/kg bw 1,2-dibromo-3-chloropropane was found to produce a minor decline in the number of litters per F_0 pair and reduced epididymis and prostate weights in the F_1 mice. These effects were considered to be relatively minor compared with the effects seen in rats (Lamb *et al*., 1997).

In female Wistar rats, subcutaneous administration of 1,2-dibromo-3-chloropropane during gestation did not affect oogenesis (Shaked *et al*., 1988).

4.4 Genetic and related effects

The genetic toxicology of 1,2-dibromo-3-chloropropane has been reviewed (Teramoto & Shirasu, 1988).

4.4.1 *Humans*

Kapp *et al.* (1979) reported the presence of Y-chromosomal non-disjunction in 1,2-dibromo-3-chloropropane-exposed workers using a quinacrine-staining technique. [There was no indication of the level of exposure to 1,2-dibromo-3-chloropropane or whether other exposures were present.] The frequency of sperm with two spots (indicating two Y chromosomes) was 1.2% (range, 0.8–1.8%) in 15 controls and 3.8% (range, 2.0–5.3%) in 18 samples from exposed men.

In preparations from human organ transplant donors, no DNA single-strand breaks were detected in testicular germ cells treated with 1,2-dibromo-3-chloropropane up to 300 μM, which is in contrast to rat cells, in which breaks were increased after exposure to 3 μM (Bjørge *et al*., 1996a,b).

4.4.2 *Experimental systems* (see Table 3 for references)

1,2-Dibromo-3-chloropropane is mutagenic to *Salmonella typhimurium* strains, particularly strain TA100 and usually in the presence of an exogenous metabolic activation system. The occasional significant responses in strain TA1535 in the absence of such an activation system are probably due to the presence of epichlorohydrin (see this volume), which was used as a stabilizer (Biles *et al*., 1978). The mutagenicity of 1,2-dibromo-3-chloropropane in *S. typhimurium* TA100 was greatly increased if the strain was modified to express the human glutathione-*S*-transferase genes A1-1 or P1-1. It

Table 3. Genetic and related effects of 1,2-dibromo-3-chloropropane

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SAF, *Salmonella typhimurium* BA13, forward mutation (Ara test)	–	+	3.9	Roldán-Arjona, *et al*. (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	+	50	Blum & Ames (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	30	Stolzenberg & Hine (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	1180	Stolzenberg & Hine (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	50	Moriya *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	236	Miller *et al*. (1986)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	2.5	McKee *et al*. (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	2.5	Ratpan & Plaumann (1988)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	24	Holme *et al*. (1989)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	1.2	Låg *et al*. (1994)
SA0, *Salmonella typhimurium* TA100 expressing human GST A1-1 or P1-1, reverse mutation	–	+	1.25	Simula *et al*. (1993)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	(+)	10.5	Prival *et al*. (1977)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	25	Biles *et al*. (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	0.5	McKee *et al*. (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	0.5	Ratpan & Plaumann (1988)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	100	McKee *et al*. (1987)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	50	Ratpan & Plaumann (1988)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	10450	Rosenkranz (1975)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	525	Prival *et al*. (1977)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	+	50	McKee *et al*. (1987)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	100	McKee *et al*. (1987)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	50	Ratpan & Plaumann (1988)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	1180	Stolzenberg & Hine (1979)

Table 3 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	50	Ratpan & Plaumann (1988)
SAS, *Salmonella typhimurium* TA1530, reverse mutation	+	NT	200	Rosenkranz (1975)
DMG, *Drosophila melanogaster*, genetic crossing over or recombination	+		2400 mg/m^3 vap. × 0.5 h	Kale & Baum (1982)
DMG, *Drosophila melanogaster*, genetic crossing over or recombination	+		23.6 feed	Vogel & Nivard (1993)
DMX, *Drosophila melanogaster,* sex-linked recessive lethal mutations	(+)		7.2% vap. 5 min	Inoue *et al*. (1982)
DMX, *Drosophila melanogaster,* sex-linked recessive lethal mutations	(+)		600 mg/m^3 vap. × 0.5 h	Kale & Baum (1982)
DMX, *Drosophila melanogaster,* sex-linked recessive lethal mutations	+		200 ppm feed	Yoon *et al*. (1985)
DMH, *Drosophila melanogaster*, heritable translocations	–		3000 mg/m^3 vap. × 0.5 h	Kale & Baum (1982)
DMH, *Drosophila melanogaster*, heritable translocations	+		200 ppm feed	Yoon *et al*. (1985)
DIA, DNA strand breaks/cross-links, rat testicular germ cells *in vitro*	+	NT	17.3	Bradley & Dysart (1985)
DIA, DNA strand breaks, rat testicular cells *in vitro*	+	NT	0.6	Brunborg *et al*. (1988)
DIA, DNA strand breaks, male Wistar rat hepatocytes *in vitro*	+	NT	0.2	Holme *et al*. (1989)
DIA, DNA strand breaks, rat testicular cells *in vitro*	+	NT	1.2	Låg *et al*. (1989a)
DIA, DNA strand breaks, male Wistar rat hepatocytes *in vitro*	+	NT	0.2	Holme *et al*. (1991)
DIA, DNA strand breaks, male New Zealand white rabbit lung cells (Clara cells, type II cells and alveolar macrophages) *in vitro*	+	NT	7.0	Becher *et al*. (1993)
DIA, DNA strand breaks, Wistar rat testicular germ cells *in vitro*	+	NT	0.7	Bjørge *et al*. (1996a)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	20	McKee *et al*. (1987)
GIA, Gene mutation, Fischer 344 rat ARL-13 hepatocytes, *hprt* locus *in vitro*	(+)	NT	95	Belitsky *et al*. (1994)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	NT	2.4	Tezuka *et al*. (1980)

Table 3 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	+	10	Loveday *et al*. (1989)
CIC, Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	+	NT	24	Tezuka *et al*. (1980)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	+	50	Loveday *et al*. (1989)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	7	McKee *et al*. (1987)
DIH, DNA strand breaks, cross-links or related damage, human testicular cells *in vitro*	–	NT	71	Bjørge *et al*. (1996a)
CIH, Chromosomal aberrations, human sperm cells *in vitro*	+	NT	NG[c]	Kapp *et al*. (1979)
BFA, Bile from dosed rats, *Salmonella typhimurium* TA1535, reverse mutation	–		5 iv × 1	Connor *et al*. (1979)
DVA, DNA strand breaks, Fischer 344 rat testicular cells *in vivo*	+		35 ip × 1	Bradley & Dysart (1985)
DVA, DNA strand breaks, rat testicular cells *in vivo*	+		20 ip × 1	Brunborg *et al*. (1988)
DVA, DNA strand breaks, rat and guinea-pig testicular cells *in vivo*	+		40 ip × 1	Låg *et al*. (1989a)
DVA, DNA strand breaks, female Sprague-Dawley rat liver cells *in vivo*	+		35 po × 2	Kitchin & Brown (1994)
DVA, DNA strand breaks, male rat liver and kidney cells *in vivo*	+		5 ip × 1	Brunborg *et al*. (1996)
DVA, DNA strand breaks, rat lung, spleen, brain, urinary bladder, stomach, duodenum, colon, bone marrow, testis *in vivo*	+		10 ip × 1	Brunborg *et al*. (1996)
UVR, Unscheduled DNA synthesis, male Fischer 344 rat spermatocytes *in vivo*	+		150 ip × 1	Bentley & Working (1988)
MST, Mouse spot test, male PW and female C57BL/6 mice	+		106 ip × 1	Sasaki *et al*. (1986)
SLP, Mouse specific locus test, postspermatogonia, male (101 × C3H) or (C3H × 101)F_1 mice	–		150 ip × 1	Russell *et al*. (1986)
SLO, Mouse specific locus test, spermatogonia, (101 × C3H) or (C3H × 101)F_1 mice	–		150 ip × 1	Russell *et al*. (1986)
MVM, Micronucleus test, CD-1 and male CCBF_1 mouse bone marrow *in vivo*	–		150 po × 1	Albanese *et al*. (1988)

Table 3 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVM, Micronucleus test, male SHR mouse bone marrow *in vivo*	+		25.7 po × 2	Belitsky *et al*. (1994)
MVM, Micronucleus test, male SHR mouse forestomach *in vivo*	(+)		54.4 po × 4	Belitsky *et al*. (1994)
MVR, Micronucleus test, male PVG and Alpk rat bone marrow *in vivo*	+		75 po × 1	Albanese *et al*. (1988)
MVR, Micronucleus test, male PVG rat bone marrow *in vivo*	+		75 po × 1	George *et al*. (1990)
CGG, Chromosomal aberrations, male rat spermatogonia *in vivo*	+		7.3 po × 5	Kapp *et al*. (1979)
DLM, Dominant lethal test, female BDF_1 mice	–		150 po × 5	Teramoto *et al*. (1980)
DLM, Dominant lethal test, male $(C3H \times 101)F_1$ mice	–		200 sc × 1	Generoso *et al*. (1985)
DLR, Dominant lethal test, male Sprague-Dawley rats	+		10 po × 5	Teramoto *et al*. (1980)
DLR, Dominant lethal test, male Sprague-Dawley rats	+		50 po × 5	Saito-Suzuki *et al*. (1982)
DLR, Dominant lethal test, male and female Sprague-Dawley rats	+		8 inh 6 h/d, 5 d/w, 14 wk	Rao *et al*. (1983)
DLR, Dominant lethal test, male Sprague-Dawley rats	+		10 po × 5	Au *et al*. (1990)
BVD, Binding (covalent) to DNA, rat liver *in vivo*	+		200 ip × 1	Humphreys *et al*. (1991)
BVD, Binding (covalent) to DNA, rat kidney and testis *in vivo*	–		200 ip × 1	Humphreys *et al*. (1991)
SPM, Sperm morphology, $(C57BL/6 \times C3H)F_1$ mice *in vivo*	–		150 ip × 5	Osterloh *et al*. (1983)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; iv, intravenous; ip, intraperitoneal; po, oral; inh, inhalation; sc, subcutaneous

[c] Cells collected from exposed workers

appears that both oxidation and conjugation are requirements for bacterial mutagenicity. Activation was proportional to cytochrome P450 concentration and was reduced by exogenous reduced glutathione (Miller *et al*., 1986). However, the synthetic glutathione conjugate of 1,2-dibromo-3-chloropropane itself was not mutagenic to *S. typhimurium* TA100 (Humphreys *et al*., 1991).

The compound is mutagenic to *Drosophila melanogaster*, in which it induced sex-linked recessive lethal mutations, mitotic recombinations and heritable translocations.

In cultured mammalian cells, several studies have demonstrated the induction of DNA strand breaks (including one study with human primary testicular cell cultures), while (usually) single studies have demonstrated increases in the frequencies of gene mutations, sister chromatid exchanges, chromosomal aberrations and cell transformation.

In vivo, it is clear that rats are more sensitive than mice to the genotoxic effects of 1,2-dibromo-3-chloropropane. DNA strand breaks were induced in cells of many organs of rats dosed by intraperitoneal injection, as well as in testicular cells of guinea-pigs. Unscheduled DNA synthesis was also induced in rat spermatocytes in one study. In-vivo mutation assays have been conducted only in mice, in which somatic cell mutations were induced in one study, but specific locus mutations were not induced in either spermatogonial stem cells or post-spermatogonial cell stages in another study. Micronuclei were induced in bone-marrow cells of rats, and of mice in one of two studies, and there was evidence of micronucleus induction in the forestomach of orally dosed mice in one study. Dominant lethal effects were induced in orally dosed rats, but not in mice dosed either orally or by subcutaneous injection. Sperm of abnormal morphology were not more frequent in 1,2-dibromo-3-chloropropane-dosed mice than in controls.

In a study of DNA adducts, intraperitoneal injection of rats with 1,2-dibromo-3-chloropropane (200 mg/kg bw) produced *N7*-guanine adducts in the liver at a level of 1 pmol/mg DNA, whereas adducts were not found in either kidney or testis.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to 1,2-dibromo-3-chloropropane has occurred during its production and use as a pesticide, nematocide and soil fumigant; however, production is believed to have ceased. It has been detected at low levels in ambient air, water and soil.

5.2 Human carcinogenicity data

Four cohort studies and one population-based case–control study have examined the risk of cancer among populations exposed to 1,2-dibromo-3-chloropropane, among other chemicals. In two of the cohort studies, an excess of lung cancer was observed based on small numbers of cases. In a third cohort study, an excess of liver and biliary tract cancers was found, while in the fourth an excess of cervical cancer and a non-significant excess of melanoma and leukaemia were observed. However, in both of the last two studies, it

was unclear what proportion of the population was exposed to 1,2-dibromo-3-chloropropane, and there was exposure to multiple pesticides. In the case–control study, there was a non-significant association of gastric cancer and leukaemia with exposure to 1,2-dibromo-3-chloropropane in groundwater.

5.3 Animal carcinogenicity data

1,2-Dibromo-3-chloropropane has been tested by oral administration and inhalation in mice and rats. After oral administration, it produced squamous-cell carcinomas of the forestomach in animals of each species and adenocarcinomas of the mammary gland in female rats. After inhalation, it induced nasal cavity and lung tumours in mice, and nasal cavity and tongue tumours in rats of each sex and pharynx in females. In fish, an increased incidence of liver tumours was found.

5.4 Other relevant data

1,2-Dibromo-3-chloropropane is metabolically activated via cytochrome P450-catalysed oxidation and glutathione conjugation to form several protein- and DNA-binding products in the rat and mouse. It is also activated in human testicular cells *in vitro*. It disturbs spermatogenesis and has caused male infertility in humans. 1,2-Dibromo-3-chloropropane is a bacterial mutagen in the presence of metabolic activation. It causes DNA damage and genotoxicity in animal cells *in vitro* and *in vivo*.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of 1,2-dibromo-3-chloropropane.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,2-dibromo-3-chloropropane.

Overall evaluation

1,2-Dibromo-3-chloropropane is *possibly carcinogenic to humans (Group 2B)*.

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1,2-DICHLOROETHANE

Data were last reviewed in IARC (1979) and the compound was classified in *IARC Monographs* Supplement 7 (1987a).

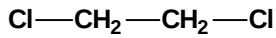
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 107-06-2
Chem. Abstr. Name: 1,2-Dichloroethane
IUPAC Systematic Name: 1,2-Dichloroethane
Synonym: Ethylene dichloride

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2H_4Cl_2$ Relative molecular mass: 98.96

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid with a pleasant odour (Budavari, 1996)
(*b*) *Boiling-point*: 83.5°C (Lide, 1995)
(*c*) *Melting-point*: –35.5°C (Lide, 1995)
(*d*) *Solubility*: Slightly soluble in water; miscible with ethanol, chloroform and diethyl ether (Lide, 1995; Budavari, 1996)
(*e*) *Vapour pressure*: 8 kPa at 20°C (Verschueren, 1996)
(*f*) *Flash-point*: 18°C, open cup (Budavari, 1996)
(*g*) *Conversion factor*: $mg/m^3 = 4.0 \times ppm$

1.2 Production and use

World production capacities in 1988 for 1,2-dichloroethane have been reported as follows (thousand tonnes): North America, 9445; western Europe, 9830; Japan, 3068; and other, 8351 (Snedecor, 1993). Production in the United States has been reported as follows (thousand tonnes): 1983, 5200; 1990, 6300; 1991, 6200; 1992, 6900; 1993, 8100 (United States National Library of Medicine, 1997). The total annual production in Canada in 1990 was estimated to be 922 thousand tonnes; more than 1000 thousand tonnes were produced in the United Kingdom in 1991 (WHO, 1995).

1,2-Dichloroethane is used primarily in the production of vinyl chloride; 99% of total demand in Canada, 90% in Japan and 88% of total production in the United States are used for this purpose. It is also used in the production of tri- and tetrachloroethylene, vinylidene chloride, ethyleneamines and trichloroethane; as a lead scavenger in antiknock fluids in gasoline; in paint, varnish and finish removers; as a component of metal-degreasing formulations; in soaps and scouring compounds, wetting and penetrating agents, organic synthesis and ore flotation; and as a solvent and fumigant. It is no longer registered for use as a fumigant on agricultural products in Canada, the United States, the United Kingdom or Belize (Lewis, 1993; WHO, 1995).

1.3 Occurrence

1.3.1 *Occupational exposure*

Current occupational exposure to 1,2-dichloroethane in North America occurs predominantly during the manufacture of other chemicals, such as vinyl chloride, where 1,2-dichloroethane is used as an intermediate. In a 1982 National Occupational Exposure Survey by the United States National Institute for Occupational Safety and Health (NIOSH), 28% of employees working with adhesives and solvents were exposed to 1,2-dichloroethane, while between 5 and 9% of workers were exposed to the substance in the medicinals and botanicals, biological products, petroleum refining and organic chemicals industries, and in museums and art galleries (United States Department of Labor, 1989).

Mean concentrations of 1,2-dichloroethane at three production plants in the United Kingdom in 1990 were 2.8, 3.2 and 6.8 mg/m^3; 95% of samples contained less than 20 mg/m^3, while maximum values at the plants were 18, 80 and 160 mg/m^3 (United Kingdom Health and Safety Executive, 1992).

1.3.2 *Environmental occurrence*

The majority of 1,2-dichloroethane released into the environment enters the atmosphere from its production and use as a chemical intermediate, solvent and lead scavenger in gasoline. It has been detected at low levels in ambient and urban air, groundwater and drinking-water samples (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 40 mg/m^3 as the threshold limit value for occupational exposures to 1,2-dichloroethane in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

The World Health Organization has established an international drinking water guideline for 1,2-dichloroethane of 30 µg/L (WHO, 1993).

2. Studies of Cancer in Humans

2.1 Cohort studies

Several studies have examined mortality or cancer incidence among chemical workers potentially exposed to 1,2-dichloroethane. Hogstedt *et al*. (1979) performed a cohort mortality study of 175 Swedish ethylene oxide production workers followed from 1961 through 1977. The workers had been employed for at least one year and were potentially exposed to 1,2-dichloroethane, ethylene oxide (IARC, 1994), ethylene chlorohydrin and bis(2-chloroethyl) ether. The mean exposure level to 1,2-dichloroethane among the most highly exposed workers was estimated to be 100 mg/m^3 during 1941–47 but to have decreased after that due to changes in production methods. There were 37 deaths [standardized mortality ratio (SMR), 1.4] and 12 cancer deaths [SMR, 1.8]. Excesses of stomach cancer ([SMR, 5.0], based on 4 cases) and leukaemia ([SMR, 11.1], based on 3 cases) were observed. It was not possible to link the excesses to any particular chemical exposure.

Austin and Schnatter (1983a) conducted a cohort study of 6588 white male workers employed at a petrochemical plant in the United States between 1941 and 1977. The study was conducted to investigate a cluster of brain tumours that was reported earlier in the same population (Alexander *et al*., 1980). There were 765 deaths (SMR, 0.8) and 150 cancer deaths (SMR, 0.9) observed. A greater than expected number (based on national rates) of brain cancers (SMR, 1.6; 95% confidence interval (CI), 0.8–2.8, based on 12 cases) was observed. Austin and Schnatter (1983b) also conducted a nested case–control study to examine the relationship between the risk of primary brain tumours and exposures at the facility. No significant association with 1,2-dichloroethane exposure was observed.

Sweeney *et al*. (1986) studied mortality among 2510 male chemical workers in the United States, followed from 1952 to 1977. Potential exposures included tetraethyl lead (IARC, 1987b), ethylene dibromide (see this volume), 1,2-dichloroethane, inorganic lead (IARC, 1987b) and vinyl chloride monomer (IARC, 1987c). There were 156 deaths (SMR, 0.7) and 38 cancer deaths (SMR, 1.0) observed. There were excesses of cancer of the larynx (SMR, 3.6; 90% CI, 0.7–11.5, based on 2 cases) and brain (SMR, 2.1; 90% CI, 0.7–4.9, based on 4 cases). The SMR for all lymphatic and haematopoietic cancers was 0.9 (90% CI, 0.3–1.9, based on 4 cases). Levels of exposure were not reported, but a NIOSH survey in 1980 found levels of exposure to 1,2-dichloroethane to be below the recommended NIOSH standard, while lead exposures were elevated. It was not possible to link mortality to any particular chemical exposure.

Benson and Teta (1993) studied the mortality among 278 chlorohydrin production workers who had ever been employed at a facility in the United States between 1940 and 1967. The follow-up period was from 1940 to 1988. This was a 10-year update of an earlier study conducted by Greenberg *et al*. (1990). There were 147 deaths (SMR, 1.0) and 40 cancer deaths (SMR, 1.3) observed. Excesses of pancreatic cancer (SMR, 4.9; 95% CI, 1.6–11.4; 8 cases) and lymphatic and haematopoietic cancers (SMR, 2.9; 95% CI, 1.3–5.8;

8 cases), which increased with duration of exposure, were observed. The workers were potentially exposed to 1,2-dichloroethane, ethylene chlorohydrin and bis(2-chloroethyl) ether. It was not possible to link the excesses to any particular chemical exposure and levels of exposure were not reported.

Olsen *et al*. (1997) studied mortality among 1361 men employed at two chlorohydrin production facilities in the United States similar to that studied by Benzon and Teta (1993). There were 300 deaths (SMR, 0.9) and 75 cancer deaths (SMR, 0.9) observed. The risks of pancreatic cancer (SMR, 0.3; 95% CI, 0.01–1.4; 1 case) and lymphatic and haematopoietic cancers (SMR, 1.3; 95% CI, 0.6–2.4; 10 cases) were less than those observed by Benson and Teta and no other cancers were observed in excess. It was not possible to link mortality to any particular chemical exposure and levels of exposure were not reported.

2.2 Ecological studies

Isacson *et al*. (1985) examined the association between cancer incidence and indices of water contamination in an ecological study conducted in the central United States. Cancer incidence rates in towns with populations between 1000 and 10 000 were compared by level of volatile organic compounds and metals in the drinking-water. Among men, significant associations between the level of 1,2-dichloroethane (≥ 0.1 ppm) and colon ($p = 0.009$) and rectal cancer ($p = 0.02$) were observed. The authors stated that 1,2-dichloroethane might be an indicator for other types of contamination rather than a causal agent.

3. Studies of Cancer in Experimental Animals

1,2-Dichloroethane was tested in one experiment in mice and in one in rats by oral administration. In mice, it produced benign and malignant tumours of the lung and malignant lymphomas in animals of both sexes, hepatocellular carcinomas in males and mammary and uterine adenocarcinomas in females. In rats, it produced carcinomas of the forestomach in male animals, benign and malignant mammary tumours in females and haemangiosarcomas in animals of both sexes. It was inadequately tested by intraperitoneal administration in mice (IARC, 1979).

3.1 Inhalation exposure

3.1.1 *Mouse*

Groups of 90 male and 90 female Swiss mice, 11 weeks of age, were exposed to concentrations of 5, 10, 50 or 250 ppm [20, 40, 200 or 1000 mg/m^3] 1,2-dichloroethane (purity, 99.82%; 1,1-dichloroethane, 0.02%; carbon tetrachloride, 0.02%; trichloroethylene, 0.02%; tetrachloroethylene, 0.03%; benzene, 0.09%) in air for 7 h per day on five days per week for 78 weeks. After several days of exposure to 250 ppm, the concentration was reduced to 150 ppm because of severe toxic effects. A group of 115 males and

134 females kept in a nearby room served as controls. At the end of the treatment period, the animals were kept until spontaneous death. The experiment lasted 119 weeks. A complete autopsy was carried out on all animals and histological examination was performed on almost all organs. Survival at 78 weeks of age was 42/115, 26/90, 34/90, 30/90 and 26/90 in control, 5-ppm, 10-ppm, 50-ppm and 150–250-ppm males and 76/134, 68/90, 50/90, 49/90 and 44/90 in control, 5-ppm, 10-ppm, 50-ppm and 150–250-ppm females, respectively. No specific types of tumour or changes in the incidence of tumours normally occurring in the strain of mice used were observed in the treated animals (Maltoni *et al*., 1980). [The Working Group noted the low survival rates, especially in males.]

Groups of 50 male and 50 female BDF_1 mice, six weeks of age, were exposed by whole-body inhalation to 0, 10, 30 or 90 ppm [0, 40, 120 or 360 mg/m^3] 1,2-dichloroethane (purity, > 99%) for 6 h per day on five days per week for 104 weeks. The maximum exposure concentration (90 ppm) was selected on the basis of the result of a 13-week study. In males, significantly increased incidence of liver haemangiosarcomas was observed at mid- and high-dose. In females, increased incidence of bronchiolar-alveolar adenomas and carcinomas, hepatocellular adenomas, adenocarcinomas of the mammary gland and endometrial stromal polyps occurred, with a significantly positive trend [statistics not specified] (Table 1) (Nagano *et al.*, 1998).

Table 1. Tumour incidence in mice administered 1,2-dichloroethane by inhalation exposure

Mice		Exposure concentration (ppm)			
		0	10	30	90
Males	Liver haemangiosarcoma	0/50	4/49	6/50	5/50
Females	Hepatocellular adenoma	1/49	1/50	1/50	6/50
	Bronchiolar-alveolar adenoma and carcinoma	5/49	1/50	4/50	11/50
	Mammary gland adenocarcinoma	1/49	2/50	1/50	6/50
	Endometrial stromal polyp	2/49	0/50	1/50	6/50

From Nagano *et al*. (1998)

3.1.2 *Rat*

Groups of 90 male and 90 female Sprague-Dawley rats, 12 weeks of age, were exposed to concentrations of 5, 10, 50 or 250 ppm [20, 40, 200 or 1000 mg/m^3] 1,2-dichloroethane (purity, 99.82%; 1,1-dichloroethane, 0.02%; carbon tetrachloride, 0.02%; trichloroethylene, 0.02%; tetrachloroethylene, 0.03%; benzene, 0.09%) in air for 7 h per day on five days per week for 78 weeks. After several days of exposure to 250 ppm, the concentration was reduced to 150 ppm because of severe toxic effects. A group of 90 males and 90 females kept in an exposure chamber under the same conditions for the same amount of

time as the exposed animals served as chamber controls. Another group of 90 males and 90 females kept in a nearby room served as untreated controls. At the end of the treatment period, the animals were kept until spontaneous death. The experiment lasted for 148 weeks. A complete autopsy was carried out on all animals and histological examination was performed on almost all organs. Survival at 104 weeks of age was 16/90, 12/90, 45/90, 13/90, 17/90 and 10/90 in control, chamber-control, 5-ppm, 10-ppm, 50-ppm and 150–250-ppm males and 36/90, 22/90, 48/90, 26/90, 29/90 and 21/90 in control, chamber-control, 5-ppm, 10-ppm, 50-ppm and 150–250-ppm females, respectively. The incidence of mammary fibromas and fibroadenomas in females was 47/90, 27/90, 56/90, 33/90, 49/90 and 47/90 in control, chamber-control, 5-ppm, 10-ppm, 50-ppm and 150–250-ppm groups, respectively. The increase in the incidence of these mammary tumours was significant (chi-square test) in the 150–250-ppm ($p < 0.001$), 50-ppm ($p < 0.01$) and 5 ppm ($p < 0.001$) groups, in comparison to chamber controls. The difference between the incidences in the two control groups was also significant ($p < 0.01$) (Maltoni *et al*., 1980). [The Working Group noted the low and variable survival rates.]

Groups of 50 male and 50 female Sprague-Dawley rats, 5.5–6 weeks of age, were exposed to concentrations of 0 or 50 ppm [200 mg/m^3] 1,2-dichloroethane (purity, > 99%) for 7 h per day on five days per week for 24 months. A complete autopsy was carried out on each animal and histological examination was performed on almost all organs and all gross lesions and tissue masses. Survival was 58% and 60% among the control and treated males and 54% and 64% among the control and treated females, respectively. There were no significant differences in the incidence of tumours between the control and treated groups (Cheever *et al*., 1990). [The Working Group noted the low exposure level.]

Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were exposed by whole-body inhalation to 0, 10, 40 or 160 ppm [0, 40, 160 or 640 mg/m^3] 1,2-dichloroethane (purity, > 99%) for 6 h per day on five days per week for 104 weeks. The maximum exposure concentration (160 ppm) was selected on the basis of the result of a 13-week study. In males, increased incidences of fibromas of the subcutis, fibroadenomas of the mammary gland and mesotheliomas of the peritoneum occurred, with a significantly positive trend [statistics not specified]. In females, increased incidences of fibromas of the subcutis and fibroadenomas, adenomas and adenocarcinomas of the mammary gland occurred, with a significantly positive trend (Table 2) (Nagano *et al.*, 1998).

3.2 Skin application

Mouse: A group of 30 female Ha:ICR Swiss mice, six to eight weeks of age, received skin applications of 126 mg/animal 1,2-dichloroethane [purity unspecified] in 0.2 mL acetone three times per week for life [survival and duration of treatment unspecified]. A group of 30 mice that received applications of 0.1 mL acetone alone served as controls. A complete autopsy was carried out and histological examinations were performed on the skin, liver, stomach, kidney and all abnormal-appearing tissues and organs. An increased incidence of lung tumours was observed in the high-dose treated group (26/30) compared with controls (11/30) ($p < 0.0005$, chi-square test). No skin tumours were observed in

Table 2. Tumour incidence in rats administered 1,2-dichloroethane by inhalation exposure

Rats		Exposure concentration (ppm)			
		0	10	40	160
Males	Mammary fibroadenoma	0/50	0/50	1/50	5/50
	Subcutaneous fibroma	6/50	9/50	12/50	15/50
	Peritoneal mesothelioma	1/50	1/50	1/50	5/50
Females	Mammary adenoma	3/50	5/50	5/50	11/50
	Mammary fibroadenoma	4/50	1/50	6/50	13/50
	Mammary adenocarcinoma	1/50	2/50	0/50	5/50
	Subcutaneous fibroma	0/50	0/50	1/50	5/50

From Nagano *et al*. (1998)

treated mice or controls (Van Duuren *et al*., 1979). [The Working Group noted the inadequate reporting.]

3.3 Multistage protocols and preneoplastic lesions

3.3.1 *Mouse*

In a two-stage mouse-skin assay, a group of 30 female Ha:ICR Swiss mice, six to eight weeks of age, received a single skin application of 126 mg per animal 1,2-dichloroethane [purity unspecified] in 0.2 mL acetone, followed 14 days later by 5 µg per animal phorbol myristyl acetate in 0.2 mL acetone three times weekly for life. Survival was described as excellent, the median survival for the various groups in the study [that included some groups exposed to chemicals other than 1,2-dichloroethane and the controls] ranging from 429 to 576 days. Animals treated with phorbol myristyl acetate alone served as controls. There were no significant differences in the occurrence of skin tumours between controls (total, 7 papillomas in 6/90 mice) and treated groups (total, 3 papillomas in 3/30 mice) (Van Duuren *et al*., 1979).

Groups of 25 male B6C3F_1 mice, 30 days of age, received drinking-water containing 10 mg/L *N*-nitrosodiethylamine (NDEA) for four weeks. Animals were then given drinking-water containing 0 (controls), 835 or 2500 mg/L 1,2-dichloroethane [purity unspecified] for 52 weeks. The highest concentration of 1,2-dichloroethane was that which failed to cause mortality in eight-week-old B6C3F_1 mice after a four-week exposure period. A complete autopsy was carried out and histological examination was performed on the liver, kidney and lung. There were no significant differences in either tumour incidence or number of tumours per mouse in any organ between the controls and 1,2-dichloroethane-treated groups. The incidences of liver tumours were 25/25, 25/25 and 23/25 in control, low-dose and high-dose mice, respectively, and the numbers of liver tumours per mouse were 29.30 ± 15.40, 34.50 ± 17.40 and 25.20 ± 16.70, respectively.

The incidences of lung tumours were 18/25, 12/25 and 23/25, respectively, and the numbers of lung tumours per mouse were 1.40 ± 1.40, 1.00 ± 1.10 and 2.60 ± 2.00, respectively (Klaunig *et al.*, 1986). [The Working Group noted that the tumour incidences in controls were too high for evaluation of a promoting effect of 1,2-dichloroethane.]

3.3.2 *Rat*

In an initiation study, one group of 10 male Osborne-Mendel rats, weighing 180–230 g, was given a two-thirds partial hepatectomy and, 24 h later, a single dose of 100 mg/kg bw 1,2-dichloroethane (purity, 97–99%) (maximum tolerated dose) in corn oil by gavage. Similar groups of animals were treated with 2 mL/kg bw corn oil alone (vehicle controls) or 30 mg/kg bw *N*-nitrosodiethylamine (NDEA; positive controls) followed by a two-thirds partial hepatectomy. Starting six days after partial hepatectomy, the rats received 500 mg/kg of diet (0.05% w/w) phenobarbital for seven weeks, then control diet for seven more days, after which time they were killed and the livers were examined histologically for γ-glutamyltranspeptidase (γ-GT)-positive foci. There was no significant increase in the number of total γ-GT-positive foci (1.02 ± 0.55 and 0.27 ± 0.19/cm^2 in the 1,2-dichloroethane group and vehicle controls, respectively). NDEA treatment increased the numbers of γ-GT-positive foci (4.04 ± 1.47/cm^2) (Milman *et al.*, 1988). [The Working Group noted the small number of animals.]

In a promotion study, groups of 10 male Osborne-Mendel rats, weighing 180–230 g, were given a single intraperitoneal injection of 30 mg/kg bw NDEA 24 h after a two-thirds partial hepatectomy. Starting six days later, the rats received daily 100 mg/kg bw 1,2-dichloroethane (purity, 97–99%) (maximum tolerated dose) in corn oil by gavage on five days per week for seven weeks. Control rats received corn oil alone instead of 1,2-dichloroethane. After the promotion phase, the rats were held for seven more days, after which they were killed and the livers were examined histologically for γ-GT-positive foci. There was no significant difference in the number of total γ-GT-positive foci between the 1,2-dichloroethane group and controls (1.54 ± 0.54 and 1.62 ± 0.33/cm^2, respectively) (Milman *et al.*, 1988). [The Working Group noted the small number of animals.]

A group of 50 male and 50 female Sprague-Dawley rats, 5.5–6 weeks of age, was exposed by inhalation to 50 ppm [200 mg/m^3] 1,2-dichloroethane (purity, > 99%) for 7 h per day on five days per week and to 500 mg/kg of diet (0.05%) disulfiram (purity, 98%) for 24 months. A complete autopsy was carried out on each animal and histopathological examination was performed on almost all organs and all gross lesions and tissue masses. In the liver, increased incidences of intrahepatic bile duct cholangiomas (0/50 untreated control males, 9/49 treated males, 0/50 untreated control females and 17/50 treated females), intrahepatic bile duct cysts (1/50 control males, 12/49 treated males, 1/50 treated females and 24/50 treated females) and neoplastic nodules in males (0/50 untreated controls and 6/49 treated) were observed in the treated group ($p < 0.05$; Fisher's exact test). The incidence of adenocarcinomas of the mammary gland in females (4/50 controls and 12/48 treated) and that of interstitial-cell tumours of the testis in males (2/50 controls and 11/50 treated) were increased in the treated group ($p < 0.05$) (Cheever *et al.*, 1990).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Case reports of reported acute toxic effects following inhalation exposure to 1,2-dichloroethane in the workplace indicate that 1,2-dichloroethane is readily absorbed by humans (Nouchi *et al.*, 1984).

The analysis of several tissues of humans who died following acute oral poisoning with 1,2-dichloroethane showed that 1,2-dichloroethane is widely distributed throughout the human body. Concentrations ranged from 1 to 50 mg/kg in the spleen and 100 to 1000 mg/kg in the stomach; levels in the liver and kidney were approximately 10 times lower than those in the stomach (Luznikov *et al.*, 1985).

Cytochrome P450 IIE1 is a major catalyst in the oxidation of 1,2-dichloroethane in human liver microsomes (Guengerich *et al.*, 1991).

4.1.2 *Experimental animals*

In rats, absorption following ingestion of 1,2-dichloroethane is rapid and complete (Reitz *et al.*, 1982). The pharmacokinetics following oral administration of 1,2-dichloroethane are dose-dependent over the range 25–150 mg/kg bw. The plasma elimination $t_{½}$ increases from 25 min to 57 min, while the area under the curve (AUC) increases 16-fold with a six-fold increase in dose. However, C_{max} is proportional to dose up to oral doses of 150 mg/kg bw (Spreafico *et al.*, 1980). There was no significant difference in kinetic parameters following single and repeated daily administrations of 50 mg/kg bw for 10 days. Gastrointestinal absorption in rats was more rapid and efficient following administration in water, compared with corn oil (Withey *et al.*, 1983).

Absorption following inhalation by experimental animals was also rapid. In rats, levels of 1,2-dichloroethane in the blood peaked (8–10 µg/mL) within 1–2 h of continuous inhalation of 600 mg/m^3 for 6 h (Reitz *et al.*, 1982).

1,2-Dichloroethane is also rapidly absorbed through the skin in mice, rats and guinea-pigs (Tsuruta, 1975, 1977). It was rapidly absorbed when applied in aqueous solution to the skin of rats *in vivo*, giving blood levels directly related to the concentration of the solution (Jakobson *et al.*, 1982; Morgan *et al.*, 1991). 1,2-Dichloroethane is widely distributed throughout the body in rats exposed via inhalation or ingestion. After inhalation, the highest concentrations were usually found in adipose tissue, although 1,2-dichloroethane was also detected in blood, liver, kidney, brain and spleen (Spreafico *et al.*, 1980).

Reitz *et al.* (1982) reported that the relative distribution of radioactivity at 48 h (assumed to be primarily in the form of metabolites) was similar in rats given ^{14}C-labelled 1,2-dichloroethane orally (single dose of 150 mg/kg bw) or by inhalation (600 mg/m^3 for 6 h). Residual radioactivity in selected tissues was 1.5–2.0 times higher after oral exposure than following inhalation. There was also a higher residual activity in the fore-

stomach after the oral exposure. The distribution pattern for macromolecular binding was similar, as determined 4 h after oral ingestion or directly after inhalation. Oral exposure produced lower (i.e., 1.5–2 times lower) levels of total macromolecular binding but higher (3–5 times) levels of DNA alkylation than inhalation, although the absolute levels were considered low.

Arfellini *et al*. (1984) reported a greater degree of binding to DNA in organs (liver, kidneys, lung and stomach) of mice than in those of rats (1.45–2.26-fold) 22 h after intraperitoneal administration of equivalent single doses of 8.7 μmol/kg bw.

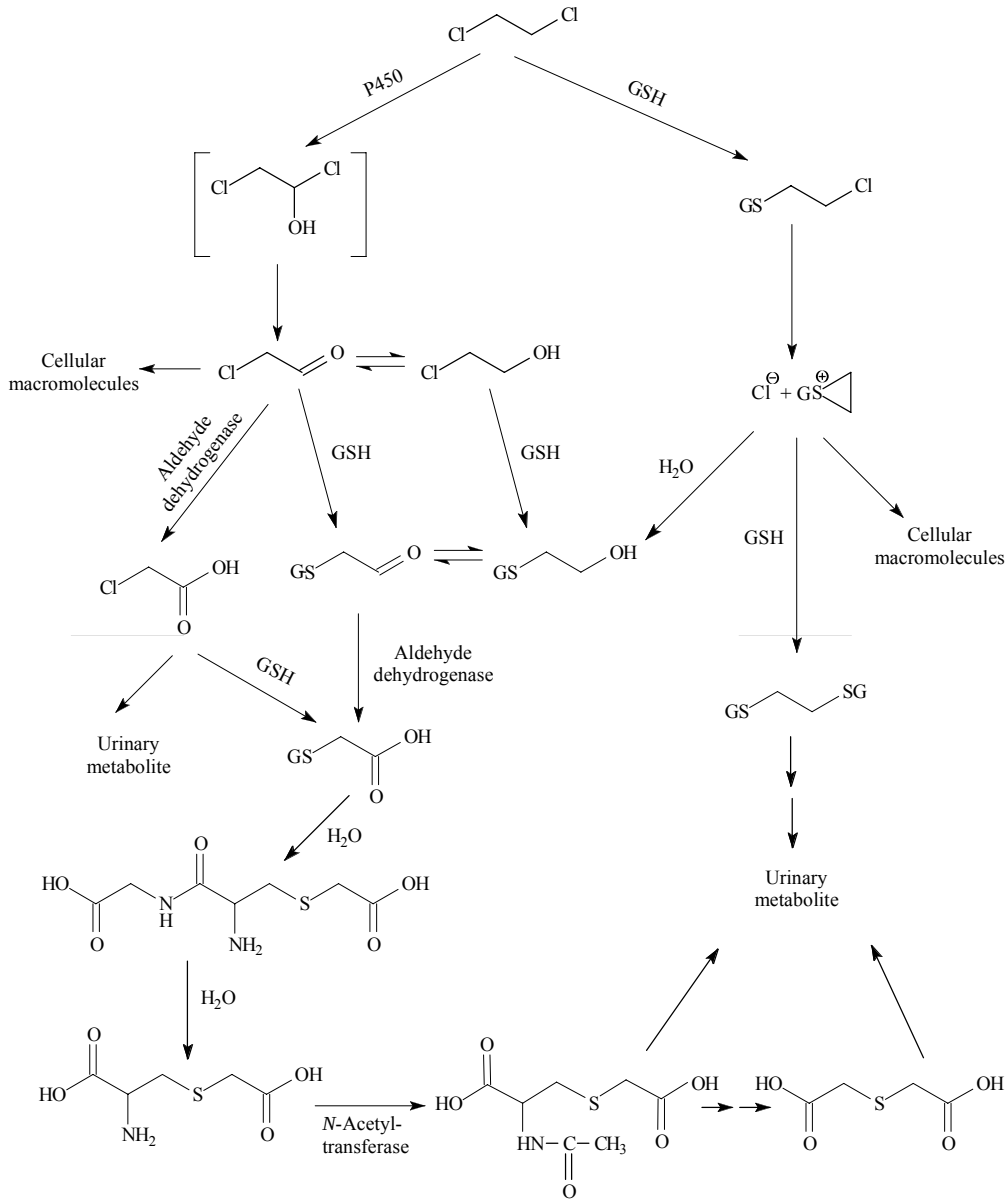
In periods from one minute to four days following intravenous administration of a single dose (0.73 mg/kg bw) of radiolabelled 1,2-dichloroethane to mice, the highest levels of radioactivity (non-volatile and bound metabolites) determined by whole-body autoradiography were present in the nasal olfactory mucosa and the tracheo-bronchial epithelium. Low levels of metabolites were also present in the epithelium of the upper alimentary tract, vagina and eyelid and in the liver and kidney. Mucosal and epithelial binding was decreased by pretreatment with metyrapone, indicating that binding might be due to oxidative metabolism. In in-vitro studies with tissues from the same strain of mice, reactive products formed from 1,2-dichloroethane were irreversibly bound to the nasal mucosa, lung and liver but not to the oesophagus, forestomach or vagina. The level of binding in the nasal mucosa was twice and in the lung 1.4 times that observed in the liver. The epithelium of the respiratory tract may be a potential target for the toxic effects of 1,2-dichloroethane due to in-situ metabolism to reactive intermediates (Brittebo *et al.*, 1989).

1,2-Dichloroethane was detected in fetal tissue of rats following maternal exposure for 5 h to airborne concentrations ranging from 612 to 8000 mg/m^3 (153–2000 ppm) on day 17 of gestation (Withey & Karpinski, 1985).

1,2-Dichloroethane is metabolized extensively in rats and mice (Mitoma *et al.*, 1985). Reitz *et al*. (1982) reported 70 and 91% transformation of 1,2-dichloroethane in the rat following oral (150 mg/kg bw) and inhalation (600 mg/m^3 for 6 h) exposures, respectively, with 85% of the metabolites appearing in the urine. The metabolism of 1,2-dichloroethane appears to be saturated or limited in rats at levels of exposure resulting in blood concentrations of 5–10 μg/mL.

Metabolism appears to occur via two principal pathways, catalysed by cytochrome P450 and by glutathione *S*-transferase (Figure 1). Cytochrome P450 enzymes catalyse oxidative transformation of 1,2-dichloroethane to 2-chloroacetaldehyde, 2-chloroacetic acid and 2-chloroethanol (Guengerich *et al.*, 1980), which are conjugated both enzymatically and non-enzymatically with glutathione (GSH). The other pathway involves direct conjugation with GSH to form *S*-(2-chloroethyl)glutathione, which is a sulfur half mustard (Schasteen & Reed, 1983; Foureman & Reed, 1987). A non-enzymatic reaction of the half mustard gives a putative alkylating agent (episulfonium ion) which may react with water to form *S*-(2-hydroxyethyl)glutathione, with thiols such as GSH to form ethene bis-glutathione, or with DNA to form adducts. With the exception of *S*-(2-chloroethyl)glutathione which forms DNA adducts, the reaction products are considered non-toxic and undergo further metabolism.

Figure 1. Proposed pathways for metabolism of 1,2-dichloroethane



Although some DNA damage has been induced via the P450 pathway *in vitro* (Banerjee *et al.*, 1980; Guengerich *et al.*, 1980; Lin *et al.*, 1985), several lines of evidence suggest that the GSH conjugation pathway is probably the major route for DNA damage (Guengerich *et al.*, 1980; Rannug, 1980; Guengerich *et al.*, 1981; Van Bladeren *et al.*, 1981; Sundheimer *et al.*, 1982; Crespi *et al.*, 1985; Storer & Conolly, 1985; Inskeep *et al.*, 1986; Koga *et al.*, 1986; Cheever *et al.*, 1990).

A single dose of 150 mg/kg bw radiolabelled 1,2-dichloroethane was administered by gavage to male and female rats 10–14 days after cessation of two years of 1,2-dichloroethane exposure via inhalation at a concentration of 50 ppm [200 mg/m^3]. The proportions of radioactivity present in the urine within 24 h were 42.5 and 33.9% in males and females, respectively, while 27.3 and 40.3% were eliminated as the unchanged parent compound in the breath. Only a very small amount of radioactivity was detected as $^{14}CO_2$ or in the faeces. In rats that had been exposed concomitantly to disulfiram during the two-year period, the proportion of unchanged 1,2-dichloroethane eliminated in the breath increased significantIy (i.e., 57.6 and 57.7%; $p < 0.05$), while the proportion eliminated in the urine decreased correspondingly (27.6 and 24.9 %). Levels of unchanged 1,2-dichloroethane in blood were significantly ($p < 0.05$) higher in rats exposed to 1,2-dichloroethane and disulfiram than in those exposed to 1,2-dichloroethane alone (Cheever *et al.*, 1990).

The pattern of elimination of metabolites was similar in rats and mice 48 h after administration of oral doses of radiolabelled 1,2-dichloroethane (100 and 150 mg/kg bw, respectively). In rats, 8.2 and 69.5% of the radiolabelled dose was recovered as CO_2 and in the excreta (principally urine), respectively, compared with 18 and 82% in mice. The overall recovery was reported to be less in rats than in mice (96 versus 110%) (Mitoma *et al.*, 1985).

In rats exposed to 600 mg/m^3 [150 ppm] 1,2-dichloroethane for 6 h or administered 150 mg/kg bw by gavage, there was no significant difference in the route of excretion of non-volatile metabolites (Reitz *et al.*, 1982). The major urinary metabolites identified following exposure of rats by either route were thiodiacetic acid (67–68%) and thiodiacetic acid sulfoxide (26–29%).

The rate of elimination following oral administration (gavage) or inhalation was rapid and 1,2-dichloroethane was no longer detected in the blood a few hours after oral or inhalation exposure and only small amounts were detected in tissues (liver, kidney, lung, spleen, forestomach, stomach and carcass) 48 h after exposure (Spreafico *et al.*, 1980; Reitz *et al.*, 1982).

The percentage of administered radioactivity excreted in the urine over a 24 h period in rats decreased with increasing single doses (0.25–8.08 mmol/kg bw 1,2-dichloroethane) administered by gavage in mineral oil (Payan *et al.*, 1993). The authors attributed these results to saturation of metabolism rather than kidney damage, as there were no variations in biochemical parameters of nephrotoxicity between the controls and groups exposed to doses up to 4.04 mmol/kg bw. Urinary levels of thiodiglycolic acid increased as a linear function of the dose of 1,2-dichloroethane until

at least 1.01 mmol/kg bw; this accounted for 63% of the total metabolites in urine at this dose.

Although 1,2-dichloroethane is eliminated more slowly from adipose tissue than from blood or other tissues (lung and liver) following exposure, it is unlikely to bioaccumulate, as no significant difference was observed between levels in blood or tissues following single or repeated (10 days) oral doses of 50 mg/kg bw in rats (Spreafico *et al.*, 1980; Cheever *et al.*, 1990).

4.2 Toxic effects

The toxicity of 1,2-dichloroethane has been reviewed (WHO, 1995).

4.2.1 *Humans*

Deaths due to ingestion or inhalation of 1,2-dichloroethane have been attributed to circulatory and respiratory failure; repeated exposures in the occupational environment have been associated with anorexia, nausea, abdominal pain, irritation of the mucous membranes, dysfunction of liver and kidney and neurological disorders (IARC, 1979).

4.2.2 *Experimental systems*

Acute exposure of rats to 1,2-dichloroethane caused disseminated haemorrhagic lesions, mainly in the liver; chronic exposure caused degeneration of the liver and tubular damage and necrosis of the kidneys (IARC, 1979). The limited organ toxicity of 1,2-dichloroethane in long-term experiments was substantiated in a long-term study (United States National Cancer Institute, 1978), in which no gross or histopathological indications of hepato- or nephrotoxicity were observed by gavage in Osborne-Mendel rats (47 or 95 mg/kg bw/day, five days per week for 78 weeks for both sexes) or B6C3F_1 mice (97 or 195 mg/kg bw/day, five days per week for 78 weeks for males; 149 or 299 mg/kg bw/day, five days per week for 78 weeks for females), although in rats of each sex and in female mice, survival was significantly reduced at the highest dose.

As a part of a long-term carcinogenicity study (Maltoni *et al.*, 1980), haematological parameters and clinical chemistry parameters reflecting liver and kidney function were studied after three, six, 12 or 18 months inhalation exposure to 5, 10, 50 or 150–250 ppm [20, 40, 200 or 600–1000 mg/m^3] 1,2-dichloroethane (Spreafico *et al.*, 1980). No consistent treatment-related effect was observed.

A single oral dose (≥ 400 mg/kg bw) of 1,2-dichloroethane to B6C3F_1 mice induced an elevation of alanine aminotransferase activity and an increase in relative liver weight, and some mortality occurred. The lowest intraperitoneal dose inducing an elevation of these enzymes was 500 mg/kg bw; intraperitoneal doses of up to 600 mg/kg bw did not kill any of the animals (n = 5). Inhalation exposure to 500 ppm [2000 mg/m^3] for 4 h was hepatotoxic to some of the mice, while at 150 ppm [600 mg/m^3] no toxicity was observed. Relative kidney weight was elevated after 300 mg/kg bw orally, 400 mg/kg bw intraperitoneally and after a 4-h exposure to 500 ppm 1,2-dichloroethane (Storer *et al.*, 1984).

In a 13-week study, using administration of 1,2-dichloroethane in the drinking-water, the highest dose used, 8000 ppm (corresponding to 515–727 mg/kg bw/day), no histological evidence of toxicity was observed in male Fischer 344/N rats or Osborne-Mendel or Sprague-Dawley rats of either sex. Minimal histological damage was observed in the kidney of female Fischer 344/N rats. Equivalent doses given by gavage to Fischer 344 rats were more toxic than those introduced in the drinking-water and caused substantial mortality. However, no histological damage to the liver or kidney was observed in the gavage experiments (Morgan *et al.*, 1990) .

In a 10-day toxicity study (Daniel *et al.*, 1994), Sprague-Dawley rats of each sex were given 1,2-dichloroethane at dose levels of 10, 30, 100 or 300 mg/kg bw per day by gavage. Although 8/10 males and all females in the high-dose group died, no haematological or clinical chemical changes were observed. The only histopathological effect was a slight inflammation of the forestomach in the 100-mg/kg bw group. In a 90-day study at dose levels of 37.5, 75 and 150 mg/kg bw per day, no treatment-related effect on mortality or gross histopathology was observed.

Mild forestomach hyperplastic changes and hyperkeratosis were observed in 2/8 male Fischer 344/N rats given 1,2-dichloroethane (350 or 700 mg/kg bw) by gavage (five days per week for two weeks), while no such changes were observed in 16 vehicle-treated animals (Ghanayem *et al.*, 1986). The difference between treated animals and controls was not significant.

A non-significant 13% decrease in the cellular glutathione content in the absence of cell lysis was observed in freshly isolated hepatocytes from Sprague-Dawley rats upon incubation with 1.2 mmol/L 1,2-dichloroethane for 1 h (Jean & Reed, 1992).

Inhalation exposure (≤ 455 ppm [1820 mg/m^3], 7 h per day for five days per week, for 30 days) of male Sprague-Dawley rats to 1,2-dichloroethane induced no histopathological changes in the liver or testis. However, when the animals were simultaneously treated with disulfiram (0.15% in the diet), bilateral testicular atrophy and periportal necrosis and cytoplasmic swelling of hepatocytes, together with moderate bile duct proliferation and periductal mononuclear infiltration were seen at the two highest 1,2-dichloroethane dose levels. Similar interaction was also observed when 1,2-dichloroethane was given by the intraperitoneal route (Igwe *et al.*, 1986). A single oral dose of 1,2-dichloroethane (6 μL/100 g) induced a slight increase in thiobarbituric-reacting substances and the activity of aspartate aminotransaminase in the serum and decreased the hepatic content of glutathione, but had no effect on alanine aminotransaminase or sorbitol dehydrogenase in male Wistar rats. Co-administration with carbon tetrachloride resulted in more than additive increases in serum thiobarbituric acid-reacting substances and all the serum enzyme activities, but did not accentuate the decrease in hepatic glutathione content (Aragno *et al.*, 1992).

When CD-1 mice were given 1,2-dichloroethane by gavage for 14 days at a level of 4.9 or 49 mg/kg bw per day (0.01 and 0.1 × LD_{50}, as determined in an acute toxicity study), the number of splenic IgM antibody-forming cells in response to sheep red blood cells showed a dose-dependent suppression (Munson *et al.*, 1982); no significant effect

was observed in the cell-mediated immune response to sheep erythrocytes. In a 90-day study (0.02, 0.2 or 2.0 mg/L in the drinking-water, calculated to yield 3, 24, or 189 mg/kg bw per day 1,2-dichloroethane), no effect on antibody-forming cell number, splenic response to the B-cell mitogen *Salmonella* lipopolysaccharide or to the T-cell mitogen concanavalin A, or vascular clearance of ^{51}Cr-labelled sheep erythrocytes was observed. Inhalation exposure of CD-1 mice to 5 or 10 ppm [20 or 40 mg/m^3] 1,2-dichloroethane for 3 h significantly decreased survival of the mice upon challenge of inhalation exposure to *Streptococcus zooepidemicus*; exposure to 10 ppm also decreased the bactericidal activity of the lungs toward *Klebsiella pneumoniae*. No effect was observed on the phagocytic or cytostatic activity of alveolar macrophages even at concentrations of 100 ppm [400 mg/m^3]. No immunotoxic effects were observed in rats (Sherwood *et al*., 1987).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

In a teratology study (Rao *et al*., 1980), rats and rabbits were exposed to 100 or 300 ppm [400 or 1200 mg/m^3] 1,2-dichloroethane for 7 h per day on days 6 through 15 (rats) or 6 through 18 (rabbits) of gestation. In rats, 10/16 dams died at the high dose, one exhibited implantation sites but all the implantantations were resorbed. At 100 ppm, 1,2-dichloroethane was not overtly toxic to the dam and did not induce fetotoxicity, teratogenicity or skeletal variations with the exception of a decrease in the number of bilobed thoracic centra. In rabbits, 3/19 dams died at the high dose; there were no adverse effects on fetal or embryonal development.

In a reproduction study (Rao *et al*., 1980), rats were exposed to 25, 75 or 150 ppm [100, 300 or 600 mg/m^3] 1,2-dichloroethane for 60 days before breeding (6 h per day, five days per week) and thereafter to similar concentrations for 6 h per day on seven days per week, with the exception of day 21 of gestation through day 4 postpartum. No effect on the reproductive performance or on the development (until day 21) of the F_1A or F_1B (bred 21 days after F_1A birth) litters was observed.

In a two-generation reproduction study (Lane *et al*., 1982), ICR Swiss mice were continuously administered 1,2-dichloroethane in the drinking-water (30, 90 or 290 mg/L with the aim of producing daily doses of 5, 15 or 50 mg/kg bw) starting five weeks before mating of the F_0 generation. No treatment-related effect on fertility, gestation, viability, pup survival, weight gain or teratogenicity was observed.

1,2-Dichloroethane administration (1.2, 1.6, 2.0 or 2.4 mmol/kg bw/day by gavage or inhalation of 150, 200, 250 or 300 ppm [600, 800, 1000 or 1200 mg/m^3] for 6 h per day on days 6 through 20 of gestation) induced no embryo- or fetotoxicity, changes in fetal growth or teratological effects. Maternal toxicity, as indicated by smaller weight gain, was observed at the highest inhalation dose level and two highest oral dose levels (Payan *et al*., 1995).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 3 for references)

1,2-Dichloroethane was mutagenic in most of the *Salmonella typhimurium* strains (TA100, TA98) tested with and without an exogenous metabolic activation system. In TA1535, mutagenic activity was dependent on addition of an exogenous metabolic system (not from frog) or specifically glutathione *S*-transferase. 1,2-Dichloroethane was mutagenic in *Drosophila melanogaster* for all end-points tested. In one study, 1,2-dichloroethane was not mutagenic in *Aspergillus nidulans*.

In vitro in animal cells, DNA repair and *hprt* gene mutations were induced by 1,2-dichloroethane. Cell transformation was observed in Syrian hamster embryo cells in a single study but not in two independent studies with BALB/c-3T3 cells. 1,2-Dichloroethane induced gene mutations in human lymphoblastoid cell lines.

In vivo in mouse liver, DNA strand breaks were induced by 1,2-dichloroethane after intraperitoneal injection or oral exposure but not after inhalation. DNA single strand-breaks were also observed in liver cells after gavage of rats. *In vivo* in mice, single studies with the spot test and sister chromatid exchange were inconclusive and positive, respectively; no micronuclei were found in bone marrow or peripheral blood cells of mice.

1,2-Dichloroethane binds *in vitro* and *in vivo* to DNA, RNA and proteins in mice and rats.

In a single study, 1,2-dichloroethane induced mainly micronuclei not staining for the presence of kinetochore (which is indicative of aneuploidy) in human MCL-5 cells that stably express cDNAs encoding human CYP1A2, CYP2A6, CYP3A4, CYP2E1 and epoxide hydrolase and in h2E1 cells, which contain a cDNA for CYP2E1. AHH-1 cells constitutively expressing CYP1A1 showed an increase in the frequency only of non-kinetochore-staining micronuclei.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

1,2-Dichloroethane is used mainly in the production of vinyl chloride. It is no longer registered as a fumigant. It has been detected at low levels in ambient and urban air, groundwater and drinking-water.

5.2 Human carcinogenicity data

Five cohort studies and one nested case–control study of brain tumours have examined the risk of cancer among workers with potential exposure to 1,2-dichloroethane. Excesses of lymphatic and haematopoietic cancers were observed in three studies and of stomach cancer in one study, while an excess of pancreatic cancer was observed in

Table 3. Genetic and related effects of 1,2-dichloroethane

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS chromotest	–	–	NG	Quillardet *et al.* (1985)
ECD, *Escherichia coli pol* A, differential toxicity (spot test)	(+)	NT	12000	Brem *et al.* (1974)
SAF, *Salmonella typhimurium* BA13 (*Ara* test), forward mutation	–	+	74	Roldán-Arjona *et al.* (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1782	King *et al.* (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	3120	Barber *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	60000	Principe *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	3960	Van Bladeren *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	NG	Milman *et al.* (1988)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)[c]	NT	20[d]	Simula *et al.* (1993)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	(+)	NT	495	Brem *et al.* (1974)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	NT	495	Brem *et al.* (1974)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	+	740	Rannug & Ramel (1977)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1782	King *et al.* (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	990	Cheh *et al.* (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	990	Guengerich *et al.* (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	1574	Barber *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	(+)	60000	Principe *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	1250	Moriya *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	NG	Milman *et al.* (1988)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1782	King *et al.* (1979)
SA7, *Salmonella typhimurium* TA1537, reverse mutation (spot test)	–	–	60000	Principe *et al.* (1979)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Milman *et al.* (1988)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	495	Brem *et al.* (1974)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1782	King *et al.* (1979)
SA8, *Salmonella typhimurium* TA1538, reverse mutation (spot test)	–	–	60000	Principe *et al.* (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1782	King *et al.* (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	11475	Barber *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	60000	Principe *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	NG	Milman *et al.* (1988)
SAS, *Salmonella typhimurium* TA1535 + SOS/*umuC"lacZ*, *umuC* gene expression	+	NT	50	Oda *et al.* (1996)
SAS, *Salmonella typhimurium*,TA1535 + GST (NM 5004) + SOS/*umuC"lacZ*, reverse mutation	+	NT	1	Oda *et al.* (1996)
ECK, *Escherichia coli* K12, forward or reverse mutation	–	–	990	King *et al.* (1979)
ANG, *Aspergillus nidulans*, genetic crossing-over	–	NT	1.4% in air	Crebelli *et al.* (1984)
STF, *Streptomyces coelicolor*, forward mutation	–	NT	60000	Principe *et al.* (1981)
ANF, *Aspergillus nidulans*, forward mutation	–	NT	300000	Principe *et al.* (1981)
ANN, *Aspergillus nidulans*, aneuploidy	+	NT	2500	Crebelli *et al.* (1988)
DMG, *Drosophila melanogaster*, interchromosomal mitotic recombination	+		200 ppm diet	Vogel & Nivard (1993)
DMM, *Drosophila melanogaster*, somatic mutation and recombination test (SMART)	+		1200 diet	Nylander *et al.* (1978)
DMM, *Drosophila melanogaster*, somatic mutation and recombination test (SMART)	+		40 mg/m^3 96 h inh	Kramers *et al.* (1991)
DMM, *Drosophila melanogaster*, somatic wing spot test	+[e]		500 ppm inh	Romert *et al.* (1990)
DMM, *Drosophila melanogaster*, somatic mutation, eye spot test	+		100 ppm, 48 h inh	Ballering *et al.* (1993)
DMM, *Drosophila melanogaster*, *vermilion* forward mutation assay	+		250 ppm inh	Ballering *et al.* (1994)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		4950 feed	King *et al.* (1979)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		8 mg/m^3 96 h inh	Kramers *et al.* (1991)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		12 000 feed	Ballering *et al.* (1993)
DMN, *Drosophila melanogaster*, ring-X chromosome loss	(+)		1200 mg/m^3 inh	Kramers *et al.* (1991)
DMN, *Drosophila melanogaster*, ring-X chromosome loss	+		12 000 48 h feed	Ballering *et al.* (1993)
RIA, DNA repair, mouse hepatocytes *in vitro*	+	NT	NG	Milman *et al.* (1988)
URP, Unscheduled DNA synthesis, rat hepatocytes *in vitro*	+	NT	NG	Milman *et al.* (1988)
GCO, Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	+	+	99	Tan & Hsie (1981)
GCO, Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	+	NT	8[d]	Zamora *et al.* (1983)
AIA, Aneuploidy, AHH-1 cells (CYP1A1 native) *in vitro*, kinetochore staining	–	NT	495	Doherty *et al.* (1996)
AIA, Aneuploidy, MCL-5 cells (cDNAs for CYP1A2, 2A6, 3A4, 2E1 and epoxide hydrolase) *in vitro*, kinetochore staining	(+)	NT	495	Doherty *et al.* (1996)
AIA, Aneuploidy, h2E1 cells (cDNA for CYP2E1) *in vitro*, kinetochore staining	–	NT	495	Doherty *et al.* (1996)
TBM, Cell transformation, BALB/c-3T3, mouse cells	–	NT	50	Tu *et al.* (1985)
TBM, Cell transformation, BALB/c-3T3, mouse cells	–	NT	NG	Milman *et al.* (1988)
T7S, Cell transformation, SA7/Syrian hamster embryo cells	+	NT	1.3% in air	Hatch *et al.* (1983)
GIH, Gene mutation, human EUE cells *in vitro*	+	NT	99	Ferreri *et al.* (1983)
GIH, Gene mutation, human lymphoblastoid cell line AHH-1 *in vitro*	+	NT	100	Crespi *et al.* (1985)
GIH, Gene mutation, human lymphoblastoid cell line TK6 *in vitro*	+	NT	500	Crespi *et al.* (1985)
MIH, Micronucleus test, AHH-1 cells (CYP1A1 native) *in vitro*	+[f]	NT	198	Doherty *et al.* (1996)
MIH, Micronucleus test, MCL-5 cells (cDNAs for CYP1A2, 2A6, 3A4, 2E1 and epoxide hydrolase) *in vitro*	+[f]	NT	198	Doherty *et al.* (1996)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MIH, Micronucleus test, h2E1 cells (cDNA for CYP2E1) *in vitro*	+[f]	NT	198	Doherty *et al.* (1996)
BFA, Bile of CBA mice, *Salmonella typhimurium* TA1535, reverse mutation	+		80 ip × 1	Rannug & Beije (1979)
HMM, Host-mediated assay, *Escherichia coli* K12 in female NMRI mouse hosts	–		198 ip × 1	King *et al.* (1979)
DVA, DNA single-strand breaks, B6C3F$_1$ mouse liver *in vivo*	+		198 ip × 1	Storer & Conolly (1983)
DVA, DNA single-strand breaks, B6C3F$_1$ mouse liver *in vivo*	+		100 po × 1	Storer *et al.* (1984)
DVA, DNA single-strand breaks, B6C3F$_1$ mouse liver *in vivo*	+		150 ip × 1	Storer *et al.* (1984)
DVA, DNA single-strand breaks, B6C3F$_1$ mouse liver *in vivo*	–		500 ppm inh 4 h	Storer *et al.* (1984)
DVA, DNA damage, CD rat liver cells *in vivo*	+		134 po × 2	Kitchin & Brown (1994)
MST, Mouse spot test, female C57BL/6J Han mice *in vivo*	?		300 ip × 1	Gocke *et al.* (1983)
SVA, Sister chromatid exchange, male Swiss albino mouse bone marrow *in vivo*	+		1 ip × 1	Giri & Que Hee (1988)
MVM, Micronucleus test, NMRI mouse bone marrow *in vivo*	–		396 ip × 2	King *et al.* (1979)
MVM, Micronucleus test, Eμ-PIM-1 transgenic mouse peripheral blood *in vivo*	–		300 po/d 41 wk	Armstrong & Galloway (1993)
DLM, Dominant lethal test, ICR Swiss mice *in vivo*	–		50 po × 7 d	Lane *et al.* (1982)
BID, Binding (covalent) to calf thymus DNA *in vitro*	–	+	99	Guengerich *et al.* (1980)
BID, Binding (covalent) to calf thymus DNA *in vitro*	+	+[g]	3.6	Arfellini *et al.* (1984)
BID, Binding (covalent) to DNA *in vitro*	–	+	6	Colacci *et al.* (1985)
BID, Binding (covalent) to DNA, mouse hepatocytes *in vitro*	+	NT	103 μg	Banerjee (1988)
BVD, Binding (covalent) to DNA, rat liver, spleen, kidney and stomach *in vivo*	+		150 po × 1	Reitz *et al.* (1982)
BVD, Binding (covalent) to DNA, Wistar rat liver, kidney, stomach and lung *in vivo*	+		0.86 ip × 1	Arfellini *et al.* (1984)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BVD, Binding (covalent) to DNA, BALB/c mouse liver, kidney, stomach and lung *in vivo*	+		0.86 ip × 1	Arfellini *et al.* (1984)
BVD, Binding (covalent) to DNA, Sprague-Dawley rat hepatocytes *in vivo*	+		150 ip × 1	Inskeep *et al.* (1986)
BVD, Binding (covalent) to DNA, Sprague-Dawley rat liver *in vivo*	+		1.38 ip × 1	Banerjee (1988)
BVD, Binding (covalent) to DNA, $B6C3F_1$ mouse liver *in vivo*	+		1.38 ip × 1	Banerjee (1988)
BVD, Binding (covalent) to DNA, Sprague-Dawley rat liver *in vivo*	+		150 po × 1	Cheever *et al.* (1990)
BVD, Binding (covalent) to DNA, Fischer 344 rat lung *in vivo*	+		34 inh 4 h	Baertsch *et al.* (1990)
BVP, Binding (covalent) to RNA and proteins, Wistar rat liver, kidney, stomach and lung *in vivo*	+		0.86 ip × 1	Arfellini *et al.* (1984)
BVP, Binding (covalent) to RNA and proteins, BALB/c mouse liver, kidney, stomach and lung *in vivo*	+		0.86 ip × 1	Arfellini *et al.* (1984)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; inh, inhalation; ip, intraperitoneal; po, oral

[c] Strains transfected with plasmids expressing human α-class glutathione *S*-transferase (GST) were more sensitive than those expressing π-class GSTs or the control TA100 strain

[d] Atmospheric concentration (µg/mL)

[e] 24-h pretreatment with buthionine sulfoximine, an inhibitor of GSH synthesis, significantly decreased the mutagenic activity, while pretreatment with phenobarbital, an inducer of glutathione *S*-transferase, significantly increased the mutagenic activity of 1,2-dichloroethane.

[f] Approximately 20% of the micronucleated cells stained positive for kinetochore at the highest dose (5 mM).

[g] S9 from rat of mouse liver, stomach, lung or kidney mediated DNA binding.

one study. All the cohort studies included workers with potential exposure to multiple agents and were not able to examine the excess risk associated with 1,2-dichloroethane.

5.3 Animal carcinogenicity data

1,2-Dichloroethane was tested in one experiment in mice and in one in rats by oral administration. In mice, it produced benign and malignant tumours of the lung and malignant lymphomas in animals of each sex, hepatocellular carcinomas in males and mammary and uterine adenocarcinomas in females. In rats, it produced carcinomas of the forestomach in males, benign and malignant mammary tumours in females and haemangiosarcomas in animals of each sex. No increase in tumour incidence was found after inhalation exposure in two experiments in rats or in one experiment in mice, but these studies were considered to be inadequate. In two other inhalation studies, one in mice and one in rats, 1,2-dichloroethane increased the incidence of tumours at various sites including the liver, lung and mammary gland.

In a multistage study measuring γ-glutamyl transpeptidase (γ-GT)-positive foci in the liver of male rats, single administration of 1,2-dichloroethane by gavage after a two-thirds partial hepatectomy followed by treatment with phenobarbital (initiation study) or repeated administration of 1,2-dichloroethane by gavage after a two-thirds partial hepatectomy and initiation by *N*-nitrosodiethylamine (promotion study) did not increase the number of γ-GT-positive foci. In a two-stage mouse-skin assay, 1,2-dichloroethane was not active as an initiator of skin carcinogenicity.

5.4 Other relevant data

1,2-Dichloroethane is easily absorbed by humans and animals and is metabolized extensively by rats and mice via cytochrome P450 and glutathione *S*-transferase.

No teratogenic effect was seen in rats, rabbits or mice.

1,2-Dichloromethane is mutagenic in bacteria, *Drosophila melanogaster* and mammalian cells. It induces DNA damage in liver cells *in vivo* and binds to DNA, RNA and proteins in animals.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of 1,2-dichloroethane.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,2-dichloroethane.

Overall evaluation

1,2-Dichloroethane is *possibly carcinogenic to humans (Group 2B)*.

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DIMETHYLCARBAMOYL CHLORIDE

Data were last reviewed in IARC (1976) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

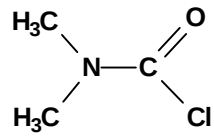
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 79-44-7

Chem. Abstr. Name: Dimethylcarbamic chloride

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_3H_6NOCl Relative molecular mass: 107.6

1.1.3 *Physical properties* (for details, see IARC, 1976)

(*a*) *Boiling point*: 64°C at 27 kPa

(*b*) *Melting point*: –33°C

(*c*) *Conversion factor*: $mg/m^3 = 4.40 \times ppm$

1.2 Production and use

Dimethylcarbamoyl chloride has been produced since 1961. It has been used as an intermediate in the manufacture of a number of pharmaceuticals and pesticides (IARC, 1976).

2. Studies of Cancer in Humans

No death from cancer was reported in an investigation of 39 dimethylcarbamoyl chloride production workers, 26 processing workers and 42 ex-workers aged 17–65 years, who were exposed for periods ranging from six months to 12 years (IARC, 1976).

3. Studies of Cancer in Experimental Animals

Dimethylcarbamoyl chloride was tested for carcinogenicity by skin application and by subcutaneous and intraperitoneal injection in female mice of one strain; it induced local tumours (IARC, 1976).

3.1 Inhalation exposure

3.1.1 *Rat*

A group of 50 male Sprague-Dawley rats was treated by whole-body exposure to an atmosphere of 1 ppm [4.4 mg/m^3] dimethylcarbamoyl chloride for 6 h per day on five days per week for six weeks (i.e., 30 exposures). The experiment included two chamber control groups, each of 150 rats. The incidence of nasal cancer corrected for mortality at 480 and 600 days in the exposed group was 12% and 17%, respectively (Snyder *et al.*, 1986). [This experiment was not fully reported.]

3.1.2 *Hamster*

A group of 100 male Syrian golden hamsters, eight weeks of age, was exposed by inhalation to 1 ppm [4.4 mg/m^3] dimethylcarbamoyl chloride for 6 h per day on five days per week for life. Two groups of 50 and 120 male hamsters served as sham-exposed and untreated controls, respectively. Neoplastic lesions of the nasal cavity were observed from 406 to 770 days. Squamous-cell carcinomas of the nasal cavity occurred in 50/99 hamsters in the treated group. No such tumour occurred in controls (Sellakumar *et al.*, 1980).

3.2 Skin application

Mouse: A group of 50 female ICR/Ha Swiss mice was treated by topical application with 2 mg dimethylcarbamoyl chloride in 0.1 mL acetone three times per week for up to 615 days. Three control groups, each of 50 mice, received acetone only for 575–665 days. No skin tumours arose in the control groups, whereas 32/50 mice in the treatment group developed tumours at the site of administration. Time to first tumour was 350 days and the tumours were identified as 1 papilloma, 27 squamous carcinomas and 4 keratoacanthomas (Van Duuren *et al.*, 1987).

In the same study, two groups of 30 female ICR/Ha Swiss mice were injected subcutaneously with 0.43 or 4.3 mg dimethylcarbamoyl chloride in 0.1 mL tricaprylin once per week for 365 days and then observed for the remainder of their lifespan. An additional group of 50 mice received a subcutaneous dose of 4.3 mg per week for 365 days and were then killed. Three control groups consisting of either 30 or 50 (two groups) mice received tricaprylin alone once per week for up to 560–660 days. Two injection-site haemangiomas arose in one of the control groups. Injection-site tumours arose in 9/30, 22/30 and 42/50 of the treated groups, respectively (Van Duuren *et al.*, 1987).

In the same study as the previous skin application experiment, 50 female ICR/Ha Swiss mice were treated by topical application with 5 mg dimethylcarbamoyl chloride in 0.1 mL

acetone on a single occasion, followed by three times weekly applications of phorbol myristyl acetate in 0.1 mL acetone [either 0.0025 or 0.005 mg per administration]. Two phorbol myristyl acetate control groups were available. Tumour incidences were: phorbol myristyl acetate, 0.0025 mg dose group, 0/50; 0.005 mg dose group, 3/30, which included 2 papillomas and 1 sarcoma; dimethylcarbamoyl chloride–phorbol myristyl acetate group 10/50, which included 2 papillomas, 7 squamous carcinomas and 1 keratoacanthoma (Van Duuren *et al.*, 1987).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

No data were available to the Working Group. However, dimethylcarbamoyl chloride is rapidly hydrolysed on contact with water to dimethylamine, HCl and CO_2.

4.2 Toxic effects

4.2.1 *Humans*

As previously summarized, one case of eye irritation and one of liver disturbances have been observed in workers exposed to dimethylcarbamoyl chloride. No other data were available to the Working Group (IARC, 1976).

4.2.2 *Experimental systems*

As previously summarized, dimethylcarbamoyl chloride when inhaled by rats damages the nasal mucous membrane, throat and lungs and causes breathing difficulties. It is irritant to the skin of rats and to skin and eye in rabbits. No evidence for sensitizing potential has been shown in guinea-pigs (IARC, 1976).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

Chromosomal analysis of peripheral lymphocytes from 10 people who had been occupationally exposed to dimethylcarbamoyl chloride (and diethylcarbamoyl chloride) for periods ranging from 4 to 17 years showed differences in the frequency of chromosomal aberrations (inclusive and exclusive gaps), when compared with a control group of 10 people matched for age, although statistical evaluation revealed no significant increase (Fleig & Thiess, 1978).

4.4.2 *Experimental systems* (see Table 1 for references)

Dimethylcarbamoyl chloride induced DNA damage and mutation in bacteria. In fungi, it induced aneuploidy, mutation, gene conversion and DNA damage. Dimethylcarbamoyl chloride induced sex-linked recessive lethal mutations in *Drosophila melanogaster* in two studies, but not in a single feeding (aqueous solution) study, in which it would have been rapidly hydrolysed; it did not induce heritable translocations in two studies using administration by injection. Unscheduled DNA synthesis was not induced in primary cultures of rat hepatocytes. In other cultured mammalian cells, dimethylcarbamoyl chloride induced DNA strand breaks, chromosomal aberrations (in Chinese hamster ovary CHO cells, but not in rat hepatocytes), mutation at the *tk* locus of mouse lymphoma L5178Y cells and transformation in Syrian hamster embryo cells; conflicting results were obtained in studies of sister-chromatid exchange induction *in vitro*. *In vivo*, dimethylcarbamoyl chloride induced micronuclei but not sister chromatid exchanges in bone marrow cells of treated mice.

In conjunction with a carcinogenicity study (described in Section 3.1), male rats were exposed by inhalation to [^{3}H]dimethylcarbamoyl chloride (2.8–7.8 mCi inhaled). The association of radioactivity with DNA purified from the nasal mucosa was 11.0 ± 5.1 dpm/μg DNA per mCi inhaled (Snyder *et al*., 1986). *In vitro* reaction of dimethylcarbamoyl chloride with calf thymus DNA resulted in the formation of 6-dimethylcarbamyloxy-2′-deoxyguanosine and 4-dimethylaminothymidine (Segal *et al.*, 1982).

DNA from both rat nasal squamous carcinomas (2) and mouse skin squamous carcinomas (4) and fibrosarcomas (4) arising in dimethylcarbamoyl chloride-treated animals failed to transform NIH 3T3 cells by DNA transfection (Garte *et al.*, 1985).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to dimethylcarbamoyl chloride may occur during its manufacture and its use as an intermediate in the manufacture of a number of pharmaceuticals and pesticides.

5.2 Human carcinogenicity data

No deaths from cancer were reported in a small study of workers exposed for periods ranging from six months to 12 years.

5.3 Animal carcinogenicity data

Dimethylcarbamoyl chloride was tested for carcinogenicity in rats and hamsters by inhalation exposure, producing malignant tumours of the nasal cavity. It was also tested in mice by skin application and by subcutaneous and intraperitoneal injection, producing local tumours.

Table 1. Genetic and related effects of dimethylcarbamoyl chloride

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ECB, *Escherichia coli* (*polA*), DNA strand breaks, cross-links or related damage; DNA repair	+	+	500	Tweats (1981)
ECD, *Escherichia coli pol* A/W3110-P3478, differential toxicity	+	NT	10	Rosenkranz & Poirier (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	+	10	Anderson & Styles (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	500	Simmon (1979a)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	2000	MacDonald (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	+	500	Martire *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	250	Nagao & Takahashi (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	50	Richold & Jones (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	250	Simmon & Sheperd (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	250	Rowland & Severn (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	1250	Ashby *et al*. (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	50	Haworth *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	167[c]	Dunkel *et al*. (1984)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	NT	+	10	Anderson & Styles (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	5	Rosenkranz & Poirier (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	500	Simmon (1979a)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	25	Brooks & Dean (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	50	Garner *et al*. (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	2500	Richold & Jones (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	5	Haworth *et al*. (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	?	+	50[c]	Dunkel *et al*. (1984)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	NT	500	Simmon (1979a)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500	MacDonald (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	(+)	–	2500	Richold & Jones (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	+	+	500	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	167[c]	Dunkel *et al*. (1984)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	NT	+	10	Anderson & Styles (1978)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	250	Rosenkranz & Poirier (1979)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	500	Simmon (1979a)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	5000	Richold & Jones (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	167[c]	Dunkel *et al*. (1984)
SA8, *Salmonella typhimurium* TA98, reverse mutation	+	+	500	Haworth *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	NT	+	10	Anderson & Styles (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	500	Simmon (1979a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	MacDonald (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	500	Richold & Jones (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	167	Dunkel *et al*. (1984)
SAS, *Salmonella typhimurium* TA1536, reverse mutation	–	NT	500	Simmon (1979a)
ECR, *Escherichia coli* (other miscellaneous strains), reverse mutation	+	+	200	Mohn *et al*. (1981)
SSB, *Saccharomyces* species, RAD strains, differential toxicity	+	+	100	Sharp & Parry (1981a)
SCG, *Saccharomyces cerevisiae* D4, gene conversion	–	–	160	Jagannath *et al*. (1981)
SCG, *Saccharomyces cerevisiae* JD1, gene conversion	+	NT	300	Sharp & Parry (1981b)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SCG, *Saccharomyces cerevisiae* D7, gene conversion	+	NT	2400	Zimmermann & Scheel (1981)
SCH, *Saccharomyces cerevisiae* D3, homozygosis by mitotic recombination or gene conversion	(+)	(+)	50000	Simmon (1979b)
SCR, *Saccharomyces cerevisiae* XV185-14C, reverse mutation	–	+	107	Mehta & Von Borstel (1981)
SZF, *Schizosaccharomyces pombe*, forward mutation	+	+	2	Loprieno (1981)
SCN, *Saccharomyces cerevisiae* D6, aneuploidy	–	+	100	Parry & Sharp (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		0.2% feed	Würgler & Graf (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		10000 ppm inj	Yoon *et al.* (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		2500 ppm inj	Foureman *et al.* (1994)
DMH, *Drosophila melanogaster*, heritable translocations	–		10000 ppm inj	Yoon *et al.* (1985)
DMH, *Drosophila melanogaster*, heritable translocations	–		2500 ppm inj	Foureman *et al.* (1994)
DIA, DNA strand breaks, cross-links or related damage, Chinese hamster lung V79 cells *in vitro*	+	NT	321	Swenberg (1981)
URP, Unscheduled DNA synthesis, primary rat hepatocytes *in vitro*	–	NT	54	Probst *et al.* (1981)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	1200	Jotz & Mitchell (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	0.04%	Evans & Mitchell (1981)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	100	Perry & Thomson (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	120	Natarajan & Van Kestern-Van Leeuwen (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO and lung DON cells *in vitro*	+	NT	1.7	Baker *et al*. (1983)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	+	20	Natarajan & Van Kestern-Van Leeuwen (1981)
CIR, Chromosomal aberrations, rat liver RL_1 cell line *in vitro*	–	NT	200	Dean (1981)
TCS, Cell transformation, Syrian hamster embryo cells	+	NT	0.1	Pienta *et al*. (1977)
HMM, Host-mediated assay, *Salmonella typhimurium* TA1535 in Swiss-Webster mice *in vivo*	+		1000 po × 1	Simmon *et al*. (1979)
SVA, Sister chromatid exchange, CBA/J mouse bone-marrow cells *in vivo*	–		100 ip × 1	Paika *et al*. (1981)
MVM, Micronucleus test, ICR mice *in vivo*	+		160 ip × 1	Kirkhart (1981)
MVM, Micronucleus test, CD-1 mice *in vivo*	–		160 ip × 2	Tsuchimoto & Matter (1981)
MVM, Micronucleus test, $B6C3F_1$ mice *in vivo*	+		130 ip × 2	Salamone *et al*. (1981)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; inj, injection; po, oral; ip, intraperitoneal

[c] Results from four laboratories

5.4 Other relevant data

No data were available on the metabolism of dimethylcarbamoyl chloride, but it rapidly decomposes on contact with water to dimethylamine, hydrochloric acid and carbon dioxide.

Dimethylcarbamoyl chloride when inhaled by rats damages the nasal mucous membrane, throat and lung.

It has a wide spectrum of genotoxic activity, which is expressed as a result of its direct alkylating activity.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of dimethylcarbamoyl chloride.

There is *sufficient evidence* in experimental animals for the carcinogenicity of dimethylcarbamoyl chloride.

Overall evaluation

Dimethylcarbamoyl chloride is *probably carcinogenic to humans (Group 2A)*.

In making the overall evaluation, the Working Group took into consideration that dimethylcarbamoyl chloride is a direct-acting alkylating agent with a wide spectrum of genotoxic activity, including activity in somatic cells *in vivo*.

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DIMETHYLFORMAMIDE

Data were last evaluated in IARC (1989).

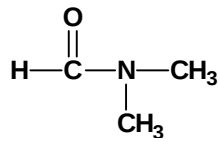
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 68-12-2
Chem. Abstr. Name: *N*,*N*-Dimethylformamide
IUPAC Systematic Name: *N*,*N*-Dimethylformamide
Synonym: DMF

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_3H_7NO Relative molecular mass: 73.09

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless to very slightly yellow liquid with a faint amine odour (Budavari, 1996)
(*b*) *Boiling-point*: 153°C (Lide, 1997)
(*c*) *Melting-point*: –60.4°C (Lide, 1997)
(*d*) *Solubility*: Miscible with water and most common organic solvents (Budavari, 1996)
(*e*) *Vapour pressure*: 3 kPa at 20°C; relative vapour density (air = 1), 2.51 (Verschueren, 1996)
(*f*) *Flash-point*: 67°C, open cup (Budavari, 1996)
(*g*) *Explosive limits*: Upper, 15.2%; lower, 2.2%, by volume in air (American Conference of Governmental Industrial Hygienists, 1993)
(*h*) *Conversion factor*: $mg/m^3 = 3.0 \times ppm$

1.2 Production and use

World production of dimethylformamide is estimated to be 125 thousand tonnes (Marsella, 1995). Information available in 1995 indicated that it was produced in 19 countries (Chemical Information Services, 1995).

Dimethylformamide has been termed the universal solvent and is used commercially as a solvent, for example, for vinyl resins, adhesives and epoxy formulations (the latter for use in laminated printed circuit boards); for purification and/or separation of acetylene, 1,3-butadiene, acid gases and aliphatic hydrocarbons; in the production of polyacrylic or cellulose triacetate fibres and pharmaceuticals. It is also used as a catalyst in carboxylation reactions; in organic synthesis; as a quench and cleaner combination for hot-dipped tin parts (e.g., for high-voltage capacitors); as an industrial paint stripper; as a carrier for gases, and in inks and dyes in printing and fibre-dyeing applications (American Conference of Governmental Industrial Hygienists, 1991; Lewis, 1993; Marsella, 1994).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 United States National Occupational Exposure Survey (NOES, 1997), as many as 125 000 workers in the United States were potentially exposed to dimethylformamide (see General Remarks). Occupational exposures to dimethylformamide may occur in the production of the chemical, other organic chemicals, resins, fibres, coatings, inks and adhesives. Exposure also may occur during use of these coatings, inks, adhesives, in the synthetic leather industry, in the tanning industry and in the repair of aircraft (Ducatman *et al.*, 1986; IARC, 1989).

1.3.2 *Environmental occurrence*

Dimethylformamide has been measured in ambient air near a fibre plant and near waste facilities. It has rarely been found in water samples in the United States, other than at sewage treatment plants or in effluents of plants likely to have been using dimethylformamide. Levels measured were very low (WHO, 1991). It has been detected at low levels in drinking-water, surface water, wastewater and ambient air samples (United States National Library of Medicine, 1997).

Exposure through the use of dimethylformamide in food processing, food packaging and pesticides may occur, but no data are available (WHO, 1991).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 30 mg/m^3 as the 8-h time-weighted average threshold limit value, with a skin notation, for occupational exposures to dimethylformamide in workplace air. Values of 10–60 mg/m^3 have been used as standards or guidelines in other countries (International Labour Office, 1991).

No international guideline for dimethylformamide in drinking-water has been established (WHO, 1993).

2. Studies of Human Cancer

2.1 Case reports

Ducatman *et al.* (1986) reported three cases of testicular germ-cell tumour that occurred during 1981–83 among 153 white men who repaired the exterior surfaces and electrical components of F4 Phantom jet aircraft in the United States. This finding led to surveys of two other repair shops at different locations. One repaired F4 Phantom jets while the other repaired different types of aircraft. Four among 680 white male workers in the F4 Phantom shop had testicular germ-cell cancers (approximately one expected) diagnosed during 1970–83. No case of testicular germ-cell cancer was found among 446 white men employed at the facility where different types of aircraft were repaired. Of the seven cases, five were seminomas and two were embryonal-cell carcinomas. All seven men had long work histories in aircraft repair. There were many common exposures to solvents in the three facilities, but the only exposure identified as unique to the F4 Phantom jet aircraft repair facilities was to a solvent mixture containing 80% dimethylformamide [20% unspecified]. Three of the cases had been exposed to this mixture with certainty and three cases had probably been exposed.

Levin *et al.* (1987), in a letter to the editor, described three cases of embryonal-cell carcinoma of the testis in workers at one leather tannery in the United States. According to the authors, all the tanneries they had surveyed used dimethylformamide, as well as a wide range of dyes and solvents. A screening effort to identify additional testicular cancers at the leather tannery with the three cases was undertaken in 1989 (Calvert *et al.*, 1990). Fifty-one of 83 workers employed at the plant between 1975 and 1989 participated. No additional case of testicular cancer was identified.

2.2 Industry-based studies

These case reports led to a cohort study of cancer among employees of the Du Pont company. Chen *et al.* (1988a) studied cancer incidence among 2530 actively employed workers with potential exposure to dimethylformamide during 1950–70 in Virginia and 1329 employees with exposure to dimethylformamide and acrylonitrile (see this volume) at an acrylic fibre manufacturing plant in South Carolina, United States. Cancer incidence rates for the company (1956–84) and national rates (1973–77) for the United States were used to calculate expected numbers of cases. [The tumour registry of the Du Pont company covers only active workers, but the Working Group noted this would be less of a limitation for testicular cancer than other tumours.] For all workers exposed to dimethylformamide (alone or with acrylonitrile), the standardized incidence ratio (SIR) based on company rates for all cancers combined was [1.1] [95% confidence interval (CI), 0.9–1.4] (88 cases). One case of testicular cancer was found among the 3859 workers exposed to dimethylformamide (alone or with acrylonitrile), with 1.7 expected based on company rates. The SIR for cancer of the buccal cavity and pharynx was [3.4] [95% CI, 1.7–6.2] (11 cases) among workers exposed to dimethylformamide, based on company rates. No such excess for any cancer was found among the 1329 workers exposed to both

dimethylformamide and acrylonitrile. There was no relationship between cancer of the buccal cavity and pharynx and intensity or duration of exposure: low exposure, SIR, 4.2 (five cases, 1.2 expected); moderate exposure, SIR, 3.0 (six cases, 2.0 expected). 'Low' exposure was defined as workplace levels consistently below 10 ppm (30 mg/m^3), while 'moderate' exposure was defined as workplace levels sometimes above 10 ppm.

Chen *et al.* (1988b) evaluated mortality in 1950–82 in the same cohort among both active and pensioned employees. Expected numbers (adjusted for age and time period) were based on company rates. For all workers exposed to dimethylformamide only, the standardized mortality ratios (SMR) were [0.9] (38 obs./40.1 exp.) for all cancers combined, [2.5] (2 obs./0.8 exp.) for buccal cavity and pharynx and [1.4] (19 obs./13.5 exp.) for lung cancer. No other cancer excesses were reported.

Walrath *et al*. (1989) conducted case–control studies of cancers of the buccal cavity and pharynx (n = 39), liver (n = 6), prostate (n = 43), testis (n = 11) and malignant melanoma of the skin (n = 39) among workers from four DuPont plants. Two plants had been previously studied for exposure to acrylonitrile and dimethylformamide (Chen *et al*., 1988a,b) but two others had not. Cancers occurring during 1956 to 1985 were identified through the Du Pont Cancer Registry from a combined cohort composed of approximately 8700 workers per year. For each case, the first two eligible controls from the employment roster were selected matched on year of birth, sex, wage/salary class and plant. The plants studied included a dimethylformamide production plant, two acrylic fibre plants that used dimethylformamide as a spinning solvent and a plant using the chemical as a solvent for inks. Potential exposure to dimethylformamide was classified as low or moderate (no worker fell in the high category) from job title/work area combinations by a team of two industrial hygienists and an epidemiologist. Dimethylformamide measurements were available from all plants. Geometric means for air measurements of dimethylformamide ranged from less than 1.0 ppm [3.0 mg/m^3] to about 10 ppm [30 mg/m^3]. Relative risks were estimated by Mantel–Haenszel matched analyses and logistic regression (adjusted for plant, pay class, year of diagnosis and age at diagnosis), using all controls. Mantel–Haenszel odds ratios for ever exposed were 0.9 (n = 15) (90% CI, 0.4–2.3) for buccal cavity and pharynx cancers, 1.7 (n = 16) (90% CI, 0.5–5.5) for malignant melanoma, 1.5 (n = 17) (90% CI, 0.7–3.3) for prostate cancer and 1.0 (n = 3) (90% CI, 0.2–4.4) for testicular cancer. Two liver cancer cases and one control were exposed to dimethylformamide giving a logistic regression odds ratio of 6.1 (90% CI, 0.4–72.0). Odds ratios for malignant melanoma by level of exposure were 1.9 (90% CI, 0.5–7.3) for low and 3.1 (90% CI, 0.8–11.9) for moderate exposure. Odds ratios for testicular cancer by level of exposure were 0.9 (90% CI, 0.1–8.6) for low and 11.6 (2 exposed cases and 2 exposed controls) (90% CI, 0.5–286) for moderate exposure.

3. Studies of Cancer in Experimental Animals

Dimethylformamide was tested for carcinogenicity by oral administration and subcutaneous injection in one strain of rats. In a study in which dimethylformamide was

administered by intraperitoneal injection in another strain of rats, a small number of uncommon tumours was observed in treated rats. All of these studies were inadequate for evaluation (IARC, 1989).

3.1 Inhalation exposure

3.1.1 *Mouse*

Groups of 78 male and 78 female Crl: CD-1 (ICR) BR mice, 55 days old, were administered dimethylformamide (purity, 99.9%) at 0, 25, 100 or 400 ppm [0, 75, 300 or 1200 mg/m^3] in air by whole-body vapour exposure for 6 h per day on five days a week for 18 months. Five males and five females per group were killed at 2 weeks, 3 months and 12 months. No compound-related effect on survival was evident. At termination, the 100- and 400-ppm males and 400-ppm females had higher liver weights. In both sexes, at the two highest exposures, centrilobular hepatocellular hypertrophy and hepatic single-cell necrosis were increased. No increased tumour incidence was observed (Malley *et al*., 1994).

3.1.2 *Rat*

Groups of 87 male and 87 female Crl:CD BR rats, 47 days old were administered dimethylformamide (purity, 99.9%) at 0, 25, 100 or 400 ppm [0, 75, 300 or 1200 mg/m^3] in air by whole-body vapour exposure for 6 h per day on five days a week for two years. Exposure to the highest concentration reduced body weight gain in both sexes but did not affect survival. The highest concentration also increased liver weights in both sexes. Ten males and ten females per group were killed at 12 months. In both sexes of the two highest concentration groups, incidences of minimal to mild centrilobular hepatocellular hypertrophy and centrilobular accumulation of lipofuscin/haemosiderin were increased. No increase in tumours occurred, but a 14.8% incidence of uterine endometrial stromal polyps in high-dose females was observed compared to 1.7% in controls [numerical data not given]. However, the range of historical control incidence for the laboratory was 2.0–15.0% (Malley *et al*., 1994).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

The penetration of dimethylformamide through excised human skin *in vitro* was evaluated by Bortsevich (1984), who showed that dimethylformamide was much better absorbed (51% in 4 h) than its aqueous solutions (15–60% v/v, < 1% in 4 h).

Percutaneous absorption *in vivo* was examined by Mráz and Nohová (1992a) in two ways, from liquid dimethylformamide and from dimethylformamide vapour. The first was evaluated by dipping one hand into undiluted dimethylformamide for up to 20 min and by

the application of 2 mmol dimethylformamide over an area of 100 cm^2 on the forearm (approximately 1.5 mg/cm^2). In both studies, the absorption rate was 9 $mg/cm^2/h$.

Percutaneous uptake of dimethylformamide vapour was evaluated in volunteers exposed to an atmosphere of 50 mg/m^3 dimethylformamide for 4 h, while wearing light clothing and breathing fresh air through masks. The percutaneous uptake of dimethylformamide increased with increasing ambient temperature and humidity and contributed some 13–36% of the urinary *N*-hydroxymethyl-*N*-methylformamide excreted during combined inhalation and percutaneous exposure to the same concentration of dimethylformamide vapour (Mráz & Nohová, 1992a).

Mráz and Nohová (1992b) placed 10 volunteers (5 men, 5 women) in atmospheres of 10, 30 and 60 mg/m^3 dimethylformamide for 8 h and measured the metabolites in urine collected for up to five days. In addition, two men and two women were exposed to 30 mg/m^3, for 8 h per day on five consecutive days. The uptake from the respiratory tract was 90% and the various urinary metabolites examined accounted for 49% of the retained dose. The half-lives of excretion and the urinary recoveries of the metabolites were: dimethylformamide, 2 h (0.3% of dose); *N*-hydroxymethyl-*N*-methylformamide, 4 h (22%); *N*-hydroxymethylformamide, 7 h (13%); and the mercapturic acid conjugate, *N*-acetyl-*S*-(*N*-methylcarbamoyl)cysteine, 23 h (13%).

A number of studies of workplace exposure to dimethylformamide have been performed to search for the most appropriate biomarkers of dimethylformamide exposure. These all showed a good and linear correlation between the amounts of dimethylformamide and *N*-hydroxymethyl-*N*-methylformamide in the urine at the end of an 8-h shift and the atmospheric concentration of dimethylformamide. Kawai *et al*. (1992) studied over 200 workers exposed to up to 9 ppm [27 mg/m^3] dimethylformamide alone or with toluene; Sakai *et al*. (1995) examined 10 workers exposed in different ways to up to 8 ppm [24 mg/m^3] dimethylformamide; while Casal Lareo and Perbellini (1995) evaluated 22 workers exposed to individual mean dimethylformamide concentrations of 10–20 mg/m^3 over three working days. The latter two studies also found that excretion of the mercapturic acid conjugate provides a good indication of the total exposure to dimethylformamide over a prolonged period, as a result of its slower excretion relative to other major metabolites.

4.1.2 *Experimental systems*

Hundley *et al*. (1993a) exposed rats and mice to atmospheres of 10, 250 and 500 ppm [30, 750 and 1500 mg/m^3] dimethylformamide for single 1-, 3- and 6-h periods or for 6 h per day on 10 days over two weeks. Dimethylformamide was not detected in the plasma after the 10 ppm dose, while at the 250 ppm dose, steady-state plasma levels were approached after 6 h of exposure; this was not the case at 500 ppm, where plasma levels increased two-fold in rats and three-fold in mice between 3 and 6 h of exposure. The area under the plasma concentration curve (AUC) values for dimethylformamide after a single 6-h exposure increased disproportionately (8-fold and 29-fold increases in rats and mice, respectively) compared with the increase in dimethylformamide exposure concentration (from 250 ppm to 500 ppm). Multiple 500 ppm dimethylformamide exposures resulted in

lower dimethylformamide AUC values for both rats (to 34%) and mice (to 23%) compared with the AUC values following a single 500 ppm exposure. These authors also presented data on plasma levels of '*N*-methylformamide', representing the total of *N*-hydroxymethyl-*N*-methylformamide and *N*-methylformamide, determined by gas chromatography. '*N*-Methylformamide' levels increased with time of exposure to single 250 ppm doses but did not increase further at 500 ppm.

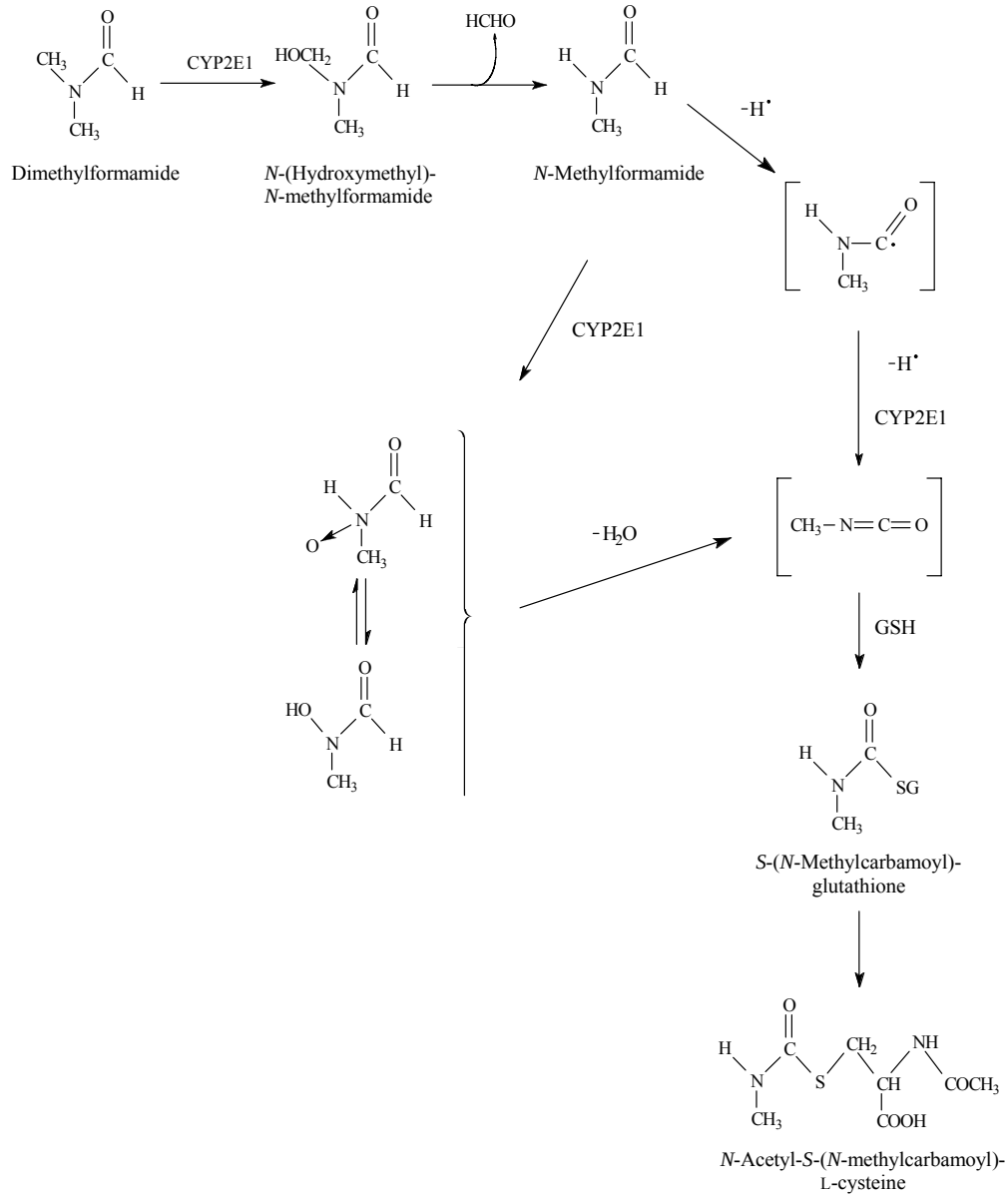
Serial blood and urine samples were collected from two cynomolgus monkeys of each sex in groups subjected to whole-body exposure to atmospheres of 30, 100 or 500 ppm [90, 300 or 1500 mg/m^3] dimethylformamide for 6 h per day on five days per week for 13 weeks (Hundley *et al.*, 1993b). As was found in rats and mice, there were disproportionate increases in plasma AUC values of 19- to 37-fold in male monkeys and 35- to 54-fold in females as the atmospheric concentrations increased five-fold from 100 to 500 ppm. Plasma half-lives ranged from 1–2 h for dimethylformamide and 4–15 h for '*N*-methylformamide'. There was rapid metabolism, the plasma '*N*-methylformamide' concentration being higher than that of dimethylformamide at 0.5 h. *N*-(Hydroxymethyl)-*N*-methylformamide formed 56–95% of the urinary metabolites, depending upon the exposure level and duration of the study.

There have been numerous studies of the metabolism of dimethylformamide in the past 25 years and these have been summarized by Gescher (1993). The major pathway of dimethylformamide metabolism is hydroxylation of one of the methyl groups, giving *N*-hydroxymethyl-*N*-methylformamide, which is unstable in many analytical manipulations and readily decomposes to *N*-methylformamide. *N*-Hydroxymethyl-*N*-methylformamide was underestimated, or not detected at all, in a number of early studies for this reason (see, for example, Kawai *et al*., 1992; Rosseel *et al*., 1993). The formation of *N*-hydroxymethyl-*N*-methylformamide is a cytochrome P450-dependent reaction mediated by CYP2E1 in rat liver microsomes. The reaction mediated by human liver microsomes was inhibited by a monospecific antibody against rat liver CYP2E1 (Mráz *et al.*, 1993).

Both *N*-hydroxymethyl-*N*-methylformamide and *N*-methylformamide formed from dimethylformamide undergo further oxidative metabolism, *N*-demethylation giving formamide and oxidation of the formyl group giving a reactive intermediate (Cross *et al*., 1990) probably methyl isocyanate, which acylates glutathione. The resulting *S*-(*N*-methylcarbamoyl)glutathione undergoes the usual further transformations to give the mercapturic acid *N*-acetyl-*S*-(*N*-methylcarbamoyl)-L-cysteine (AMCC), which is a major metabolite of dimethylformamide in animals and humans. The formyl group oxidation which is the key step in the formation of AMCC is mediated by CYP2E1 (Mráz *et al*., 1993) (Figure 1).

There occur marked differences between rodent species and humans in the proportions of a dose excreted as these various major metabolites, and the dose size introduces further variables. Mráz *et al*. (1989) gave male Sprague-Dawley rats, BALB/c mice and Syrian hamsters 0.1, 0.7 and 7 mmol/kg bw dimethylformamide (approximately 7, 50 and 500 mg/kg bw) by intraperitoneal injection and collected urine for 60 h (rat), 24 h (mice) and 36 h (hamster). In all cases, dimethylformamide and AMCC were very minor urinary metabolites, while the amounts of substances analysed as '*N*-methylformamide'

Figure 1. Metabolism of dimethylformamide



From Cross *et al.* (1990) and Gescher (1993)

and a formamide precursor varied with dose, with 8–47% of dose as the former and 8–37% as the latter. In comparison, human subjects exposed to dimethylformamide by inhalation excreted 16–49% of the dose as '*N*-methylformamide', 8–24% as 'formamide' and 10–23% as AMCC.

These data suggest a quantitative difference in the formation of AMCC between species, most likely in the formation of the reactive carbamoylating intermediate which acylates glutathione.

Purified CYP2E1 from mouse and rat liver microsomes in a reconstituted system is a very active catalyst of dimethylformamide oxidation, the turnover being about 10 nmol/min per nmol P450 for both species; however, the affinities are very different. The K_m values for mouse and rat CYP2E1, respectively, were about 0.08 mM and 1.1 mM (Chieli *et al.*, 1995).

4.2 Toxic effects

4.2.1 *Humans*

An outbreak of liver disease in a fabric-coating factory was investigated by Redlich *et al*. (1988). Dimethylformamide was used as a solvent for fabric coating in poorly ventilated areas without appropriate skin protection. Overall, 36 of 58 workers had elevations of either aspartate aminotransferase or alanine aminotransferase serum activity. Among 46 workers, the following symptoms were reported: anorexia, abdominal pain or nausea by 31 workers; headaches and dizziness by 18 workers; alcohol intolerance (facial flushing and palpitations) by 11 workers. Liver biopsies of workers exposed to several organic solvents, predominantly to dimethylformamide, showed focal hepatocellular necrosis and microvesicular steatosis with prominence of smooth endoplasmic reticulum, complex lysosomes and pleomorphic mitochondria with crystalline inclusions (Redlich *et al*., 1990). Among workers with longer exposure, no signs of liver fibrosis were found.

In 183 out of 204 employees in a synthetic leather factory, Wang *et al*. (1991) found a significant corrrelation between high exposure concentrations of dimethylformamide (25–60 ppm) and elevated serum alanine aminotransferase and creatine phosphokinase levels. Furthermore, high dimethylformamide exposure concentrations were correlated with symptoms such as dizziness, anorexia, nausea and epigastric pain.

In a group of 318 workers exposed to dimethylformamide levels of up to 7 ppm (geometric mean [21 mg/m^3]), no significant alterations in haematological and biochemical blood parameters were found compared with a non-exposed group (143 controls) (Cai *et al*., 1992). However, a dose-dependent increase in complaints of subjective symptoms was found, including nausea and abdominal pain in particular during work. Furthermore, the prevalence of alcohol intolerance was also elevated depending on the calculated dose. A prolonged susceptibility to alcohol-induced flushing after dimethylformamide exposure was reported in a case report by Cox and Mustchin (1991). A cluster of toxic liver disease among workers exposed to dimethylformamide was reported by Fleming *et al*. (1990). Thirty-five out of 45 exposed production workers had abnormalities of their liver transaminases, compared with one of 12 unexposed, nonproduction workers.

4.2.2 *Experimental systems*

In acetone-pretreated male CD-1 mice, dimethylformamide, given as a single intraperitoneal dose of 1000 mg/kg bw, resulted in liver necrosis and a strong increase in serum alanine aminotransferase activity (Chieli, 1995). In contrast, no signs of hepatotoxicity were found in non-pretreated mice given the same dose or in pretreated or non-pretreated male Sprague-Dawley rats given up to 2000 mg/kg bw as a single intraperitoneal dose. These differences are probably related to the highly different substrate affinities of CYP2E1 in rats and mice (see Section 4.1.2). The hepatotoxicity of dimethylformamide was also investigated by Imazu *et al*. (1992) who treated male Wistar rats by daily subcutaneous injections of 0.5 mL/kg bw dimethylformamide for one week. Treated rats showed a significant increase in serum glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, cholinesterase and total cholesterol. Hepatic microsomal cytochrome P450 and hepatic glutathione reductase activity were decreased, while glutathione *S*-transferase activity using 1-chloro-2,4-dinitrobenzene as a substrate was induced by about 66%. In male Wistar rats, Van der Bulcke *et al*. (1994) found increased serum sorbitol dehydrogenase activity after intraperitoneal administration of 4.1 but not of 1.4 mmol/kg bw dimethylformamide. They also found that dimethylformamide is more hepatotoxic than either of two of its metabolites, *N*-hydroxymethyl-*N*-methylformamide and *N*-methylformamide, which are similar and express their hepatotoxicity earlier.

Cynomolgus monkeys showed no measurable adverse effect following inhalation of 500 ppm [1500 mg/m^3] dimethylformamide for 6 h per day on five days per week for two weeks (Hurtt *et al*., 1991). In a 13-week inhalation study, cynomolgus monkeys received whole-body exposures of 0, 30, 100 or 500 ppm [0, 90, 300 or 1500 mg/m^3] dimethylformamide for 6 h per day on five days per week (Hurtt *et al*., 1992). No exposure-related effect on body weight or a number of haematological parameters and serum chemistry including transaminases occurred.

In a number of human and rodent cell lines (Hoosein *et al*., 1988a,b; Levine *et al*., 1989; Guilbaud *et al*., 1990; Grunt *et al*., 1992; Levine & Chakrabarty, 1992), relatively high concentrations of dimethylformamide (in the range of 0.5–1% in the medium), initiated differentiation and led to simultaneous growth inhibition. These effects upon the differentiation state were shown to be associated in certain confluent, transformed cell cultures with a reduction of c-*myc* levels (Mulder *et al*., 1989).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

SPF (Mol:Wist) rats were administered up to 2 mL/kg bw dimethylformamide per day applied in a porous dressing placed on shaved skin either on gestation days 6–15 or on gestation days 1–20 (Hansen & Meyer, 1990). Body weight, weight gain and preg-

nancy rate were reduced in those rats receiving 2 mL/kg bw per day on days 6–15. A reduction in the number of live fetuses and in fetal weight, as well as an increase in post-implantation loss, were also observed at this dose level. Similar but more pronounced effects were observed in rats treated on days 1–20 with the same daily dose. The lowest effect level in this study was 1 mL/kg bw per day.

In a 13-week inhalation study, male cynomolgus monkeys received whole-body exposures of 0, 30, 100 or 500 ppm [0, 90, 300 or 1500 mg/m^3] dimethylformamide for 6 h per day on five days per week (Hurtt *et al.*, 1992). No significant effect on semen volume, percentage of motile sperm, sperm count or abnormal sperm morphology was found.

4.4 Genetic and related effects

4.4.1 *Humans*

In a study of chromosomal aberrations in peripheral blood lymphocytes, 20 workers exposed to mono-, di- and trimethylamines as well as dimethylformamide in the then German Democratic Republic, the mean workplace concentrations during one year before blood sampling were: 12.3 mg/m^3 (range, 5.6–26.4) dimethylformamide, 5.3 mg/m^3 (range, 1.2–10.1) monomethylformamide and 0.63 mg/m^3 (range, 0.01–3.3) dimethylamine, which were within the maximal admissible range in the country. Eighteen unexposed employees from the same factory were used as controls. The frequency of chromosomal gaps and breaks was 1.4% in the exposed group compared with 0.4% in the controls (Berger *et al.*, 1985). The authors commented that the value in the control group was low, in comparison with other studies. [The Working Group noted that the possible effect of smoking was not taken into account.]

Chromosomal aberrations in peripheral lymphocytes were also reported in a study of about 40 workers who had been occupationally exposed to trace quantities of 2-butanone (methyl ethyl ketone), butyl acetate, toluene, cyclohexanone and xylene in addition to dimethylformamide. Blood samples were taken at two four-month intervals, when exposure was to an average of 180 and 150 mg/m^3 dimethylformamide, respectively. The frequencies of chromosomal aberrations were 3.82% and 2.74% at these two sampling times. Subsequent sampling at three six-month intervals, when average dimethylformamide exposures were to 50, 40 and 35 mg/m^3, gave lower aberration frequencies of 1.59%, 1.58% and 1.49%. Aberration frequencies in two control groups were 1.61% and 1.10% (Koudela & Spazier, 1981).

It was reported in an abstract that there was no evidence for an increased frequency of chromosomal aberrations in peripheral lymphocytes of a group of workers exposed to dimethylformamide [details not given] (Šrám *et al.*, 1985).

Seiji *et al.* (1992) studied the effects of occupational exposure to dimethylformamide on sister chromatid exchange rates in peripheral lymphocytes from 22 dimethylformamide-exposed women (aged 22–52 years) in comparison with 22 sex-, age- and residence-matched controls. All subjects were non-smokers and non-drinkers of alcohol as confirmed by medical interview. The 22 pairs were divided into three subgroups according to the intensity of their exposure to dimethylformamide: high exposure (8 pairs with mean exposure of

5.8 ppm [17.4 mg/m^3]), medium exposure (5 pairs at 0.7 ppm [2.1 mg/m^3] in combination with toluene at 0.9 ppm) and low exposure (9 pairs at 0.3 ppm [0.9 mg/m^3]). Sister chromatid exchange frequencies per cell were significantly higher in the high- and medium-exposure groups than in their matched pairs (8.26 ± 1.76 *vs* 5.63 ± 1.56 and 7.24 ± 1.53 *vs* 4.66, respectively), but not in the low-exposure group (5.67 ± 1.35 *vs* 6.57 ± 1.12) (Seiji *et al.*, 1992). [The Working Group noted the incomplete reporting of the data.]

4.4.2 *Experimental systems* (see Table 1 for references)

Dimethylformamide was one of 42 chemicals selected for study in the International Collaborative Program for the Evaluation of Short-term Tests for Carcinogens, in which 30 assay systems were included and more than 50 laboratories contributed data (de Serres & Ashby, 1981). Since then, the database has been expanded. In most of the in-vitro studies, dimethylformamide was tested in both the presence and absence of an exogenous metabolic system. It was reported to induce mutation in *Salmonella typhimurium* TA1538 and TA98 in one test with metabolic activation, but the response occurred at a single, intermediate dose and, in many other studies, dimethylformamide did not induce gene mutation in any strain of *S. typhimurium* or in *Escherichia coli* WP2*uvrA* and did not induce differential toxicity indicative of DNA damage in bacteria. In one study, dimethylformamide enhanced the mutagenicity of tryptophan-pyrolysate in *S. typhimurium* TA98 in the presence of an exogenous metabolic system (Arimoto *et al.*, 1982). It induced aneuploidy in *Saccharomyces cerevisiae* D6 in both the presence and absence of an exogenous metabolic system in a single study and gave positive results in another study for mitotic recombination in yeast, but most results for gene mutation or mitotic recombination were negative.

Dimethylformamide induced a slight increase in unscheduled DNA synthesis in primary rat hepatocyte cultures in one study but not in two others or in studies with mouse and Syrian hamster hepatocytes.

Dimethylformamide did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster* in experiments where it was used as a solvent for other substances to be tested and the responses were, therefore, compared with those of untreated controls.

Dimethylformamide was not mutagenic in L5178Y *tk*$^{+/-}$ mouse lymphoma cells in three studies, while an increased mutation frequency of about two-fold was observed at the highest dose level in one experiment.

Gene mutations were not induced in a single study with human fibroblasts. In no study were sister chromatid exchanges induced in either Chinese hamster or human cells and no chromosomal aberrations were induced in rodent cells. Chromosomal aberrations were reported to be induced in one study with cultured human lymphocytes, at a dose level of 0.007 μg/mL, but not in another study at a dose level of 80 000 μg/mL.

In mouse experiments *in vivo*, dimethylformamide did not induce sister chromatid exchanges in mouse bone-marrow cells in a single study or micronuclei in mouse bone-marrow cells in four studies, in which intraperitoneal doses up to 2000 mg/kg bw were used; in one study, micronuclei were reported to be induced at a dose of 1 mg/kg bw.

Table 1. Genetic and related effects of *N,N*-dimethylformamide

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS repair test, *Salmonella typhimurium* TA1535/pSK1002	–	–	32	Nakamura *et al.* (1987)
ECL, *Escherichia coli pol* A/W3110-P3478, differential toxicity (liquid suspension test)	–	–	2300	Rosenkranz *et al.* (1981)
ERD, *Escherichia coli* DNA-repair deficient strains, differential toxicity	–	–	NG	Tweats (1981)
ERD, *Escherichia coli* DNA-repair deficient strains, differential toxicity	–	NT	NG	Ichinotsubo *et al.* (1981b)
ERD, *Escherichia coli* DNA-repair deficient strains, differential toxicity	–	–	NG	Green (1981)
BSD, *Bacillus subtilis rec* strains, differential toxicity	–	–	19000	Kada (1981)
SAF, *Salmonella typhimurium*, forward mutation	NT	–	1000	Skopek *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	–	1250	Purchase *et al.* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Baker & Bonin (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	?	500	Hubbard *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	NT	NG	Ichinotsubo *et al.* (1981a)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500	MacDonald (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Nagao & Takahashi (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	Richold & Jones (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1000	Rowland & Severn (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Simmon & Shepherd (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1250	Trueman (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	250	Venitt & Crofton-Sleigh (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	94	Antoine *et al.* (1983)

Table 1 (cond)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	–	1000	Falck *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	Mortelmans *et al.* (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	NT	–	1250	Purchase *et al.* (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Baker & Bonin (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1000	Gatehouse (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	Richold & Jones (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1000	Rowland & Severn (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	NG	Simmon & Shepherd (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1250	Trueman (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	94	Antoine *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	NT	–	1000	Falck *et al.* (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	Mortelmans *et al.* (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Baker & Bonin (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1000	Gatehouse (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	MacDonald (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Nagao & Takahashi (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Richold & Jones (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1000	Rowland & Severn (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Simmon & Shepherd (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1250	Trueman (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	94	Antoine *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	NT	–	500	Falck *et al.* (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Mortelmans *et al.* (1986)

Table 1 (cond)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA8, *Salmonella typhimurium* TA1538, reverse mutation	NT	–	1250	Purchase *et al.* (1978)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	500	Baker & Bonin (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	5000	Richold & Jones (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1000	Rowland & Severn (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	NG	Simmon & Shepherd (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	+	NG	Trueman (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	94	Antoine *et al.* (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	NT	–	1000	Falck *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	NT	-	1250	Purchase *et al.* (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Baker & Bonin (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	Gatehouse (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	?	500	Hubbard *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	NG	Ichinotsubo *et al.* (1981a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	MacDonald (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Nagao & Takahashi (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Richold & Jones (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	Rowland & Severn (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Simmon & Shepherd (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	250	Trueman (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250	Venitt & Crofton-Sleigh (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	94	Antoine *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	NT	–	1000	Falck *et al.* (1985)

Table 1 (cond)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Mortelmans *et al.* (1986)
SAS, *Salmonella typhimurium* (other miscellaneous strains), reverse mutation	NT	–	73000	Green & Savage (1978)
SAS, *Salmonella typhimurium*, TA92, reverse mutation	–	–	1000	Brooks & Dean (1981)
ECF, *Escherichia coli* K-122/343/113 forward or reverse mutation	NT	–	4000	Mohn *et al.* (1981)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	–	500	Gatehouse (1981)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	–	NG	Matsushima *et al.* (1981)
ECW, *Escherichia coli* WP2 *uvr*ApKM101, reverse mutation	–	–	NG	Matsushima *et al.* (1981)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	–	250	Venitt & Crofton-Sleigh (1981)
ECW, *Escherichia coli* WP2 *uvr*ApKM101, reverse mutation	–	–	250	Venitt & Crofton-Sleigh (1981)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	NT	–	1000	Falck *et al.* (1985)
SSB, *Saccharomyces cerevisiae* 'race XII', DNA damage in DNA-repair strains	–	–	1000	Kassinova *et al.* (1981)
SSD, *Saccharomyces cerevisiae* rad strains, differential toxicity in DNA repair-deficient strains	+	+	500	Sharp & Parry (1981a)
SCH, *Saccharomyces cerevisiae*, 'race XII', homozygosis by mitotic recombination or gene conversion	–	–	1000	Kassinova *et al.* (1981)
SCH, *Saccharomyces cerevisiae* D4, homozygosis by mitotic recombination or gene conversion	–	–	167	Jagannath *et al.* (1981)
SCH, *Saccharomyces cerevisiae* D7, homozygosis by mitotic recombination or gene conversion	+	+	4700	Zimmermann & Scheel (1981)
SCH, *Saccharomyces cerevisiae* JD1, homozygosis by mitotic recombination or gene conversion	–	–	500	Sharp & Parry (1981b)

Table 1 (cond)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SCF, *Saccharomyces cerevisiae* XV185-14C, forward mutation	–	–	800	Mehta & von Borstel (1981)
SZF, *Schizosaccharomyces pombe*, forward mutation	–	–	20	Loprieno (1981)
SCN, *Saccharomyces cerevisiae* D6, aneuploidy	+	+	100	Parry & Sharp (1981)
ASM, *Arabidopsis* species, mutation	–	NT	300000	Gichner & Veleminsky (1987)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		900	Wurgler & Graf (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		40 000 ppm feed	Foureman *et al.* (1994)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		40 000 ppm inj	Foureman *et al.* (1994)
URP, Unscheduled DNA synthesis, Fischer 344 rat primary hepatocytes *in vitro*	(+)	NT	700	Williams (1977)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	7300	Williams & Laspia (1979)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	70	Ito (1982)
UIA, Unscheduled DNA synthesis, mouse and hamster hepatocytes *in vitro*	–	NT	700	McQueen *et al.* (1983)
UIA, Unscheduled DNA synthesis, mouse hepatocytes *in vitro*	–	NT	70	Klaunig *et al.* (1984)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	–	3000	Jotz & Mitchell (1981)
G5T Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	(+)	–	5000	McGregor *et al.* (1988)

Table 1 (cond)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
G5T Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	–	4700	Mitchell *et al.* (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	–	4700	Myhr & Caspary (1988)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	900	Evans & Mitchell (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	6300	Natarajan & van Kesteren-van Leeuwen (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	100	Perry & Thomson (1981)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	6300	Natarajan & van Kesteren-van Leeuwen (1981)
CIR, Chromosomal aberrations, rat liver RL_1 cells *in vitro*	–	NT	300	Dean (1981)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	–	NT	10 000	Pienta *et al.* (1977)
GIH, Gene mutation, diphtheria toxin HF Dip^r, human fibroblasts *in vitro*	–	–	500	Gupta & Goldstein (1981)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	NT	80000	Antoine *et al.* (1983)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	0.007	Koudela & Spazier (1979)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	80000	Antoine *et al.* (1983)
SVA, Sister chromatid exchange, CBA/J mouse bone-marrow cells *in vivo*	–		2500 ip × 1	Paika *et al.* (1981)
MVM, Micronucleus test, ICR mice *in vivo*	–		1600 ip × 1	Kirkhart (1981)
MVM, Micronucleus test, $B6C3F_1$ mice *in vivo*	–		2.5 ip × 1	Salamone *et al.* (1981)
MVM, Micronucleus test, CD mice *in vivo*	–		1500 ip × 2	Tsuchimoto & Matter (1981)

Table 1 (cond)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVM, Micronucleus test, BALB/c mice *in vivo*	–		2000 ip × 1	Antoine *et al.* (1983)
MVM, Micronucleus test, mice *in vivo*	+		1 ip × 1	Ye (1987)
TVI, Cell transformation, Syrian hamster embryo cells treated *in vivo*	–		3 ip × 1	Quarles *et al.* (1979)
ICR, Inhibition of intercellular communication, Chinese hamster lung V79 fibroblasts *in vitro*	+	NT	3800	Chen *et al.* (1984)
SPM, Sperm morphology, (CBA × BALB/c) F_1 mice *in vivo*	–		900 ip × 5	Topham (1981)
SPM, Sperm morphology, BALB/c mice *in vivo*	–		667 ip × 1	Antoine *et al.* (1983)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; inj, injection; ip, intraperitoneal

As reported in an abstract, no dominant-lethal effect was observed in groups of ten Sprague-Dawley rats exposed by inhalation to 300 ppm [900 mg/m^3] dimethylformamide for 6 h per day for five consecutive days (Lewis, 1979).

No morphologically transformed colonies were observed in Syrian hamster embryo cell cultures, either after treatment *in vitro* or after exposure of the dams to dimethylformamide (3 mL/kg bw) by intraperitoneal injection.

Dimethylformamide inhibited intercellular communication (as measured by metabolic co-operation) between Chinese hamster V79 *hprt* $^{+/-}$ cells.

The Working Group was also aware of inhalation studies with dimethylformamide conducted for the United States National Institute of Occupational Health. These involved exposure to 400 ppm [1200 mg/m^3] for 7 h in a rat bone-marrow cell cytogenetic study, for 7 h per day for five days in a rat bone-marrow cell cytogenetic study, a male rat dominant lethal assay and a mouse sperm morphology assay and for 2.25 h in a *Drosophila melanogaster* sex-linked recessive lethal assay. All results were negative.

4.4.3 *Mechanistic considerations*

Dimethylformamide does not appear to be genotoxic as judged from results of a variety of in-vitro and in-vivo assays. The positive data for cytogenetic damage in humans occupationally exposed to it are not very convincing. If dimethylformamide is carcinogenic, it is extremely unlikely that it owes its carcinogenicity to a genotoxic mechanism.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposures to dimethylformamide occur during its production and during the production of inks, adhesives, resins, fibres, pharmaceuticals, synthetic leather, and its use as a purification or separation solvent in organic synthesis. It has been detected in ambient air and water.

5.2 Human carcinogenicity data

Case reports of testicular cancer in aircraft repair and leather tannery facilities suggested possible association with dimethylformamide. Further research has failed to confirm this relationship. A screening effort at a leather tannery, where a cancer cluster had been noted, identified no additional cases. Mortality and cancer incidence studies and nested case–control investigations of testicular cancer and several other anatomical sites at several facilities with exposure to dimethylformamide noted no convincing associations.

5.3 Animal carcinogenicity data

Dimethylformamide was adequately tested for carcinogenicity by inhalation in one study in mice and one study in rats. No increase in tumours was found.

5.4 Other relevant data

Acute exposure of humans or experimental animals to relatively high concentrations of dimethylformamide causes hepatotoxicity as a major toxic effect.

Reports on chromosomal damage in workers exposed to dimethylformamide either failed to take into account smoking as a bias factor or were documented incompletely.

Dimethylformamide has been extensively tested in a broad range of in-vitro and in-vivo genotoxicity assays. Results have been consistently negative in well controlled studies.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of dimethylformamide.

There is *evidence suggesting lack of carcinogenicity* of dimethylformamide in experimental animals.

Overall evaluation

Dimethylformamide is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

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DIMETHYL SULFATE

Data were last reviewed in IARC (1974) and the compound was classified in *IARC Monographs* Supplement 7 (1987a).

1. Exposure Data

1.1 Chemical and physical data

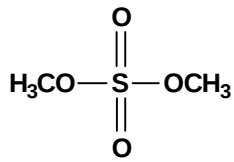
1.1.1 *Nomenclature*

Chem. Abstr. No.: 77-78-1

Chem. Abstr. Name: Sulfuric acid, dimethyl ester

Synonyms: Dimethyl monosulfate; methyl sulfate

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2H_6O_4S$ Relative molecular mass: 126.13

1.1.3 *Chemical and physical properties of the pure substance*

From IARC (1974)

(*a*) *Description*: Colourless, oily liquid

(*b*) *Boiling point*: 188°C (with decomposition); 76°C at 2 kPa

(*c*) *Melting point*: –27°C

(*d*) *Solubility*: Miscible with many polar organic solvents and aromatic hydrocarbons, but sparingly soluble in carbon disulfide and aliphatic hydrocarbons

(*e*) *Vapour pressure*: 13 Pa at room temperature

(*f*) *Stability*: Stable at room temperature; hydrolysis in water is rapid.

(*g*) *Reactivity*: An active alkylating agent

(*h*) *Conversion factor*: $mg/m^3 = 5.16 \times ppm$

1.2 Production and use

Dimethyl sulfate has been produced commercially since at least the 1920s. It is used mainly as a methylating agent for converting active-hydrogen compounds such as phenols, amines and thiols to the corresponding methyl derivatives.

No information was available on production. During 1967–70, only five companies worldwide reported manufacturing it (IARC, 1974).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997) as many as 10 000 workers in the United States were potentially exposed to dimethyl sulfate (see General Remarks). No information was available as to the operations in which these exposures might have occurred.

1.3.2 *Environmental occurrence*

No information on environmental exposures was available to the Working Group.

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 0.52 mg/m^3 as the 8-h time weighted average threshold limit value for occupational exposures to dimethyl sulfate. Values used as standards or guidelines have ranged from 0.05 to 0.50 mg/m^3 in other countries (International Labour Office, 1991).

2. Studies of Cancer in Humans

As previously summarized, four cases of bronchial carcinoma were reported in men exposed occupationally to dimethyl sulfate (IARC, 1974). Additional case reports have since appeared: a case of pulmonary carcinoma in a man exposed for seven years to 'small amounts' of dimethyl sulfate but to larger amounts of bis(chloromethyl)ether and chloromethyl methyl ether (IARC, 1987b), and a case of choroidal melanoma in a man exposed for six years to dimethyl sulfate (IARC, 1987a).

3. Studies of Cancer in Experimental Animals

Dimethyl sulfate has been tested for carcinogenicity in rats by inhalation, subcutaneous and intravenous injection, and following prenatal exposure. It produced local sarcomas and tumours of the nervous system (IARC, 1974).

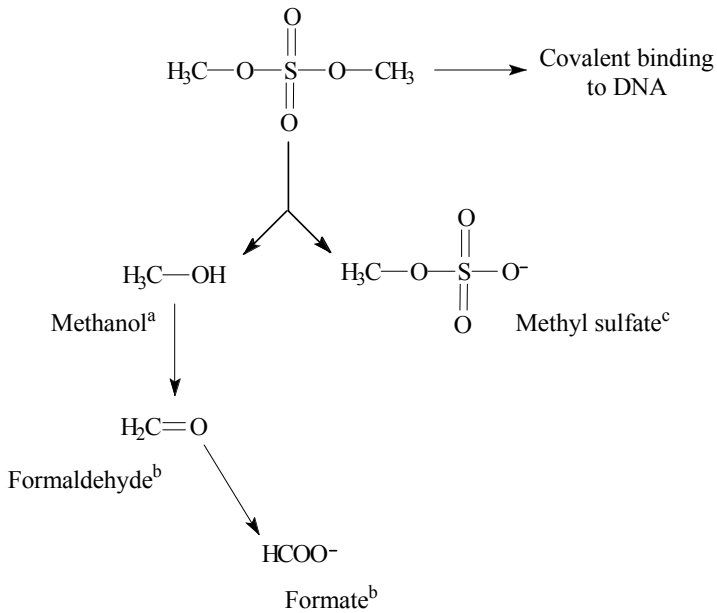
4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

As previously summarized, after an intravenous injection of 75 mg/kg bw in the rat, dimethyl sulfate was no longer detectable in blood after three minutes. No other data

were available to the Working Group (IARC, 1974). Dimethyl sulfate rapidly decomposes on contact with water to methanol and methyl sulfate (Figure 1) (Mathison *et al.*, 1995).

Figure 1. Dimethyl sulfate biotransformation and decomposition pathways *in vivo*



From Mathison *et al.* (1995)

[a] Primary products expected from hydrolysis of dimethyl sulfate

[b] Minor metabolites expected to be produced following further oxidation or hydrolysis of methanol

[c] Methyl sulfate does not further decompose to sulfate or function as a DNA methylating intermediate.

4.2 Toxic effects

4.2.1 *Humans*

Exposure to dimethyl sulfate causes corrosion or irritation to the skin, eyes and respiratory tract, with inflammation and tissue necrosis upon acute exposure. Death is commonly a result of respiratory failure (Molodkina *et al.*, 1985; Wang *et al.*, 1988; Ip *et al.*, 1989).

4.2.2 *Experimental systems*

No data were available to the Working Group.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Groups of adult female (C3H/R1 × 101/R1)F_1 mice were treated with 25 mg/kg bw dimethyl sulfate once by intraperitoneal injection of 25 mg/kg bw within four days before mating or at 1, 6, 9 or 25 h after mating with untreated males. Control groups were treated with vehicle only (1 mL water) four days before mating or 6 or 25 h after mating. Control and treated females were killed and their uterine contents examined 17–18 days after mating. Resorptions were significantly increased ($p < 0.01$) following treatment at 1, 6, 9 and 25 h after mating (63%, 57%, 50% and 34%, respectively) in comparison with before mating and 6 h and 25 h after mating control group frequencies of 4.8, 4.3% and 5.3%, respectively. Treatment before mating had no effect on the frequency of resorptions. The frequency of midgestational deaths was significantly increased at 1 h (6%) compared with control group frequencies of 0.3–0.6%. Late gestational deaths were significantly increased following the 1, 6 and 9 h treatments (11%, 10% and 5%, respectively), compared with a control group frequency of 0.3%. No effect was observed at other times. The incidences of live fetuses with malformations were (numbers of fetuses examined in parentheses): before mating control, 1.0% (298), treated (with, exceptionally, 75 mg/kg bw), 2.6 % (269); pooled after mating controls, 1.1% (650), treated at 1 h, 30% (40); treated at 6 h, 25% (120); treated at 9 h, 13% (134); treated at 25 h, 2% (187). In contrast to other alkylating agents with similar DNA-binding properties but different effects upon exposed zygotes, there appeared to be no site-specific alkylation product identifiable as the critical target. The authors speculated that the effects were due to an epigenetic disruption in the normal programming of gene expression during early embryogenesis (Generoso *et al*., 1991).

4.4 Genetic and related effects

The genetic effects of dimethyl sulfate have been reviewed by Hoffmann (1980).

4.4.1 *Humans*

Chromosome aberrations have been reported in lymphocytes of workers exposed to 100 mg/m^3 dimethyl sulfate (Molodkina *et al.*, 1985).

4.4.2 *Experimental systems* (see Table 1 for references)

Dimethyl sulfate induced mutation in bacteria and DNA damage in prophage. It forms a variety of alkylated bases, including *N*7-methylguanine, *N*3-methyladenine and *N*7-methyladenine with DNA *in vitro.*

In single studies, dimethyl sulfate induced somatic mutations in *Drosophila melanogaster* and in stamen hairs of *Tradescantia* clone BNL 4430.

In experiments conducted with mammalian cells *in vitro*, in the absence of exogenous metabolic activation, dimethyl sulfate induced morphological transformation, chromosomal aberrations, sister chromatid exchanges and gene mutations; it induced DNA strand breaks and formed *N*7-methylguanine, *N*3-methyladenine and *N*7-methyladenine in DNA.

Dimethyl sulfate forms *N*7-methylguanine in DNA when administered to rats *in vivo*. Urine collected from rats up to 48 h after exposure to airborne concentrations of ^{3}H-

Table 1. Genetic and related effects of dimethyl sulfate

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage, induction, SOS repair test, DNA strand breaks, cross-links or related damage	+	NT	252	Tudek *et al.* (1992)
PRB, Prophage, induction, SOS repair test, DNA strand breaks, cross-links or related damage	+	NT	1% in air	Dianov *et al.* (1991)
ECD, *Escherichia coli pol A*, differential toxicity	+	NT	6500	Fluck *et al.* (1976)
SAF, *Salmonella typhimurium*, forward mutation	+	NT	2.02	Skopek & Thilly (1983)
SAS, *Salmonella typhimurium* TA1535/pUC8, reverse mutation	+	NT	1.9	Tomicic & Franekic (1996)
SAS, *Salmonella typhimurium* hisG46/pUC8, reverse mutation	+	NT	1.9	Tomicic & Franekic (1996)
SAS, *Salmonella typhimurium* hisG428/pUC8, reverse mutation	+	NT	1.9	Tomicic & Franekic (1996)
SAS, *Salmonella typhimurium* MT101/UC8, reverse mutation	+	NT	1.9	Tomicic & Franekic (1996)
SAS, *Salmonella typhimurium* JK947, reverse mutation	+	NT	50	Lee *et al.* (1994)
ECF, *Escherichia coli* B, forward mutation	+	NT	126	Alderson (1964)
ECF, *Escherichia coli* NR3835, *LacI* gene, forward mutation	+	NT	164	Zielenska *et al.* (1989)
SCH, *Saccharomyces cerevisiae*, homozygosis	(+)	NT	240	Pavlov & Khromov-Borisov (1981)
SCR, *Saccharomyces cerevisiae*, reverse mutation	(+)	NT	240	Pavlov & Khromov-Borisov (1981)
TSM, *Tradescantia* clone BNL 4430, stamen hair mutation	+	NT	163	Shima & Ichikawa (1995)
DMM, *Drosophila melanogaster*, somatic mutation	+		5044 feed	Vogel (1989)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DMX, *Drosophila melanogaster*, sex-linked lethal recessive mutations	+		48 feed	Alderson (1964)
DIA, DNA strand breaks/cross-links, L1220 mouse leukaemic lymphoblastoid cells *in vitro*	+	NT	0.25	Durkacz *et al.* (1981)
DIA, DNA strand breaks *in vitro*	+	NT	252	Kubinski *et al.* (1981)
DIA, DNA strand breaks in PM2 DNA *in vitro*	+	NT	6.3	Mhaskar *et al.* (1981)
DIA, DNA strand breaks/cross-links, rat hepatocytes *in vitro*	+	NT	4	Sina *et al.* (1983)
DIA, DNA strand breaks, rat hepatocytes *in vitro*	+	NT	0.8	Sargent *et al.* (1991)
DIA, DNA strand breaks, Fischer 344 rat hepatocytes *in vitro*	+	NT	3.8	Bradley *et al.* (1987)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	NT	12.60	Probst *et al.* (1981)
GCO, Gene mutation, Chinese hamster ovary CHO cells *in vitro*	+	NT	1.3	Couch *et al.* (1978)
GCO, Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	+	NT	5	Tan *et al.* (1983)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	+	NT	8	Newbold *et al.* (1980)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	+	NT	6.3	Natarajan *et al.* (1984)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	+	NT	5	Nishi *et al.* (1984)
G9O, Gene mutation, Chinese hamster lung V79 cells, ouabain resistance *in vitro*	+	NT	NG	Newbold *et al.* (1980)
SIC, Sister chromatid exchange, Chinese hamster lung CP-1 cells *in vitro*	(+)	NT	6.3	Palitti & Becchetti (1977)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	NT	1.3	Natarajan *et al.* (1984)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	NT	6.3	Natarajan *et al.* (1984)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	NT	1.3	Connell & Medcalf (1982)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	NT	5	Nishi *et al.* (1984)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CIC, Chromosomal aberrations, Chinese hamster lung Cl-1 cells *in vitro*	(+)	NT	6.3	Palitti & Becchetti (1977)
CIC, Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	+	NT	6.3	Connell & Medcalf (1982)
CIC, Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	+	NT	6.3	Natarajan *et al.* (1984)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	NT	6.3	Natarajan *et al.* (1984)
TCL, Cell transformation, immortalized hamster dermal fibroblasts (4DH2)	+	NT	10	Shiner *et al.* (1988)
DIH, DNA strand breaks, human fibroblasts *in vitro*	+	NT	19	Teo *et al.* (1983)
DIH, DNA strand breaks/cross-links, human KB cells (line of HeLa cells) *in vitro*	+	NT	50	Walker (1984)
DIH, DNA strand breaks, human fibroblasts *in vitro*	+	NT	3.1	Yamada *et al.* (1996)
DIH, DNA strand breaks, human fibroblasts *in vitro*	+	NT	63	Klaude *et al.* (1996)
SHT, Sister chromatid exchange, transformed human cells *in vitro*	+	NT	1.5	Wolff *et al.* (1977)
CBA, Chromosomal aberrations, white rat bone-marrow cells *in vivo*	+		325 ip × 1	Sharma *et al.* (1980)
COE, Chromosomal aberrations, NMRI mouse embryos *in vivo*	+		25 ip × 1	Braun *et al.* (1986)
AVA, Aneuploidy, rat bone-marrow cells *in vivo*	+		325 ip × 1	Sharma *et al.* (1980)
BID, Formation of *N*3-methylguanine, O^6-methylguanosine in DNA *in vitro*	+	NT	NG	Lawley *et al.* (1972)
BID, Alkylated purines, Chinese hamster lung V79 cells *in vitro*	+	NT	100	Fox & Brennand (1980)
BID, Formation of *N*7-methylguanine, *N*3-methyladenine, O^6-methylguanine, *N*3-methylguanine in DNA of Chinese hamster lung V79 cells *in vitro*	+	NT	8	Newbold *et al.* (1980)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BID, Binding (covalent) to calf thymus DNA *in vitro*	+	NT	5675	Randerath *et al.* (1981)
BID, Formation of *N*7-methylguanine, O^6-methylguanine, *N*3-methyladenine in DNA of Chinese hamster lung V79 cells, *in vitro*	+	NT	10	Connell & Medcalf (1982)
BID, Formation of *N*7-methylguanine, *N*7-methyladenine, *N*3-methyladenine in DNA of Chinese hamster C4DH2 cells, *in vitro*	+	NT	10	Shiner *et al.* (1988)
BID, Formation of *N*7-methylguanine in DNA *in vitro*	+	NT	1576	Park *et al.* (1989)
BID, Formation of *N*7-methylguanine in DNA *in vitro*	+	NT	32	Tudek *et al.* (1992)
BVD, Formation of *N*7-methylguanine in DNA and RNA from rat liver *in vivo*	+		80 inj × 1	Swann & Magee (1968)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; NG, not given; inj, injection; ip, intraperitoneal

labelled dimethyl sulfate of 0.32 and 16.3 µg/L contained *N*7-methylguanine, *N*3-methyladenine and *N*1-methyladenine (Löfroth *et al.*, 1974). Dimethyl sulfate (lowest effective dose 25 mg/kg bw i.p.) induced chromosomal aberrations in NMRI mouse embryos at day 10 of gestation following its transplacental administration.

4.4.3 *Mechanistic considerations*

Dimethyl sulfate is a monofunctional alkylating agent that reacts with DNA through a bimolecular substitution (S_N2) reaction, forming a transition complex with strong nucleophiles, particularly base nitrogens such as the N7 position of guanine and the N3 position of adenine. It reacts far less extensively with weaker nucleophilic centres in DNA, such as O^6-position of guanine (Lawley, 1974). Thus, *N*7-methylguanine, and *N*3-methyladenine are the major DNA adducts formed when dimethyl sulfate reacts with DNA *in vitro* or *in vivo*, *O*^{6}-methylguanine being formed at very low levels (Singer & Grunberger, 1983). Of these adducts, only *O*^{6}-methylguanine is firmly established as directly mispairing, resulting in GC→AT transition mutations (Singer & Grunberger, 1983). Experiments conducted in mammalian cells *in vitro* (Newbold *et al.*, 1980; Connell & Medcalf, 1982; Natarajan *et al.*, 1984; Shiner *et al.*, 1988) and carcinogenicity studies *in vivo* (Lawley, 1984) suggest that S_N2 alkylating agents such as dimethyl sulfate are weak carcinogens because they yield low levels of mispairing adducts such as *O*^{6}-methylguanine, and that their cytotoxic, mutagenic and carcinogenic activities owe more to the indirect effects of depurination, DNA strand breakage and chromosomal damage. This is in contrast to S_N1 alkylating agents, such as *N*-methyl-*N*-nitrosourea, which produces relatively high levels of mispairing adducts such as *O*^{6}-methylguanine, induces high levels of gene mutations at low cytotoxicity, and is a potent carcinogen.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to dimethyl sulfate may occur during its manufacture and its use as a methylating agent.

5.2 Human carcinogenicity data

No epidemiological studies were available to the Working Group. A small number of cases of, mainly, bronchial carcinoma has been reported.

5.3 Animal carcinogenicity data

Dimethyl sulfate was tested for carcinogenicity in rats by inhalation, subcutaneous and intravenous injection, and following prenatal exposure. It produced local sarcomas and tumours of the nervous system.

5.4 Other relevant data

Dimethyl sulfate rapidly decomposes on contact with water, as a result of which it very rapidly disappears from the circulation of dosed rats.

It is corrosive or irritant to the skin, eyes and respiratory tract of exposed people, and may result in death caused by respiratory failure.

Dimethyl sulfate is embryotoxic to rats and causes malformations among surviving foetuses.

Workers exposed to dimethyl sulfate have developed chromosomal aberrations in their circulating lymphocytes. Dimethyl sulfate has been subjected to a broad range of in-vitro tests for genotoxic activity, in which positive results were consistently found without the need for exogenous metabolic activation systems. It has also consistently produced positive responses in the small number of in-vivo tests to which it has been subjected. It forms a variety of alkylated bases with DNA *in vitro* and the same alkylated bases are formed *in vivo*.

5.5 Evaluation

There is *inadequate evidence* for the carcinogenicity in humans of dimethyl sulfate.

There is *sufficient evidence* for the carcinogenicity in experimental animals of dimethyl sulfate.

Overall evaluation

Dimethyl sulfate is *probably carcinogenic to humans (Group 2A)*.

In making the overall evaluation, the Working Group took into consideration that dimethyl sulfate is a potent genotoxic chemical which can directly alkylate DNA both *in vitro* and *in vivo*.

6. References

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1,4-DIOXANE

Data were last reviewed in IARC (1976) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

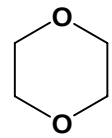
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 123-91-1
Chem. Abstr. Name: 1,4-Dioxane
IUPAC Systematic Name: *para*-Dioxane
Synonym: 1,4-Diethylene dioxide

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_4H_8O_2$ Relative molecular mass: 88.11

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Flammable liquid with faint pleasant odour (Budavari, 1996)
(*b*) *Boiling-point*: 101.1°C (American Conference of Governmental Industrial Hygienists, 1991)
(*c*) *Melting-point*: 11.8°C (American Conference of Governmental Industrial Hygienists, 1991)
(*d*) *Solubility*: Soluble in water and most organic solvents (Budavari, 1996)
(*e*) *Vapour pressure*: 4 Pa at 20°C; relative vapour density (air = 1), 3.30 (Verschueren, 1996)
(*f*) *Flash point*: 12.22°C , closed cup; 18.33°C, open cup (American Conference of Governmental Industrial Hygienists, 1991)
(*g*) *Explosive limits*: upper, 22%; lower, 2% by volume in air (American Conference of Governmental Industrial Hygienists, 1991)
(*h*) *Conversion factor*: $mg/m^3 = 3.60 \times ppm$

1.2 Production and use

Production of 1,4-dioxane in the United States in 1982 was approximately three thousand tonnes (United States National Library of Medicine, 1997).

1,4-Dioxane is used as a solvent in a wide range of organic products: lacquers; paints; varnishes; paint and varnish removers; wetting and dispersing agent in textile products, dye baths, and stain and printing compositions; cleaning and detergent preparations; cements; cosmetics; deodorants; fumigants; emulsions; and polishing compositions. It is also used as a stabilizer for chlorinated solvents (Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

No data were available to the Working Group.

1.3.2 *Environmental occurrence*

1,4-Dioxane has been detected in ambient air samples at low levels at several sites in the United States (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 90 mg/m^3 as the threshold limit value (TLV) for occupational exposures to 1,4-dioxane in the workplace air. Until 1981, the ACGIH TLV was 180 mg/m^3 (American Conference of Governmental Industrial Hygienists, 1991). Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for 1,4-dioxane in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

In a prospective mortality study of 165 workers who had been exposed to low concentrations of 1,4-dioxane since 1954, seven deaths had occurred in the manufacturing department by 1975, two of which were from cancer. Expected numbers, based on Texas mortality rates, were 4.9 and 0.9, respectively. In the processing department, five deaths were observed versus 4.9 expected, of which one was from cancer (0.8 expected) (Buffler *et al.*, 1978).

3. Studies of Cancer in Experimental Animals

1,4-Dioxane was tested in rats and guinea-pigs by oral administration: it produced malignant tumours of the nasal cavity and liver in rats and tumours of the liver and gallbladder in guinea-pigs. It was also active as a promoter in a two-stage skin carcino-

genesis study in mice. No carcinogenic effect was observed in one inhalation study in rats (IARC, 1976).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female Crj:BDF_1 mice [age unspecified] were administered 1,4-dioxane (purity, > 99%) at 0, 500, 2000 or 8000 mg/L (ppm) in the drinking-water for 104 weeks. Survival of the two high-dose female groups was reduced. 1,4-Dioxane increased the incidence of hepatocellular adenomas and carcinomas combined in males from 22/50 in controls, to 36/50 low-dose, 45/50 mid-dose and 44/50 high-dose [$p < 0.01$ for all comparisons, Fisher's exact test] and in females from 4/50 in controls, to 36/50 low-dose, 50/50 mid-dose and 47/50 high-dose [$p < 0.01$ for all comparisons, Fisher's exact test]. One nasal cavity tumour occurred in a high-dose female (Yamazaki *et al*., 1994).

3.1.2 *Rat*

Groups of 50 male and 50 female F344/DuCrj rats [age not given] were administered 1,4-dioxane (purity > 99%) at 0, 200, 1000 or 5000 mg/L (ppm) in the drinking-water for 104 weeks. Survival of exposed males and females was reduced. In males, combined hepatocellular adenoma and carcinoma occurred in 0/50 controls, 2/50 low-dose, 4/50 mid-dose and 38/50 high-dose animals [$p < 0.01$, Fisher's exact test]. In females, combined hepatocellular adenomas and carcinomas occurred in 1/50 controls, 0/50 low-dose, 5/50 mid-dose and 48/50 high-dose rats [$p < 0.01$, Fisher's exact test]. Mesotheliomas of the peritoneum were found in 28/50 high-dose males compared with 2/50 controls [$p < 0.01$, Fisher's exact test]. The incidence of subcutaneous fibromas and mammary fibroadenomas in high-dose males was greater than in the control group (12/50 and 4/40 versus 5/50 and 1/50, respectively). In females, nasal cavity tumours were found in 7/50 high-dose rats compared with 0/50 controls [$p < 0.05$, Fisher's exact test], and mammary adenomas were found in 16/50 high-dose rats compared with 6/50 controls [$p < 0.01$, Fisher's exact test] (Yamazaki *et al*., 1994).

3.2 Intraperitoneal injection

Mouse: Groups of 30 male A/J mice, six to eight weeks of age, were administered 1,4-dioxane [purity unspecified] by intraperitoneal injection three times per week for eight weeks for total doses of 0, 400, 1000 and 2000 mg/kg bw. The high dose increased the multiplicity of lung tumours to 0.97 per mouse ($p < 0.05$) compared with 0.28 per mouse in controls given vehicle alone (Maronpot *et al*., 1986).

In a mouse-lung adenoma assay, 1,4-dioxane produced a significant increase in the incidence of lung tumours in males given an intermediate intraperitoneal dose; no such increase was noted in males given a lower or higher intraperitoneal dose or in females given three intraperitoneal doses or in either males or females given 1,4-dioxane orally (Stoner *et al.*, 1986).

3.3 Administration with known carcinogens

Rat: Groups of 8–11 male Sprague-Dawley rats and 19 controls, weighing 200 g, were administered 1,4-dioxane (purity, 99.5%) by gavage once a day on five days per week for seven weeks at a dose of 0, 100 or 1000 mg/kg bw beginning five days after partial hepatectomy and injection of a single dose of 30 mg/kg bw *N*-nitrosodiethylamine (NDEA) to initiate hepatocarcinogenesis. The high dose increased the multiplicity of hepatic foci to 4.7 per cm^2 ($p < 0.01$) compared with 1.3 per cm^2 with NDEA initiation alone. In two other groups of rats, 100 or 1000 mg/kg 1,4-dioxane alone did not induce foci (Lundberg *et al*., 1987).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

The toxicology of 1,4-dioxane has been reviewed and evaluated comprehensively (DeRosa *et al*., 1996), including a full summary of its disposition in animals and humans. 1,4-Dioxane is rapidly absorbed and metabolized and does not accumulate in the body, but the saturation of metabolism at high doses is of toxicological relevance.

4.1.1 *Humans*

(*a*) *Inhalation exposure*

Young *et al*. (1977) exposed four volunteers to 50 ppm [180 mg/m^3] 1,4-dioxane vapour for 6 h. It was rapidly taken up, with plasma levels reaching a plateau after 3 h. The major metabolite, β-hydroxyethoxyacetic acid (HEAA), was detected during the exposure period. At the end of the exposure, plasma levels of 1,4-dioxane fell with a half-life of 59 min. HEAA plasma levels reached their peak 1 h after the end of the exposure and fell thereafter with a half-life of 48 min. The absorption rate of 1,4-dioxane under these conditions was 76.1 mg/h and the total dose was 5.4 mg/kg. The dominant route of elimination was oxidation to HEAA, which is rapidly cleared in the urine; 47% of the dose was excreted as HEAA during exposure and excretion was complete within 8 h of the end. The excretion half-life of HEAA was 2.7 h and its renal clearance was 121 mL/min, which indicates clearance by glomerular filtration, as creatinine clearance in these subjects was 124 mL/min. Renal clearance of 1,4-dioxane was 0.34 mL/min, compared with its metabolic clearance of 75 mL/min.

During workplace exposures of 1.6 ppm [5.8 mg/m^3] 1,4-dioxane for 7.5 h, at the end of the workday, the levels of HEAA were 118-fold those of 1,4-dioxane (urinary concentrations of 414 and 3.5 μmol/L, respectively), showing rapid and very extensive metabolism (Young *et al*., 1976).

(*b*) *Dermal absorption*

The penetration of 1,4-dioxane through human skin is poor. In-vitro studies show that 3.2% of an applied dose passes through excised skin under occlusion and only 0.3% when not occluded (ECETOC, 1983).

4.1.2 *Experimental systems*

Young *et al*. (1978) exposed rats by inhalation to 50 ppm [180 mg/m^3] 1,4-dioxane for 6 h, resulting in an estimated absorbed dose of 71.9 mg/kg which was recovered as 6.8 μg 1,4-dioxane and 21.3 mg HEAA in the 0–48 h urine. These data are consistent with quantitative absorption of 1,4-dioxane after inhalation. These authors also administered orally 10, 100 or 1000 mg/kg bw [^{14}C]1,4-dioxane to rats. In each case, absorption was complete within 24 h. Of the low dose, 99% was excreted in the urine within 24 h; this fell to 86% of the 100-mg/kg bw and 76% of the 1000 mg/kg bw dose within 72 h. This reduction in urinary excretion was compensated by exhalation as unchanged 1,4-dioxane at a rate of 0.43% at 10 mg/kg bw, 5% at 100 mg/kg bw and 25% at 1000 mg/kg bw.

The principal route of metabolism of dioxane is C-oxidation, giving a lactone which exists principally in the open-chain form of HEAA. The proportion detected as the lactone (1,4-dioxane-2-one) depends upon the analytical techniques used (Braun & Young, 1977; Woo *et al*., 1978). A small percentage of the dose (2–3%) is excreted as $^{14}CO_2$, presumably arising from β-oxidation of HEAA.

Young *et al*. (1978) also gave rats oral daily doses of 10 or 1000 mg/kg bw [^{14}C]1,4-dioxane for 17 days. Urine was collected for 20 days and 99% and 82% of the 10 and 1000 mg/kg doses were recovered, respectively, with 1% and 9% exhaled as dioxane and 4% and 7% as $^{14}CO_2$.

The single-dose data show that the formation of HEAA is saturated as the dose is increased, throwing emphasis upon alternate pathways of elimination. Comparison of the single- and repeated-dose data suggests that the conversion of 1,4-dioxane to HEAA is induced by repeated administration.

The saturation of the clearance of 1,4-dioxane as a function of dose was shown clearly after intravenous administration of 3, 10, 30, 100 and 1000 mg/kg bw to rats (Young *et al*., 1978). At 3 and 10 mg/kg, the elimination half-life of 1,4-dioxane was 1.1 h but, as the dose increased, this became progressively longer. The clearance decreased from 3.33 mL/min at 3 mg/kg bw to 0.25 mL/min at 100 mg/kg bw, this being due to decreased metabolic clearance. At 10 mg/kg bw, 5% of a dose of [^{14}C]1,4-dioxane was excreted unchanged in urine and expired air, while at 1000 mg/kg, excretion of unchanged 1,4-dioxane rose to 38%. The major metabolite HEAA accounted for 92% of the 10 mg/kg dose and 60% at 1000 mg/kg bw.

These findings are complemented by those of Woo *et al*. (1977a), who gave rats 1000, 2000, 3000 or 4000 mg/kg bw [^{14}C]1,4-dioxane by intraperitoneal injection and found that saturation of the formation of HEAA occurred at about 3000 mg/kg.

The metabolism of 1,4-dioxane to HEAA has the characteristics of a mixed-function oxidase-mediated reaction (Woo *et al*., 1977b, 1978) and the administration of 1,4-

dioxane to rats resulted in increased hepatic microsomal aniline hydroxylase and aminopyrine *N*-demethylase activities (Dietz *et al*., 1982).

4.2 Toxic effects

4.2.1 *Humans*

In a cohort of workers exposed to various concentrations of 1,4-dioxane at the workplace (0.02–47.8 mg/m^3), Thiess *et al*. (1976) observed no clinical effects or changes in mortality related to the exposure.

4.2.2 *Experimental systems*

In a study by Kociba *et al*. (1974), rats of each sex received 0.01, 0.1 or 1% 1,4-dioxane in the drinking-water for up to 716 days. At 16 months, about 50% of the 1% dose group survived. Histopathological examination of the animals revealed degenerative and necrotic alterations in the liver parenchyma and in renal tubules. These changes were observed to a lower extent in the 0.1% dose group. No increased DNA repair was found in the liver or in nasoturbinate or maxilloturbinate nasal epithelial cells isolated from male Fischer 344 rats receiving 2% or 1% 1,4-dioxane in the drinking-water. The dose of 1% 1,4-dioxane in the drinking-water for five days did not increase relative liver weight or hepatic palmitoyl coenzyme A-reductase activity (Goldsworthy *et al*., 1991). While a single dose of 1000 mg/kg bw 1,4-dioxane given by gavage did not enhance hepatic DNA synthesis, administration in the drinking-water for two weeks led to an approximately two-fold increase in the hepatic labelling index. Similarly, Stott *et al*. (1981) reported a 1.5-fold increase in hepatic DNA synthesis, and a minimal centrilobular hepatocellular swelling in male Sprague-Dawley rats given 1000 mg/kg bw 1,4-dioxane daily by gavage over 11 weeks; the relative liver weight was enhanced in these animals, while gavage of 10 mg/kg bw did not result in such effects in the liver. In female Sprague-Dawley rats treated orally with 850, 2550 or 4200 mg/kg bw 1,4-dioxane, 21 and 4 h before killing, Kitchin and Brown (1990) found a marked increase in hepatic ornithine decarboxylase activity, whereas alanine aminotransferase activity and the level of reduced glutathione in the liver were unchanged. Doses of 2550 and 4200 mg/kg bw resulted in significant induction of total hepatic cytochrome P450.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

In Sprague-Dawley rats administered 1,4-dioxane by gavage (0, 0.25, 0.5 and 1 mL/kg bw per day) on days 6–15 of gestation, no effect on implantation number, number of live fetuses, postimplantation loss or the rate of malformations was found (Giavini *et al*., 1985). At 1 mL/kg bw per day, embryotoxicity and slight maternal toxicity, manifested by reduced weight gain, were observed.

4.4 Genetic and related effects

The toxicity (including genotoxicity) of 1,4-dioxane has been reviewed (DeRosa *et al*., 1996).

4.4.1 *Humans*

In lymphocytes obtained from six workers employed in 1,4-dioxane production and exposed to unspecified airborne levels of the compound for 6–15 years, no increase in chromosomal aberrations was found relative to that observed in an equal number of controls (Thiess *et al.*, 1996).

4.4.2 *Experimental systems* (see Table 1 for references)

1,4-Dioxane with or without metabolic activation did not induce differential DNA repair in *Escherichia coli* K-12 *uvrB/rec A* and was not mutagenic in *Salmonella typhimurium* or in L5178Y mouse lymphoma cells. In Chinese hamster ovary CHO cells, it did not cause chromosomal aberrations, although it did cause a slight increase in sister chromatid exchange in the absence of metabolic activation. It has also been reported to cause morphological transformation of BALB/c 3T3 mouse cells.

Oral administration of 1,4-dioxane to rats caused DNA strand breaks in liver cells. However, no covalent DNA binding was detected in rat liver. No induction of unscheduled DNA synthesis was observed in rat hepatocytes after either in-vivo treatment or in-vitro cell treatment with 1,4-dioxane, even when the animals had previously been exposed to 1% 1,4-dioxane for one week. In the same study, no induction of unscheduled DNA synthesis in rat nasal epithelial cells was observed.

Of three studies on the induction of bone-marrow micronuclei, one was negative for male C57BL/6 and CBA mice, one was inconclusive for male $B6C3F_1$ mice, while the third gave a clear positive result for male and female C57BL/6 mice and a negative result for male BALB/c mice, suggesting overall possible weak, strain-specific clastogenic activity.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to 1,4-dioxane may occur during its manufacture and its use as a solvent in a wide range of organic products. It has been detected in ambient air.

5.2 Human carcinogenicity data

Deaths from cancer were not elevated in a single, small prospective study of workers exposed to low concentrations of dioxane.

5.3 Animal carcinogenicity data

1,4-Dioxane was tested for carcinogenicity by oral administration in mice, rats and guinea-pigs. It produced an increased incidence of hepatocellular adenomas and

Table 1. Genetic and related effects of 1,4-dioxane

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ERD, *Escherichia coli* K12 *uvrB/recA* strains, differential toxicity	–	–	101315	Hellmér & Bolcsfoldi (1992)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	51500	Stott *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	Haworth *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Khudoley *et al*. (1987)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	–	–	NG	Khudoley *et al*. (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	51500	Stott *et al*. (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	Haworth *et al*. (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	NG	Khudoley *et al*. (1987)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	51500	Stott *et al*. (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Khudoley *et al*. (1987)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	51500	Stott *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	51500	Stott *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Khudoley *et al*. (1987)
SCN, *Saccharomyces cerevisiae* D61M, aneuploidy	–	NT	4.75% in air	Zimmermann *et al*. (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		35000 ppm feed	Yoon *et al*. (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		50000 ppm inj	Yoon *et al*. (1985)

Table 1 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DIA, DNA strand breaks, cross-links or related damage, rat hepatocytes *in vitro*	+	NT	26.4	Sina *et al*. (1983)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–[c]		88	Goldsworthy *et al*. (1991)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	–	5000	McGregor *et al*. (1991)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	(+)	–	10520	Galloway *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	10520	Galloway *et al*. (1987)
TBM, Cell transformation, BALB/c 3T3 mouse cells	+	NT	2000	Sheu *et al*. (1988)
DVA, DNA strand breaks, Sprague-Dawley rat liver cells *in vivo*	+		2550 po × 1	Kitchin & Brown (1990)
UPR, Unscheduled DNA synthesis, male Sprague-Dawley rat hepatocytes *in vivo*	–		1000 po × 1	Goldsworthy *et al*. (1991)
UVR, Unscheduled DNA synthesis, male Fischer 344 rat nasal epithelial cells *in vivo*	–[c]		1000 po × 1	Goldsworthy *et al*. (1991)
MVM, Micronucleus test, male and female C57BL/6 mouse bone-marrow cells *in vivo*	+		900 po × 1	Mirkova (1994)
MVM, Micronucleus test, male BALB/c mouse bone-marrow cells *in vivo*	–		5000 po × 1	Mirkova (1994)
MVM, Micronucleus test, male $B6C3F_1$ mouse bone-marrow cells *in vivo*	?		2000 ip × 3	McFee *et al*. (1994)

Table 1 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVM, Micronucleus test, male C57BL/6 mouse bone-marrow cells *in vivo*	–		3600 po × 1	Tinwell & Ashby (1994)
MVM, Micronucleus test, male CBA mouse bone-marrow cells *in vivo*	–		1800 po × 1	Tinwell & Ashby (1994)
BVD, Binding (covalent) to DNA, rat liver cells *in vivo*	–		1000 po × 1	Stott *et al.* (1981)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; inj, injection; po, oral; ip, intraperitoneal

[c] With or without pretreatment with 1% dioxane in drinking-water for one week

carcinomas in mice, tumours of the nasal cavity, liver subcutaneous tissues, mammary gland and peritoneal mesotheliomas in rats and tumours of the liver and gall-bladder in guinea-pigs. No increase in tumours was seen in rats following inhalation exposure. In the mouse-lung adenoma assay, intraperitoneal injection of 1,4-dioxane increased the incidence of lung tumours in males; no such effect was seen following oral administration. In a two-stage liver foci assay in rats, 1,4-dioxane showed promoting activity.

5.4 Other relevant data

1,4-Dioxane is rapidly absorbed upon inhalation or after oral administration, but its penetration of skin is poor. The major metabolite is β-hydroxyethoxyacetic acid, which is rapidly excreted. In rats, the elimination of 1,4-dioxane and its metabolites is progressively delayed as doses are increased, indicating saturation of metabolism.

No clinical signs or changes in mortality were found in a cohort of exposed workers. In rats, 1,4-dioxane produces degenerative and necrotic changes in liver and renal tubules. High doses can significantly increase the total hepatic cytochrome P450 content.

No reproductive effects of 1,4-dioxane exposure of rats have been reported.

Most of the broad of tests for genotoxic activity have produced negative results, but positive results were obtained in a cell transformation assay and conflicting results were obtained in mouse bone-marrow cell tests for micronucleus induction.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of 1,4-dioxane.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,4-dioxane.

Overall evaluation

1,4-Dioxane is *possibly carcinogenic to humans (Group 2B)*.

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EPICHLOROHYDRIN

Data were last reviewed in IARC (1976) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

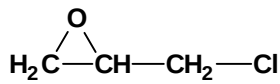
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 106-89-8
Chem. Abstr. Name: (Chloromethyl)oxirane
IUPAC Systematic Name: 1-Chloro-2,3-epoxypropane
Synonym: Chloropropylene oxide

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_3H_5ClO Relative molecular mass: 92.53

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid (Verschueren, 1996)
(*b*) *Boiling-point*: 117°C (Verschueren, 1996)
(*c*) *Melting-point*: –48°C (Verschueren, 1996); –25°C (Lewis, 1993; Budavari, 1996)
(*d*) *Solubility:* Insoluble in water; miscible with ethanol, diethyl ether, chloroform, trichloroethylene and carbon tetrachloride; immiscible with petroleum hydrocarbons (Budavari, 1996)
(*e*) *Vapour pressure:* 1.6 kPa at 20°C; relative vapour density (air = 1), 3.3 (Verschueren, 1996)
(*f*) *Flash-point:* 40°C, open cup (Budavari, 1996)
(*g*) *Explosive limits:* upper, 21.0%; lower, 3.8% (American Conference of Governmental Industrial Hygienists, 1991)
(*h*) *Conversion factor:* $mg/m^3 = 3.78 \times ppm$

1.2 Production and use

Total world production figures for epichlorohydrin are not available. In the United States, production increased from 156 thousand tonnes in 1973 to 250 thousand tonnes in 1975 and 213 thousand tonnes in 1978. Epichlorohydrin was also produced in Czechoslovakia, France, Germany, the Netherlands and the USSR (WHO, 1984).

Epichlorohydrin is a major raw material for epoxy and phenoxy resins, and is used in the manufacture of glycerine, in curing propylene-based rubbers, as a solvent for cellulose esters and ethers, and in resins with high wet-strength for the paper industry (Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1990–93 CAREX database for 15 countries of the European Union (Kauppinen *et al.*, 1998) and the 1981–83 National Occupational Exposure Survey (NOES) in the United States (NOES, 1997), approximately 25 000 workers in Europe and as many as 80 000 workers in the United States were potentially exposed to epichlorohydrin (see General Remarks). Occupational exposures to epichlorohydrin may occur in its use as a solvent and in resin production and use, the manufacture of glycerine and use of propylene-based rubbers.

1.3.2 *Environmental occurrence*

Epichlorohydrin may be released to the atmosphere and in wastewater during its production and use in manufacture of epoxy resins, glycerine and other chemicals and other uses. It has been detected at low levels in wastewater, groundwater and ambient water samples (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 1.9 mg/m^3 as the 8-h time-weighted average threshold limit value for occupational exposures to epichlorohydrin in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

The World Health Organization has established a provisional international drinking-water guideline for epichlorohydrin of 0.4 μg/L (WHO, 1993).

2. Studies of Cancer in Humans

2.1 Industry-based studies

Delzell *et al*. (1989) conducted a cohort study of workers at a dye and resin manufacturing plant. The full cohort consisted of 2642 male workers who had been employed at the facility for at least six months between 1952 and 1985. The study follow-up was from 1952 to 1985 and was 94% complete; 106 cancer deaths were observed (97 expected).

Seven cancers were observed (7.3 expected) among 230 workers in the plastics and additives production area where there was potential for exposure to epichlorohydrin. An excess of lung cancer was observed among the 44 workers who had been employed in the production of epichlorohydrin, which had been manufactured at the plant between 1961 and 1965 (levels of exposure not reported) (standardized mortality ratio (SMR), [4.4]; 4 observed versus 0.91 expected; $p = 0.03$).

Tsai *et al.* (1996) reported on a small cohort of workers in the United States who were potentially exposed to epichlorohydrin and isopropanol. Enterline (1982) and Enterline *et al.* (1990) had previously reported on this cohort, which consisted of 863 workers employed at two chemical manufacturing facilities between 1948 and 1965. Exposure was classified by a panel of industrial hygienists and current and former employees as nil, light, moderate or heavy. Exposures during 'early production periods' were estimated to be 10–20 ppm [38–76 mg/m^3]. Results from the latest follow-up were reported for 1960–93 with comparisons made with local county mortality rates. There were 175 deaths (SMR, 0.6; 95% confidence interval (CI), 0.5–0.7) and 60 cancer deaths observed (SMR, 0.8; 95% CI, 0.6–1.0). A number greater than expected of cancers of the prostate (SMR, 2.3; 95% CI, 1.0–4.5; $n = 8$) and malignant melanomas (SMR, 3.2; 95% CI, 0.7–9.4; $n = 3$) were observed among workers at least 20 years after first exposure, but the relative risks did not vary with estimated level of exposure. The SMR for lung cancer was 0.7 (95% CI, 0.5–1.1; 23 cases) in the total population and did not increase with level of exposure or time since first exposure.

Olsen *et al*. (1994) reported on the results of a retrospective cohort mortality study of workers in the United States with potential exposure to epichlorohydrin and allyl chloride (see this volume). The cohort consisted of 1064 men employed in the epoxy resin, glycerine and allyl chloride/epichlorohydrin production areas of a large chemical facility between 1957 and 1986. Follow-up was carried out until 1989. Mortality was compared with national rates and company rates for other facilities. Average exposures to epichlorohydrin were estimated to be generally below 1 ppm [3.8 mg/m^3] in the epoxy resin area, in the allyl chloride/epichlorohydrin area and, after 1970, in the glycerine area. Exposures to epichlorohydrin were estimated to be between 1 and 5 ppm [3.8 and 18.9 mg/m^3] in the glycerine area before 1970 and occasionally in some jobs in the allyl chloride/epichlorohydrin area, although respiratory protection may have been worn by these workers. There were 66 deaths (SMR, 0.8; 95% CI, 0.6–1.0). Ten cancers were observed (SMR, 0.5; 95% CI, 0.2–0.9, compared with national rates) in the entire cohort and no associations between site-specific cancer risks and exposure to epichlorohydrin were observed.

Nested case–control studies for lung (Barbone *et al*., 1992) and central nervous system (Barbone *et al*., 1994) neoplasms were conducted using the full cohort of dye and resin manufacturing workers reported on by Delzell *et al*. (1989). Exposure was assessed on an ordinal scale based on job titles, work areas, and potential for contact. When the work histories of 51 lung cancer cases were compared with those of 102 controls matched for year of birth, an association was observed with potential epichlorohydrin

exposure (odds ratio, 1.7; 95% CI, 0.7–4.1) after adjustment for smoking. However, no association was observed with duration or cumulative level of exposure. For 11 central nervous system tumour cases compared with 44 similarly matched controls, an association was observed with potential exposure to epichlorohydrin (odds ratio, 4.2; 95% CI, 0.7–26) and the magnitude of this association increased with both duration of exposure (p = 0.11 for trend test) and cumulative level of exposure (p = 0.08 for trend test). Two of the four epichlorohydrin-exposed central nervous system tumour cases had meningiomas.

Bond *et al*. (1986) conducted a nested case–control study of lung cancer among a cohort of 19 608 male chemical workers in the United States (Bond *et al*., 1985). Further details of the study are reported in Section 2.2 of the monograph on carbon tetrachloride in this volume. Ever having been exposed to epichlorohydrin was associated with a decreased risk of lung cancer (odds ratio, 0.3; 95% CI, 0.1–0.9; 5 exposed cases).

3. Studies of Cancer in Experimental Animals

Epichlorohydrin was tested for carcinogenicity in mice by subcutaneous injection: it produced local sarcomas. It was active as an initiator in a two-stage carcinogenesis study in mice (IARC, 1976).

3.1 Oral administration

Rat: Groups of 18 male outbred Wistar rats, six weeks of age, were administered 0, 375, 750 or 1500 mg/L (ppm) epichlorohydrin [purity unspecified] in the drinking-water for 81 weeks, at which time the experiment was terminated. All rats were necropsied and tissues examined histologically. Forestomach lesions ranging from hyperplasia or papilloma to carcinoma occurred in treated rats: hyperplasia, 0/10, 7/9, 9/10 and 12/12; papilloma, 0/10, 0/9, 1/10 and 7/12; carcinoma, 0/10, 0/9, 1/10 and 2/12 in the control, low-dose, mid-dose and high-dose groups, respectively. Tumours at other sites were not reported (Konishi *et al*., 1980).

Groups of 50 female weanling Wistar rats were administered 0, 2 or 10 mg/kg bw epichlorohydrin (purity, 99.5%) daily by gavage on five days per week for two years. All surviving animals were killed. The incidence of forestomach hyperplasia, papilloma and carcinoma was increased in both sexes (Table 1). The incidence of tumours at other sites was not increased (Wester *et al*., 1985).

3.2 Inhalation exposure

Rat: Groups of 100 male Sprague-Dawley rats, eight weeks of age, were exposed by whole-body inhalation to 0, 10 or 30 ppm (0, 38 or 113 mg/m^3) epichlorohydrin (99% pure) for 6 h per day on five days per week for lifetime. Two further groups of 100 and 40 male rats were exposed to 100 ppm (380 mg/m^3) for 6 h per day on 30 days followed by observation for lifetime. A group of 100 male controls was sham-exposed and a group

Table 1. Incidence of forestomach lesions in Wistar rats treated with epichlorohydrin

Sex	Lesion	Control	Dose of epichlorohydrin	
			2 mg/kg bw	10 mg/kg bw
Males	Hyperplasia	5/50	24/40	6/49
	Papilloma	1/50	6/49	4/49
	Carcinoma		6/49	35/49
Females	Hyperplasia	3/47	12/44	7/39
	Papilloma	2/47	3/44	
	Carcinoma		2/44	24/39

From Wester *et al*. (1985)

of 50 controls was untreated. In rats exposed to 10 ppm epichlorohydrin, no neoplastic changes were reported. In the 30-ppm group, one rat had a nasal papilloma and one a squamous-cell carcinoma of the nasal cavity after 402 and 752 days, respectively. In rats exposed 30 times to 100 ppm and observed for lifespan, 17 rats developed 15 squamous-cell carcinomas and two papillomas of the nasal epithelium between 330 and 933 days from the start of exposure. One bronchial papilloma was observed at day 583 after the start of exposure. Four exposed rats had pituitary adenomas and one rat had a squamous-cell carcinoma of the forestomach. No tumour of these types was found in controls (Laskin *et al*., 1980).

3.3 Intraperitoneal administration

Mouse: In a strain A lung adenoma assay, intraperitoneal injection of total doses of 20, 50 or 100 mg/kg bw epichlorohydrin given three times per week for eight weeks significantly increased the number of lung tumours per mouse in males treated with the highest dose (0.80 ± 0.68, compared with 0.47 ± 0.63 in controls; $p < 0.01$) but not in other groups (Stoner *et al*., 1986).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Incubation of epichlorohydrin in the presence of human bronchial and lung parenchymal tissues led to a decrease in its mutagenicity, suggesting rapid inactivation (Petruzzelli *et al*., 1989), probably via thiol binding (De Flora *et al*., 1984).

Recently, a biomonitoring method for epichlorohydrin by measuring *N*-(2,3-dihydroxypropyl)valine in haemoglobin has been developed. The adduct level is increased in cigarette smokers. The same adduct can be detected in rats after intraperitoneal administration of 40 mg/kg bw epichlorohydrin (Landin *et al*., 1996).

4.1.2 *Experimental systems*

Early toxicokinetic studies were summarized by Šrám *et al*. (1981). In rats, epichlorohydrin is rapidly absorbed via oral or inhalation routes and practically all of the compound is eliminated via urine as metabolites or via lungs as CO_2.

After an oral dose of 6 mg/kg bw to rats, approximately 38% of the dose was exhaled as CO_2, 50% was excreted as metabolites in the urine and 3% was present in faeces (Gingell *et al*., 1985). Concentrations were highest in liver, kidney and forestomach. The initial metabolic reactions are conjugation of the epoxide with glutathione, which is probably a chemical, not enzymatic, reaction, and hydration of the epoxide by epoxide hydrolase. The major metabolites in urine are *N*-acetyl-*S*-(3-chloro-2-hydroxypropyl)-L-cysteine (36% of the dose) and 3-chloro-1,2-propanediol (α-chlorohydrin) (4%).

The absorption and elimination of epichlorohydrin in mice are rapid after oral administration. The diol metabolite, 3-chloro-1,2-propanediol, was detected in plasma (Rossi *et al*., 1983a).

4.2 Toxic effects

4.2.1 *Humans*

Fomin (1966) found that exposure to epichlorohydrin at a concentration of 0.3 mg/m^3, which represents a threshold value for the smell of that substance for the most sensitive human subjects, produced changes in the electroencephalogram pattern, whereas a concentration of 0.2 mg/m^3 was inactive.

Several cases of severe skin burns have resulted from local contact with epichlorohydrin (Hine & Rowe, 1963). Six workers with occupational exposure to epichlorohydrin, four of whom worked in an epoxy resin plant, were diagnosed with contact dermatitis, apparently due to epichlorohydrin (van Joost, 1988). A 46-year-old worker in a pharmaceutical plant quickly developed pronounced swelling and erythema of the face, dorsum of the hands and neck after 11 months of epichlorohydrin exposure, which regressed completely after a two-week absence from work (Rebandel & Rudzki, 1990). There was a recurrence of the skin changes three days after returning to work. The patient was also exposed to other reagents in the process of propranolol and oxprenolol synthesis. One case of severe epichlorohydrin poisoning occurred in a 39-year-old laboratory assistant; initial irritation of the eyes and throat was followed by chronic asthmatic bronchitis; successive biopsies established a high degree of fatty infiltration of the liver (Schultz, 1964).

Several hours after having been exposed for about 30 min to fumes of epichlorohydrin, a 53-year-old worker complained of burning of the nose and throat, coughing, chest congestion, running nose, eye tenderness and headache, followed by nausea (United States National Institute for Occupational Safety and Health, 1976).

Studies of effects of co-exposures to epichlorohydrin and allyl chloride on heart disease mortality are described in the monograph on allyl chloride (see this volume).

4.2.2 *Experimental systems*

The intraperitoneal LD_{50} values of epichlorohydrin range from 120 to 170 mg/kg bw for rats, mice, guinea-pigs and rabbits. Oral LD_{50} values in mice and rats are 240 and 260 mg/kg bw, respectively. The LD_{50} following oral percutaneous administration to rabbits is 760 mg/kg bw. The median time to death of mice inhaling an air–vapour mixture containing 7200 mg/m^3 epichlorohydrin was 9 min (Lawrence *et al*., 1972).

Epichlorohydrin can cause central nervous depression and irritation of the respiratory tract; death is generally due to depression of the respiratory centre (Hine & Rowe, 1963). Nephrotoxicity is a cumulative effect of epichlorohydrin poisoning (Hine & Rowe, 1963; Pallade *et al*., 1968); renal insufficiency occurred within 24–48 h in approximately 80% of rats that had been given 125 mg/kg bw of the compound (Pallade *et al*., 1968). Epichlorohydrin produces extreme irritation when tested intradermally, dermally or intraocularly in rabbits (Lawrence *et al*., 1972). It caused skin sensitization in 60% (9/15) of female albino guinea-pigs tested using a 24-h occluded patch test with a 1.0% concentration in ethanol applied two weeks after a sensitivity induction protocol that consisted of three intradermal injections (5% w/v in ethanol) and one topical application using a 48-h occluded patch (5% w/v in ethanol) (Thorgeirsson & Fregert, 1977).

In a 12-week, subacute toxicity test in rats given intraperitoneal injections of epichlorohydrin, treatment led to a dose-related decrease in haemoglobin values; an increase in segmented neutrophils was seen with doses of 56 mg/kg bw and a reduction in the proportion of lymphocytes occurred at doses of 22 and 56 mg/kg bw (Lawrence *et al*., 1972). An increased leukocyte count was observed in animals exposed chronically to vapours of epichlorohydrin in air at concentrations of 2 mg/m^3 (Fomin, 1966). The maximum tolerated dose in a 13-week subacute study in rats following oral administration of epichlorohydrin was 45 mg/kg bw per day (Oser *et al*., 1975).

Daniel *et al*. (1996) treated adult male and female Sprague-Dawley rats with epichlorohydrin by gavage at dose levels of 3, 7, 19 and 46 mg/kg bw per day for 10 consecutive days and dose levels of 1, 5 and 25 mg/kg bw per day for 90 days. Although mortality was not affected by treatment, other adverse effects were observed. Significant decreases in both final mean body weight and total body weight gain were observed for both sexes at the highest dose level (46 mg/kg bw/day) in the 10-day dosing study; however, this was not observed in the 90-day study. Significant increases in relative kidney weights were seen at the two highest doses (19 and 46 mg/kg bw/day) for both sexes at the end of the 10-day dosing study and in the high-dose group (25 mg/kg bw/day) of each sex at the end of the 90-day study. Relative liver weights were significantly increased in the female high-dose group (46 mg/kg bw/day) and in the two highest-dose groups (19 and 46 mg/kg bw/day) for males in the 10-day dosing study. Increased relative liver weights were also observed in the highest-dose group of each sex at the end of the 90-day dosing study. In addition, relative testis weights were

increased in males at the highest dose in the 10-day study. All other relative organ weights were unchanged in both sexes relative to controls. Significant decreases in erythrocyte count, haemoglobin and haematocrit levels were found in the male high-dose group after 10 and 90 days of epichlorohydrin dosing. In both sexes and in both the 10- and 90-day gavage studies, induction of dose-related lesions of the forestomach was observed. Histopathological examination revealed a range of inflammatory and epithelial alterations in both sexes. The most pronounced effect was a dose-related increase in mucosal hyperplasia and hyperkeratosis. The authors suggested that the lowest observable adverse effect level (LOAEL) for oral exposure for both sexes of Sprague-Dawley rats to epichlorohydrin is 3 mg/kg bw per day for 10 days and 1 mg/kg bw per day for a 90-day oral exposure.

Groups of 20 male and 20 female B6C3F_1 mice, Fischer 344 rats and Sprague-Dawley rats were exposed for 6 h per day on five days per week during a 90-day period to 0, 5, 25 or 50 ppm [0, 19, 95 or 189 mg/m^3] epichlorohydrin vapour. The following clinical signs were evaluated: body weight, haematology, urine analysis, blood serum urea nitrogen, serum alkaline phosphatase activity, serum glutamic pyruvic transaminase activity, serum glutamic oxaloacetic transaminase activity, serum glucose and gross pathology. In addition to histological examination, organ weights and organ:body weight ratios of brain, heart, liver, kidneys, testes, spleen and thymus were determined; the nasal turbinates were the most sensitive organ. Dose-related microscopic changes were seen in the nasal turbinates at 25 and 50 ppm. Other parameters evaluated showed minimal treatment-related effects at the 50 ppm level. No treatment-related effect was detected at the 5 ppm level of exposure (Quast *et al.*, 1979).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

Venable *et al*. (1980) studied the fertility status of male employees engaged in the manufacture of glycerine (exposure to epichlorohydrin, allyl chloride and 1,3-dichloropropene). This study included 64 exposed workers and 63 control volunteers. Reproductive medical histories were taken, and laboratory studies included blood hormone analysis and analysis of semen specimens (volume, viscosity, percentage progressive sperm, percentage motile sperm, sperm count (MM/cc), percentage viable sperm and percentage normal sperm forms). The results showed no detrimental effect on fertility due to exposure to epichlorohydrin. Milby and Whorton (1980) also reported no sperm-count suppression among workers exposed to epichlorohydrin, in contrast to parallel observations on workers exposed to 1,2-dibromo-3-chloropropane.

4.3.2 *Experimental systems*

Repeated oral administration of 15 mg/kg bw epichlorohydrin produced reversible infertility in male rats within seven days: fertility was restored after dosing had been discontinued for approximately one week (Hahn, 1970). In male mice given single intraperitoneal doses of 5, 10 or 20 mg/kg bw epichlorohydrin, single oral doses of 20 or

40 mg/kg bw, five daily intraperitoneal doses of 1 and 5 mg/kg bw or five daily oral doses of 4 and 20 mg/kg bw, fertility was reduced in some groups but no dose–response relationship was observed (Šrám *et al*., 1976). Toth *et al*. (1989) found that male Long-Evans rats exposed by gavage to 50 mg/kg bw per day for 21 days (a period covering development of the late-stage spermatids and their transit through the cauda epididymis) had totally impaired fertility. Fertility was not evaluated at lower doses. This effect was said to be consistent with the spermatozoal metabolic lesions reported for α-chlorohydrin, a metabolite of epichlorohydrin.

Marks *et al*. (1982) evaluated the teratogenic effect of epichlorohydrin administered by gavage to CD-1 mice and CD rats during days 6–15 of gestation. Rats were given doses of 40, 80 and 160 mg/kg bw per day and mice were given 80, 120 and 160 mg/kg bw per day. Epichlorohydrin caused a significant reduction in the weight gain of pregnant rats at 80 mg/kg per day compared with the control group. However, there was no evidence of teratogenicity in the rat fetuses even at the highest dose level (160 mg/kg bw/day), which caused the death of some of the treated dams. Epichlorohydrin did not produce a significant increase in the average percentage of malformed mouse fetuses even at 160 mg/kg bw per day, a dose that killed three of 32 treated dams. The highest two doses in the mouse study (120 and 160 mg/kg bw per day) caused a significant ($p < 0.05$) reduction in average fetal weight compared with controls.

4.4 Genetic and related effects

4.4.1 *Humans*

Chromosomal aberrations were observed in three studies of lymphocytes of workers occupationally exposed to concentrations of epichlorohydrin ranging from 0.5 to 5.0 mg/m^3 (Kučerová *et al.*, 1977; Šrám *et al.*, 1980) and in one other study in which epichlorohydrin concentrations were not given (Picciano, 1979).

4.4.2 *Experimental systems* (see Table 2 for references)

The genetic and related effects of epichlorohydrin have been reviewed (Giri, 1997).

Epichlorohydrin induced DNA damage in *Escherichia coli* and *Bacillus subtilis*. It was mutagenic to *Salmonella typhimurium* and *E. coli* in the presence and absence of exogenous metabolic activation. Epichlorohydrin induced gene mutation in *Krebsiella pneumoniae* without exogenous metabolic activation. It induced DNA damage, gene conversion, recombination, aneuploidy and mutation in *Saccharomyces cerevisiae* and gene mutations in *Schizosaccharomyces pombe* and *Neurospora crassa*. It was mutagenic in the *Drosophila melanogaster* sex-linked recessive lethal mutation assay.

Epichlorohydrin induced DNA single-strand breaks but not unscheduled DNA synthesis in mammalian cell cultures. It induced gene mutations in mouse lymphoma L5178Y cells and gene mutations, sister chromatid exchanges and chromosomal aberrations in Chinese hamster cells *in vitro*.

Diphtheria toxin-resistant mutants were observed in human epithelial type EUE cells but not in human lung fibroblasts exposed to epichlorohydrin *in vitro*. Epichlorohydrin

Table 2. Genetic and related effects of epichlorohydrin

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
PRB, Induction of SOS response in *S. typhimurium* TA1535/pSK1002	+	NT	60	Nakamura *et al*. (1987)
ECD, *Escherichia coli pol A*, differential toxicity	+	+	250	Tweats (1981)
ECL, *Escherichia coli pol A*, differential toxicity	+	NT	10	Rosenkranz & Leifer (1980)
BSD, *Bacillus subtilis rec* strains, differential toxicity	–	NT	10500	Elmore *et al*. (1976)
BSD, *Bacillus subtilis rec* strains, differential toxicity	–	(+)	92300	Laumbach *et al*. (1977)
BSD, *Bacillus subtilis rec* strains, differential toxicity	+	–	0.1	Kada *et al*. (1980)
SAF, *Salmonella typhimurium*, forward mutation	NT	+	1000	Skopek *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	92.5	Elmore *et al*. (1976)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	100	Šrám *et al*. (1976)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	220	Laumbach *et al*. (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	13.9	Andersen *et al*. (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	0.04	Bridges (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	0.03	Simmon (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	250	Wade *et al*. (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	462	Bartsch *et al*. (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	27.8	Hemminki & Falck (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	46.2	Stolzenberg & Hine (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	25	Connor *et al*. (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	90	Eder *et al*. (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	25	Martire *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	31	Nagao & Takahashi (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	0.5	Richold & Jones (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	–	9.25	Voogd *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	46	Bartsch *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	+	200	Imamura *et al*. (1983)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	250	Hughes *et al*. (1987)
SA2, *Salmonella typhimurium* TA102, reverse mutation	+	+	250	Hughes *et al*. (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	11.7	Andersen *et al*. (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	0.06	Biles *et al*. (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	0.2	Bridges (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	250	Wade *et al*. (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	46.2	Stolzenberg & Hine (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	25	Rowland & Severn (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	5	Simmon & Shepherd (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	–	250	Richold & Jones (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	46	Bartsch *et al*. (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	250	De Flora *et al*. (1984)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	(+)	–	250	Richold & Jones (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	(+)	–	250	Richold & Jones (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	4625	Stolzenberg & Hine (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	50	Richold & Jones (1981)
SAS, *Salmonella typhimurium* G46, reverse mutation	+	NT	1000	Šrám *et al*. (1976)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	+	NT	27.8	Hemminki & Falck (1979)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	+	NT	NG	Hemminki *et al*. (1980)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	+	+	10	Gatehouse (1981)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	+	+	120	Matsushima *et al*. (1981)
EC2, *Escherichia coli* WP2, reverse mutation	+	+	295	Matsushima *et al*. (1981)
ECR, *Escherichia coli* WP2 *uvrA*/pkM101, reverse mutation	+	+	120	Matsushima *et al*. (1981)
ECR, *Escherichia coli* 3431M31 *uvrB*, reverse mutation	+	+	200	Mohn *et al*. (1981)
KPF, *Klebsiella pneumoniae*, forward mutation	+	–	18	Voogd *et al*. (1981)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
KPF, *Klebsiella pneumoniae*, forward mutation	+	NT	9	Knaap *et al*. (1982)
SSD, *Saccharomyces cerevisiae* rad strains, differential toxicity	+	+	100	Sharp & Parry (1981a)
SCG, *Saccharomyces cerevisiae* D7, gene conversion	+	NT	6010	Vashishat *et al*. (1980)
SCG, *Saccharomyces cerevisiae* D4, gene conversion	–	–	166	Jagannath *et al*. (1981)
SCG, *Saccharomyces cerevisiae* JD1, gene conversion	+	NT	50	Sharp & Parry (1981b)
SCG, *Saccharomyces cerevisiae* D7, gene conversion	+	NT	100	Zimmermann & Scheel (1981)
SCH, *Saccharomyces cerevisiae* D7, homozygosis	+	NT	6010	Vashishat *et al*. (1980)
SCH, *Saccharomyces cerevisiae* 'race XII', homozygosis	–	(+)	100	Kassinova *et al*. (1981)
SCR, *Saccharomyces cerevisiae* D7, reverse mutation	+	NT	6010	Vashishat *et al*. (1980)
SCR, *Saccharomyces cerevisiae* XV185-14C, reverse mutation	+	NT	48	Mehta & von Borstel (1981)
SZF, *Schizosaccharomyces pombe*, forward mutation	+	+	18.5	Migliore *et al*. (1982)
SZF, *Schizosaccharomyces pombe*, forward mutation	+	NT	92	Rossi *et al*. (1983a)
SZF, *Schizosaccharomyces pombe*, forward mutation	–	+	1	Loprieno (1981)
SZF, *Schizosaccharomyces pombe*, forward mutation	+	+	74	Rossi *et al*. (1983b)
SZR, *Schizosaccharomyces pombe*, reverse mutation	+	NT	180	Heslot (1962)
NCR, *Neurospora crassa*, reverse mutation	(+)	NT	14000	Kolmark & Giles (1955)
SCN, *Saccharomyces cerevisiae* D6, aneuploidy	+	NT	50	Parry & Sharp (1981)
ASM, *Arabidopsis* species, mutation	+	NT	NG	Acedo & Rédei (1982)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		472	Vogel *et al*. (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		0.2%	Wurgler & Graf (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		472 inj × 1	Knaap *et al*. (1982)
DIA, DNA single-strand breaks, rat hepatocytes *in vitro*	+	NT	28	Sina *et al*. (1983)
DIA, DNA single-strand breaks, mouse lymphoma L5178Y cells *in vitro*	+	NT	96	Garberg *et al*. (1988)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	4.6	Probst *et al*. (1981)
GCO, Gene mutation, Chinese hamster ovary CHO cells *in vitro*	+	NT	25	Amacher & Zelljadt (1984)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	100	Nishi *et al*. (1984)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	68.3	Jotz & Mitchell (1981)
G51, Gene mutation, mouse lymphoma L5178Y cells, *hprt* locus *in vitro*	+	NT	46	Knaap *et al*. (1982)
G51, Gene mutation, mouse lymphoma L5178Y cells, ouabain resistance *in vitro*	+	NT	24	Amacher & Dunn (1985)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	4.8	Evans & Mitchell (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	100	Natarajan & van Kesteren-van Leeuwen (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	–	10	Perry & Thomson (1981)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	NT	500	Nishi *et al*. (1984)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	(+)	9.25	von der Hude *et al*. (1987)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	NT	23	von der Hude *et al*. (1991)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	NT	9.2	Sasaki *et al*. (1980)
CIC, Chromosomal aberrations, Chinese hamster lung CHL fibroblasts *in vitro*	+	NT	47	Ishidate *et al*. (1981)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	+	100	Natarajan & van Kesteren-van Leeuwen (1981)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	NT	15	Asita (1989)
CIA, Chromosomal aberrations, rat epithelial-like liver cells *in vitro*	–	NT	20	Dean & Hodson-Walker (1979)
GIH, Gene mutation, human HSC172 lung fibroblasts, diphtheria toxin resistance *in vitro*	–	–	100	Gupta & Goldstein (1981)
GIH, Gene mutation, human epithelial-type EUE cells, diphtheria toxin resistance *in vitro*	+	NT	46	Perocco *et al*. (1983)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	+	9	White (1980)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.0009	Carbone *et al*. (1981)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	4.6	Norppa *et al*. (1981)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	0.09	Kucerová & Polívková (1976)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	0.009	Šrám *et al*. (1976)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	18.5	Norppa *et al*. (1981)
HMM, Host-mediated assay, *Salmonella typhimurium* TA60, G46 in ICR mouse peritoneal fluid	+		100 im × 1	Šrám *et al*. (1976)
HMM, Host-mediated assay, *Schizosaccharomyces pombe* in CD1 and C57BL × CD1 mice	–		200 ip × 1	Rossi *et al*. (1983c)
HMM, Host-mediated assay, *Escherichia coli* K12 in NMRI mice	–[c]		240 po × 1	Hellmér & Bolcsfoldi (1992)
SVA, Sister chromatid exchange, CBA/J mouse bone marrow *in vivo*	+[d]		6 ip × 1	Paika *et al*. (1981)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
MVM, Micronucleus test, ICR mice *in vivo*	–		100 ip × 2	Kirkhart (1981)
MVM, Micronucleus test, B6C3F_1 mice *in vivo*	–		160 ip × 2	Salamone *et al*. (1981)
MVM, Micronucleus test, CD-1 mice *in vivo*	–		100 ip × 2	Tsuchimoto & Matter (1981)
MVM, Micronucleus test, ddY mice *in vivo*	–		200 ip × 2	Asita *et al*. (1992)
CBA, Chromosomal aberrations, ICR mouse bone marrow *in vivo*	+		1 ip × 1	Šrám *et al*. (1976)
CBA, Chromosomal aberrations, CD-1 mouse bone marrow *in vivo*	–		200 po × 1	Rossi *et al*. (1983a)
DLM, Dominant lethal test, ICR/Ha Swiss mice	–		150 ip × 1	Epstein *et al*. (1972)
DLM, Dominant lethal test, ICR mice	–[e]		20 po × 5	Šrám *et al*. (1976)
BID, DNA binding (covalent), calf thymus DNA *in vitro*	+	NT	15	Hemminki (1979)
BVD, DNA binding, BALB/c mouse and Wistar rat liver, lung, kidney and stomach *in vivo*	+		0.6 ip × 1	Prodi *et al*. (1986)
SPM, Sperm morphology, CBA × BALB/c mice *in vivo*	–		200 ip × 5	Topham (1980)
SPR, Sperm morphology, Wistar rats *in vivo*	+		50 po × 1	Cassidy *et al*. (1983)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; im, intramuscular; inj, injection; ip, intraperitoneal; po, oral

[c] Positive when mice were treated intraperitoneally with 180 mg/kg bw/day epichlorohydrin

[d] Positive only when mice received partial hepatectomy before treatment

[e] Negative also after a single intraperitoneal dose of 20 mg/kg bw or a single oral dose of 40 mg/kg bw

also increased the frequency of sister chromatid exchanges and chromosomal aberrations in cultures of human lymphocytes.

In a single study, epichlorohydrin bound to DNA of mice and rats treated *in vivo*. One study reported that sister chromatid exchanges were induced in the bone marrow of partially hepatectomized CBA/J mice treated with epichlorohydrin by a single intraperitoneal injection. Sister chromatid exchange frequencies in mice that did not receive partial hepatectomy before treatment with epichlorohydrin were comparable to the control frequencies. One of two studies reported that epichlorohydrin induced chromosomal aberrations in mouse bone marrow. Positive results were also reported for epichlorohydrin in the mouse host-mediated assay in one of three studies. In single studies, epichlorohydrin caused sperm head abnormalities in rats but not mice. It did not induce micronuclei or dominant lethal mutations in mice *in vivo*.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to epichlorohydrin may occur during the production and use of resins, glycerine and propylene-based rubbers and its use as a solvent. It has been detected at low levels in water.

5.2 Human carcinogenicity data

The risk of cancer has been investigated among four populations exposed to epichlorohydrin. In one cohort study, an excess of lung cancer was observed among the small number of workers employed in the production of epichlorohydrin. A nested case–control study within this population found a weak association between epichlorohydrin and lung cancer but risk was not related to level of exposure. In another nested case–control study based on the same cohort, a weak association with central nervous system tumours was observed which appeared to be related to the level of exposure. A small excess of lung cancer was observed in another cohort, but in a third no excess of cancer was observed. In a case–control study of lung cancer nested within a further cohort of chemical workers, a significantly decreased risk of lung cancer was associated with epichlorohydrin exposure. All results were based on relatively small numbers.

5.3 Animal carcinogenicity data

Epichlorohydrin was tested in rats by oral administration, inducing papillomas and carcinomas of the forestomach, and by inhalation, inducing papillomas and carcinomas of the nasal cavity. It was also tested in mice by skin application and by subcutaneous and intraperitoneal injection; it gave negative results after continuous skin painting but was active as an initiator on skin. It produced local sarcomas after subcutaneous injection and was active in a mouse-lung tumour bioassay by intraperitoneal injection.

5.4 Other relevant data

Epichlorohydrin is itself a reactive epoxide and is metabolized by binding to glutathione and by hydration via epoxide hydrolase. The same haemoglobin adduct has been detected in humans and rats. In man, epichlorohydrin causes local damage upon contact exposure. In rodents, toxicity to kidneys, liver and forestomach has been observed. After inhalation, the most sensitive target organ is the nasal turbinates. Epichlorohydrin induces genetic damage in most bacterial and mammalian tests *in vitro* or *in vivo*, not requiring the presence of a metabolic activation system.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of epichlorohydrin.

There is *sufficient evidence* in experimental animals for the carcinogenicity of epichlorohydrin.

Overall evaluation

Epichlorohydrin is *probably carcinogenic to humans (Group 2A)*.

In making the overall evaluation, the Working Group took into consideration the known chemical reactivity of epichlorohydrin and its direct activity in a wide range of genetic tests.

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1,2-EPOXYBUTANE

Data were last evaluated in IARC (1989).

1. Exposure Data

1.1 Chemical and physical properties

1.1.1 *Nomenclature*

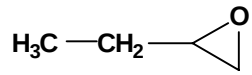
Chem. Abstr. Services Reg. No.: 106-88-7

Chem. Abstr. Name: Ethyloxirane

IUPAC Systematic Name: 1,2-Butylene oxide

Synonyms: 1-Butene oxide; 1,2-butene oxide; 1,2-butylene epoxide; α-butylene oxide; 1-butylene oxide; epoxybutane; ethyl ethylene oxide; 2-ethyloxirane

1.1.2 *Structural and molecular formula and relative molecular mass*



C_4H_8O Relative molecular mass: 72.12

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Clear, colourless liquid with pungent odour (Dow Chemical Co., 1988)

(*b*) *Boiling-point*: 63.3°C (Lide, 1997)

(*c*) *Melting-point*: –60°C (Verschueren, 1996)

(*d*) *Solubility*: Soluble in water (82.4 mg/L at 25°C); miscible with diethyl ether; very soluble in acetone, ethanol and most organic solvents (Verschueren, 1996; Lide, 1997)

(*e*) *Density*: d_{20}^{20} 0.83

(*f*) *Vapour pressure*: 18.6 kPa at 20°C (Dow Chemical Co., 1988); relative vapour density (air = 1), 2.49 (Verschueren, 1996)

(*g*) *Flash-point*: –22°C (closed-cup) (Dow Chemical Co., 1988)

(*h*) *Reactivity*: Extremely inflammable; reacts with water and other sources of labile hydrogen, especially in the presence of acids, bases or other oxidizing substances. Reactive monomer which can polymerize exothermically. Undergoes atmospheric hydrolysis; atmospheric half-life for oxidation estimated to be

six days (Hine *et al*., 1981; Dow Chemical Co., 1988; United States National Toxicology Program, 1988)

(*i*) *Conversion factor*: $mg/m^3 = 2.95 \times ppm$

1.2 Production and use

It has been reported that 3600 tonnes of 1,2-epoxybutane were produced in the United States in 1978 (United States National Toxicology Program, 1988). Data on production elsewhere in the world were not available. Information available in 1995 indicated that it was produced in Germany, Japan and the United States (Chemical Information Services, 1995).

1,2-Epoxybutane is widely used as a stabilizer for chlorinated hydrocarbon solvents. It is also used as a chemical intermediate for the production of butylene glycols and their derivatives (polybutylene glycols, mixed poly glycols and glycol ethers and esters), butanolamines, surface-active agents and other products, such as gasoline additives (Hine *et al*., 1981; Parmeggiani, 1983; Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 47 900 workers in the United States were potentially exposed to 1,2-epoxybutane (see General Remarks). Occupational exposures to 1,2-epoxybutane may occur in its production and use as a monomer and chemical intermediate and as a stabilizer in chlorinated solvents.

1.3.2 *Environmental occurrence*

No information on environmental occurrence of 1,2-epoxybutane was available to the Working Group.

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not proposed any occupational exposure limit for 1,2-epoxybutane in workplace air. However, manufacturers in the United States have recommended a voluntary standard of 40 ppm [118 mg/m^3] for an 8-h time-weighted average exposure limit (United States National Toxicology Program, 1988).

No international guideline for 1,2-epoxybutane in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

1,2-Epoxybutane was tested for carcinogenicity by inhalation exposure in one study in mice and in one study in rats, producing nasal papillary adenomas in rats of both sexes and pulmonary alveolar/bronchiolar tumours in male rats. It did not induce skin tumours when tested by skin application in one study in mice. Oral administration of trichloroethylene containing 1,2-epoxybutane to mice induced squamous-cell carcinomas of the forestomach, whereas administration of trichloroethylene alone did not (IARC, 1989).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

1,2-Epoxybutane caused inflammatory and degenerative changes in the nasal mucosa and myeloid hyperplasia in the bone marrow in rats and mice (IARC, 1989).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

1,2-Epoxybutane did not cause prenatal toxicity in rats or rabbits (IARC, 1989).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

The genetic activity of 1,2-epoxybutane has been reviewed (Ehrenberg & Hussain, 1981). It is a direct-acting alkylating agent.

1,2-Epoxybutane has been shown to induce SOS repair activity in *Salmonella typhimurium* TA1525/pSK1002 and to produce differential killing zones in various *pol*- and *rec*-proficient and -deficient strains of *Escherichia coli*. It induced streptomycin-resistant

Table 1. Genetic and related effects of 1,2-epoxybutane

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
PRB, Prophage, induction/SOS response/strand-breaks/or cross-links, *Salmonella typhimurium* TA1525/pSK1002	+	NT	780	Nakamura *et al*. (1987)
ECL, *Escherichia coli pol A*, differential toxicity	+	NT	50	Rosenkranz & Poirier (1979)
ECL, *Escherichia coli pol A*, differential toxicity	+	NT	20000	McCarroll *et al*. (1981)
ERD, *Escherichia coli rec*, differential toxicity	+	NT	4300	McCarroll *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	NT	2100	McCann *et al*. (1975)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	7	Speck & Rosenkranz (1976)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	2100	Henschler *et al*. (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	2.5	De Flora (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	NG	McMahon *et al*. (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	250	Simmon (1979a)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	2000	De Flora (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	NG	De Flora *et al*. (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	167	Dunkel *et al*. (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	1100	Gervasi *et al*. (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	500	Canter *et al*. (1986)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	NT	360	Rosman *et al*. (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	500	US National Toxicology Program (1988)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	500	McGregor *et al*. (1989)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	+	NT	17000	Chen *et al*. (1975)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	2100	McCann *et al*. (1975)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	42	Rosenkranz & Poirier (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	250	Simmon (1979a)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	2000	De Flora (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	1250	Weinstein *et al*. (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	NG	De Flora *et al*. (1984)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	167	Dunkel *et al*. (1984)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	500	Canter *et al*. (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	90	Rosman *et al*. (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	500	US National Toxicology Program (1988)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	50	McGregor *et al*. (1989)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	250	Simmon (1979a)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	20000	De Flora (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	167	Dunkel *et al*. (1984)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Canter *et al*. (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	US National Toxicology Program (1988)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	250	Simmon (1979b)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	167	Dunkel *et al*. (1984)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	5000	US National Toxicology Program (1988)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	20000	De Flora (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250	Simmon (1979a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	20000	De Flora (1981)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	2200	Gervasi *et al*. (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Canter *et al*. (1986)
SAS, *Salmonella typhimurium* TA100-FR1, reverse mutation	+	NT	3.5	Rosenkranz & Speck (1975)
SAS, *Salmonella typhimurium* TA1536, reverse mutation	–	–	250	Simmon (1979a)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	+	NT	NG	McMahon *et al*. (1979)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	–	–	167	Dunkel *et al*. (1984)
KPF, *Klebsiella pneumoniae*, forward mutation	(+)	+	72	Voogd *et al*. (1981)
KPF, *Klebsiella pneumoniae*, forward mutation	+	NT	72	Knaap *et al*. (1982)
SCH, *Saccharomyces cerevisiae* D3, homozygosis	+	+	5000	Simmon (1979b)
SZF, *Schizosaccharomyces pombe* P1, forward mutation	+	+	29	Migliore *et al*. (1982)
NCR, *Neurospora crassa*, reverse mutation	(+)	NT	14	Kolmark & Giles (1955)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		8400 inj × 1	Knaap *et al*. (1982)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		50000 ppm feed	US National Toxicology Program (1988)
DMH, *Drosophila melanogaster*, heritable translocations	+		50000 ppm feed	US National Toxicology Program (1988)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	1000	Williams *et al*. (1982)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	63	Amacher *et al*. (1980)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	400	McGregor *et al*. (1987)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	55	Mitchell *et al*. (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	50	Myhr & Caspary (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	50	US National Toxicology Program (1988)
G51, Gene mutation, mouse lymphoma L5178Y cells, *hprt* locus *in vitro*	+	NT	360	Knaap *et al*. (1982)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	16	US National Toxicology Program (1988)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	16	Anderson *et al*. (1990)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	(+)	(+)	500	US National Toxicology Program (1988)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	(+)	(+)	500	Anderson *et al*. (1990)
TBM, Cell transformation, BALB/c 3T3 mouse cells	–	NT	50	Dunkel *et al*. (1981)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	NG	Pienta *et al*. (1981)
TFS, Cell transformation, Syrian hamster embryo cells, focus assay	(+)	NT	50	Dunkel *et al*. (1981)
TRR, Cell transformation, RLV/Fischer 344 rat embryo cells	+	NT	10	Price & Mishra (1980)
TRR, Cell transformation, RLV/Fischer 344 rat embryo cells	+	NT	700	Dunkel *et al*. (1981)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; inj, injection

mutants in *Klebsiella pneumoniae*. It was shown to be mutagenic to *E. coli* WP2 *uvr*A$^-$ in one of two studies. In *S. typhimurium*, it induced base–pair substitutions (strains TA100 and TA1535) but not frameshift mutations in the presence or absence of exogenous metabolic activation. 1,2-Epoxybutane induced forward mutation in *Schizosaccharomyces pombe* P1 and mitotic recombination in *Saccharomyces cerevisiae* D3. It was weakly mutagenic at the adenine locus in *Neurospora crassa*. It induced sex-linked recessive lethal mutations and translocations in *Drosophila melanogaster* after either feeding or injection.

It did not induce unscheduled DNA synthesis in rat primary hepatocytes but did induce mutation in L5178Y TK$^{+/-}$ mouse lymphoma cells in the absence or presence of an exogenous metabolic system. In one study, 1,2-epoxybutane gave marginally positive results for induction of 6-thioguanine-resistant mutations in L5178Y cells. It increased the frequency of sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary CHO cells with or without exogenous metabolic activation. It induced morphological transformation in Syrian hamster embryo cells and virally enhanced Fischer 344 rat embryo cells but not in BALB/c 3T3 cells.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to 1,2-epoxybutane may occur in its production and use as a monomer, chemical intermediate and stabilizer.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

1,2-Epoxybutane was tested for carcinogenicity by inhalation exposure in one study in mice and in one study in rats, producing nasal papillary adenomas in rats of both sexes and pulmonary alveolar/bronchiolar tumours in male rats. It did not induce skin tumours when tested by skin application in one study in mice.

5.4 Other relevant data

1,2-Epoxybutane induced morphological transformation, sister chromatid exchanges, chromosomal aberrations and mutation in cultured animal cells; however, in a single study, it did not induce unscheduled DNA synthesis in rat primary hepatocytes. It induced sex-linked recessive lethal mutations and translocations in *Drosophila melanogaster*, mitotic recombination in yeast, and mutations in yeast and fungi. 1,2-Epoxybutane induced DNA damage and mutations in bacteria.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of 1,2-epoxybutane were available.

There is *limited evidence* in experimental animals for the carcinogenicity of 1,2-epoxybutane.

Overall evaluation

1,2-Epoxybutane is *possibly carcinogenic to humans (Group 2B)*.

In making the overall evaluation, the Working Group took into consideration that 1,2-epoxybutane is a direct-acting alkylating agent which is mutagenic in a range of test systems.

6. References

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ETHYLENE DIBROMIDE (1,2-DIBROMOETHANE)

Data were last reviewed in IARC (1977) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 106-93-4
Chem. Abstr. Name: 1,2-Dibromoethane
IUPAC Systematic Name: 1,2-Dibromoethane
Synonym: EDB

1.1.2 *Structural and molecular formulae and relative molecular mass*

$C_2H_4Br_2$ Relative molecular mass: 187.86

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid with a sweetish, chloroform-like odour (Lewis, 1993; Budavari, 1996)
(*b*) *Boiling-point*: 131.6°C (Lide, 1995)
(*c*) *Melting-point*: 9.9°C (Lide, 1995)
(*d*) *Solubility*: Miscible with acetone, benzene, diethyl ether and ethanol; slightly soluble in water (0.43 g/100 mL at 30°C) (Lide, 1995; Verschueren, 1996)
(*e*) *Vapour pressure*: 1.5 kPa at 25°C; relative vapour density (air = 1), 6.5 (Budavari, 1996; Verschueren, 1996)
(*f*) *Conversion factor*: $mg/m^3 = 7.69 \times ppm$

1.2 Production and use

Production of ethylene dibromide in the United States in 1982 was reported to be 77 100 tonnes (United States National Library of Medicine, 1997).

Ethylene dibromide has been used as a scavenger for lead in gasoline, as a general solvent, in waterproofing preparations, in organic synthesis and as a fumigant for grain and tree crops (Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 9000 workers in the United States were potentially exposed to ethylene dibromide (see General Remarks). Occupational exposures to ethylene dibromide occur in pest control occupations, petroleum refining and waterproofing. In addition, car mechanics and other workers handling leaded gasoline may be dermally exposed to ethylene dibromide.

1.3.2 *Environmental occurrence*

Ethylene dibromide enters the atmosphere primarily from fugitive emissions and exhaust associated with its use as a scavenger in leaded gasoline. Another important but localized source is emissions from fumigation centres for citrus and grain and soil fumigation operations. It has been detected at low levels in groundwater, drinking-water, wastewater, ambient water, urban air and ambient air samples (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not proposed any occupational exposure limit for ethylene dibromide in workplace air. The ACGIH (1991) lists ethylene dibromide as an animal carcinogen. Until 1979, the 8-h time-weighted average threshold limit value was 154 mg/m^3. Values ranging between 1 mg/m^3 and 145 mg/m^3 have been used as standards or guidelines in many countries (International Labour Office, 1991).

The World Health Organization has determined that there are no adequate data to permit recommendation of a health-based guideline value for ethylene dibromide in drinking-water (WHO, 1993).

2. Studies of Cancer in Humans

In one study, the mortality of 161 men exposed to ethylene dibromide in two factories since the mid-1920s and 1942, respectively, was investigated. By January 1976, 36 workers had died, seven of them from cancer (5.8 expected) (Ott *et al*., 1980). In another study, the mortality of 2510 male workers employed at a chemical plant was investigated. Ethylene dibromide was one of the several chemicals used and was apparently a minor component of the mixed exposure. No significant excess of cancer at any site was found (Sweeney *et al*., 1986).

In the United States, ethylene dibromide has been used as a fumigant in the grain industry since the 1940s. Alavanja *et al*. (1990) analysed mortality during 1955–85 in 22 938 white men who were enrolled in the life insurance programme of the American Federation of Grain Millers. Among a subset of 9660 who worked in flour mills (where

pesticides were used more frequently), 1914 deaths were recorded, giving a standardized mortality ratio (SMR) of 0.9 based on national rates ($p < 0.05$). These included 25 deaths from leukaemia (SMR, 1.4, not significant) and 21 from non-Hodgkin lymphoma (SMR, 1.5, not significant). In a nested case–control study, having ever been employed in a flour mill was significantly associated with mortality from non-Hodgkin lymphoma (21 cases; odds ratio, 4.2; 95% confidence interval (CI), 1.2–14.2) and pancreatic cancer (33 cases; odds ratio, 2.2; 95% CI, 1.1–4.3) but not leukaemia (25 cases; odds ratio, 1.8; 95% CI, 0.8–3.9). [The Working Group noted that interpretation was difficult in the absence of information about individual exposures to specific fumigants.]

3. Studies of Cancer in Experimental Animals

Ethylene dibromide was administered orally to mice and rats and produced squamous-cell carcinomas of the forestomach (IARC, 1977).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F$_1$ mice, five weeks of age, were administered daily time-weighted average doses of 62 and 107 mg/kg bw technical-grade ethylene dibromide (purity, 99.1%) in corn oil by gavage on five days per week for 53 weeks followed by observation for 24–37 weeks. A group of 20 males and 20 females received corn oil alone and served as vehicle controls and a further group of 20 males and 20 females served as untreated controls. Squamous-cell carcinomas of the forestomach were observed in both sexes (males: vehicle control, 0/20; low dose, 45/50; high dose, 29/49; females, 0/20, 46/49, 28/50). The incidence of alveolar/bronchiolar adenomas was significantly higher in treated mice of each sex than in vehicle controls (males, 0/20, 4/45, 10/47 ($p = 0.02$); females, 0/20, 11/43 ($p = 0.009$), 6/46) (United States National Cancer Institute, 1978).

Groups of 30 male and 30 female B6C3F$_1$ mice were administered 4 mmol/L ethylene dibromide (purity, > 99%), a dose equivalent to 116 mg/kg bw for males and 103 mg/kg bw for females) in distilled drinking-water for 450 days. A control group of 60 males and 60 females was given distilled drinking-water. Ethylene dibromide induced squamous-cell carcinomas of the forestomach in 26/28 males and 27/29 females and squamous-cell papilloma of the oesophagus in 3/30 females compared with none in 45 male and 50 female controls (Van Duuren *et al*., 1985).

3.1.2 *Rat*

Groups of 50 male and 50 female Osborne-Mendel rats, five weeks of age, were administered daily time-weighted average doses of 38 or 41 (males) and 37 or 39 (females) mg/kg bw technical-grade ethylene dibromide (purity, 99.1%) in corn oil by gavage on five days per week for 36–57 weeks followed by observation for 2–13 weeks.

A group of 20 males and 20 females received corn oil alone and served as vehicle controls. Squamous-cell carcinomas of the forestomach were observed in 45/50 low-dose males, 33/50 high-dose males, 40/50 low-dose females and 29/50 high-dose females, while none was observed in controls. The lesions, seen as early as week 12, were locally invasive and eventually metastasized. A significantly higher incidence of haemangiosarcomas of the spleen was observed in low-dose males (0/20 controls, 10/50 low-dose and 3/49 high-dose) (United States National Cancer Institute, 1978).

3.2 Inhalation exposure

3.2.1 *Mouse*

Groups of 50 male and 50 female B6C3F_1 mice, five weeks of age, were exposed by whole-body inhalation to air containing 0 (control), 10 or 40 ppm [0, 77 or 308 mg/m^3] ethylene dibromide (purity, 99.3–99.4%) for 78–106 weeks. The incidence of alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas was significantly higher in exposed male and female mice than in controls. The incidence of haemangiosarcomas of the circulatory system, fibrosarcomas in subcutaneous tissue, carcinomas of the nasal cavity and adenocarcinomas of the mammary gland was significantly increased in females (see Table 1) (United States National Toxicology Program, 1982).

3.2.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, five weeks of age, were exposed by whole-body inhalation to air containing 0 (control), 10 or 40 ppm [0, 77 or 308 mg/m^3] ethylene dibromide (purity, 99.3–99.4%) for 88–106 weeks. The incidence of carcinomas, adenocarcinomas and adenomas of the nasal cavity and haemangiosarcomas of the circulatory system was significantly increased in exposed male and female rats. The incidence of mesotheliomas of the tunica vaginalis and adenomatous polyps of the nasal cavity in males and of fibroadenomas of the mammary gland and alveolar/bronchiolar adenomas and carcinomas (combined) in females was also significantly increased (see Table 2) (United States National Toxicology Program, 1982).

Groups of 48 male and 48 female Sprague-Dawley weanling rats were exposed by whole-body inhalation to 0 or 20 ppm [154 mg/m^3] ethylene dibromide (purity, 99%) for 7 h per day on five days per week for 18 months. Rats inhaling 20 ppm ethylene dibromide vapour had significantly higher mortality than the controls. Among treated rats, 10/48 males and 6/48 females developed haemangiosarcomas of the spleen compared with 0/48 male and 0/48 female controls. Mammary tumours (benign and malignant combined) occurred in 25/48 treated females compared with 2/48 controls. Subcutaneous mesenchymal tumours were found in 11/48 males compared with 3/48 controls (Wong *et al*., 1982).

3.3 Skin application

Mouse: Groups of 30 female Ha:ICR Swiss mice, six to eight weeks of age, received thrice-weekly skin applications of 25 or 50 mg per animal ethylene dibromide (purity,

Table 1. Incidence of tumours in mice exposed to ethylene dibromide by inhalation exposure

Tumour type	Males			Females		
	0	10 ppm	40 ppm	0	10 ppm	40 ppm
Lung, alveolar/bronchiolar						
Adenoma	0/41	0/48	11/46**	3/49	7/49	13/50*
Carcinoma	0/41	3/48	19/46**	1/49	5/49	37/50**
Circulatory system						
Haemangiosarcoma				0/50	11/50**	23/50**
Subcutaneous tissue						
Fibrosarcoma				0/50	5/50*	11/50**
Nasal cavity						
Carcinoma				0/50	0/50	6/50*
Mammary gland						
Adenocarcinoma				2/50	14/50**	8/50*

From United States National Toxicology Program (1982)
* $p < 0.05$
** $p \leq 0.001$

Table 2. Incidence of tumours in rats exposed to ethylene dibromide by inhalation exposure

Tumour type	Males			Females		
	0	10 ppm	40 ppm	0	10 ppm	40 ppm
Nasal cavity						
Adenoma	0/50	11/50**	0/50	0/50	11/50**	3/50
Carcinoma	0/50	0/50	21/50**	0/50	0/50	25/50**
Adenocarcinoma	0/50	20/50**	28/50**	0/50	20/50**	29/50**
Circulatory system						
Haemangiosarcoma	0/50	1/50	15/50**	0/50	0/50	5/50*
Tunica vaginalis						
Mesothelioma	0/50	7/50**	25/50**			
Mammary gland						
Fibroadenoma				4/50	29/50**	24/50**
Lung alveolar/bronchiolar						
Adenoma and carcinoma				0/50	0/48	5/47*

From United States National Toxicology Program (1982)
* $p < 0.05$
** $p \leq 0.001$

> 99%) in 0.2 mL acetone on the shaved dorsal skin, or applications of acetone alone or served as untreated controls. The times to the first appearance of skin tumour (papilloma) were 434 days for the 25-mg group and 395 days for the 50-mg group. In comparison with controls, both groups showed a significantly increased incidence of lung papillary adenomas (24/30 low-dose, 26/30 high-dose) and, in the 50-mg group, a significant increase in the incidence of skin papillomas (8/30) (Van Duuren *et al*., 1979).

3.4 Other systems

Fish: Groups of 200 (males and females combined) Shasta strain rainbow trout, eight weeks of age, were fed a diet containing 0 or 2000 ppm ethylene dibromide [purity unspecified]. Eighty fish were killed after nine months of feeding the test diet and 120 fish were killed after 18 months. Liver neoplasms (adenoma and carcinoma combined), occurred at nine months in 0/66 and 1/65 fish (males and females combined) in the control and ethylene dibromide-treated groups, respectively; at 18 months, the incidences were 0/113 and 6/117, respectively. The incidences of stomach papillomas at nine months were 0/66 and 0/65 for the control and ethylene dibromide-treated groups, respectively and at 18 months were 0/113 and 36/117, respectively. A higher incidence of stomach tumours was seen in males than in females ($p < 0.05$, Mantel–Haenszel test) (Hendricks *et al*., 1995).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

Various aspects of the toxicokinetics and metabolism of ethylene dibromide have recently been reviewed (Guengerich, 1994; WHO, 1996).

4.1.1 *Humans*

Human liver preparations metabolize ethylene dibromide to water-soluble and irreversibly protein- and DNA-bound metabolites by both cytochrome P450 and glutathione *S*-transferase (GST) enzymes (Wiersma *et al*., 1986). DNA adduct formation occurs also in isolated human hepatocytes (Cmarik *et al*., 1990).

There is convincing evidence that CYP2E1 is a major enzyme metabolizing ethylene dibromide. Among heterologously expressed human cytochromes P450, only CYP2E1 (low K_m enzyme), CYP2B6 and CYP2A6 (high K_m enzymes) metabolized ethylene dibromide to 2-bromoacetaldehyde (Wormhoudt *et al*., 1996), CYP2E1 having the highest intrinsic clearance. Interindividual variation in P450-catalysed microsomal metabolism, reflecting presumably variable amounts of CYP2E1 enzyme, was almost 50-fold.

Human fetal liver cytosol and several GST forms from human fetal liver catalyse the conjugation of ethylene dibromide (Kulkarni *et al*., 1992; Mitra *et al*., 1992). The α-class GST enzymes from human liver are especially active in the conjugation of ethylene dibromide (Cmarik *et al*., 1990).

4.1.2 *Experimental systems*

After intraperitoneal administration of radiolabelled ethylene dibromide to guinea-pigs (30 mg/kg bw) or mice (40 mg/kg bw), the largest portion of the radioactivity was excreted in urine. The highest levels of radioactivity were found in kidney, liver and stomach. Enzymatic reaction with glutathione (GSH) *in vitro* and *in vivo* as well as excretion of glutathione-derived metabolites in urine of rats and mice have been demonstrated (IARC, 1977).

Ethylene dibromide was absorbed rapidly through the skin of guinea-pigs and reached maximal blood levels at 1 h (Jakobson *et al*., 1982). In rats, 24-h urinary excretion of radiolabelled ethylene dibromide was > 70 % and the highest amount at 24 h was found in the liver and kidneys (Plotnick *et al*., 1979).

The metabolic pathways of ethylene dibromide are known in detail. In rodents, the major routes are oxidation by CYP2E1 and conjugation by GST (Guengerich, 1994; Wormhoudt *et al*., 1996). The primary metabolite formed by CYP2E1 is 2-bromoacetaldehyde, which can be conjugated with glutathione and enter the mercapturic acid pathway (Guengerich, 1994). The excretion of thiodiacetic acid in urine has been suggested as a biomarker for P450-catalysed oxidation (Wormhoudt *et al*., 1997).

The CYP2E1-catalysed pathway is responsible for the major part of protein binding and consequent tissue toxicity, although glutathione conjugates also play a role (Khan *et al*., 1993; Wormhoudt *et al*., 1996). The ratio between the oxidation pathway and the GST pathway in rodents *in vitro* and *in vivo* is about 4. Debromination during oxidative metabolism may result in increased bromine concentrations, which may be of significance in initiating lipid peroxidation (Guha *et al*., 1993).

The GSH conjugation pathway is responsible for the formation of DNA adducts and bacterial mutagenicity (Sipes *et al*., 1986). The major (> 95%) adduct is *S*-[2-(*N*7-guanyl)ethyl]glutathione (Cmarik *et al*., 1990). Three minor guanyl or adenyl adducts (1% or less) are also formed. Various forms of GST differ in their catalytic activities. The amount of the major adduct formed *in vivo* in liver and kidney DNA is directly proportional to the dose in rats (Kim & Guengerich, 1989). The amount of the adduct can be modulated by inducers of GST or inhibitors of CYP2E1 (Kim & Guengerich, 1990; Guengerich, 1994), with resultant consequences for hepatic tumorigenesis (Wong *et al*., 1982). More DNA adduct was formed in the livers of rats than in those of mice (Kim & Guengerich, 1990).

In whole-body autoradiographic studies, covalently bound radioactivity from ethylene dibromide was detected in the surface epithelia of the entire respiratory and the upper alimentary tracts of mice and rats (Brandt, 1986), in the epithelia of the oral cavity, oesophagus and forestomach of fetal mouse (Kowalski *et al*., 1986), and in vaginal epithelium of mice and rats (Brittebo *et al*., 1987).

Covalent binding of ethylene dibromide to albumin has been demonstrated after in-vivo administration of ethylene dibromide to rats and after in-vitro incubation of ethylene dibromide with human albumin (Kaphalia & Ansari, 1992).

4.1.3 *Comparison of human and rodent data*

Both human and rodent livers contain significant levels of enzymes necessary for the two major pathways of ethylene dibromide metabolism, although some qualitative (especially with respect to GST) and quantitative (CYP2E1) differences may exist.

The rate of metabolism of ethylene dibromide by human liver cytosol (three individuals examined) is about half that in rat cytosol (Kim & Guengerich, 1990).

A physiologically based pharmacokinetic model for predicting ethylene dibromide kinetics and consequent toxicity, based on in-vitro metabolic parameters of rodents and humans and on the use of scaling factors, has been presented (Ploemen *et al*., 1997). Its most important prediction is that the GST pathway is significantly active even at low ethylene dibromide concentrations, which has important implications for risk assessment.

4.2 Toxic effects

4.2.1 *Humans*

Several cases of fatalities following acute exposure of humans to ethylene dibromide have been reported. Two workers died following inhalation exposure while cleaning a tank used to temporarily store fertilizer mixtures in the field during application. Neither worker had respiratory or skin protection. The air inside the tank was sampled approximately 20 h after the accident and ethylene dibromide concentrations ranged from 15 to 41 ppm [115–315 mg/m^3] with an average of 28 ppm [215 mg/m^3]. The oxygen concentration inside the tank was 21%. The first worker was exposed for approximately 5 min and the second for approximately 20–30 min. The first worker died approximately 12 h after exposure and the second died 64 h after entering the tank. These two cases provided evidence that ethylene dibromide can produce metabolic acidosis, acute renal and hepatic failure and necrosis of skeletal muscle and many other organs (Letz *et al*., 1984).

Another fatal poisoning occurred in a woman who intentionally ingested a capsule containing 6480 mg ethylene dibromide [140 mg/kg]. On admission to hospital, the patient was drowsy, disoriented and jaundiced with mild hepatomegaly. She died eight days later and a post-mortem liver biopsy revealed congestion and focal liver cell necrosis (Singh *et al*., 1993).

4.2.2 *Experimental systems*

Male and female Fischer 344 rats were exposed to 0, 3, 10 or 40 ppm [0, 23, 77 or 308 mg/m^3] ethylene dibromide for 6 h per day on five days per week for 13 weeks for a total of 67–68 exposures in 95–96 days. Animals were killed after one, six or 13 weeks of exposure and after a recovery period of 88–89 days. At 10 ppm, ethylene dibromide caused slight epithelial hyperplasia of the nasal turbinates in animals killed after one, six or 13 weeks of exposure. However, 88 days after the last exposure, nasal turbinate changes were not observed. Rats exposed to 40 ppm ethylene dibromide had increased liver and kidney weights, hyperplasia and non-keratinizing squamous metaplasia of the respiratory epithelium of the nasal turbinates. After the recovery period of 88 days, the turbinates had reverted to normal histology. The most sensitive response associated with

repeated subchronic exposure of rats to 10 or 40 ppm ethylene dibromide involved pathological changes in the respiratory epithelium of the nasal turbinates (Nitschke *et al*., 1981). In these studies, 3 ppm was defined as the no-observable-effect level (NOEL).

Male and female Fischer 344 rats and B6C3F_1 mice were exposed to 3, 15 or 75 ppm [23, 115 or 577 mg/m^3] ethylene dibromide for 6 h per day on five days per week for 13 weeks. Rats and mice examined after 13 weeks of exposure showed severe necrosis and atrophy of the olfactory epithelium in the nasal cavity after inhalation of 75 ppm ethylene dibromide. Lower concentrations induced squamous-cell metaplasia, hyperplasia and cytomegaly of the epithelium of the respiratory nasal turbinates. Metaplasia, hyperplasia and epithelial cytomegaly were also seen in other respiratory tissues (larynx, trachea, bronchi, bronchioles) at this dose (Reznik *et al*., 1980).

The characteristics of the nasal lesions in mice following chronic inhalation of ethylene dibromide were investigated. Male and female B6C3F_1 mice were exposed to 10 or 40 ppm [77 or 308 mg/m^3] ethylene dibromide for 6 h per day on five days per week for 103 (10 ppm) or 90 (40 ppm) weeks. The incidence of hyperplastic lesions was related to the dose of ethylene dibromide and was equivalent in males and females. Lesions consisted of focal areas of cuboidal to columnar cells arranged in a glandular pattern with foci of hyperplastic squamous epithelium also seen occasionally. Lesions were usually located in the anterior (respiratory turbinates) of the nasal cavities. A broad spectrum of proliferative lesions was observed (Stinson *et al*., 1981).

Female B6C3F_1 mice were administered 100, 125, 160 or 200 mg/kg bw ethylene dibromide in corn oil by gavage daily for 14 days. Host resistance was not altered after challenge with a variety of agents. Decreases were seen in relative thymus and spleen weights, red blood cells, haemoglobin, haematocrit and responses of immunological cells in culture. Increases in relative weights of liver and kidney were seen. The authors concluded that even in animals exhibiting clinical signs of toxicity, short-term exposure to ethylene dibromide did not alter the immune integrity of mice as measured by host resistance assays *in vivo*. However, in-vitro assessments of immune integrity were altered in a dose-dependent fashion (Ratajczak *et al*., 1994).

Male and female Fischer 344 rats were given intraperitoneal injections of 40 mg/kg bw ethylene dibromide in corn oil twice daily for two consecutive days and were killed on the third day. No hepatotoxic effects were observed and impairment of renal function, as measured by in-vitro accumulation of *para*-aminohippurate by slices of renal cortex, was only observed only in male rats (Kluwe *et al*., 1981a).

Induction of renal cell proliferation following administration by gavage of a single dose of 100 mg/kg bw ethylene dibromide in corn oil was investigated in male Wistar rats. Incorporation of ^{3}H measured in extracted DNA was used to quantitate renal cell proliferation and was five times greater than in controls 20–30 h after treatment. No tubular necrosis was observed on histological examination (Ledda-Columbano *et al*., 1987).

Livers of male Sprague-Dawley rats were evaluated for foci and nodules either 90 days or 16 months after one or two oral doses of 75 mg/kg bw ethylene dibromide. Doses

were given within a 24-h period. Cell proliferation was stimulated by partial hepatectomy at approximately one day or 90 days after dosing and 0.05% phenobarbital in drinking-water for four months beginning at one year. At 90 days, no changes were noted. At 16 months, the incidence of nodules in the animals receiving two doses of ethylene dibromide was twice that of animals receiving one dose and three times that of the control group. Animals receiving ethylene dibromide had higher incidence of eosinophilic foci and γ-glutamyltranspeptidase-positive foci, suggesting that both hepatocyte foci and nodules can be initiated by limited exposure to ethylene dibromide (Moslen, 1984).

The cell cycle-dependent expression of proto-oncogenes in response to the proliferative stimuli induced by the mitogenic action of ethylene dibromide was investigated. Male Wistar rats were given a single dose of 100 mg/kg bw ethylene dibromide in corn oil by gavage. Hepatic cell proliferation was assessed using a single injection of tritiated thymidine 22 h after ethylene dibromide administration. Although there was a measurable increase in cell proliferation as measured by incorporation of tritiated thymidine into hepatic DNA extracted after 1 h, there was no increase in the expression of c-*fos* mRNA, although there was elevated expression of c-*myc* mRNA. Increased expression of c-Ha-*ras* mRNA and c-Ki-*ras* mRNA was also observed (Coni *et al*., 1990, 1993).

Male ICR mice and Fischer 344 rats were given a single intraperitoneal injection of 33, 100 or 330 mg/kg ethylene dibromide in corn oil and were killed 2 h later. Tissues were removed and assayed for non-protein sulfhydryl content (largely glutathione). Hepatic and renal non-protein sulfhydryl concentrations were depleted in mice in a dose-related manner. Lung, testis and stomach non-protein sulfhydryl concentrations were also decreased. The degree of depletion was not as great in the other organs as in kidney and liver, being significant only at the highest dose. In general, the conclusion of the authors was that there was a poor correlation between reported organ sensitivities to ethylene dibromide and tissue-specific depletion of non-protein sulfhydryls (Kluwe *et al*., 1981b).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

A retrospective assessment of the potential antifertility influence of ethylene dibromide was conducted by studying the reproductive performance of men exposed to ethylene dibromide in the workplace. Data were obtained from four chemical plants manufacturing ethylene dibromide located in the southern part of the United States (Arkansas and Texas). Exposures in the plants ranged from less than 0.5 ppm to 5 ppm [3.8–38 mg/m^3]. Evaluations were made exclusively on the basis of the men's reproductive histories of live births to their wives, subsequent to their occupational exposure. The number of live births was compared with the expected number derived from national fertility tables. One of the four plants studied showed a significant decrease in fertility; however, when data from the four plants were combined, there was no significant effect of ethylene dibromide exposure on reproductive performance (Wong *et al*., 1979).

The effect of long-term exposure to ethylene dibromide on semen quality was studied among 46 men employed in the papaya fumigation industry in Hawaii, United States,

with an average duration of exposure of five years and an average exposure to ethylene dibromide of 8 ppb [0.06 mg/m^3] as an 8-h time-weighted average, with peak exposures up to 262 ppb [2.0 mg/m^3]. The comparison group was 43 unexposed men from a sugar refinery. Significant decreases in sperm count, viable and motile sperm and increases in sperm with morphological abnormalities were observed among exposed men. The authors suggested that exposure to ethylene dibromide may increase the risk of reproductive impairment in workers at exposure levels near the recommended limit of 45 ppb [0.35 mg/m^3] and far below the current permissible exposure limit of 20 ppm [154 mg/m^3] (Ratcliffe *et al*., 1987).

A longitudinal study was conducted in 10 forestry employees and six unexposed men in Colorado, United States, with an exposure time of approximately six weeks. Sperm velocity decreased in all 10 exposed men and in only two unexposed men. Semen volume was also decreased. The time-weighted average exposure of these men was 60 ppb [0.46 mg/m^3] with peak exposures in the order of 2165 ppb [16.6 mg/m^3]. The authors suggested that the exposure may have effected the accessory sex glands and that ethylene dibromide may have multiple sites of action (Schrader *et al*., 1988).

4.3.2 *Experimental systems*

The effect of ethylene dibromide on reproduction was studied in male and female CD rats exposed to 0, 19, 39 or 89 ppm [0, 146, 300, 684 mg/m^3] ethylene dibromide for 7 h per day on five days per week for 10 weeks. Morbidity and mortality were observed at the highest concentration. Males in this group had reduced testicular weight, reduced serum testosterone concentration and failed to impregnate any females during a two-week mating period. Atrophy of the testes, epididymis, prostate and seminal vesicles was also observed. Reproductive performance of males exposed to the lower doses (19 or 39 ppm) was not impaired. Females in the highest-dose group did not cycle normally until several days after termination of exposure. However, the reproductive performance of females in the lower-dose groups was normal (Short *et al*., 1979).

Male New Zealand white rabbits were given subcutaneous injections of 15, 30 or 45 mg/kg bw ethylene dibromide per day for five days. Semen samples were taken before exposure, during treatment and during 12 weeks after exposure and analysed for serum concentration, number, morphology, viability and motion parameters. Fertility was assessed by artificial insemination. Mortality, hepatotoxicity and alterations in measured semen parameters were observed in the highest-dose group. Fertility and fetal structural development were unaffected. The authors noted that semen parameters (velocity, percentage motility, amplitude of lateral head displacement) were affected only at doses close to the LD_{50} (55 mg/kg) (Williams *et al*., 1991).

The effect of exposure to ethylene dibromide on oestrous cycling was investigated in female B6C3F$_1$ mice given by gavage 31.25, 62.5 or 125 mg/kg bw on five days per week for 12 weeks. Vaginal smears showed that the oestrous cycle was significantly longer at the highest dose (Ratajczak *et al*., 1995). The effect of inhaled ethylene dibromide during the gestation period in rats and mice was investigated by exposing pregnant CD rats and

CD-1 mice to 20, 38 and 80 ppm [154, 292, and 615 mg/m^3] ethylene dibromide for 23 h per day over 10 days, beginning on day 6 of gestation. Rats and mice were killed on gestational days 20 and 18, respectively. Ethylene dibromide was more toxic to pregnant mice than pregnant rats. All of the mice exposed to 80 ppm died during the study. A significant increase in adult mortality occurred in rats exposed to 80 ppm and in mice exposed to 38 ppm or 80 ppm ethylene dibromide. Ethylene dibromide produced adverse effects on maternal welfare as measured by weight change, feed consumption and survival in both species at all doses tested. Fetal mortality was increased in rats exposed to 80 ppm and in mice exposed to 38 ppm. Reduced body weights were observed in fetuses from rats exposed to 38 ppm and in mice exposed to 20 or 38 ppm ethylene dibromide. Signs of fetal toxicity occurred at ethylene dibromide concentrations that adversely affected the dam (Short *et al*., 1978).

The effects of ethylene dibromide exposure in male rats were studied through behavioural assessments of their F_1 progeny. Fischer 344 male rats were treated by subacute intraperitoneal injection of a daily dose of 1.25, 2.5, 5 or 10 mg/kg bw ethylene dibromide on five successive days. Four weeks or nine weeks after the last injection, males were crossed with virgin females. Behavioural assessment of motor reflexes and motor coordination were examined in the offspring up to 21 days of age. Significant differences in the development of motor coordination and motor activity were observed in the F_1 progeny (Fanini *et al*., 1984). In a review of experimental male-mediated behavioural and neurochemical disorders, Nelson *et al*. (1996) noted that, although the above study is suggestive of effects in offspring following paternal exposures, only one laboratory has studied these effects.

4.4 Genetic and related effects

4.4.1 *Humans*

There have been two studies of ethylene dibromide workers for cytogenetic effects upon peripheral lymphocytes. In one of these (Steenland *et al.*, 1985), full working shift breathing zone samples of 14 sprayers of felled pine trees in Colorado, United States, indicated an average eight-hour time weighted average concentration of ethylene dibromide of 60 ppb [0.46 mg/m^3], with a range of 5 to 281 ppb [0.04 to 2.16 mg/m^3]; short-term samples taken over 4 to 15 min in the breathing zone during times of peak exposures averaged 463 ppb [3.6 mg/m^3], with a range of 8 to 2165 ppb [0.06 to 17 mg/m^3]. Exposure was for a few months and blood samples were taken before and after exposure. Six nonexposed controls were available who provided blood samples at the same time. In the other study (Steenland *et al.*, 1986), full working shift breathing zone samples of 60 papaya-packing workers at six different plants in Hawaii, United States, indicated geometric mean exposures to ethylene dibromide ranging from 16 to 175 ppb [0.12 to 1.35 mg/m^3]. Controls consisted of 42 sugar mill workers from a plant in the same area. In this study, there was control for sex, age, smoking, alcohol use, prescription and nonprescription drug use and recent illness. There were no increases in levels of either sister chromatid exchanges or total chromosomal aberrations as a result of exposure in either study.

4.4.2 *Experimental systems* (see Table 3 for references)

Ethylene dibromide was mutagenic in bacteria, *Streptomyces coelicolor*, *Aspergillus nidulans*. *Salmonella typhimurium* TA1535 expressing human GST1-1 showed greatly enhanced mutagenicity when treated with ethylene dibromide. Ethylene dibromide was highly mutagenic in *Salmonella typhimurium* NM5004, which has high levels of GST and inducible *umuC* gene expression (Oda *et al*., 1996).

Ethylene dibromide induced delayed sex-linked recessive lethal mutations in spermatozoa and spermatids of adult *Drosophila* males. Mutations were detected in F_3 generations as well as in the conventional F_2 generations.

Ethylene dibromide was mutagenic to *Drosophila melanogaster* and studies in repair-proficient and -deficient strains suggested that the compound is mutagenic through modification of ring nitrogens of purines (N7 of guanine and N1 of adenine).

Ethylene dibromide induced gene mutations, sister chromatid exchanges, chromosomal aberrations and cell transformation in animal cells. It induced mutations in two human lymphoblastoid cell lines, AHH-1 and TK6 in the absence of exogenous metabolic activation. Administration of radiolabelled ethylene dibromide to Wistar rats and BALB/c mice resulted in binding to DNA, RNA and proteins. [The nature of the binding was not characterized.]

Ethylene dibromide gave rise to micronuclei in binucleated peripheral human lymphocytes after a 4-h exposure, whereas a comparable effect in mononucleated cells was observed only after continual exposure.

Ethylene dibromide caused a dose-dependent increase in liver DNA alkaline-labile sites and single-strand breaks (as determined by alkaline elution assay) in female Sprague-Dawley rats. It was positive in an unscheduled DNA synthesis assay in rat spermatocytes *in vitro* but was negative in the spermatocytes of rats dosed *in vivo*. Ethylene dibromide gave positive results in an amphibian (*Pleurodeles waltl*) micronucleus test but gave negative results in dominant lethal tests.

The binding of ethylene dibromide to DNA of human and rat hepatocytes is mediated by GST-catalysed conjugation to glutathione (see Section 4.1.2).

Administration of a single intraperitoneal dose of ethylene dibromide gave rise to *S*-[2-(*N*7-guanyl)ethyl]glutathione DNA adducts in livers of several strains of rats (Fischer 344, Sprague-Dawley and Osborne-Mendel) and mice ($B6C3F_1$, ICR and A/J), with levels in rats being four to five times higher than those in mice.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to ethylene dibromide (1,2-dibromoethane) may occur in pest control, petroleum refining and waterproofing. Dermal exposure is possible when handling leaded gasoline containing ethylene dibromide. It has been detected at low levels in air and water.

Table 3. Genetic and related effects of ethylene dibromide

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links	+	NT	65 µg/assay	Quillardet *et al.* (1985)
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage	+	NT	442	Nakamura *et al.* (1987)
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage	+	NT	0.16% in air	Ong *et al.* (1987)
PRB, SOS *umu* test, *Salmonella typhimurium* NM5004 expressing GST 5-5	+	NT	1.9	Oda *et al.* (1996)
PRB, SOS *umu* test, *Salmonella typhimurium* TA1535/pSK1002	–	NT	19	Oda *et al.* (1996)
ERD, *Escherichia coli pol*A-deficient, differential toxicity	(+)	NT	22000	Brem *et al.* (1974)
SAF, *Salmonella typhimurium* BA13, forward mutation	+	+	54.5	Roldán-Arjona *et al.* (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	NT	2174	McCann *et al.* (1975)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	188	van Bladeren *et al.* (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	94	Stolzenberg & Hine (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	1300	Barber *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	1100	Principe *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	250	Moriya *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	50	Dunkel *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	0.725 in air	Simula *et al.* (1993)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	25	Novotná & Duverger-van Bogaert (1994)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	+	NT	470 µg/disk	Brem *et al.* (1974)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	NT	1880 µg/disk	Brem *et al.* (1974)

Table 3 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	NT	2174	McCann *et al.* (1975)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	47	Rannug *et al.* (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	10	Elliott & Ashby (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	1300	Barber *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	1100	Principe *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	94	Kerklaan *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	17	Dunkel *et al.* (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	25	Novotná & Duverger-van Bogaert (1994)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	220000	Principe *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1667	Dunkel *et al.* (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	1880 µg/disk	Brem *et al*. (1974)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	220000	Principe *et al.* (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1667	Dunkel *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	1300	Barber *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	220000	Principe *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	167	Dunkel *et al.* (1985)
SAS, *Salmonella typhimurium* TA1535 with decreased GSH levels, reverse mutation	+	NT	94	Kerklaan *et al.* (1983)
SAS, *Salmonella typhimurium* TA100 expressing GSTA1-1 or GST1-1, reverse mutation	+	NT	0.544 in air	Simula *et al*. (1993)
SAS, *Salmonella typhimurium* TA1535 expressing GST1-1, reverse mutation	+[c]	NT	18.8	Thier *et al*. (1996)
ECF, *Escherichia coli* (excluding K12), forward mutation	(+)	NT	NG	Izutani *et al.* (1980)
ECK, *Escherichia coli* K12, forward or reverse mutation	+	+	75	Mohn *et al.* (1984)

Table 3 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	+	+	167	Dunkel *et al.* (1985)
EC2, *Escherichia coli* WP2, reverse mutation	(+)	(+)	8800	Scott *et al.* (1978)
STF, *Streptomyces coelicolor*, forward mutation	+	NT	220000	Principe *et al.* (1981)
ANR, *Aspergillus nidulans*, reverse mutation	(+)	(+)	8800	Scott *et al.* (1978)
ANR, *Aspergillus nidulans*, reverse mutation	+	NT	110000	Principe *et al.* (1981)
DMM, *Drosophila melanogaster*, somatic mutation (and recombination)	+		7 ppm inh	Ballering *et al*. (1993)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		56 inh	Vogel & Chandler (1974)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		125 ppm/h inh	Kale & Baum (1979a)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		2.3 ppm/h inh	Kale & Baum (1979b)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		31 ppm/h inh	Kale & Baum (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		1 ppm 3 h inh	Kale & Baum (1983)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		94 feed	Ballering *et al*. (1993)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		25 ppm feed	Foureman *et al.* (1994)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		94 feed	Ballering *et al*. (1994)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		125 ppm inh	Kale & Kale (1995)
DIA, DNA strand breaks, cross-links or related damage, rat hepatocytes *in vitro*	+	NT	5.6	Sina *et al.* (1983)
DIA, DNA strand breaks, cross-links or related damage, rat testicular germ cells *in vitro*	+	NT	117	Bradley & Dysart (1985)
URP, Unscheduled DNA synthesis, Fischer 344 rat primary hepatocytes *in vitro*	+	NT	22	Williams *et al.* (1982)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	NT	9.4	Working *et al*. (1986)
UIA, Unscheduled DNA synthesis, rat spermatocytes *in vitro*	+	NT	18.8	Working *et al*. (1986)
GCO, Gene mutation, Chinese hamster ovary CHO cells *in vitro*	+	+	9.4	Tan & Hsie (1981)

Table 3 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
GCO, Gene mutation, Chinese hamster ovary CHO cells *in vitro*	+	+	7.5	Brimer *et al.* (1982)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	(+)	50	Clive *et al.* (1979)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	NT	94	Tezuka *et al.* (1980)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	5	Ivett *et al*. (1989)
CIC, Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	+	NT	380	Tezuka *et al.* (1980)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	+	125	Ivett *et al*. (1989)
TBM, Cell transformation, BALB/c 3T3 mouse cells	+	+	23	Perocco *et al.* (1991)
TBM, Cell transformation, BALB/c 3T3 mouse cells	+	NT	3	Colacci *et al*. (1995)
GIH, Gene mutation, human epithelial-like (EUE) cells *in vitro*	+	NT	19	Ferreri *et al.* (1983)
GIH, Gene mutation, human lymphoblastoid cell lines (AHH-1 and TK6) *in vitro*	+	NT	5	Crespi *et al*. (1985)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	1.8	Tucker *et al.* (1984)
MIH, Micronucleus test, human lymphocytes *in vitro*	+	NT	188	Channarayappa *et al.* (1992)
DVA, DNA strand breaks, cross-links or related damage, rat liver cells *in vivo*	+		75 po × 1	Nachtomi & Sarma (1977)
DVA, DNA strand breaks, cross-links or related damage, Swiss-Webster mouse liver cells *in vivo*	+		50 ip × 1	White *et al.* (1981)
DVA, DNA strand breaks, cross-links or related damage, $B6C3F_1$ mouse liver *in vivo*	+		90 ip × 1	Storer & Conolly. (1983)
DVA, DNA strand breaks, cross-links or related damage, male Fischer 344 rat testicular germ cells *in vivo*	+		234 ip × 1	Bradley & Dysart (1985)
DVA, DNA strand breaks, cross-links or related damage, female Sprague-Dawley rat liver cells *in vivo*	+		1.8 po × 1	Kitchin & Brown (1994)

Table 3 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
RVA, DNA repair exclusive of unscheduled DNA synthesis, Swiss Webster mouse liver *in vivo*	–		50 ip × 1	White *et al.* (1981)
UPR, Unscheduled DNA synthesis, male Fischer 344 rat hepatocytes *in vivo*	+		100 ip × 1	Working *et al.* (1986)
UPR, Unscheduled DNA synthesis, male Fischer 344 rat hepatocytes *in vivo*	(+)		100 po × 1	Working *et al.* (1986)
UVR, Unscheduled DNA synthesis, male Fischer 344 rat spermatocytes *in vivo*	–		100 ip × 1	Working *et al.* (1986)
UVR, Unscheduled DNA synthesis, male Fischer 344 rat spermatocytes *in vivo*	–		100 po × 1	Working *et al.* (1986)
UVR, Unscheduled DNA synthesis, male Fischer 344 rat spermatocytes *in vivo*	–		150 ip × 1	Bentley & Working (1988)
SVA, Sister chromatid exchange, CD1 mouse bone-marrow cells *in vivo*	(+)		84 ip × 1	Krishna *et al.* (1985)
Micronucleus test, *Pleurodeles waltl in vivo*	+		1 feed	Fernandez *et al.* (1993)
MVM, Micronucleus test, CD1 mouse bone-marrow cells *in vivo*	–		168 ip × 1	Krishna *et al.* (1985)
MVM, Micronucleus test, ddY mice *in vivo*	–		200 ip × 1	Asita *et al.* (1992)
CBA, Chromosomal aberrations, CD1 mouse bone-marrow cells *in vivo*	–		168 ip × 1	Krishna *et al.* (1985)
DLM, Dominant lethal test, ICR/Ha Swiss mice	–		100 po × 1	Epstein *et al.* (1972)
DLM, Dominant lethal test, BDF_1 mice	–		150 po × 5	Teramoto *et al.* (1980)
DLM, Dominant lethal test, male DBA/2J mice	–		100 ip × 1	Barnett *et al.* (1992)
DLR, Dominant lethal test, Sprague-Dawley rats	–		30 po × 5	Teramoto *et al.* (1980)
DLR, Dominant lethal test, Fischer 344 rats	–		75 inj × 1	Teaf *et al.* (1990)
BID, Binding (covalent) to DNA *in vitro*	+	+	10.7	Arfellini *et al.* (1984)
BID, Binding (covalent) to DNA *in vitro*	NT	+	10	Colacci *et al.* (1985)
BID, Binding (covalent) to DNA *in vitro*	+	NT	94	Inskeep *et al.* (1986)

Table 3 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BID, Binding (covalent) to calf thymus DNA *in vitro*	NT	+	11	Prodi *et al.* (1986)
BID, Binding (covalent) to DNA, rat hepatocytes *in vitro*	+	NT	94	Cmarik *et al.* (1990)
BIH, Binding (covalent) to DNA, human hepatocytes *in vitro*	+	NT	94	Cmarik *et al.* (1990)
BIP, Binding (covalent) to human albumin, *in vitro*	NT	+	28.9	Kaphalia & Ansari (1992)
BIP, Binding (covalent) to RNA or protein *in vitro*	+	+	10.7	Arfellini *et al.* (1984)
BVD, Binding (covalent) to liver, kidney, stomach lung DNA, BALB/c mice *in vivo*	+		1.6 ip × 1	Arfellini *et al.* (1984)
BVD, Binding (covalent) to liver, kidney, stomach lung DNA, Wistar rats *in vivo*	+		1.6 ip × 1	Arfellini *et al.* (1984)
BVD, Binding (covalent) to DNA, Sprague-Dawley rat hepatocytes *in vivo*	+		37 ip × 1	Inskeep *et al.* (1986)
BVD, Binding (covalent) to DNA, Sprague-Dawley rat hepatocytes *in vivo*	+		37 ip × 1	Kim & Guengerich (1990)
BVD, Binding (covalent) to DNA, Fischer 344 rat hepatocytes *in vivo*	+		37 ip × 1	Kim & Guengerich (1990)
BVD, Binding (covalent) to DNA, Osborne-Mendel rat hepatocytes *in vivo*	+		37 ip x 1	Kim & Guengerich (1990)
BVD, Binding (covalent) to DNA, ICR Swiss mouse hepatocytes *in vivo*	+		37 ip × 1	Kim & Guengerich (1990)
BVD, Binding (covalent) to DNA, B6C3F$_1$ mouse hepatocytes *in vivo*	+		37 ip × 1	Kim & Guengerich (1990)
BVD, Binding (covalent) to liver, kidney, stomach, lung DNA, Wistar rats *in vivo*	+		1.2 ip × 1	Prodi *et al.* (1986)
BVD, Binding (covalent) to liver, kidney, stomach, lung DNA, BALB/c mice *in vivo*	+		1.2 ip × 1	Prodi *et al.* (1986)

Table 3 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BVP, Binding (covalent) to RNA or protein, BALB/c mouse liver, kidney, stomach, lung *in vivo*	+		1.6 ip × 1	Arfellini *et al*. (1984)
BVP, Binding (covalent) to RNA or protein, Wistar rat liver, kidney, stomach, lung *in vivo*	+		1.6 ip × 1	Arfellini *et al*. (1984)
BVP, Binding (covalent) to RNA, or proteins, BALB/c mice *in vivo*	+		1.2 ip × 1	Prodi *et al.* (1986)
BVP, Binding (covalent) to RNA, or proteins, Wistar rats *in vivo*	+		1.2 ip × 1	Prodi *et al.* (1986)
BVP, Binding (covalent) to albumin, Sprague-Dawley rats *in vivo*	+		25 po × 2	Kaphalia & Ansari (1992)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; inh, inhalation; po, oral; ip, intraperitoneal; inj, injection

[c] Results were negative for strain TA1535 not expressing GST1-1 at doses of up to 0.5 mM (94 µg/mL)

5.2 Human carcinogenicity data

Three cohort studies have included workers exposed to ethylene dibromide, but because of their low statistical power and/or lack of information about individual exposures, little can be concluded about the carcinogenicity of this compound in humans.

5.3 Animal carcinogenicity data

Ethylene dibromide has been tested for carcinogenicity by oral administration in mice, rats and fish, by inhalation in mice and rats and by skin application in mice. Following its oral administration, it produced squamous-cell carcinomas of the forestomach in rodents of both species, an increased incidence of alveolar/bronchiolar lung tumours in mice of each sex, haemangiosarcomas in male rats, oesophageal papillomas in female mice and liver and stomach tumours in fish. Following its inhalation, ethylene dibromide produced adenomas and carcinomas of the nasal cavity, haemangiosarcomas, mammary gland tumours, subcutaneous mesenchymal tumours, an increased incidence of alveolar/bronchiolar lung tumours in animals of each species and an increased incidence of peritoneal mesotheliomas in male rats. It induced skin and lung tumours in mice after skin application.

5.4 Other relevant data

In rodents and humans, ethylene dibromide is metabolized both by cytochrome P450 and GST enzymes; the latter seem to be responsible for DNA adduct formation. In rodents, covalently bound radioactivity has been detected in the epithelial lining of a number of organs.

In humans, acute high-dose exposure leads to liver and kidney damage. In rodents, inhalation exposure causes primarily proliferative lesions in nasal cavities. After intragastric administration, liver and kidney were the main target organs. Some evidence of adverse effects on reproduction was observed both in humans and rodents.

Ethylene dibromide is mutagenic in bacteria and *Drosophila*, and in rodent and human cells *in vitro*. It induced DNA breakage but not chromosomal aberrations or micronuclei *in vivo* in rodents. It gave negative results in dominant lethal tests in mice and rats. It did not induce either chromosomal aberrations or sister chromatid exchange in humans *in vivo*.

Ethylene dibromide binds to DNA *in vitro* and *in vivo* in rodents.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of ethylene dibromide.

There is *sufficient evidence* in experimental animals for the carcinogenicity of ethylene dibromide.

Overall evaluation

Ethylene dibromide is *probably carcinogenic to humans (Group 2A)*.

In making the overall evaluation, the Working Group took into consideration that ethylene dibromide is genotoxic in a broad range of in-vitro and in-vivo assays and binds covalently with DNA *in vivo*.

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HYDROGEN PEROXIDE

Data were last reviewed in IARC (1985) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

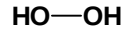
Chem. Abstr. Serv. Reg. No.: 7722-84-1

Chem. Abstr. Name: Hydrogen peroxide

IUPAC Systematic Name: Hydrogen peroxide

Synonyms: Dihydrogen dioxide; hydrogen dioxide; hydrogen oxide; hydroperoxide; peroxide

1.1.2 *Structural and molecular formulae and relative molecular mass*



H_2O_2 Relative molecular mass: 34.0

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid with a bitter taste (Budavari, 1996; Lide, 997)

(*b*) *Boiling-point:* 150.2°C (Lide, 1997)

(*c*) *Melting-point:* –0.43°C (Lide, 1997)

(*d*) *Solubility:* Very soluble in water; soluble in diethyl ether; insoluble in petroleum ether (Budavari, 1996; Lide, 1997)

(*e*) *Vapour pressure:* 665 Pa at 30°C (American Conference of Governmental Industrial Hygienists, 1992)

(*f*) *Reactivity*: May decompose violently if traces of impurities are present; decomposed by many organic solvents (Budavari, 1996)

(*g*) *Conversion factor*: $mg/m^3 = 1.39 \times ppm$

1.2 Production and use

Production capacity of hydrogen peroxide in North America (including plants in the United States, Canada and Mexico) in 1995 was reported to be 547 thousand tonnes; that in the United States in 1992 was reported to be 348 thousand tonnes and, in Canada, 143

thousand tonnes. Worldwide capacity for hydrogen peroxide is estimated at 1800–1900 thousand tonnes per year (Anon., 1992, 1995; Hess, 1995).

Hydrogen peroxide is an oxidizing agent widely used for the bleaching or deodorizing of textiles, wood pulp, hair, fur and foods; in the treatment of water and sewage; as a disinfectant; as a component of rocket fuels; and in the manufacture of paper and pulp, foam rubber and many chemicals and chemical products. It has also been used in the synthesis of organic and inorganic peroxides; in the manufacture of glycerol, plasticizers and antichlors; in epoxidation, hydroxylation, oxidation, and reduction reactions; for viscosity control for starch and cellulose derivatives; for refining and cleaning metals; in dyeing and electroplating; and as a laboratory reagent, seed disinfectant and neutralizing agent in wine distillation (IARC, 1985; American Conference of Governmental Industrial Hygienists, 1992; Lewis, 1993).

Other uses for hydrogen peroxide in the United States are in the removal of hydrogen sulfide from the steam produced by geothermal power plants, during the mining and processing of uranium, pickling of copper and copper alloys, cleaning metals (germanium) and silicon semiconductors used in the electronics industry, and a variety of small-volume applications in photography, cosmetics (e.g., hair bleaches and dyes, mouthwashes), antiseptics and cleansing agents, food and wine processing and treatment of package liners in aseptic packaging (IARC, 1985).

The consumption pattern for hydrogen peroxide in the United States in 1995 was (%): pulp and paper, 50; environmental uses, including water treatment, 17; chemical synthesis, 15; textiles, 9; and miscellaneous, including mining, electronic, food and cosmetic uses, and the distributor market, 9 (Anon., 1995).

1.3 Occurrence

1.3.1 *Occupational exposure*

Occupational exposures may occur in the production of hydrogen peroxide, in wastewater treatment, metal cleaning, and chemical synthesis, and in the textile, pulp and paper, geothermal energy and mining industries (IARC, 1985).

1.3.2 *Environmental occurrence*

Gaseous hydrogen peroxide is a key component and product of the earth's lower atmospheric photochemical reactions, in both clean and polluted atmospheres. Atmospheric hydrogen peroxide is believed to be generated exclusively by gas-phase photochemical reactions (IARC, 1985). Low concentrations of hydrogen peroxide have been measured in the gas-phase and in cloud water in the United States (United States National Library of Medicine, 1998). It has been found in rain and surface water, in human and plant tissues, in foods and beverages and in bacteria (IARC, 1985).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 1.4 mg/m^3 as the 8-h time-weighted average threshold limit value for

occupational exposures to hydrogen peroxide in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for hydrogen peroxide in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

In the Montreal case–control study carried out by Siemiatycki (1991) (see the monograph on dichloromethane in this volume), the investigators estimated the associations between 293 workplace substances and several types of cancer. Hydrogen peroxide was one of the substances. About 0.7% of the study subjects had ever been exposed to hydrogen peroxide. Among the main occupations to which this exposure was attributed were hairdressers, textile bleachers and furriers. For all types of cancer examined (oesophagus, stomach, colon, rectum, pancreas, lung, prostate, bladder, kidney, skin melanoma, lymphoma), there was no indication of an excess risk due to hydrogen peroxide exposure. [The interpretation of the null results has to take into account the small numbers and possibly low exposure levels.]

3. Studies of Cancer in Experimental Animals

Hydrogen peroxide had been tested for carcinogenicity in mice, by oral administration in drinking-water, by skin application and by subcutaneous administration. Adenomas and carcinomas of the duodenum were reported following its oral administration. The other studies were inadequate for an evaluation of carcinogenicity. One study by skin application indicated that hydrogen peroxide has no promoting activity (IARC, 1985).

3.1 Topical administration

Hamster: Groups of 25 male and 25 female Syrian golden hamsters, 8–10 weeks of age, were administered hydrogen peroxide at a concentration of 0.75% in dentifrice introduced into the buccal cheek pouches five times per week for 20 weeks. The hydrogen peroxide-containing dentifrice induced no neoplasms in 37 animals surviving to 20 weeks (Marshall *et al*., 1996). [The Working Group noted the unusual vehicle and the short duration of the study.]

3.2 Administration with known carcinogens

3.2.1 *Hamster*

Groups of 30–40 male and female Syrian golden hamsters, eight weeks of age, were administered hydrogen peroxide [purity unspecified] by topical application to the cheek pouch of 20 μL of a 30% solution on five days per week for 24 weeks, after which they

were maintained for up to 16 months. Another group received hydrogen peroxide for 24 weeks after an initiating dose of 4-(nitrosomethylamino)-1-(3-pyridyl)-1-butanone, after which they were also maintained for up to 16 months. In the group given hydrogen peroxide after initiation, 1/31 animals developed a cheek pouch adenoma, compared with 1/15 with initiator alone (Padma *et al*., 1989).

3.2.2 *Trout*

Groups of 52–93 Shasta rainbow trout embryos, 23 days of age, were exposed to *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine to initiate hepatic carcinogenesis and four weeks after hatching were administered hydrogen peroxide [purity unspecified] at 0, 600 or 3000 ppm [mg/kg] in diets containing two levels of vitamin E for 10 months. Hydrogen peroxide increased the incidence of liver tumours, mainly mixed hepatocholangiocellular carcinomas, in a dose-related manner, especially in fish given the higher level of vitamin E, from about 15% in fish exposed only to the initiator to about 25% with low-dose hydrogen peroxide and to about 45% with high-dose hydrogen peroxide ($p < 0.02$) (Kelly *et al*., 1992). [The Working Group noted the complexity of oral administration in the diet and the presence of other variables in the diets.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Glutathione peroxidase, responsible for decomposing hydrogen peroxide, is present in normal human tissues. Hydrogen peroxide has been detected in serum and in intact liver (IARC, 1985).

4.1.2 *Experimental systems*

Hydrogen peroxide is formed intracellularly by mitochondria, endoplasmic reticulum, peroxisomes and soluble enzymes, where it results from oxidase-catalysed reactions or superoxide dismutase-catalysed superoxide breakdown. It is decomposed by catalase or glutathione peroxidase. Levels of hydrogen peroxide are particularly high in rat kidney, reflecting the high peroxisomal content, and polymorphonuclear leukocytes during phagocytosis (IARC, 1985). These levels are markedly increased in rat liver homogenates after in-vivo administration of peroxisome proliferators (Tamura *et al*., 1990).

The presence of oxygen bubbles in the tongue and jugular veins following sublingual application of 3–30% hydrogen peroxide solutions to dogs, cats and rabbits suggests that significant amounts of hydrogen peroxide were absorbed. Ingested hydrogen peroxide can increase the oxygen content of blood, also indicating absorption by the intestine. It can penetrate the epidermis and mucous membranes and decomposes in the underlying

tissues. Within 1 h, 33% of the ^{18}O of a 19% solution of $H_2{}^{18}O_2$ was recovered in expired air following sublingual application to cats (IARC, 1985).

4.2 Toxic effects

The toxicity of hydrogen peroxide has been reviewed (Li, 1996).

4.2.1 *Humans*

A characteristic whitening of the skin occurs after topical application of hydrogen peroxide, which is believed to be the result of oxygen bubbles acting microembolically in the capillaries. Human erythrocytes exhibit increased osmotic fragility when incubated with hydrogen peroxide; this is related to lipid peroxidation. Erythrocytes from individuals with enzyme deficiencies related to oxygen radical metabolism, such as those with acatalasaemia, favism, paroxysmal nocturnal haemoglobinuria, erythropoietic protoporphyria or thalassaemia, or with glutathione-metabolizing enzyme or vitamin E deficiencies, are unusually sensitive to hydrogen peroxide-induced haemolysis (IARC, 1985).

4.2.2 *Experimental systems*

Hydrogen peroxide, administered extrinsically or produced intrinsically, generates hydroxyl radicals and induces lipid peroxidation and may lead to DNA damage and cell death. In in-vitro studies, these effects may be prevented by antioxidants or iron chelators (IARC, 1985). In line with these findings, hydrogen peroxide evoked a dose-dependent increase in dichlorofluorescein fluorescence intensity in Hep G_2 cells, and this effect was completely blocked by catalase or a water-soluble vitamin E (Trolox C) (Wu *et al*., 1997). Low (10^{-8} mol/L), but not high ($\geq 10^{-5}$ mol/L) concentrations of hydrogen peroxide stimulated the growth of immortalized hamster BHK-2 cells, H-*ras* transformed RFAGT1 rat cells (Burdon *et al*., 1990) and BHK-21 fibroblasts *in vitro* (Burdon *et al*., 1996).

Hydrogen peroxide induced squamous metaplasia in hamster tracheal explants at concentrations of 50–100 μmol/L, while cytotoxicity was observed only at concentrations ≥ 500 μmol/L. Squamous metaplasia was prevented by exogenous addition of catalase (Radosevich & Weitzman, 1989).

At a concentration of 700 μmol/L, hydrogen peroxide induced necrosis of immortalized rat embryo fibroblasts, while at a concentration of 150 μmol/L, it induced apoptosis (Guénal *et al*., 1997). In primary human diploid fibroblasts, low concentrations (50–100 μmol/L) of hydrogen peroxide induced a senescence-like state, while higher concentrations (300–400 μmol/L) induced apoptosis (Bladier *et al*., 1997). Apoptosis was also observed in BHK-21 fibroblasts at hydrogen peroxide concentrations of ≥ 100 μmol/L (Burdon *et al*., 1996).

Hydrogen peroxide (50 μmol/L) induced transcription of the early growth response 1 gene (*EGR*1) in a human HL-525 myeloid leukaemia cell line; this was prevented by *N*-acetyl-L-cysteine (Datta *et al*., 1993).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Hydrogen peroxide induced DNA damage in bacteria and mutation in *Salmonella typhimurium* and *Escherichia coli* in the absence of exogenous metabolic activation. It was not mutagenic in *S. typhimurium* in the presence of exogenous metabolic activation. It induced forward mutation in *Saccharomyces cerevisiae* and was mutagenic to *Aspergillus nidulans* and *Neurospora crassa*. In a single study, sex-linked recessive lethal mutations were not induced in *Drosophila* following larval injections with 3% hydrogen peroxide.

Hydrogen peroxide induced DNA damage in Chinese hamster cell cultures. It induced a weak mutagenic response at the *hprt* locus in one study using L5178Y mouse lymphoma cell sublines (LY-R and LY-S). Only one of six studies reviewed reported that hydrogen peroxide induced gene mutation in Chinese hamster V79 cells at the *hprt* locus. Hydrogen peroxide induced sister chromatid exchanges in Chinese hamster cell cultures (Chinese hamster ovary CHO or lung V79) and inhibited gap junctional intercellular communication in WB-Fischer 344 rat liver epithelial cells. It did not bind covalently to DNA in mouse keratinocytes *in vitro*. It did induce chromosomal aberrations in Chinese hamster cells and in ascites tumour cells of mice treated *in vivo*. In a single study *in vivo*, hydrogen peroxide did not increase the frequency of chromosomal aberrations in rat bone marrow.

DNA single-strand breaks and fragmentations were observed in human lymphocytes and respiratory tract epithelial cells and in cultures of transformed human cells. Hydrogen peroxide induced unscheduled DNA synthesis and chromosomal aberrations in human fibroblast cells *in vitro*. It induced sister chromatid exchanges or chromosomal aberrations in human lymphocyte cultures and gave inconclusive results for induction of aneuploidy.

Hydrogen peroxide transformed mouse myeloid progenitor cells (FDC-P1) from interleukin-3 dependence to factor independence, but only at cytotoxic concentrations (≥ 12/5 μmol/L). Such a transformation was not induced by non-specific insults to the cells, such as sodium fluoride or heat shock treatment. The transformed cells produced tumours when injected into pre-irradiated mice (Crawford & Greenberger, 1991). Hydrogen peroxide (10 μmol/L) induced overexpression of the proto-oncogene c-*jun* in hamster tracheal epithelial (HTE) cells; c-*jun* overexpression led to proliferation and increased growth rate, as well as increased anchorage-independence of HTE cells (Timblin *et al*., 1995).

Table 1. Genetic and related effects of hydrogen peroxide

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
PRB, Prophage, induction/SOS response/strand-breaks/or cross-links	+	NT	0.5	Müller & Janz (1993)
PRB, Prophage, induction/SOS response/strand-breaks/or cross-links	+	NT	1	Northrop (1958)
PRB, Prophage, induction/SOS response/strand-breaks/or cross-links	+	NT	45	Nakamura *et al*. (1987)
BRD, *Escherichia coli*, differential toxicity	+	NT	20	Hartman & Eisenstark (1978)
BRD, *Escherichia coli*, differential toxicity	+	NT	340	Ananthaswamy & Eisenstark (1977)
SAF, *Salmonella typhimurium* BA13, forward mutation	+	NT	0.2	Ariza *et al*. (1988)
SAF, *Salmonella typhimurium* (SV50), forward mutation	+	NT	0.22	Xu *et al*. (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	NT	340	Stich *et al*. (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	NT	136	Norkus *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	0.9	Xu *et al*. (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	NT	5	Fujita *et al*. (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	–	5780	Kensese & Smith (1989)
SA2, *Salmonella typhimurium* TA102, reverse mutation	(+)	–	5780	Kensese & Smith (1989)
SA2, *Salmonella typhimurium* TA102, reverse mutation	(+)	NT	20.4	Abu-Shakra & Zeiger (1990)
SA4, *Salmonella typhimurium* TA104, reverse mutation	+	NT	10	Abu-Shakra & Zeiger (1990)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	+	–	4046	Kensese & Smith (1989)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	(+)	–	5780	Kensese & Smith (1989)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	340	Stich *et al*. (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	0.9	Xu *et al*. (1984)
SA9, *Salmonella typhimurium* TA98, reverse mutation	(+)	–	5780	Kensese & Smith (1989)
SAS, *Salmonella typhimurium* hisC3108, reverse mutation	+	NT	30	Ames *et al*. (1981)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SAS, *Salmonella typhimurium* TA96, reverse mutation	+	NT	50	Levin *et al*. (1982)
SAS, *Salmonella typhimurium* TA97, reverse mutation	(+)	–	2890	Kensese & Smith (1989)
SAS, *Salmonella typhimurium* TA97, reverse mutation	+	NT	4.25	Abu-Shakra & Zeiger (1990)
SAS, *Salmonella typhimurium* SB1106p, reverse mutation	+	NT	5.1	Abu-Shakra & Zeiger (1990)
SAS, *Salmonella typhimurium* SB1111, reverse mutation	(+)	NT	10	Abu-Shakra & Zeiger (1990)
SAS, *Salmonella typhimurium* SB1106, reverse mutation	+	NT	10	Abu-Shakra & Zeiger (1990)
ECF, *Escherichia coli* (excluding K12), forward mutation	+	NT	3	Abril & Pueyo (1990)
ECR, *Escherichia coli* WP2, reverse mutation	+	NT	2160	Demerec *et al*. (1951)
BSM, *Bacillus subtilis*, multigene test	+	NT	7.2	Sacks & MacGregor (1982)
MAF, *Micrococcus aureus*, forward mutation	+	NT	6	Clark (1953)
SCF, *Saccharomyces cerevisiae ade2*, forward mutation	+	NT	100	Thacker (1976)
SCF, *Saccharomyces cerevisiae ade2*, forward mutation	+	NT	2000	Thacker & Parker (1976)
SGR, *Streptomyces griseoflavus*, reverse mutation	–	NT	1440	Mashima & Ikeda (1958)
ANR, *Aspergillus chevalieres*, reverse mutation	(+)	NT	1440	Nanda *et al*. (1975)
NCF, *Neurospora crassa*, forward mutation	(+)	NT	9180	Han (1997)
NCR, *Neurospora crassa*, reverse mutation	+	NT	7140	Dickey *et al*. (1949)
NCR, *Neurospora crassa*, reverse mutation	+	NT	6800	Jensen *et al*. (1951)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		43200 inj	Dipaolo (1952)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
DIA, DNA single-strand breaks, Chinese hamster lung V79 cells *in vitro*	(+)[c]	NT	12	Bradley *et al*. (1979)
DIA, DNA single-strand breaks, rat hepatocytes *in vitro*	+	NT	3.4	Olson (1988)
DIA, DNA single-strand breaks, Chinese hamster ovary CHO cells *in vitro*	+	NT	3.4	Cantoni *et al*. (1989)
DIA, DNA single-strand breaks, Chinese hamster lung V79-379A fibroblasts *in vitro*	+	NT	0.34	Prise *et al*. (1989)
DIA, DNA single-strand breaks, Chinese hamster ovary CHO cells *in vitro*	+	NT	0.85	Cantoni *et al*. (1992)
DIA, DNA single-strand breaks, Chinese hamster ovary CHO cells *in vitro*	+	NT	0.68	Iliakis *et al*. (1992)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	12	Bradley *et al*. (1979)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	20	Bradley & Erickson (1981)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	3.4	Tsuda (1981)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	7	Nishi *et al*. (1984)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	13.6	Speit (1986)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	+	NT	17	Ziegler-Skylakakis & Andrae (1987)
G9O, Gene mutation, Chinese hamster lung V79 cells, ouabain resistance *in vitro*	–	NT	3.4	Tsuda (1981)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
G51, Gene mutation, mouse lymphoma L5178Y cell subline LY-R, *hprt* locus *in vitro*	(+)	NT	0.17	Kruszewski *et al*. (1994)
G51, Gene mutation, mouse lymphoma L5178Y cell subline LY-S, *hprt* locus *in vitro*	(+)	NT	0.34	Kruszewski *et al*. (1994)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	(+)	NT	12	Bradley *et al*. (1979)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	NT	0.13	MacRae & Stich (1979)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	(+)	NT	17	Wilmer & Natarajan (1981)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	NT	3.4	Speit *et al*. (1982)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	(+)	0.34	Mehnert *et al*. (1984a)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	(+)	0.34	Mehnert *et al*. (1984a)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	(+)	NT	7	Nishi *et al*. (1984)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	NT	0.68	Speit (1986)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO AU × 91 cells *in vitro*	+	NT	1.4	Tucker *et al*. (1989)
MIA, Micronucleus test, C57BL/6J mouse splenocytes *in vitro*	–	NT	0.68	Dreosti *et al*. (1990)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	(+)	NT	10	Stich *et al*. (1978)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
CIC, Chromosomal aberrations, Chinese hamster DON-6 cells *in vitro*	+	NT	34	Sasaki *et al*. (1980)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO-K1 cells *in vitro*	+	NT	3.4	Tsuda (1981)
CIC, Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	+	NT	3.4	Tsuda (1981)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	(+)	NT	340	Wilmer & Natarajan (1981)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	(+)	NT	1	Hanham *et al*. (1983)
CIM, Chromosomal aberrations, newborn BALB/c mouse back-skin cells *in vitro*	+	NT	0.34	Tsuda (1981)
CIS, Chromosomal aberrations, Syrian hamster lung cells *in vitro*	+	NT	3.4	Tsuda (1981)
DIH, DNA single-strand breaks, transformed human WI-38 & XP cells *in vitro*	(+)	NT	3.4	Hoffmann & Meneghini (1979)
DIH, DNA single-strand breaks, human D98/AH2 cells *in vitro*	+	NT	2	Wang *et al*. (1980)
DIH, DNA single-strand breaks, human epithelioid P3 cells *in vitro*	+	NT	0.21	Peak *et al*. (1991)
DIH, DNA single-strand breaks, human cells *in vitro*	+	NT	0.85	Meyers *et al*. (1993)
DIH, DNA single-strand breaks, human leukocytes *in vitro*	+	NT	17	Rueff *et al*. (1993)
DIH, DNA damage, human bronchial epithelium (HBEI) cells *in vitro*	+	NT	1.7	Spencer *et al*. (1995)
DIH, DNA damage, human bronchial epithelium (BEAS and NHBE) cells *in vitro*	+	NT	0.68	Lee *et al*. (1996)
DIH, DNA damage, human lymphobastoid (GM1899A) cells *in vitro*	+[d]	NT	0.34	Duthie & Collins (1997)
UHF, Unscheduled DNA synthesis, human fibroblasts *in vitro*	+	NT	20	Stich *et al*. (1978)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
UHF, Unscheduled DNA synthesis, human fibroblasts *in vitro*	+	NT	9	Coppinger *et al*. (1983)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	(+)	2.7	Mehnert *et al*. (1984b)
CHF, Chromosomal aberrations, human fibroblasts *in vitro*	+	NT	0.07	Parshad *et al*. (1980)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	0.17	Smith *et al*. (1990)
CIH, Chromosomal aberrations, human embryonic fibroblasts *in vitro*	+	NT	0.34	Oya *et al*. (1986)
CIH, Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	510	Rueff *et al*. (1993)
AIH, Aneuploidy, human lymphocytes *in vitro*	?	NT	0.17	Smith *et al*. (1990)
CBA, Chromosomal aberrations, rat bone-marrow cells *in vivo*	–		NG	Kawachi *et al*. (1980)
CVA, Chromosomal aberrations, mouse ascites tumour cells *in vivo*	+		340 µg/mouse	Schöneich (1967)
CVA, Chromosomal aberrations, mouse ascites tumour cells *in vivo*	+		170 µg/mouse	Schöneich *et al*. (1970)
BID, DNA binding (covalent), 8-hydroxydeoxyguanosine, BALB/c mouse keratinocytes *in vitro*	–	NT	680	Beehler *et al*. (1992)
ICR, Inhibition of cell communication, WB-Fischer 344 rat liver epithelial cells *in vitro*	+	NT	3.4	Upham *et al*. (1997)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; inj, injection; NG, not given

[c] Negative for DNA–DNA and DNA–protein cross-links

[d] Positive at 50 µM (1.7 µg/mL) for HeLa, CaCo-2 colon cells and HepG2 liver cells.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Hydrogen peroxide is produced in moderately high volume and is widely used. Its primary uses are as a chemical intermediate, as a bleaching agent in the textile and paper and pulp industry and in water treatment operations. It occurs naturally at low levels in the air and water, in human and plant tissues and bacteria, and in food and beverages.

5.2 Human carcinogenicity data

No adequate data on the carcinogenicity of hydrogen peroxide were available to the Working Group.

5.3 Animal carcinogenicity data

Hydrogen peroxide was tested in mice by oral administration, skin application and subcutaneous administration and in hamsters by topical application to oral mucosa. In mice, adenomas and carcinomas of the duodenum were found following oral administration. The other studies in mice and the study in hamsters were inadequate for evaluation. One study in mice and one study in hamsters showed no promoting activity of hydrogen peroxide.

5.4 Other relevant data

Hydrogen peroxide is formed intracellularly as a result of certain enzymatic reactions. Hydrogen peroxide, either from this source or externally applied, generates hydroxyl radicals that initiate lipid peroxidation chain reactions within exposed cells and can lead to DNA damage and cell death. DNA damage has been demonstrated in bacteria and in cultured mammalian cells. In addition, hydrogen peroxide induced mutations in bacteria, yeast and other fungi and there is some evidence that it can do so in Chinese hamster V79 and mouse lymphoma L5178Y cells at the *hprt* locus. Chromosomal aberrations and sister chromatid exchanges are induced in both human and other mammalian cells *in vitro*, but it did not induce chromosomal aberrations in the bone-marrow cells of exposed rats.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of hydrogen peroxide.

There is *limited evidence* in experimental animals for the carcinogenicity of hydrogen peroxide.

Overall evaluation

Hydrogen peroxide is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

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HYDROQUINONE

Data were last reviewed in IARC (1977) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

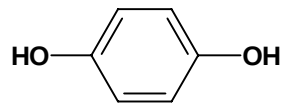
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 123-31-9
Chem. Abstr. Name: 1,4-Benzenediol
IUPAC Systematic Name: Hydroquinone
Synonym: Benzoquinol

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_6H_6O_2$ Relative molecular mass: 110.11

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Hexagonal prisms (Verschueren, 1996)
(*b*) *Boiling-point*: 287°C (Lide, 1997)
(*c*) *Melting-point*: 172.3°C (Lide, 1997)
(*d*) *Solubility*: Soluble in water, ethanol and diethyl ether (Lewis, 1993)
(*e*) *Vapour pressure*: 532 Pa at 150°C; relative vapour density (air = 1), 3.81 (Verschueren, 1996)
(*f*) *Flash-point:* 165°C, closed cup (American Conference of Governmental Industrial Hygienists, 1992)
(*g*) *Conversion factor*: $mg/m^3 = 4.5 \times ppm$

1.2 Production and use

In 1992, world production of hydroquinone was approximately 35 thousand tonnes (United States, 16; Europe, 11; Japan, 6; Central and South America and Asian countries other than Japan, 2) (WHO, 1994).

Hydroquinone is used as a photographic developer (with black-and-white film), a dye intermediate, a stabilizer in paints, varnishes, motor fuels and oils, an antioxidant for fats and oils, an inhibitor of polymerization and in the treatment of skin hyperpigmentation (Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 100 000 workers in the United States were potentially exposed to hydroquinone (see General Remarks). Occupational exposures to hydroquinone may occur in its production and use in the production of dyes, paints, motor fuels and oils, and some polymers. Dermal contact with hydroquinone may occur in the development of black-and-white photographs.

1.3.2 *Environmental occurrence*

Hydroquinone is both a natural and an anthropogenic compound. It occurs naturally as a conjugate with β-D-glucopyranoside in the leaves, bark and fruit of a number of plants, especially the ericaceous shrubs such as cranberry, cowberry, bearberry and blueberry. It may be released to the environment as a fugitive emission during its production, formulation and use as a chemical intermediate, photographic chemical and stabilizer (United States National Library of Medicine, 1997). Users of skin-bleaching formulations may be exposed to hydroquinone.

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 2 mg/m^3 as the 8-h time-weighted average threshold limit value for occupational exposures to hydroquinone in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for hydroquinone in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

One of the most prominent uses of hydroquinone is in photographic development and it is possible that work as a photographic processor often involved hydroquinone exposure in the past. Several studies have examined cancer risks among photographic processors. However, the Working Group did not use these except where the report provided some information indicating that the workers concerned had indeed been exposed to hydroquinone.

2.1 Cohort studies

Pifer *et al*. (1995) reported a cohort mortality study of 879 workers (22 895 person–years of follow-up) at a Tennessee (United States) plant in which hydroquinone was manufactured and used over several decades. Job history records were linked to extensive industrial hygiene data and expertise to estimate cumulative exposure to hydroquinone. Average hydroquinone dust levels ranged from 0.1 to 6.0 mg/m^3, with levels over 2 mg/m^3 for most of the period of operation of the plant. Mean employment duration was 13.7 years and mean follow-up from first exposure was 26.8 years. Relative risk estimates (standardized mortality ratios (SMRs)) for this cohort were derived by comparison with the general population of Tennessee as well as with an occupational cohort not exposed to hydroquinone (a plant of the same company, located in New York State). The SMR for all causes of death combined (n = 168) was significantly below 1.0, as was the SMR for all cancers combined (n = 33). Only two sites, colon (n = 5) and lung (n = 14) had more than three observed cases. Most site-specific SMRs were well below 1.0. The results were similar with both comparison populations. The dose–response analyses of selected cancer sites did not reveal any meaningful trend or heterogeneity. [The numbers for individual cancer sites were small and the power to detect effects was weak. The Working Group noted that this cohort had systematically lower SMRs than the comparison industrial cohort.]

Nielsen *et al*. (1996) carried out a cohort incidence study among 837 Danish lithographers born between 1933 and 1942 and registered with the Danish Union of Lithographers in 1974 or later. Questionnaires were sent to cohort members in 1989 to obtain information on job exposures; usable responses were received from 620 workers. About one-quarter of the cohort members reported working regularly with hydroquinone for photographic development. The entire cohort was traced in the Danish Cancer Registry from 1974 to 1989. Relative risk estimates (standardized incidence ratios (SIRs)) for this cohort were derived by comparison with the general population of Denmark. There were a total of 24 cancers registered, giving an SIR of 0.9. For no site except skin were there more than three cases. Five cases of malignant melanoma occurred, with 1.5 expected (SIR, 3.4; 95% confidence interval, 1.2–7.5). Among these five, two had reportedly been exposed to hydroquinone.

3. Studies of Cancer in Experimental Animals

In skin painting studies in mice, hydroquinone was inactive as an initiator of skin carcinogenesis. In bladder implantation studies, hydroquinone in cholesterol pellets increased the incidence of bladder carcinomas in mice (IARC, 1977).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 55 male and 55 female B6C3F$_1$ mice, eight to 10 weeks of age, were administered 0, 50 or 100 mg/kg bw hydroquinone (purity, > 99%) by gavage on five days per

week for 103 weeks. Mean body weights of high-dose mice at the end of the study were lower than those of vehicle controls, and the relative liver weights were increased for exposed males and high-dose females. Survival in treated mice was similar to that in controls. No increase in tumours was found in exposed males. In females, hepatocellular adenomas were found in 2/55 controls, 15/55 low-dose group ($p = 0.001$) and 12/55 high-dose group ($p = 0.005$) (United States National Toxicology Program, 1989).

Groups of 28–30 male and 28–30 female B6C3F$_1$ mice, six weeks of age, were given hydroquinone (purity, > 99%) in the diet at concentrations of 0 or 0.8% for 96 weeks. The final body weight was reduced in hydroquinone-treated females. The incidence of hepatocellular adenoma was increased to 14/30 in exposed males ($p < 0.05$) compared with 6/28 in controls. Incidence of no other tumour type was significantly increased by exposure in males, although three renal adenomas occurred. No increase in tumour incidence was found in females (Shibata *et al*., 1991).

3.1.2 *Rat*

Groups of 55 male and 55 female Fischer 344/N rats, seven to nine weeks of age, were administered 0, 25 or 50 mg/kg bw hydroquinone (purity, > 99%) by gavage on five days per week for 103 weeks. Mean body weights of exposed males were reduced and the relative kidney weights for high-dose males were greater than those for vehicle controls. Survival was reduced in exposed animals. In exposed males, renal tubule cell adenomas developed in 4/55 low-dose group ($p = 0.069$) and 8/55 high-dose group ($p = 0.003$) compared with 0/55 controls. In exposed females, mononuclear cell leukaemia developed in 15/55 low-dose group ($p = 0.048$) and 22/55 high-dose group ($p = 0.003$) compared with 9/55 controls. The historical incidence of leukaemia for water/vehicle control female rats was 25 ± 15% (United States National Toxicology Program, 1989). [The Working Group noted that the incidences of leukaemia in the exposed group were within the historical control range.]

Groups of 30 male and 30 female Fischer 344 rats, six weeks of age, were given hydroquinone (purity, > 99%) in the diet at concentrations of 0 or 0.8% for 104 weeks. Body weight gain was decreased in both exposed males and females. Chronic nephropathy was more severe in males given hydroquinone. In the kidneys of exposed male rats, the incidence of tubule hyperplasia was 30/30 (100%) and that of adenomas was 14/30 (47%; $p < 0.01$), compared with 1/30 (3%) and 0/30 (0%), respectively in unexposed controls. Incidence of no other tumour type was increased by exposure (Shibata *et al*., 1991).

3.2 Administration with known carcinogens

3.2.1 *Rat*

Groups of 15 male Fischer 344 rats, six weeks of age, were administered 0 or 0.05% *N*-nitrosobutyl-*N*-(4-hydroxybutyl)amine in the drinking-water for two weeks followed by ureteric ligation one week later to initiate bladder carcinogenesis. Hydroquinone [purity unspecified] was administered at concentrations of 0 or 0.2% in the diet for 22

weeks and all animals were killed at week 24. Hydroquinone alone induced no bladder lesions. When hydroquinone was given after initiation, no increase in bladder lesions was observed (Miyata *et al*., 1985).

Groups of 10 or 15 male Fischer 344 rats, six weeks of age, were given hydroquinone (purity > 99%) at concentrations of 0 or 0.8% in the diet for 51 weeks, while other groups of 15 or 16 animals were fed 0 or 0.8% hydroquinone for 51 weeks starting one week after exposure to 150 mg/kg bw *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine by oral gavage to initiate stomach carcinogenesis. The body weights of rats given hydroquinone after initiator were lower than those given only initiator. Hydroquinone alone did not induce forestomach lesions, nor did it enhance the incidence of forestomach or glandular stomach lesions induced by the initiator (Hirose *et al*., 1989).

Groups of 7–10 male Sprague-Dawley rats, weighing 200 g, were given hydroquinone (purity, > 99%) in the diet at concentrations of 0, 100 or 200 mg/kg for six weeks beginning one week after partial hepatectomy and intraperitoneal injection of 300 mg/kg bw *N*-nitrosodiethylamine to initiate liver carcinogenesis. One group underwent only partial hepatectomy and was fed the high dose of hydroquinone. In the hepatectomized group exposed only to hydroquinone, no liver enzyme-altered (γ-glutamyltranspeptidase) foci were induced. Hydroquinone after initiation increased the multiplicity of foci from 0.08 per cm^2 to 0.68 in the low-dose group and to 0.34 in the high-dose group [statistical analysis not given]. In a second experiment, groups of 10 rats underwent the regimen to initiate liver carcinogenesis and received 0 or 1 mg/kg bw hydroquinone by oral gavage on five days per week for seven weeks. Hydroquinone did not increase the multiplicity of enzyme-altered foci, but their area was increased from a mean of 1.00×10^{-4} cm^2 to 1.30×10^{-4} cm^2 ($p < 0.05$) and their volume from 1.49×10^{-4} cm^3 to 3.12×10^{-4} cm^3 ($p < 0.01$) (Stenius *et al*., 1989).

Groups of 15 or 12 male Fischer 344 rats, seven to eight weeks of age, were given hydroquinone (purity, > 99%) at concentrations of 0 or 0.8% in the diet for 49 weeks alone or starting one week after six intraperitoneal injections of 25 mg/kg bw *N*-nitrosomethyl-*n*-amylamine to initiate upper digestive tract carcinogenesis. Hydroquinone alone reduced weight gain. In animals given hydroquinone after carcinogen, the incidence of oesophageal carcinoma was 4/12 rats (not significant) compared with 0/11 in the group given initiator only, and the multiplicity was increased to 0.33 tumours per rat ($p < 0.05$) compared with 0 in the controls (Yamaguchi *et al*., 1989).

Groups of 10 or 20 male Fischer 344/Du Crj rats [age unspecified] were given hydroquinone [purity unspecified] at concentrations of 0 or 0.8% in the diet for 30 weeks either alone or after exposure to 0.1% *N*-nitroso-bis(2-hydroxypropyl)amine in the drinking-water for two weeks to initiate carcinogenesis in several organs. No unexposed controls were included. Body weight was reduced by hydroquinone given after the initiator and liver weight was increased compared with the group given initiator only. Hydroquinone alone induced no lung or thyroid tumours. Rats given initiator developed low incidences of tumours in the thyroid, lung, urinary bladder and kidney. None of these incidences was increased by hydroquinone (Hasegawa *et al*., 1990).

Groups of 10 or 20 male Fischer 344 rats, six weeks of age, were given hydroquinone (purity, > 99%) in the diet at a concentration of 0.8% for 36 weeks alone or after exposure to 0.05% *N*-nitrosobutyl-*N*-(4-hydroxybutyl)amine in the drinking-water for four weeks to initiate bladder carcinogenesis. Hydroquinone alone did not affect body weight or bladder weight. Hydroquinone exposure alone did not induce bladder tumours and feeding of hydroquinone after initiator did not increase the incidence or multiplicity of bladder neoplasms induced by the initiatior alone (Kurata *et al*., 1990).

Groups of 15 or 20 male Wistar/Crj rats, six weeks of age, were given hydroquinone [purity unspecified] at concentrations of 0 or 0.8% in the diet for 36 weeks starting one week after exposure to 0.1% *N*-nitrosoethyl-*N*-hydroxyethylamine in the drinking-water for three weeks to initiate liver and kidney carcinogenesis. The final body weights of rats given hydroquinone were lower than those of animals given only basal diet or initiation. The relative liver and kidney weights of rats receiving hydroquinone were higher than those of the basal diet group. Hydroquinone alone did not induce preneoplastic or neoplastic liver or kidney lesions. In the kidney, hydroquinone exposure after initiation increased the multiplicity of renal cell tumours to 5.22 per rat ($p < 0.01$) compared with 2.58 after initiation only and increased the multiplicity of microadenomas to 2.77 ($p < 0.05$) compared with 0.94 after initiation only (Okazaki *et al*., 1993).

3.2.2 *Hamster*

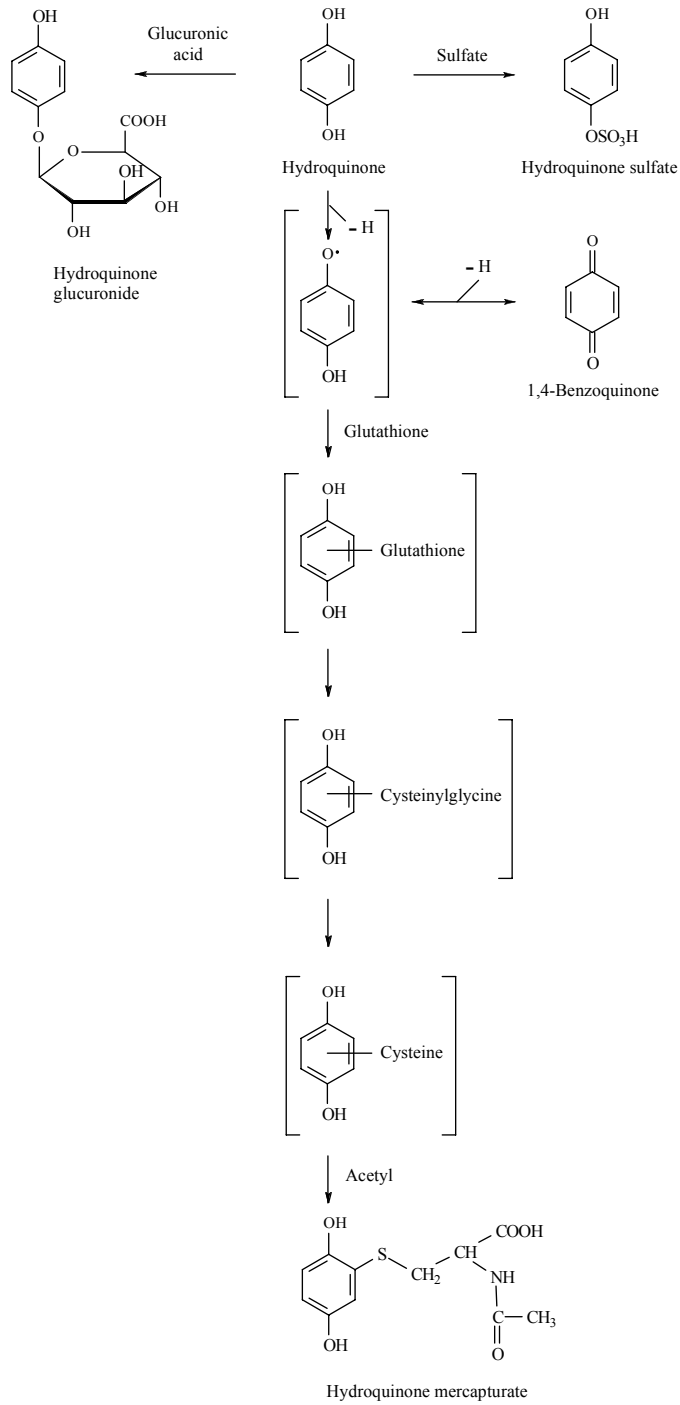
Groups of female Syrian golden hamsters, six weeks of age, were given hydroquinone (purity, > 99%) at concentrations of 0 or 1.5% in the diet for 16 weeks either alone (10 and 15 hamsters) or after two subcutaneous injections of 70 mg/kg bw *N*-nitrosobis(2-oxopropyl)amine (20 hamsters) to initiate pancreatic carcinogenesis. Hydroquinone alone did not affect body weights or liver or pancreas weights compared with untreated controls. Given after the initiator, hydroquinone did not affect body weight or liver weight, but reduced pancreas weight compared with hamsters given only initiator. Hydroquinone alone did not induce neoplastic lesions in the pancreas or liver. In hamsters given hydroquinone after initiator, the multiplicity of pancreatic lesions was reduced (Maruyama *et al*., 1991).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

The major metabolism of hydroquinone is to the sulfate and, at higher exposure, glucuronide conjugates. Oxidation to 1,4-benzoquinone results in a reactive metabolite, that may form mono- or polyglutathione conjugates (see Figure 1).

Figure 1. Proposed metabolism of hydroquinone



From WHO (1994)

4.1.1 *Humans*

Rates of percutaneous absorption of hydroquinone in 5% aqueous solution through human stratum corneum *in vitro* were approximately half those through full-thickness rat skin; the human skin penetration rate was classified as 'slow' (Barber *et al*., 1995). The data allowed calculation of skin absorbance in workers in photographic development.

Rates of hydroquinone glucuronidation in human liver microsomes showed a two- to three-fold variation between individual liver samples; they were somewhat higher than in the rat, and lower than in the mouse liver (Seaton *et al*., 1995). A compartmental pharmacokinetic model was derived to describe the pharmacokinetics of hydroquinone *in vivo* in humans, rats and mice, incorporating hydroquinone glucuronidation rates; sulfation of hydroquinone was not included in this model. NAD(P)H:quinone acceptor oxidoreductases protect against reactive quinones by reducing them to the hydroquinone; this enzyme seems to be absent in some individuals, which will lead to loss of such protection and make them more sensitive to hydroquinone toxicity (Ross, 1996).

4.1.2 *Experimental systems*

Percutaneous absorption of hydroquinone from an aqueous solution was studied in full-thickness rat skin *in vitro*; the permeability constant was 2.3×10^{-5} cm/h, which was approximately two-fold faster than that of human skin (Barber *et al*., 1995).

The disposition of [^{14}C]hydroquinone after oral administration to Sprague-Dawley rats was studied by Divincenzo *et al*. (1984). Whether mixed with the diet or administered as a single dose, the compound was almost completely excreted in urine, with up to 4% in the faeces. By far the major metabolites were the sulfate and glucuronide conjugates, with a small amount of unconjugated hydroquinone. Apparently no analysis for mercapturates was performed. These results were confirmed by Saito and Takeichi (1995), who also demonstrated a wide tissue distribution of hydroquinone. Hill *et al*. (1993) found appreciable amounts of hydroquinone–glutathione conjugates in bile after intraperitoneal administration of hydroquinone to rats that had been pretreated with AT-125, an inhibitor of γ-glutamyltranspeptidase: both mono-, di- and triglutathione conjugates were found, as well as a mercapturate in urine. More than 4% of the dose was recovered as glutathione conjugates, indicating considerable formation of the highly toxic 1,4-benzoquinone (see this volume) metabolite. Nerland and Pierce (1990) identified the hydroquinone mercapturate (*N*-acetyl-*S*-(2,5-dihydroxyphenol)-L-cysteine) in untreated rats after administration of hydroquinone.

A simple compartmental pharmacokinetic model was proposed by Seaton *et al*. (1995) to describe the pharmacokinetics of hydroquinone in mice, rats and humans. The model did not include hydroquinone sulfation, which does occur in rats and possibly in mice, although glucuronidation is the major reaction. Phenol and hydroquinone may mutually inhibit their sulfation if both are present simultaneously in the rat (Legathe *et al*., 1994).

Hydroquinone can be converted to the very reactive 1,4-benzoquinone by several enzymes. A major activity is myeloperoxidase (Subrahmanyam *et al*., 1991), which is

stimulated by phenol and some other phenols. Microsomal cytochrome P450 may also play a role (Hill *et al*., 1993). Macrophage peroxidase activity converting hydroxyquinone to 1,4-benzoquinone may be important in the myelotoxicity of benzene (Schlosser & Kalf, 1989; Smith *et al*., 1989; Snyder & Hedli, 1996). Copper(II) ions strongly enhance this process, in which hydrogen peroxide and other reactive oxygen species may be involved (Eyer, 1991; Rao, 1991; Li & Trush, 1993a,b).

Hydroquinone forms DNA adducts in the peroxidase-containing promyelocytic HL-60 cell line; this process is enhanced by addition of hydrogen peroxide or cumene hydroperoxide (Lévay & Bodell, 1996), presumably because the hydroquinone is oxidized by a cellular peroxidase to a reactive, DNA-binding metabolite.

4.1.3 *Comparison of human and rodent data*

The metabolism of hydroquinone seems very similar in man and rodents: sulfate and glucuronide conjugates are the major metabolites. Through the 1,4-benzoquinone metabolite, a reactive intermediate can be formed, in particular in macrophages by peroxidases, that may be trapped by conjugation with glutathione. The reactive intermediate may form DNA adducts, and may also be responsible for kidney toxicity.

4.2 Toxic effects

The toxicity of hydroquinone has been reviewed (WHO, 1994).

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Long-term feeding of hydroquinone to rats led to aplastic anaemia, liver cord-cell atrophy and ulceration of the gastric mucosa. A single high dose was reported to induce renal tubule necrosis in rats (IARC, 1977).

In a carcinogenicity study (United States National Toxicology Program, 1989; Kari *et al*., 1992), nephropathy was observed in nearly all male and most female rats of all dosed groups and vehicle controls. The nephropathy was characterized by degeneration and regeneration of tubule epithelium, atrophy and dilatation of some tubules, hyaline casts in the tubule lamina, glomerulosclerosis, interstitial fibrosis, and chronic inflammation. In males, the nephropathy was more severe in the high-dose (50 mg/kg bw per day) group, while in females no dose-dependence was observed. Nephropathy was observed in males also in 13-week studies. Presence of hyaline droplets was not reported. In another carcinogenicity study (Shibata *et al*., 1991), the prevalence and severity of chronic nephropathy was more marked in dosed males than in females. It was stated that the nephropathy observed was not of the α_{2u}-globulin nephropathy type. In a reanalysis of the histology of the United States National Toxicology Program study, it was observed that the atypical tubule hyperplasias and adenomas were located in areas of severe chronic progressive nephropathy (Hard *et al*., 1997).

After six weeks of oral administration (50 mg/kg bw per day) of hydroquinone to male Fischer 344 rats, modestly elevated urinary excretion of alanine aminopeptidase, alkaline phosphatase, γ-glutamyltranspeptidase and *N*-acetylglucosaminidase was observed (English *et al*., 1994a). No such indication of renal toxicity was observed in female Fischer 344 rats or male Sprague-Dawley rats. Interstitial inflammation and degenerative/regenerative tubule foci were more frequent in high-dose (25 or 50 mg/kg bw/day) male Fischer 344 rats. Similarly, the proportion of proliferating cells, measured by bromodeoxyuridine (BrdU) labelling, was elevated in the proximal tubules in male Fischer 344 rats given the highest dose (50 mg/kg bw per day), while no consistent change in the labelling was observed in the renal tubules from male Sprague-Dawley or female Fischer 344 rats. On the other hand, after a single dose of hydroquinone (Boatman *et al*., 1996), female Fischer 344 rats were more sensitive to hydroquinone-induced nephrotoxicity, as measured by urinary excretion of alanine aminopeptidase, *N*-acetylglucosaminidase, alkaline phosphatase, γ-glutamyltranspeptidase, glucose and creatinine, by urinary osmolality or by blood levels of urea nitrogen. In these acute experiments, no nephrotoxicity was observed in Sprague-Dawley rats.

In 14-day studies (United States National Toxicology Program, 1989), tremors, convulsions and death following gavage were observed at doses ≥ 500 mg/kg bw per day. In 13-week studies, lethargy, tremor and convulsions leading to death were also observed at doses ≥ 200 mg/kg bw per day.

In a two-year study (United States National Toxicology Program, 1989; Kari *et al*., 1992), dose-dependent hepatic morphological changes (anisokaryosis, elevated frequency of multinucleated cells) were observed in male mice. In a long-term feeding study (0.8% in the diet) (Shibata *et al*., 1991), hepatic centrilobular hypertrophy was observed in males and forestomach hyperplasia in both males and females, while no non-neoplastic changes in the kidney were reported.

Administration of hydroquinone (0.5% in the diet) for 20 weeks did not induce hyperplasia or papillomatous lesions in the forestomach in Syrian golden hamsters (Hirose *et al*., 1986). In male Fischer 344 rats, oral administration of hydroquinone for eight weeks (0.8% in the diet) did not induce hyperplasia or DNA synthesis, as measured by BrdU-labelling index in the forestomach epithelium. No cell proliferation, increased DNA synthesis or increase in pepsinogen-isoenzyme-1-altered neoplastic foci was observed in the pyloric mucosa (Shibata *et al*., 1990).

A large number of studies have been performed on the effects of hydroquinone on bone marrow, in order to elucidate the mechanisms of the myelodepressive and leukaemogenic activity of benzene.

Hydroquinone decreased interleukin (IL)-1 secretion and protein and RNA synthesis of isolated human peripheral blood monocytes induced by *Escherichia coli* lipopolysaccharide at micromolar concentrations (Carbonnelle *et al*., 1995). Hydroquinone (4 μmol/L) inhibited the growth of bone marrow cells from female C57BL/6 × DBA/2 mice (Seidel *et al*., 1991) and from male Swiss Webster and C57BL/6J mice (10 μmol/L) (Neun *et al*., 1992). Hydroquinone (50, 75 or 100 mg/kg bw, single intraperitoneal admi-

nistration) decreased the incorporation of ^{59}Fe into erythrocytes in a dose-dependent fashion in female Swiss albino mice (Snyder *et al*., 1989).

Hydroquinone induced apoptosis in HL60 human promyelocytic leukaemia cells and $CD34^+$ human bone-marrow progenitor cells at concentrations (25 and 50 μmol/L, respectively) at which necrosis was negligible (Hiraku & Kawanishi, 1996; Moran *et al*., 1996). Hydroquinone (1 μmol/L) inhibited the phorbol myristyl acetate- and 1,25-dihydroxyvitamin D_3-induced differentiation of HL-60 cells to macrophages, but had no effect on IL-1-induced differentiation or on cell proliferation. Similarly, it did not affect the differentiation of HL-60 cells to granulocytes (Oliveira & Kalf, 1992). Hydroquinone (2 μmol/L) induced granulocytic differentiation of 32D.3(G) myeloblasts; it also stimulated granulocytic differentiation of myeloblasts *in vivo* in C57BL/6J mice after intraperitoneal injection of 25–50 mg/kg bw twice daily for two days (Hazel *et al*., 1996a) and increased the number of femoral granulocyte/macrophage colony-forming cells in mice after intraperitoneal injection (50–75 mg/kg bw twice daily for 11 days) (Henschler *et al*., 1996). Hydroquinone (at 1 μmol/L) enhanced the colony-forming response of murine bone-marrow cells stimulated with recombinant granulocyte/macrophage colony-stimulating factor (rGM-CSF) (Irons *et al*., 1992) and of factor-dependent cells Paterson (FDCP)–mix (at 10^{-9} mol/L) induced by granulocyte/macrophage colony-stimulating factor (Henschler *et al*., 1996). In human $CD34^+$ cells, a similar effect was observed at 10^{-21} mol/L concentrations of hydroquinone (Irons & Stillman, 1996a,b).

On the other hand, hydroquinone (3 μmol/L) prevented the staurosporine-induced apoptosis of HL-60 and the IL-3-dependent murine myeloblastic (32D) cell line; it also prevented apoptosis of the 32D cells observed in the absence of IL-3. The myeloperoxidase inhibitor indomethacin opposed the effect of hydroquinone on staurosporine-induced apoptosis of HL-60 cells (Hazel *et al*., 1995, 1996b). Pretreatment of human leukaemia cells ML-1 with buthionine sulfoximine (100 μmol/L for 24 h), in order to decrease their glutathione content, increased the susceptibility of these cells to hydroquinone-induced inhibition of differentiation caused by phorbol acetate; pretreatment with 1,2-dithiole-3-thione, which induces reduced glutathione synthesis, prevented the differentiation inhibition of hydroquinone. Treatment of DBA/2 mice with 1,2-dithiole-3-thione, which increased the activity of quinone reductase of bone-marrow stromal cells by 50%, decreased the susceptibility of these cells towards hydroquinone (Trush *et al*., 1996).

Hydroquinone (50 μmol/L) induced a cytosol-to-membrane translocation of protein kinase C, followed by inactivation of the enzyme activity, in cultured LL/2 lung carcinoma cells (Gopalakrishna *et al*., 1994).

Hydroquinone (1–10 μmol/L) induced fluorescence from 2′,7′-dichlorofluorescin acetate in HL-60 human leukaemia cells; this was interpreted to indicate intracellular generation of hydrogen peroxide and other peroxides (Hiraku & Kawanishi, 1996). Hydroquinone (200 mg/kg bw, as a single oral dose) administered to male Sprague-Dawley rats induced a three-fold increase in urinary excretion of malonaldehyde, increased hepatic ornithine decarboxylase activity from a control value of 16.8 pmol/mg/h

to 86.5 pmol/mg/h and, *in vitro*, 0.3 mmol/L induced a rapid depletion (30%) of the glutathione content of isolated hepatocytes (Stenius *et al*., 1989). Hydroquinone (10 μmol/L) induced formation of 8-hydroxydeoxyguanosine in the DNA of HL-60 cells *in vitro*, but not in bone-marrow cells of $B6C3F_1$ mice *in vivo* after a single intraperitoneal dose of 75 mg/kg bw (Kolachana *et al*., 1993). An increase in urinary excretion of 8-hydroxyguanine was observed in rats given a single intraperitoneal dose of 11 mg/kg bw hydroquinone (Suzuki *et al*., 1995).

Hydroquinone (≥ 0.25 μmol/L) prevented the elimination by apoptosis of G418-resistant, transformed Swiss 3T3 MxCl1 cells by co-cultured TGF-β-treated C3H 10T½ Cl8 cells (Schaeffer *et al*., 1995).

In a study on the immunotoxic effects of cigarette tar components, hydroquinone, at a concentration that did not affect the viability of the cells (50 μmol/L), decreased IL-2-dependent DNA synthesis and cell proliferation by > 90% in cultured human T lymphoblasts (Li *et al*., 1997). Hydroquinone inhibited Fc-receptor-mediated phagocytosis in mouse peritoneal macrophages only at rather high concentrations (100 μmol/L) (Manning *et al*., 1994).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

In a developmental toxicity study in COBS-CD-BR rats dosed by gavage, hydroquinone (30, 100 or 300 mg/kg bw per day on days 6 through 15 of gestation) did not induce malformation, gross variations or skeletal variations, with the exception of an increase in the incidence of total common vertebral variations at the highest dose. At the highest dose, slight reductions of mean fetal body weight and of maternal body weight gain were also observed (Krasavagne *et al*., 1992).

In New Zealand white rabbits administered 25–150 mg/kg bw per day hydroquinone by gavage on days 6 through 18 of gestation, the only treatment-related changes observed were nonsignificant increases in minor skeletal malformations (vertebral/rib defects, angulated hyoid arch) and microphthalmia at the highest dose level, at which maternal weight gain was also decreased (Murphy *et al*., 1992).

In a two-generation reproductive toxicity study in rats, no adverse effect was observed on feed consumption, survival, reproductive parameters, pup weight, sex distribution, survival, gross lesions or microscopic anatomy after oral doses of 15–150 mg/kg bw per day (Blacker *et al*., 1993).

Hydroquinone had no adverse effect upon cultured whole rat conceptuses at a concentration of 50 μmol/L, but killed all embryos at 100 μmol/L (Chapman *et al*., 1994).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Hydroquinone did not induce SOS repair and did not increase the numbers of mutants when tested against commonly used strains of *Salmonella typhimurium*. However, it was shown to be mutagenic to *S. typhimurium* TA104 and TA102, which are sensitive to oxidative mutagens. The activity demonstrated with TA104 was almost completely inhibited by co-incubation with superoxide dismutase and catalase and is consistent with superoxide and hydrogen peroxide being the mutagen(s). Hydroquinone induced gene conversion and mutations in *Saccharomyces cerevisiae*. It did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster*.

In cultured mammalian cells, hydroquinone induced DNA single-strand breaks in rat hepatocytes, gene mutations, chromosomal aberrations and sister chromatid exchanges. Positive results were obtained in a cell transformation assay using Syrian hamster embryo cells. Increased frequencies of CREST-positive micronuclei (indicating chromosome loss) and CREST-negative micronuclei (indicating chromosome breakage) were observed following exposure of Chinese hamster lung cells to hydroquinone in one extensive study; only kinetochore-negative micronuclei were found in another study. The formation of micronuclei was dependent on arachidonic acid supplementation. The micronuclei induced in the presence of a superoxide-generation system (hypoxanthine and xanthine oxidase) consisted exclusively of CREST-negative micronuclei and their formation was completely inhibited by pre-treatment with catalase. In addition, glutathione treatment inhibited both CREST-positive and negative micronuclei (Dobo & Eastmond, 1994).

In vitro in human cells, induction of DNA strand breaks was shown to be dependent on the presence of Cu(II). Hydroquinone induced sister chromatid exchanges and chromosomal aberrations without an exogenous metabolic system. The metabolic activation system was not required for the induction of micronuclei in human lymphocytes where kinetochore-positive micronuclei were found.

In vivo in mouse bone marrow, hydroquinone induced micronuclei and chromosomal aberrations in several studies but not sister chromatid exchanges in a single study. Hyperploidy and chromosome loss (as demonstrated by centromere-positive micronuclei) but not polyploidy were also found in mouse bone marrow. In mouse spermatocytes, chromosomal aberrations and hyperploidy were observed.

Hydroquinone inhibited intercellular communication in Chinese hamster cells *in vitro*. Topoisomerase II (Frantz *et al*., 1996; Hutt & Kalf, 1996) but not topoisomerase I (Chen & Eastmond, 1995b) activity was inhibited *in vitro* by hydroquinone treatment.

Hydroquinone binding to calf thymus DNA and cysteine is enhanced by oxidation (prostaglandin H synthetase or cumene hydroperoxide) and inhibited by indomethacin

Table 1. Genetic and related effects of hydroquinone

Test system	Results [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
PRB, SOS repair activity, *Salmonella typhimurium* TA1535/pSK1002, *umu* test	–	–	3300	Nakamura *et al*. (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	333	Haworth *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	125	Sakai *et al*. (1985)
SA2, *Salmonella typhimurium* TA102, reverse mutation	+	NT	NG	Hakura *et al*. (1996)
SA4, *Salmonella typhimurium* TA104, reverse mutation	+	NT	25	Hakura *et al*. (1996)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	333	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	333	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	333	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	125	Sakai *et al*. (1985)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	125	Sakai *et al*. (1985)
SCG, *Saccharomyces cerevisiae* MP1, gene conversion	+	NT	1320	Fahrig (1984)
SCH, *Saccharomyces cerevisiae* MP1, homozygosis by mitotic recombination or gene conversion	–	NT	1320	Fahrig (1984)
SCF, *Saccharomyces cerevisiae* MP1, forward mutation	+	NT	1320	Fahrig (1984)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	?		28 000 ppm feed	Foureman *et al*. (1994)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutation	–		1500 ppm inj × 1	Foureman *et al*. (1994)
DIA, DNA single strand breaks, cross-links or related damage, LYS mouse lymphoma cells, alkaline elution *in vitro*	–	NT	11	Pellack-Walker & Blumer (1986)
DIA, DNA single strand breaks, isolated rat hepatocytes, alkaline elution *in vitro*	+	NT	33	Walles (1992)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitgro*	+	NT	2.5	McGregor *et al*. (1988a,b)
GIA, Gene mutation, Syrian hamster embryo cells, *hprt* locus *in vitro*	+	NT	1.1	Tsutsui *et al*. (1997)

Table 1 (contd)

Test system	Results [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
GIA, Gene mutation, Syrian hamster embryo cells, ouabain resistance *in vitro*	+	NT	1.1	Tsutsui *et al*. (1997)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	0.5	Galloway *et al*. (1987)
SIS, Sister chromatid exchange, Syrian hamster embryo cells *in vitro*	+	NT	0.11	Tsutsui *et al*. (1997)
MIA, Micronucleus test, Chinese hamster embryonic lung CL-1 cells *in vitro*	+[c]	NT	1	Antoccia *et al*. (1991)
MIA, Micronucleus test, Chinese hamster lung V79 cells *in vitro*	(+)	NT	31.6	Seelbach *et al*. (1993)
MIA, Micronucleus test, Chinese hamster lung V79 cells *in vitro*	+	NT	2.8	Ellard & Parry (1993)
MIA, Micronucleus test, Chinese hamster XEM2 (V79 exp CYP1A1) cells *in vitro*	+	NT	2.8	Ellard & Parry (1993)
MIA, Micronucleus test, Chinese hamster SD1 (V79 exp CYP2B1) cells *in vitro*	+	NT	2.8	Ellard & Parry (1993)
MIA, Micronucleus test, Chinese hamster lung V79 cells *in vitro*	NT	+[d]	11.5	Dobo & Eastmond (1994)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	+	450	Galloway *et al*. (1987)
CIS, Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	+	NT	3.3	Tsutsui *et al*. (1997)
AIA, Aneuploidy, DON:Wg3h Chinese hamster cells, dislocating metaphase chromosomes *in vitro*	+	NT	10	Warr *et al*. (1993)
AIA, Aneuploidy, LUC2 Chinese hamster cells, *in vitro*	–	NT	5	Warr *et al*. (1993)
AIA, Aneuploidy, Syrian hamster embryo cells *in vitro*	–	NT	3.3	Tsutsui *et al*. (1997)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	0.33	Tsutsui *et al*. (1997)
DIH, DNA strand breaks, cross-links or related damage, human lymphocytes, comet assay *in vitro*	?	+	11	Anderson *et al*. (1995)
DIH, DNA strand breaks, human promyelocytic HL60 cells, pulse field electrophoresis *in vitro*	+	NT	1.1	Hiraku & Kawanishi (1996)

Table 1 (contd)

Test system	Results [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	4.4	Morimoto & Wolff (1980)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	+	110	Morimoto *et al*. (1983)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	6	Erexson *et al*. (1985)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	?[e]	NT	4.4	Knadle (1985)
MIH, Micronucleus test, human lymphocytes (kinetochore-positive) *in vitro*	+	NT	2.8	Yager *et al*. (1990)
MIH, Micronucleus test, human lymphocytes (kinetochore-positive) *in vitro*	+	NT	8.2	Robertson *et al*. (1991)
MIH, Micronucleus test, human lymphocytes *in vitro*	?	?	1	Van Hummelen & Kirsch-Volders (1992)
MIH, Micronucleus test, human lymphocytes *in vitro*	(+)[f]	NT	20	Ferguson *et al*. (1993)
MIH, Micronucleus test, human lymphocytes *in vitro*	–	+	50	Vian *et al*. (1995)
CHL, Chromosomal aberrations, human lymphocytes (fluorescence in-situ hybridization; FISH) *in vitro*	+	NT	11	Eastmond *et al*. (1994)
AIH, Aneuploidy, human lymphocytes *in vitro*, MN multicolour chromosome staining (FISH)	+	NT	8.3	Eastmond *et al*. (1994)
SVA, Sister chromatid exchange, (C57BL/Cnc × C3H/Cne)F_1 mouse bone marrow *in vivo*	–		120 ip × 1	Pacchierotti *et al*. (1991)
MVM, Micronucleus test, NMRI mouse bone marrow *in vivo*	+		50 sc × 6	Tunek *et al*. (1982)
MVM, Micronucleus test, Swiss CD-1 mouse bone marrow *in vivo*	+		80 ip × 1	Ciranni *et al*. (1988)
MVM, Micronucleus test, Swiss CD-1 mouse bone marrow *in vivo*	(+)		80 po × 1	Ciranni *et al*. (1988)
MVM, Micronucleus test, (101/E1 × C3H/E1)F_1 mouse bone marrow *in vivo*	+		50 ip × 1	Adler & Kliesch (1990)
MVM, Micronucleus test, (101/E1 × C3H/E1)F_1mouse bone marrow *in vivo*	+		15 ip × 3	Adler & Kliesch (1990)
MVM, Micronucleus test, Swiss CD-1 mouse bone marrow *in vivo*	(+)		60 ip × 1	Barale *et al*. (1990)
MVM, Micronucleus test, (102/E1 × C3H/E1)F_1 mouse bone marrow *in vivo*	+		50 ip × 1	Adler *et al*. (1991)
MVM, Micronucleus test, (102/E1 × C3H/E1)F_1 mouse bone marrow *in vivo*	+[c]		100 ip × 1	Miller *et al*. (1991)

Table 1 (contd)

Test system	Results [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
MVM, Micronucleus test, (C57BL/Cnc × C3H/Cne)F_1 mouse bone marrow *in vivo*	+		40 ip × 1	Pacchierotti *et al*. (1991)
MVM, Micronucleus test, Swiss CD-1 mouse bone marrow *in vivo*	+		20 ip × 1	Marrazinni *et al*. (1994a)
MVM, Micronucleus test, Swiss CD-1 mouse bone marrow *in vivo*	+		80 ip × 1	Marrazinni *et al*. (1994b)
MVM, Micronucleus test, CD-1 mouse bone marrow *in vivo*	+		60 ip × 3	Chen & Eastmond (1995a)
CBA, Chromosomal aberrations, (102/E1 × C3H/E1)F_1 mouse bone marrow *in vivo*	+		75 ip × 1	Xu & Adler (1990)
CBA, Chromosomal aberrations, Swiss CD-1 mouse bone marrow *in vivo*	+		80 ip × 1	Marrazinni *et al*. (1994b)
CCC, Chromosomal aberrations, (102/E1 × C3H/E1)F_1 mouse spermatocytes treated *in vivo*	+		40 ip × 1	Ciranni & Adler (1991)
CGG, Chromosomal aberrations, (102/E1 × C3H/E1)F_1 mouse spermatogonia treated *in vivo*	+		40 ip × 1	Ciranni & Adler (1991)
AVA, Aneuploidy, (102/E1 × C3H/E1)F_1 mouse bone marrow polyploidy *in vivo*	–		100 ip × 1	Xu & Adler (1990)
AVA, Aneuploidy, (C57BL/Cnc × C3H/Cne)F_1 mouse bone marrow hyperploidy *in vivo*	+		80 ip × 1	Pacchierotti *et al*. (1991)
AVA, Aneuploidy, (C57BL/Cnc × C3H/Cne)F_1 mouse bone marrow polyploidy *in vivo*	–		120 ip × 1	Pacchierotti *et al*. (1991)
AVA, Aneuploidy, (C57BL/Cnc × C3H/Cne)F_1 mouse spermatocytes hyperploidy *in vivo*	+		80 ip × 1	Leopardi *et al*. (1993)
AVA, Aneuploidy, Swiss CD-1 mouse bone marrow polyploidy *in vivo*	–		80 ip × 1	Marrazinni *et al*. (1994b)
AVA, Aneuploidy, Swiss CD-1 mouse bone marrow hyperploidy *in vivo*	+		80 ip × 1	Marrazinni *et al*. (1994b)

Table 1 (contd)

Test system	Results [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
AVA, Aneuploidy, CD-1 mouse bone marrow *in vivo*, MN multicolour chromosome staining (FISH)	+		60 ip × 3	Chen & Eastmond (1995a)
BID, Binding (covalent) to DNA, mouse P388D_1 cells *in vitro*	+	NT	5.5	Kalf *et al*. (1990)
BID, Binding (covalent) to calf thymus DNA *in vitro*	+	NT	5.5	Leanderson & Tagesson (1990)
BID, Binding (covalent) to DNA, cultured rat Zymbal glands *in vitro*	+	NT	750	Reddy *et al*. (1990)
BID, Binding (covalent) to calf thymus DNA *in vitro*	–	+[g]	11	Schlosser *et al*. (1990)
BID, Binding (covalent) to DNA, human promyelocytic HL-60 cells *in vitro*	+	NT	5.5	Lévay *et al*. (1991)
BID, Binding (covalent) to DNA, male B6C3F_1 mouse bone-marrow cells *in vitro*	+	NT	11	Lévay *et al*. (1993)
BID, Binding (covalent) to DNA, human bone-marrow macrophages *in vitro*	+	NT	11	Lévay *et al*. (1993)
BID, Binding (covalent) to DNA, human promyelocytic HL-60 cells *in vitro*	+	NT	27.5	Pathak *et al*. (1995)
BID, Binding (covalent) to DNA, B6C3F_1 mouse bone marrow *in vitro*	+	NT	27.5	Pathak *et al*. (1995)
BID, Binding (covalent) to DNA, human promyelocytic HL-60 cells *in vitro*	+	NT	5.5	Lévay & Bodell (1996)
BVD, Binding (covalent) to DNA, Sprague-Dawley rat Zymbal gland, liver or spleen *in vivo*	–		150 po × 4	Reddy *et al*. (1990)
BVD, Binding (covalent) to DNA, Fischer 344 rat kidneys *in vivo*	–		50 po, 5 d/wk, 6 wk	English *et al*. (1994b)
ICR, Inhibition of intercellular communication, V79MZ Chinese hamster cells *in vitro*	+	NT	0.055	Vang *et al*. (1993)

Table 1 (contd)

Test system	Results [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
DNA single-strand breaks on supercoiled Bluescript plasmid DNA	–	+	11	Schlosser *et al*. (1990)
Binding (covalent) to porcine brain tubulin [porcine brain tubulin assembly assay] *in vitro*	–	NT	2750	Brunner *et al*. (1991)
Inhibition of assembly of bovine microtubules *in vitro*	(+)	NT	110	Wallin & Hartley-Hasp (1993)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; po, oral; NG, not given; inj, injection; ip, intraperitoneal; sc, subcutaneous

[c] No increase in % kinetochore-positive micronuclei compared with controls

[d] Supplemented with arachidonic acid; increase in both kinetochore-positive and –negative micronucleated cells compared with controls (CREST-labelling procedure)

[e] Positive if glutathione depleted with diethyl maleate

[f] Size ratio of micronuclei to nucleus is not significant different from controls.

[g] With prostaglandin H synthetase for oxidation

(Kalf *et al*., 1990; Schlosser *et al*., 1990). Hydroquinone bound weakly to isolated bovine microtubules but not to porcine brain tubulin *in vitro* and to DNA in most of the in-vitro studies, in single studies with rat Zymbal glands in culture and in mice bone marrow *in vitro*. *In vivo*, hydroquinone did not bind to DNA from Zymbal gland, liver, spleen or kidneys of rat treated orally; it did not induce DNA strand breaks in plasmid DNA.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to hydroquinone may occur during its production, its use as an inhibitor, antioxidant and intermediate in the production of dyes, paints, motor fuels and oils, and in black-and-white photographic processing. Hydroquinone occurs naturally in certain plant species. It is used as a topical treatment for skin hyperpigmentation.

5.2 Human carcinogenicity data

A cohort of workers with definite and lengthy exposure to hydroquinone had low cancer rates compared with two comparison populations; the reason for the lower than expected rates is unclear. A cohort of lithographers, some of whom had worked with hydroquinone, had an excess of malignant melanoma based on five cases; only two of the cases had reported exposure to hydroquinone.

5.3 Animal carcinogenicity data

Hydroquinone was tested for carcinogenicity in two studies in mice and two studies in rats by oral administration. It was also tested in rats for promoting activity in assays for bladder, stomach, liver, lung, oesophagus and kidney carcinogenesis and in one study in hamsters for pancreatic carcinogenesis.

In mice, hydroquinone induced hepatocellular adenomas in females in one study and in males in another study. In rats it induced renal tubule adenomas in males in two studies.

Hydroquinone had no promoting activity in most assays; an increase in the multiplicity of oesophageal tumours was observed in one study and in the multiplicity of renal cell tumours in another study. No promoting effect on pancreatic carcinogenesis was observed in the study in hamsters.

5.4 Other relevant data

Hydroquinone is metabolized mainly to conjugates, but a small percentage may be converted to 1,4-benzoquinone, conjugated with glutathione or form DNA adducts *in vitro*.

It caused toxicity in several organs, notably the kidney and forestomach.

Hydroquinone was mutagenic in many in-vitro systems using a variety of end-points. Also, after intraperitoneal administration, it caused genotoxicity or chromosomal aberrations in bone marrow.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of hydroquinone.

There is *limited evidence* in experimental animals for the carcinogenicity of hydroquinone.

Overall evaluation

Hydroquinone is *not classifiable as to its carcinogenicity to humans (Group 3)*.

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METHYL BROMIDE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987a).

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 74-83-9
Chem. Abstr. Name: Bromomethane
IUPAC Systematic Name: Bromomethane
Synonym: Monobromomethane

1.1.2 *Structural and molecular formulae and relative molecular mass*

CH_3Br

CH_3Br Relative molecular mass: 94.94

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless gas with a chloroform-like odour at high concentrations (Budavari, 1996)
(*b*) *Boiling-point*: 3.5°C (Lide, 1997)
(*c*) *Melting-point*: –93.7°C (Lide, 1997)
(*d*) *Solubility*: Slightly soluble in water, very soluble in organic solvents (American Conference of Governmental Industrial Hygienists, 1992)
(*e*) *Vapour pressure*: 166 kPa at 20°C; relative vapour density (air = 1), 3.27 (Lewis, 1993)
(*f*) *Explosive limits*: Upper, 15%; lower, 10% by volume in air (American Conference of Governmental Industrial Hygienists, 1992)
(*g*) *Conversion factor*: $mg/m^3 = 3.88 \times ppm$

1.2 Production and use

Production of methyl bromide in the United States was estimated to be 20 400 tonnes in 1984 (United States National Library of Medicine, 1997). Information available in 1995 indicated that it was produced in 10 countries (Chemical Information Services, 1995).

Methyl bromide is used as a soil and space fumigant; as a pesticide on potatoes, tomatoes and other crops; in organic synthesis; and as an extraction solvent for vegetable oil (Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 5000 workers in the United States were potentially exposed to methyl bromide (see General Remarks). Occupational exposures may occur in its production, in pest control for vegetables and fruits and in fumigation of soil.

1.3.2 *Environmental occurrence*

Methyl bromide is produced by a variety of marine organisms. The bulk of the methyl bromide detected in the environment is believed to be released from oceans. Release to the environment also results from the use of methyl bromide as a soil and space fumigant and its occurrence in vehicle exhaust. Methyl bromide is frequently detected in ambient air and, at low levels, in surface water, drinking water and groundwater (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 3.9 mg/m^3 as the 8-h time-weighted average threshold limit value, with a skin notation, for occupational exposures to methyl bromide in workplace air. Values of 1–60 mg/m^3 have been used as standards or guidelines in other countries (International Labour Office, 1991).

No international guideline for methyl bromide in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

Wong *et al.* (1984) studied the mortality of a cohort of 3579 white male workers with potential exposure to brominated compounds at three chemical manufacturing plants and at a research establishment between 1935 and 1976. The exposures included 1,2-dibromo-3-chloropropane (DBCP) (see this volume), tris(2,3-dibromopropyl)phosphate (Tris) (see this volume), polybrominated biphenyls (PBBs) (IARC, 1987b), various organic and inorganic bromides and DDT (IARC, 1991). Among a subgroup of 665 men exposed to organic brominated compounds other than DBCP, Tris and PBBs, and with potential exposure to methyl bromide, 51 deaths occurred versus 44.77 expected (standardized mortality ratio (SMR), 1.1; 95% confidence interval (CI), 0.9–15.0). Ten deaths from cancer were observed versus 7.86 expected, yielding a SMR of 1.3 (95% CI, 0.6–2.3). In this group of workers, there were two deaths from testicular cancer versus 0.11 expected

(SMR, 17.8; 95% CI, 2.0–64.9). An investigation of the work histories showed that methyl bromide was the only common potential exposure of these two cases. These men died at the ages of 17 and 33 years, respectively. [The Working Group noted that no information was available on duration of exposure or on time between first exposure and death from testicular cancer.]

A number of studies have analysed cancer mortality or incidence in pesticide applicators, some of whom may have been exposed to methyl bromide. However, none have provided estimates of risk in relation to methyl bromide specifically.

3. Studies of Cancer in Experimental Animals

In one 90-day study, methyl bromide was tested in rats by oral administration. An increased incidence of squamous-cell carcinomas of the forestomach was observed in animals of each sex (IARC, 1986).

3.1 Oral administration

Rat: In a study to investigate further the findings of a previously reported 90-day study, groups of 15 male Wistar rats, six weeks of age, were administered 50 mg/kg bw methyl bromide (purity, > 99%) in arachis oil by gavage on five days per week for 13, 17, 21 or 25 weeks, at which times the surviving animals were killed. Further groups received methyl bromide for 13, 17 or 21 weeks followed by observation up to 25 weeks. Control animals received arachis oil for 13 or 25 weeks. In rats exposed for 25 weeks, one squamous-cell carcinoma of the forestomach occurred. Hyperplasia of the forestomach occurred in all treated groups but the hyperplasia regressed by 25 weeks in the groups in which treatment stopped earlier (Boorman *et al*., 1986).

3.2 Inhalation exposure

3.2.1 *Mouse*

Groups of 70 male and 70 female B6C3F_1 mice, six weeks of age, were administered methyl bromide (purity, 99.8%) by whole-body inhalation at concentrations of 0 (controls), 10, 33 or 100 ppm [0, 4, 129 or 389 mg/m^3] for 6 h per day on five days per week. The control, low- and mid-dose groups were exposed for 103 weeks. In the high-dose group, exposure to methyl bromide was stopped after 20 weeks because of high mortality in this group and the remaining mice were exposed to air only for the rest of the study. Ten mice of each sex from each group were killed at six and 15 months. All surviving animals were killed at weeks 105–106. Necropsy was performed on all animals and all organs were examined histologically. Survival at termination was 40/50, 37/50, 40/50 and 16/70 in males and 36/50, 41/50, 45/49 and 40/60 in females in the control, low-, mid- and high-dose groups, respectively. No treatment-related increase in the incidence of tumours was observed in males or females (United States National Toxicology Program, 1992).

Groups of 50 male and 50 female BDF_1 (C57BL/6 × DBA/2) mice, six weeks of age, were administered methyl bromide (purity, > 99.9%) by whole-body inhalation at concentrations of 0 (controls), 4, 16 or 64 ppm [0, 16, 62 or 249 mg/mg^3] for 6 h per day on five days per week for 104 weeks. At 105 weeks, all surviving animals were killed. Necropsy was performed on all animals and all organs were examined histologically. Survival at 104 weeks was 41/50, 36/50, 33/50 and 45/50 in males and 32/50, 23/50, 24/49 and 35/49 in females in the control, low-, mid- and high-dose groups, respectively. No increased incidence of tumours related to treatment was observed (Gotoh *et al*., 1994).

3.2.2 *Rat*

Groups of 50 male and 50 female Wistar rats, six weeks of age, were administered methyl bromide (purity, 98.8%) by whole-body inhalation at concentrations of 0 (controls), 3, 30 or 90 ppm [0, 12, 117 or 350 mg/m^3] for 6 h per day on five days per week for 29 months. Additional satellite groups of 10 males and 10 females were used for interim killings at weeks 14, 53 and 105. Survival in males at week 114 of the experiment was 25/50, 34/50, 29/50 and 14/50 and at week 128 was 15/50, 25/50, 16/50 and 8/50 in control, low-, mid- and high-dose animals. Survival in females at week 114 was 27/50, 32/50, 25/50 and 21/50 and that at week 129 was 14/50, 23/50, 17/50 and 7/50, respectively. By week 114, mortality in males in the high-dose group was significantly higher than that in controls ($p < 0.05$, Fisher's exact test, one-sided). No increased incidence of tumours was observed (Reuzel *et al*., 1991).

Groups of 50 male and 50 female Fischer 344/DuCrj rats, six weeks of age, were administered methyl bromide (purity, > 99.9%) by whole-body inhalation at concentrations of 0 (controls), 4, 20 or 100 ppm [0, 16, 78 or 389 mg/m^3] for 6 h per day on five days per week for 104 weeks. At week 105, all surviving animals were killed. Necropsy was performed on all animals and all organs were examined histologically. Survival at week 104 was 34/50, 34/50, 31/50 and 33/50 in control, low-, mid- and high-dose males and 42/49, 38/50, 39/50 and 41/50 in control, low-, mid- and high-dose females, respectively. The incidence of adenomas of the pituitary gland was significantly increased in high-dose males compared with controls (16/50, 23/50, 19/50 and 30/50 in control, low-, mid- and high-dose, respectively; $p < 0.01$, chi-square test). In females, no increase in the incidence of tumours related to treatment was observed (Gotoh *et al*., 1994).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

The metabolism of methyl bromide has been reviewed (International Programme on Chemical Safety (WHO), 1995).

4.1.1 *Humans*

No study describing toxicokinetics of methyl bromide in humans *in vivo* was available for evaluation.

In human erythrocytes *in vitro*, methyl bromide is consumed, probably with formation of a glutathione conjugate. The reaction involves a glutathione *S*-transferase enzyme that metabolizes methyl halides. This enzyme has not been found in erythrocytes of mouse, rat, cattle, sheep, pig or rhesus monkey. The enzyme is present only in part of the human population: among 45 people investigated, only 27 conjugated glutathione with methyl bromide. The enzyme in erythrocytes of conjugators is different from other glutathione *S*-transferases with respect to substrate specificity, affinity chromatography, and inhibition characteristics; it has been designated as glutathione *S*-transferase θ (Hallier *et al.*, 1990; Schröder *et al.*, 1992; Hallier *et al.*, 1993; Pemble *et al.*, 1994; Schröder *et al.*, 1996).

The interindividual differences in the ability of humans to conjugate methyl bromide suggest that the polymorphic human glutathione *S*-transferase enzyme present in erythrocytes is relevant for the disposition of methyl bromide in humans. Iwasaki *et al.* (1989) described a field study of methyl bromide workers in Japan, whose levels of the methyl bromide-derived haemoglobin adduct (*S*-methylcysteine in haemoglobin) were measured. In a subgroup of seven workers with the highest exposure levels (filling of spray cans and gas cylinders), three had high adduct levels (the highest levels in the whole study), whereas the four other workers of the same exposure subgroup had levels that were close to the background in nonexposed persons (Iwasaki, 1988a,b; Iwasaki *et al.*, 1989).

4.1.2 *Experimental animals*

Studies on rats and dogs have shown that inhaled methyl bromide is rapidly absorbed through the lungs. In rats, it is also rapidly absorbed following oral exposure.

After absorption, methyl bromide or metabolites are rapidly distributed to many tissues including the lung, adrenal gland, kidney, liver, nasal turbinates, brain, testis and adipose tissue. In an inhalation study in rats, the methyl bromide concentrations in tissues reached a maximum after 1 h of exposure, but decreased rapidly. Methyl bromide is probably metabolized by glutathione conjugation, the formed *S*-methylglutathione being sequentially catabolized to *S*-methyl-L-cysteine and then to carbon dioxide.

Methylation of proteins and lipids has been observed in the tissues of several species, including humans, after exposure via inhalation. Methylated DNA bases have also been detected following exposure of rodents *in vivo* or rodent cells *in vitro* to methyl bromide.

In inhalation studies using ^{14}C-labelled methyl bromide, exhalation of $^{14}CO_2$ was the major route of elimination of ^{14}C. A smaller amount of ^{14}C was excreted in the urine. Following oral administration, urinary excretion was the major route of elimination of ^{14}C (IARC, 1986).

After exposure of male CD rats (nose only) to 55 ppm [213 mg/m^3] [^{14}C]methyl bromide for 3 min, 43% of the radioactivity was exhaled during an observation period of 32 h (Jaskot *et al.*, 1988).

4.2 Toxic effects

The toxicity of methyl bromide has been reviewed (WHO, 1995; Yang *et al*., 1995).

4.2.1 *Humans*

More than 950 methyl bromide poisonings have been reported, involving fatalities, systemic poisoning, irritation to skin, eyes and respiratory tract, and damage to the central nervous system, liver and kidney (IARC, 1986). Several reports on poisonings after short- and long-term exposure to methyl bromide, some of them fatal, have also been published (Behrens & Dukes, 1986; Goldman *et al*., 1987; O'Neal, 1987; Zwaveling *et al*., 1987; Herzstein & Cullen, 1990; Polkowski *et al*., 1990; Kishi *et al*., 1991; Hustinx *et al*., 1993; Deschamps & Turpin, 1996; Garnier *et al*., 1996; Langård *et al*., 1996; De Haro *et al*., 1997).

4.2.2 *Experimental systems*

Signs of methyl bromide toxicity following acute exposure include irritation of the eyes and respiratory tract, tremor, incoordination, depression of the central nervous system and convulsions. Long-term exposure induces pulmonary congestion, central nervous system effects, and renal and hepatic lesions. After oral administration to rats, hyperplasia and hyperkeratosis (and squamous-cell carcinomas) of the forestomach were observed (IARC, 1986).

Methyl bromide, given by gavage (50 mg/kg on five days per week) for 13 weeks to Wistar rats induced inflammation, acanthosis, fibrosis and a high incidence of pseudo-epitheliomatous hyperplasia in the forestomach; these changes were aggravated upon continued administration for a total of 25 weeks, by which time all of the 11 rats examined showed hyperplastic changes (Boorman *et al*., 1986). In groups in which the treatment was discontinued after 13 weeks, the changes regressed, but adhesions, fibrosis and mild acanthosis persisted for 12 weeks (week 25 of the experiment).

Following inhalation exposure of male Sprague-Dawley rats for 4 h per day on five days per week to either 150 ppm [580 mg/m^3] for 11 weeks or 200, 300 or 400 ppm [780, 1160 or 1550 mg/m^3] for six weeks, mortality occurred at exposure levels ≥ 300 ppm. Observed effects included necrotic areas in the brain and heart, fatty degeneration in the liver, isolated acinar cell necroses in the pancreas, and, at the highest concentration, atrophic changes in the testis. Olfactory epithelium was not studied (Kato *et al*., 1986).

Following short-term inhalation exposure to 160 ppm [620 mg/m^3] methyl bromide (6 h per day on five days per week for up to six weeks), B6C3F$_1$ mice were found to be more sensitive than Fischer 344/N rats: 50% of male mice died after eight exposures and 50% of female mice after six exposures, while similar mortality was observed in male rats only after 14 exposures. Neuronal necrosis and testicular degeneration were observed in both species; nephrosis was observed in nearly all mice, while necrosis of the olfactory epithelium was more marked in rats. Myocardial degeneration occurred in rats and to a lesser degree in male mice. In the adrenal cortex, there was cytoplasmic vacuolation in rats and inner zone atrophy in female mice (Eustis *et al*., 1988).

In a carcinogenicity study of methyl bromide (see Section 3.2.1), survival of $B6C3F_1$ mice was decreased in males exposed to 100 ppm [390 mg/m^3], the highest concentration. Olfactory necrosis and metaplasia, cardiac degeneration and chronic cardiomyopathy, cerebral and cerebellar degeneration and sternal dysplasia were observed in both males and females at the highest concentration and were more frequent in males (United States National Toxicology Program, 1992).

In an inhalation study in which Wistar rats were exposed to 3, 30 or 90 ppm [12, 120 or 350 mg/m^3] for 6 h per day on five days per week for 29 months, a dose-dependent increase in basal-cell hyperplasia of the olfactory epithelium was observed in both sexes; this could be observed after 12 months and did not appreciably increase in frequency or severity by 24 or 29 months. In the highest-dose group, there was an increased incidence of heart thrombi in both females and males; myocardial degeneration was observed in females and cartilaginous metaplasia in both sexes. The incidence of oesophageal hyperkeratosis was elevated in treated males and females, but reached significance only in males at the highest dose group. Hyperkeratosis of the stomach was more frequent in the highest-dose group, but was not in significant excess (Reuzel *et al.*, 1991).

Extensive destruction of the olfactory epithelium was observed in male Fischer 344 rats exposed to 200 ppm [780 mg/m^3] methyl bromide for 6 h per day for five days. By day 3, despite continued exposure, there was replacement of the olfactory epithelium by a squamous-cell layer, followed by progressive reorganization toward the normal architecture, and by week 10, 75–80% of the epithelium appeared histologically normal. Olfactory epithelial-cell replication was maximal on day 3 of exposure, with a labelling index of 14.7% compared with 0.7% in the controls (Hurtt *et al.*, 1988). Degeneration and subsequent regeneration were also observed in an inhalation experiment with Fischer 344 rats exposed to 175 ppm [680 mg/m^3] 6 h twice, separated by a 28-day interval (Bolon *et al.*, 1991).

Nasal olfactory cell degeneration was observed at exposure levels ≥ 175 ppm [680 mg/m^3], when Fischer 344 rats were exposed to methyl bromide for 6 h per day for five days. A dose-dependent vacuolar degeneration of the zona fasciculata of the adrenal glands and cerebellar granule cell degeneration were also observed, while hepatocellular degeneration was confined to dose levels ≥ 250 ppm [970 mg/m^3]; cerebral cortical degeneration and (minor) testicular damage were observed only at the highest dose level, 325 ppm [1260 mg/m^3] (Hurtt *et al.*, 1987).

When food fumigated with methyl bromide (total bromine content 80, 200 or 500 ppm; methyl bromide < 20 ppm) was administered to male and female Fischer 344 rats for two years, no toxicologically important changes in clinical, chemical, haematological or histological parameters were observed. There was, however, a minor (3–6%) decrement in the weight gain among the males after 60 weeks (Mitsumori *et al.*, 1990).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

The developmental toxicity of methyl bromide was studied in rats and rabbits. Male and female Wistar rats were exposed by inhalation to methyl bromide for 7 h per day on five days per week for three weeks before mating, and the females were also exposed through 19 days of gestation. New Zealand white rabbits were inseminated and exposed for 7 h daily on days 1–24 of gestation. The target concentrations were 20 and 70 ppm [80 and 270 mg/m^3] for both species. None of the rats died during the experiment, while 24/25 rabbits inhaling the high dose died. There were minor variations in the weight development of the rats during gestation, which were, however, inconsequential by the end of gestation. Methyl bromide had no effect on the pregnancy rate, embryonic viability, or weight or length development of the fetuses; neither did it induce terata in either species (Sikov *et al.*, 1981).

Inhalation exposure of rats to 160 ppm [620 mg/m^3] or 400 ppm [1550 mg/m^3] methyl bromide for ≥ 6 weeks caused testicular degeneration (Kato *et al.*, 1986; Eustis *et al.*, 1988). However, when male Fischer 344 rats were exposed to 200 ppm [780 mg/m^3] methyl bromide for 6 h per day for five days and followed for two months, no effect was observed at any time during or after the exposure on testis weight, daily sperm production, cauda epididymal sperm count, sperm morphology, percentage motile sperm, linear sperm velocity, or epididymal or testicular histology (Hurtt & Working, 1988).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Methyl bromide induced SOS repair in *Salmonella typhimurium* and gene mutation in *Salmonella typhimurium* TA100 and TA1535; it was also mutagenic to *Escherichia coli* WP2 *uvrA*, plants and *Drosophila*. It did not induce unscheduled DNA synthesis in cultured rat hepatocytes. It induced sister chromatid exchanges *in vitro* in lymphocytes from human donors who were classified as non-conjugators of methyl bromide with glutathione.

Methyl bromide induced micronuclei in bone-marrow and peripheral blood cells of rats and mice.

Methyl bromide binds covalently to DNA *in vitro* and *in vivo* in various organs in rats and mice.

Table 1. Genetic and related effects of methyl bromide

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS repair *umu*-test, *Salmonella typhimurium* TA1535/pSK1002	+	+	116	Ong *et al.* (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	0.4	Simmon *et al*. (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	0.5	Moriya *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	1.9	Kramers *et al*. (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	0.1% atm	JETOC (1997)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	NG	Moriya *et al*. (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	0.1% atm	JETOC (1997)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Moriya *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	0.5% in air	JETOC (1997)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	NG	Moriya *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Moriya *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Kramers *et al*. (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	0.5% in air	JETOC (1997)
ECF, *Escherichia coli* SD-4, forward mutation	+	NT	570	Djalali-Behzad *et al*. (1981)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	+	+	0.2% atm	JETOC (1997)
EC2, *Escherichia coli* WP2, reverse mutation	+	+	NG	Moriya *et al*. (1983)
KPF, *Klebsiella pneumoniae*, forward mutation	+	NT	4.75	Kramers *et al*. (1985)
HSM, *Hordeum* species, mutation	(+)	NT	130	Ehrenberg *et al*. (1974)
DMM, *Drosophila melanogaster*, somatic wing-spot assay, mitotic recombination	+		8 inh	Katz (1987)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		0.38 inh	Kramers *et al*. (1985)
URP, Unscheduled DNA synthesis, male Wistar rat primary hepatocytes *in vitro*	–	NT	30	Kramers *et al*. (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	0.1	Kramers *et al*. (1985)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
G51, Gene mutation, mouse lymphoma L5178Y cells, all other loci *in vitro*	+	NT	0.1	Kramers *et al*. (1985)
T7S, Cell transformation, SA7/Syrian hamster embryo cells	–	NT	30 µg/mL	Hatch *et al*. (1983)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	(+)	NT	167 µg/mL 10 sec	Tucker *et al*. (1986)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	+	5	Garry *et al*. (1990)
SHL, Sister chromatid exchange, human lymphocytes from glutathione conjugators *in vitro*	–	NT	19.5 µg/mL 1 h	Hallier *et al*. (1993)
SHL, Sister chromatid exchange, human lymphocytes from glutathione non-conjugators *in vitro*	+	NT	19.5 µg/mL 1 h	Hallier *et al*. (1993)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	–	(+)	95	Garry *et al*. (1990)
MVM, Micronucleus test, BDF_1 mouse bone marrow and peripheral blood *in vivo*	+		600 mg/m^3 inh 6 h × 14	Ikawa *et al*. (1986)
MVR, Micronucleus test, Fischer 344 rat bone marrow *in vivo*	+		1300 mg/m^3 inh 6 h × 14	Ikawa *et al*. (1986)
BID, Binding (covalent) to calf thymus DNA *in vitro*	+	NT	48	Starratt & Bond (1988)
BVD, Binding (covalent) to DNA, CBA mouse liver and spleen *in vivo*	+		6.5 inh 1 h × 1	Djalali-Behzad *et al*. (1981)
BVD, Binding (covalent) to DNA, Fischer 344 rat liver, lung, stomach and forestomach *in vivo*	+		ca. 3.3 po × 1	Gansewendt *et al*. (1991)
BVD, Binding (covalent) to DNA, Fischer 344 rat liver, lung, stomach and forestomach *in vivo*	+		3.8 inh 6 h × 1	Gansewendt *et al*. (1991)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; atm, atmosphere; NG, not given; inh, inhalation; po, oral

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to methyl bromide may occur in its production, in pest control and in fumigation of soil. Methyl bromide is naturally produced in oceans. It is commonly detected in ambient air and at low levels in water.

5.2 Human carcinogenicity data

One cohort study of workers at three chemical manufacturing plants included a subgroup with potential exposure to methyl bromide, among whom there were two deaths from testicular cancer (0.11 expected).

5.3 Animal carcinogenicity data

Methyl bromide was tested by oral administration in rats and by inhalation in mice and rats. In one 90-day study by oral administration in rats, methyl bromide was reported to produce squamous-cell carcinomas of the forestomach. In a second, 25-week study designed to investigate further the findings of the previous study, early hyperplastic lesions of the forestomach developed after 25 weeks of continuous treatment by gavage. In two inhalation studies in mice, no significant increase in the incidence of tumours was observed. In one inhalation study in rats, an increase in the incidence of adenomas of the pituitary gland was observed in high-dose male rats. In another study in rats, no increase in tumour incidence was observed.

5.4 Other relevant data

Methyl bromide is metabolized by glutathione conjugation and excreted as carbon dioxide. In animal studies, it caused toxicity and irritation and organ toxicity in many organs. It binds covalently to DNA *in vitro* and also in various organs in the rat *in vivo*. Methyl bromide is mutagenic in bacteria; it induces gene mutations and sister chromatid exchanges *in vitro* in mammalian cells. Methyl bromide gave positive results for several genetic activity end-points in *Drosophila*.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of methyl bromide.

There is *limited evidence* in experimental animals for the carcinogenicity of methyl bromide.

Overall evaluation

Methyl bromide is *not classifiable as to its carcinogenicity to humans (Group 3)*.

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METHYL CHLORIDE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987a).

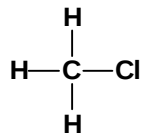
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 74-87-3
Chem. Abstr. Name: Chloromethane
IUPAC Systematic Name: Chloromethane
Synonym: Monochloromethane

1.1.2 *Structural and molecular formulae and relative molecular mass*



CH_3Cl Relative molecular mass: 50.49

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description:* Colourless gas with an ethereal odour and sweet taste (Budavari, 1996)
(*b*) *Boiling-point*: –24.0°C (Lide, 1997)
(*c*) *Melting-point*: –97.7°C (Lide, 1997)
(*d*) *Solubility*: Slightly soluble in water (303 mL/100 mL at 20°C); soluble in ethanol; miscible with acetone and diethyl ether (Budavari, 1996; Lide, 1997)
(*e*) *Vapour pressure*: 488 kPa at 20°C; relative vapour density (air = 1), 1.8 (Holbrook, 1993; Verschueren, 1996)
(*f*) *Reactivity*: Reacts with active metals (aluminium, magnesium, zinc) (Lewis, 1993)
(*g*) *Explosive limits*: Upper, 17.2%; lower, 8.1% by volume in air (American Conference of Governmental Industrial Hygienists, 1992)
(*h*) *Octanol/water partition coefficient (P):* log *P*, 0.91 (Hansch *et al*., 1995)
(i) *Conversion factor*: $mg/m^3 = 2.1 \times ppm$

1.2 Production and use

Production capacity for methyl chloride in the United States was reported to be 438 thousand tonnes in 1992 and 417 thousand tonnes in 1995 (Anon., 1992, 1995).

Methyl chloride is used in the production of tetramethyllead antiknock compounds for gasoline and methyl silicone resins and polymers, and as a catalyst carrier in low-temperature polymerization (e.g., butyl rubber), a refrigerant, a fluid for thermometric and thermostatic equipment, a methylating agent in organic synthesis, an extractant and low-temperature solvent, a herbicide, a topical antiseptic, and a slowing agent (IARC, 1986; Lewis, 1993).

The use pattern for methyl chloride in the United States in 1992 and 1995 was (%): methyl chlorosilanes used as intermediates for silicones, 80; methyl cellulose manufacture, 6; quaternary ammonium compounds, 5; agricultural chemicals, 5; butyl rubber production, 2; and miscellaneous, 2 (Anon., 1992, 1995).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 10 000 workers in the United States were potentially exposed to methyl chloride (see General Remarks). Occupational exposures to methyl chloride may occur in its production and in the production of silicones, methyl cellulose, quaternary ammonium compounds and other chemical agents. Data on workplace exposures to methyl chloride have been presented in a previous monograph (IARC, 1986).

1.3.2 *Environmental occurrence*

Thousands of tonnes of methyl chloride are produced naturally every day, primarily in the oceans. Other significant natural sources include forest and brush fires and volcanoes. Although the atmospheric budget of methyl chloride can be accounted for by volatilization from the oceanic reservoir, its production and use in the manufacture of silicones and other chemicals and as a solvent and propellant can make a significant impact on the local atmospheric concentration of methyl chloride. It has been detected at low levels in drinking-water, groundwater, surface water, seawater, effluents, sediments, in the atmosphere, in fish samples and in human milk samples (Holbrook, 1993; United States National Library of Medicine, 1998). Tobacco smoke contains methyl chloride (IARC, 1986).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 103 mg/m^3 as the 8-h time-weighted average threshold limit value for occupational exposures to methyl chloride in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for methyl chloride in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

Holmes *et al.* (1986) conducted a small study of 852 butyl rubber-manufacturing workers employed at some time between 1943 and 1978 in the United States who could have been exposed to methyl chloride. For all cancers, they observed standardized mortality ratios (SMRs) of 0.7 (95% confidence interval (CI), 0.4–1.0; $n = 19$) for white men and 0.6 (95% CI, 0.3–1.1; $n = 11$) for black men. SMRs for lung cancer were 0.7 (95% CI, 0.3–1.4; $n = 7$) for white men and 1.2 (95% CI, 0.4–2.6; $n = 6$) for black men.

Ott *et al.* (1985) conducted a cohort mortality study of 1919 men employed for one or more years between 1940 and 1969 at a chemical manufacturing facility in the United States. This cohort included 226 workers assigned to a unit which produced chlorinated methanes (methyl chloride, dichloromethane (see this volume), chloroform (see IARC, 1987b), carbon tetrachloride (see this volume) and tetrachloroethylene (see this volume)). Exposure levels were not reported. The follow-up period was from 1940 to 1979 and follow-up was 94% complete. The SMR for all causes was 0.6 (95% CI, 0.5–0.9; $n = 42$) based on United States rates and that for all cancers was 0.7 (95% CI, 0.3–1.3; $n = 9$). There were three pancreatic cancer cases (0.9 expected), two of whom had worked for less than five years and the third for six years. [The Working Group noted that the mixture of exposures and the lack of information regarding exposure levels limits the ability to draw conclusions regarding the carcinogenicity of methyl chloride.]

3. Studies of Cancer in Experimental Animals

A study in which methyl chloride was tested for carcinogenicity in mice and rats by inhalation exposure was reported only in an abstract. Although an excess of kidney tumours was reported in male mice exposed to the highest dose, the incomplete reporting precluded an evaluation of this finding. The results in female mice and in male and female rats were reported to be negative (IARC, 1986).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Following inhalation of labelled methyl chloride as a single breath, 29% of the dose was exhaled within 1 h. Among six volunteers inhaling methyl chloride, blood concentrations were proportional to the exposure concentration, but for two volunteers the concentrations were two to three times higher than for the others. The four with lower

concentrations eliminated methyl chloride more rapidly, their metabolic rate constants (K_m) being five- to seven-fold higher than for the other two (e.g., 0.284 versus 0.039 at an exposure concentration of 21 mg/m^3 [10 ppm]) (IARC, 1986).

S-Methylglutathione was identified in erythrocytes incubated with [^{14}C]methyl chloride, but no methylation of haemoglobin was detected. *S*-Methylcysteine bound to serum albumin was identified following incubation of plasma with methyl chloride (IARC, 1986). In one study, erythrocytes from 12/20 donors metabolized methyl chloride to *S*-methylglutathione, in contrast to the findings with a number of non-human species (see below) (Peter *et al*., 1989a,b). Selective inhibition experiments suggest that CYP2E1 is a major catalyst of the oxidation of methyl chloride in human liver (Guengerich *et al*., 1991).

4.1.2 *Experimental systems*

Immediately following inhalation of labelled methyl chloride by rats, up to 20% of the label was incorporated into tissue macromolecules. After 6 h, the total level of non-volatile label was highest in liver and kidney and lower in testes. Within 24 h, about 64% of the label was exhaled, 32% found in urine and about 4% in faeces. About 50% of the radiolabel was expired as [^{14}C]CO_2. Following oral administration, radioactivity in hepatic proteins was associated with methionine and serine.

Urinary metabolites are *S*-methylthioacetic acid sulfoxide, *N*-acetyl-*S*-methyl-L-cysteine and *N*-(methylthioacetyl)glycine, which are metabolites of *S*-methyl-L-cysteine and *S*-methylglutathione. These last two compounds were found after incubation of methyl chloride with rodent liver, kidney and brain homogenates. The methyl group of methyl chloride is metabolized via *S*-methyl-L-cysteine to formate which is found in urine and blood of rats, whereas formaldehyde is found in rat liver microsomes and blood of mice and rabbits (IARC, 1986).

Erythrocytes from rats, mice, bovines, pigs, sheep and rhesus monkeys were unable to metabolize methyl chloride, in contrast to the conjugation reaction described for erythrocytes from a majority of human samples (Peter *et al*., 1989a,b). However, CYP2E1 present in kidney microsomes of male mice oxidized methyl chloride to formaldehyde and the quantity formed was dependent upon the hormonal status of the animals. Significantly lower oxidation rates were found with female mouse kidney microsomes for both methyl chloride and chlorzoxazone, a specific substrate for CYP2E1. In liver microsomes, there was no sex difference in methyl chloride oxidation rates, which were about two-fold higher than those with male mouse kidney preparations. Rat kidney microsomes did not convert methyl chloride into formaldehyde (Dekant *et al*., 1995).

Similar sex and species differences have been described for glutathione *S*-transferase activity. The activities of glutathione-*S*-transferase (using dichloronitrobenzene as a substrate) were two- to three-fold higher in the livers of male B6C3F$_1$ mice, compared with female mice and Fischer 344 rats of both sexes, and about seven-fold higher than in male mouse kidney. Neither hepatic nor renal formaldehyde dehydrogenase showed any sex difference in either species, but the activities in mouse liver were about two-fold

higher than those in rat liver. Exposure of mice to 1000 ppm [2100 mg/m^3] methyl chloride for 8 h did not result in any increase in formaldehyde concentration in either liver or kidney, leading the authors to conclude that formaldehyde is unlikely to be the cause of renal carcinogenicity in male mice (Jäger *et al*., 1988). This supports the suggestion that it is the glutathione pathway which is toxicologically significant, since glutathione depletion has been shown to reduce the toxicity of methyl chloride (Chellman *et al*., 1986a).

4.2 Toxic effects

4.2.1 *Humans*

Liver cirrhosis has been described as an effect of long-term exposure to methyl chloride fumes. Non-fatal cases also developed renal damage and nervous system dysfunction (IARC, 1986).

4.2.2 *Experimental systems*

Long-term exposure of many animal species to methyl chloride induced renal damage, hyperaemia, lung haemorrhage and various nervous system effects, ranging from apathy and anorexia to convulsions or paralysis. In mice and rats, exposure by inhalation induced renal and hepatocellular necrosis and degeneration and testicular damage. Adrenal degeneration occurred in rats and cerebellar lesions were induced in mice and guinea-pigs.

Inhalation of methyl chloride decreased non-protein thiol concentrations in rodent liver, kidney, lung, brain and testis. It also induced lipid peroxidation in mice (IARC, 1986).

The effect of glutathione depletion upon methyl chloride toxicity has been assessed in inhalation experiments in male B6C3F$_1$ mice that were pretreated with buthionine sulfoximine, an inhibitor of glutathione synthesis, or diethyl maleate. Depletion reduced the lethality of methyl chloride and reduced its toxicity to liver (as indicated by serum alanine aminotransferase activity), central nervous system (as indicated by cerebellar histology) and kidney (as indicated by cortical cell regeneration following necrosis) (Chellman *et al*., 1986a). Methyl chloride toxicity was also reduced in Fischer 344 rats by treatment with 3-amino-1-[*meta*-(trifluoromethyl)phenyl]-2-pyrazoline, an inhibitor of cyclooxygenase/lipoxygenase. Thus, intraperitoneal injection of the inhibitor at 10 mg/kg bw 1 h before and 1 h after exposure to 15 750 mg/m^3 methyl chloride for 6 h per day for two days reduced lethality from 8/12 to 0/6 and epididymal granuloma formation from 4/4 to 0/6. The effect of the inhibitor on the toxicity of 10 500 mg/m^3 methyl chloride for 6 h per day for five days was to abolish hepatocellular cloudy swelling, renal cortical degeneration, necrosis of the internal granular layer of the cerebellum and degenerative changes in the testes and epididymis; only vacuolar degeneration of the adrenal cortex persisted. Neither the distribution nor the metabolism (quantities of expired methyl chloride or radiolabelled CO_2 or urine) of [^{14}C]methyl chloride was significantly altered by the anti-inflammatory agent (Chellman *et al*., 1986b).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Exposure by inhalation to methyl chloride causes fetal growth retardation and impaired male reproductive capacity in rats and malformations of the heart in fetal mice (IARC, 1986). The preimplantation losses described in rats in which the males were exposed to 6300 mg/m^3 methyl chloride for 6 h per day for five days were due to a failure of fertilization rather than preimplantation embryonic death. A concentration of 2100 mg/m^3 had no effect upon fertilization (Working & Bus, 1986).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Methyl chloride was mutagenic to bacteria and induced chromosomal aberrations in plants. It induced unscheduled DNA synthesis in cultured rat hepatocytes and, in rats exposed *in vivo*, there was a small increase in unscheduled DNA synthesis in hepatocytes but not in tracheal epithelial cells or spermatocytes. DNA strand breaks were induced by methyl chloride in the kidney cells of exposed mice. In cultured mammalian cells, it induced mutations and sister chromatid exchanges and enhanced viral cell transformation. It induced dominant lethal effects in rats. The last effect appears to be due to a failure of the males to fertilize the females, rather than to preimplantation embryonic death and can be partially inhibited by treatment with an anti-inflammatory agent (Chellman *et al.*, 1986c).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to methyl chloride may occur in its production, and in the production of silicones and various other chemical products. Methyl chloride is produced naturally, primarily in oceans, and it is widely detected in ambient air and water.

5.2 Human carcinogenicity data

Two small cohort studies evaluated the mortality experience of workers employed in facilities using or producing methyl chloride. No clear mortality excess occurred, and the small size and mixed exposures of these studies limited their utility for assessing the carcinogenicity of methyl chloride.

Table 1. Genetic and related effects of methyl chloride

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SAF, *Salmonella typhimurium* TM677, forward mutation, 8-azaguanine resistance	+	NT	10% atm	Fostel *et al*. (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	2.5% atm	Simmon *et al*. (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	1% atm	JETOC (1997)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	0.5% atm	Andrews *et al*. (1976)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	0.1% atm	JETOC (1997)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	0.5% atm	JETOC (1997)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	0.5% atm	JETOC (1997)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	+	+	10% atm	JETOC (1997)
TSC, *Tradescantia* species, pollen grains, chromosomal aberrations	+	NT	0.92% atm	Smith & Lotfy (1954)
T7S, Cell transformation, SA7 virus/Syrian hamster embryo cells *in vitro*	+	NT	0.6% atm	Hatch *et al*. (1983)
URP, Unscheduled DNA synthesis, Fischer 344 rat primary hepatocytes *in vitro*	+	NT	1% atm	Working *et al*. (1986)
UIA, Unscheduled DNA synthesis, Fischer 344 rat primary spermatocytes *in vitro*	+	NT	1% atm	Working *et al*. (1986)
DIH, DNA strand breaks, cross-links human lymphoblast line *in vitro*	–	NT	5% atm	Fostel *et al*. (1985)
GIH, Gene mutation, human lymphoblast line, *tk* locus *in vitro*	+	NT	2% atm	Fostel *et al*. (1985)
SIH, Sister chromatid exchange, human lymphoblast line *in vitro*	+	NT	1% atm	Fostel *et al*. (1985)
DVA, DNA strand breaks, cross-links in male $B6C3F_1$ mouse kidney cells *in vivo*	+		1000 ppm inh 8 h × 1	Ristau *et al*. (1990)
UPR, Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	(+)[c]		15000 ppm inh 3 h × 1	Working *et al*. (1986)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
UVR, Unscheduled DNA synthesis, Fischer 344 rat spermatocytes *in vivo*	–[c]		15000 ppm inh 3 h × 1	Working *et al.* (1986)
UVR, Unscheduled DNA synthesis, Fischer 344 rat tracheal epithelial cells *in vivo*	–[c]		15000 ppm inh 3 h × 1	Working *et al.* (1986)
DLR, Dominant lethal test, Fischer 344 rats *in vivo*	+		3000 ppm inh 6 h/d × 5	Working *et al.* (1985)
DLR, Dominant lethal test, Fischer 344 rats *in vivo*	+		3000 ppm inh 6 h/d × 5	Chellman *et al.* (1986c)
BVD, Binding (covalent) to DNA, Fischer 344 rat liver cells *in vivo*	+		9 µmol po × 1	Xu *et al.* (1993)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; atm, atmosphere; inh, inhalation; po, oral
[c] Negative for exposure to 3500 ppm, 6 h/d, up to five days

5.3 Animal carcinogenicity data

No adequate data were available to the Working Group.

5.4 Other relevant data

The toxicokinetics of methyl chloride have been studied in human volunteers. It can be converted by human erythrocytes to *S*-methylglutathione, a metabolite also observed in animal studies; alternatively, it is metabolized by CYP2E1. Carbon dioxide is a major metabolite.

Methyl chloride causes toxicity in rodents in the liver, kidney and central nervous system. It may deplete glutathione in tissues.

Methyl chloride is mutagenic to bacteria. It was genotoxic in a number of mammalian cell systems *in vitro* and gave positive results in the dominant lethal test in rats *in vivo*.

5.5 Evaluation

There is *inadequate evidence* for the carcinogenicity of methyl chloride to humans.

There is *inadequate evidence* for the carcinogenicity of methyl chloride in experimental animals.

Overall evaluation

Methyl chloride is *not classifiable as to its carcinogenicity to humans (Group 3)*.

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PHENOL

Data were last evaluated in IARC (1989).

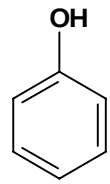
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 108-95-2
Chem. Abstr. Name: Phenol
IUPAC Systematic Name: Phenol
Synonyms: Carbolic acid; hydroxybenzene

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_6H_6O Relative molecular mass: 94.11

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless, acicular crystals with characteristic sweet and acrid odour (Budavari, 1996)
(*b*) *Boiling-point*: 181.8°C (Lide, 1997)
(*c*) *Melting-point*: 40.9°C (Lide, 1997)
(*d*) *Solubility*: Soluble in ethanol, water, diethyl ether, chloroform, glycerol, carbon disulfide, petrolatum and alkalis (Budavari, 1996)
(*e*) *Vapour pressure*: 47 Pa at 25°C; relative vapour density (air = 1), 3.24 (American Conference of Governmental Industrial Hygienists, 1991)
(*f*) *Flash point*: 79°C, closed cup (Budavari, 1996)
(*g*) *Explosive limits*: upper, 8.6%; lower, 1.7% by volume in air (American Conference of Governmental Industrial Hygienists, 1991)
(*h*) *Conversion factor*: $mg/m^3 = 3.85 \times ppm$

1.2 Production and use

The estimated worldwide synthetic phenol capacity in 1994 was approximately 5200 thousand tonnes; estimated capacities by region were reported as (thousand tonnes): Mexico and South America, 155; Europe, 1967; Japan, 800; Asia, 256; China, 126; and the United States, 1870 (Wallace, 1996). Production in the United States in 1993 was reported to be 1 544 222 tonnes (United States International Trade Commission, 1994).

Phenol has a wide range of uses, including in the preparation of phenolic and epoxy resins (bisphenol-A), nylon-6 (caprolactam), 2,4-D, selective solvents for refining lubricating oils, adipic acid, salicylic acid, phenolphthalein, pentachlorophenol and other derivatives; in germicidal paints; as a laboratory reagent and in dyes and indicators; and as a slimicide, biocide and general disinfectant (Lewis, 1993). The world demand for phenol by use in 1993 was reported as (%): phenolic resins, 35; bisphenol-A, 30; caprolactam, 15; alkylphenols, 7; aniline, 5; and others, 8 (Wallace, 1996).

1.3 Occurrence

1.3.1 *Occupational exposure*

Data on levels of occupational exposure to phenol have been presented in a previous monograph (IARC, 1989).

1.3.2 *Environmental occurrence*

Phenol is present in plant and animal organic wastes as a result of decomposition. The level of phenol present in poultry manure, for example, has been shown to increase as degradation proceeds. Phenol is an important industrial chemical and enters the environment in air emissions and wastewater connected with its use as a chemical intermediate, disinfectant and antiseptic (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 19 mg/m^3 as the threshold limit value for occupational exposures to phenol in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for phenol in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

2.1 Industry-based studies

In the nested case–control study among rubber workers in the United States (Wilcosky *et al*., 1984), described in greater detail in the monograph on dichloromethane (see this volume), one of the substances evaluated was phenol, which was analysed as a potential risk factor in relation to each of five cancer types. None of the odds ratios was

significant; the only one greater than 1.0 was that for stomach cancer (odds ratio, 1.4; *n* = 6) in white men. The odds ratio for lung cancer in white men was 1.0 (*n* = 13).

Dosemeci *et al*. (1991) reported results concerning phenol from a cohort study in the United States initiated to assess risks due to formaldehyde. This report concerned 14 861 workers employed before 1966 in five facilities producing or using phenol as well as formaldehyde. Subjects were traced to 1980. More than 360 000 person–years of follow-up accrued. Job history records were linked to extensive industrial hygiene data and expertise to assess possible exposure to formaldehyde and phenol. Relative risk estimates (standardized mortality ratios (SMRs)) for white male workers exposed to phenol were derived by comparison with the general United States population. The SMR for all causes of death combined was close to 1.0, as was the SMR for all cancers combined. Exposed workers had no excess of cancer at any of the following sites: buccal cavity and pharynx, stomach, colon, liver, pancreas, skin, prostate, testis, brain or leukaemia. There were slight, unremarkable excesses for cancers of the larynx (SMR, 1.1; 95% CI, 0.5–2.3; *n* = 7), lung (SMR, 1.1; 95% CI, 0.9–1.3; *n* = 146), urinary bladder (SMR, 1.1; 95% CI, 0.6–1.4; *n* = 13), kidney (SMR, 1.3; 95% CI, 0.7–2.1; *n* = 13) and rectum (SMR, 1.4; 95% CI, 0.8–2.2; *n* = 18). Only for oesophageal cancer (SMR, 1.6; 95% CI, 0.9–2.6; *n* = 15) and Hodgkin's disease (odds ratio, 1.7; 95% CI, 0.8–3.1; *n* = 10) were the excesses noteworthy, albeit not significant. Nor was there any stronger evidence of a cancer risk when the exposed group was compared with an internal comparison group of workers unexposed to phenol. When the phenol-exposed group was separated into subgroups by cumulative exposure, the SMRs were [2.1 (95% CI, 1.0–3.7; *n* = 11)] for oesophageal cancer, [1.1 (95% CI, 0.9–1.4; *n* = 78)] for lung cancer and [0.9 (95% CI, 0.1–3.3; *n* = 2)] for Hodgkin's disease for medium and high exposure combined. [The Working Group noted that workers typically had multiple exposures.]

Kauppinen *et al*. (1993) carried out a case–control study of respiratory tract cancer nested within a cohort of 7307 Finnish male woodworkers (IARC, 1995) from 35 plants (including plywood, particle-board, sawmill and formaldehyde (IARC, 1995) glue plants). Each case of respiratory tract cancer within the cohort identified in the Finnish Cancer Registry and diagnosed between 1957 and 1982 (*n* = 136) was matched by year of birth with three controls (*n* = 408) from the cohort. Job history records were supplemented by interviews with subjects or next-of-kin, and were linked to a specially devised plant- and period-specific job–exposure matrix which included 12 substances, one of which was phenol. The interview, achieved for 65% of subjects, also requested smoking data. Several logistic regression models were run, varying the treatment of induction period, smoking status and duration of exposure. Any exposure to phenol, without adjustment for induction period or smoking, gave an odds ratio of 3.2 (90% CI, 1.8–5.6; *n* = 14) for lung cancer. Estimates were slightly higher when a 10-year induction period was included in the model (odds ratio, 3.5; 90% CI, 1.8–7.0; *n* = 6). Adjustment for smoking did not eliminate the association (odds ratio, 2.5; 90% CI, 1.2–5.0; *n* = 9). Long-term workers (more than five years' exposure) (odds ratio, 1.4; 90% CI, 0.6–3.6; *n* = 7) had lower risk than short-term workers (one month to five years' exposure) (odds

ratio, 3.3; 90% CI, 1.0–11.0; *n* = 7). While workers exposed to phenol tended also to be exposed to other substances, none of those substances showed as strong an association with respiratory tract cancer as did phenol. In particular, although all phenol-exposed workers were also exposed to formaldehyde, workers exposed to formaldehyde but not to phenol had no excess risk of respiratory tract cancer (odds ratio, 1.0).

2.2 Community-based studies

In Siemiatycki's (1991) population-based case–control study of cancer in Montreal, Canada (see monograph on dichloromethane in this volume), phenol was one of the substances evaluated; 1% of the entire study population had been exposed to it at some time. Among the main occupations to which phenol exposure was attributed in this study were electric motor repairmen and foundry workers. The publication reported an association between phenol and pancreatic cancer (odds ratio, 4.8; 90% CI, 1.8–12.7; *n* = 4); for no other site was cancer risk associated with phenol exposure. [The Working Group noted that detailed results for other sites were not provided, because they were based on small numbers, and that workers typically had multiple exposures.]

3. Studies of Cancer in Experimental Animals

Phenol was tested for carcinogenicity by oral administration in drinking-water in one strain of mice and one strain of rats. No treatment-related increase in the incidence of tumours was observed in mice or in female rats. In male rats, an increase in the incidence of leukaemia was observed at the lower dose but not at the higher dose. Phenol was tested extensively in the two-stage mouse skin model and showed promoting activity (IARC, 1989).

3.1 Skin application

Mouse: Groups of five male TG.AC or FVB/N non-carrier mice, six to seven weeks of age, were administered 3 mg phenol (reagent grade) per animal in acetone by skin application twice per week for up to 20 weeks. A skin papilloma occurred in an exposed TG.AC mouse, whereas none occurred in controls (not considered to be significant) (Spalding *et al*., 1993).

3.2 Administration with known carcinogens

3.2.1 *Mouse*

Groups of 22–24 female CC57 Br mice, weighing 12–14 g, were administered phenol ('chemically pure') twice a week orally [method not stated] for total doses of 0, 0.02 or 1.0 mg in three modes; phenol was given for 2.5 months and 1 mg per animal benzo[*a*]pyrene subsequently for 2.5 months; 1 mg per animal benzo[*a*]pyrene was given for 2.5 months followed by phenol for 2.5 months; or the two were given concurrently for 2.5 months. The high dose of phenol given in combination with benzo[*a*]pyrene pro-

duced a 27.2% incidence of malignant forestomach tumours ($p < 0.01$) compared with 4.6% when benzo[*a*]pyrene was given alone. In groups given 1.0 mg phenol either before or after the initiator, the incidence of malignant forestomach tumours was reduced from that in mice given only the initiator (Yanysheva *et al*., 1992).

Groups of 7–10 male Sprague-Dawley rats, weighing 200 g, were administered phenol (purity, > 99.5%) at doses of 0 or 100 mg/kg bw by gavage on five days per week for six weeks beginning one week after partial hepatectomy and intraperitoneal injection of 30 mg/kg bw *N*-nitrosodiethylamine to initiate liver carcinogenesis. Phenol did not increase the multiplicity of enzyme-altered (γ-glutamyltranspeptidase) foci compared with that in a group subjected only to initiation (Stenius *et al*., 1989).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

The major route of phenol metabolism is conjugation with sulfate and, at high dose, with glucuronic acid. In addition, hydroquinone (see this volume) is formed, which is excreted as a sulfate or glucuronide conjugate. Several glutathione conjugates can be formed from the reactive 1,4-benzoquinone formed from hydroquinone (Figure 1).

4.1.1 *Humans*

In a case of lethal human phenol intoxication (a phenol-containing disinfectant was ingested), the phenol concentration in brain, kidney, liver and muscle was determined several hours after death. The concentration in the brain was highest, followed by the kidney; the concentrations in liver and muscle were half that in the brain (Lo Dico *et al*., 1989).

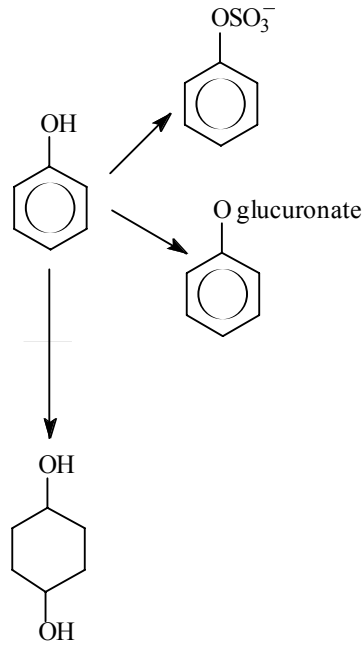
Studies in flow-through diffusion cells showed that full-thickness rat skin absorbed [^{14}C]phenol at a slightly faster rate than human skin (Hotchkiss *et al*., 1992), which absorbs phenol reasonably well (Bucks *et al*., 1990).

The sulfation of phenol and the glucuronidation of its hydroquinone metabolite were measured in human liver cytosols and microsomes, respectively. The rate of phenol sulfation varied between 0.31 and 0.92 nmol/mg protein/min; this is slightly higher than the rate for mice (0.46) and lower than that for rats (1.20). The rate of hydroquinone glucuronidation was between 0.10 and 0.28 nmol/mg protein/min, slightly higher than that for rats (0.08) and lower than that for mice (0.22). These enzyme-kinetic data were subsequently used to simulate phenol metabolism in mice, rats and humans *in vivo*, using a compartmental pharmacokinetic model with benzene as phenol precursor (Seaton *et al*., 1995).

4.1.2 *Experimental systems*

Absorption of phenol in a flow-through diffusion cell *in vitro*, using full-thickness rat skin, indicated relatively rapid absorption through rat skin: 27% was absorbed in

Figure 1. Metabolism of phenol



Hydroquinone[a]

[a]For the metabolism of hydroquinone, see Figure 1 in the monograph on hydroquinone in this volume.

72 h; the rate for human skin was somewhat lower (19%) in the same system (Hotchkiss *et al*., 1992). Studies on the disposition of phenol after oral, dermal, intravenous and intratracheal administration to rats confirmed earlier results (Hughes & Hall, 1995): even after dermal application, phenol is rapidly excreted in urine, mainly as phenyl sulfate with smaller amounts of phenyl glucuronide. At higher phenol doses, biliary excretion of phenyl glucuronide in particular becomes more important, and a 2-*S*-glutathionylhydroquinone metabolite was observed (Scott & Lunte, 1993). The latter is probably formed from 1,4-benzoquinone (see this volume), the oxidized hydroquinone metabolite, which reacts spontaneously at a high rate with glutathione. The glutathione conjugate can undergo redox cycling, which may cause toxicity (Puckett-Vaughn *et al*., 1993). When phenol and hydroquinone are administered simultaneously to mice, their conjugation may be mutually decreased by competition for the same sulfotransferase enzyme, resulting in slower elimination, and possibly increased formation of 1,4-benzoquinone; the latter may be responsible for bone-marrow toxicity (Legathe *et al*., 1994). The formation and pharmacokinetics of phenol and hydroquinone during benzene exposure in rats, mice and humans have been simulated by Seaton *et al*. (1995).

Phenol is converted by rat liver microsomes to a reactive metabolite that binds covalently to protein; the most likely metabolites involved in this are hydroquinone and, at

a lower rate, catechol, the covalent binding of which does not require NADPH (Wallin *et al*., 1985). 1,4-Benzoquinone is responsible for the inactivation of CYP2E1; this does not require reactive oxygen species, but is a direct effect (Gut *et al*., 1996). Peroxidases (e.g., from macrophages), may also catalyse the formation of reactive products from phenol (Schlosser *et al*., 1989), in which 1,4-benzoquinone plays a critical role. The conversion of hydroquinone to 1,4-benzoquinone *in vitro* was stimulated by phenol (Smith *et al*., 1989). A small percentage of phenol is converted *in vitro* to trihydroxybenzene or, after ring opening, to muconic acid (Schlosser *et al*., 1993).

Incubation of mouse peritoneal macrophage lysate with bovine serum albumin and [^{14}C]phenol or [^{14}C]hydroquinone resulted in covalent binding of ^{14}C to protein dependent on hydrogen peroxide and inhibited by the peroxidase inhibitor aminotriazole or by the –SH nucleophile antioxidant cysteine. The conversion of [^{14}C]phenol to protein- and calf thymus DNA-binding metabolite(s) was also catalysed by purified prostaglandin H synthase and was dependent on either hydrogen peroxide or arachidonic acid (Schlosser *et al*., 1989). Phenol (100 μmol/L) induced formation of 8-hydroxydeoxyguanosine in HL60 cell DNA *in vitro*, but not in bone-marrow cells of B6C3F$_1$ mice *in vivo* after a single intraperitoneal dose of 75 mg/kg (Kolachana *et al*., 1993).

4.1.3 *Comparison of human and rodent data*

The metabolism of phenol in humans and in rats or mice is very similar: at low doses, mainly sulfate conjugates of phenol and hydroquinone are excreted in urine. Whether the reactive intermediate 1,4-benzoquinone plays an important role *in vivo* at low exposure is uncertain; as long as sufficient glutathione is available, this will probably rapidly trap the 1,4-benzoquinone and protect the cell from damage. Urinary excretion of mercapturates reflects formation of the glutathione conjugates. When at higher dose this protection fails, toxicity may become overt. Whether the covalent binding observed *in vitro* has relevance *in vivo* is uncertain.

4.2 Toxic effects

The toxicity of phenol has been reviewed (WHO, 1994).

4.2.1 *Humans*

Phenol poisoning can occur in humans after skin absorption, inhalation of vapours or ingestion. Acute local effects are severe tissue irritation and necrosis. At high doses, the most prominent systemic effect is central nervous system depression (IARC, 1989).

4.2.2 *Experimental systems*

Phenol causes irritation, dermatitis, central nervous system effects and liver and kidney toxicity in experimental animals (IARC, 1989).

Phenol induced fluorescence from 2′,7′-dichlorofluorescin in HL60 human leukaemia cells *in vitro* at concentrations that were not cytotoxic; this was interpreted to indicate generation of reactive oxygen species (Shen *et al*., 1996). When phenol was incu-

bated with hydrogen peroxide and horseradish peroxidase, disappearance of polyunsaturated *cis*-parinaric fatty acid was observed in a cell-free system, and also when *cis*-parinaric acid was incorporated into cellular lipids of HL60 cells; the reaction was inhibited by ascorbate and glutathione. The authors interpreted this to demonstrate the generation from phenol of phenoxy radicals capable of direct oxidation of polyunsaturated fatty acid (Ritov *et al*., 1996).

In contrast to catechol and hydroquinone, phenol was a weak inducer of apoptosis in HL60 human promyelocytic leukaemia cells, and had an apoptotic effect only at the highest concentration tested (0.75 mmol/L) (Moran *et al*., 1996). Phenol (≤ 10 mmol/L) had no effect on the colony formation of granulocytes/macrophages induced by a recombinant granulocyte/macrophage colony-stimulating factor of murine bone-marrow cells (Irons *et al*., 1992).

In a study on the immunotoxic effects of cigarette tar components, it was shown that phenol (≤ 1 mmol/L) had no effect on interleukin-2-dependent DNA synthesis or cell proliferation in cultured human lymphoblasts (Li *et al*., 1997).

Phenol (25, 50, 75 or 100 mg/kg, single intraperitoneal administration) decreased the incorporation of ^{59}Fe by erythrocytes in a dose-dependent fashion in female Swiss mice, when administered with hydroquinone (50 mg/kg, single intraperitoneal administration) (Snyder *et al*., 1989). Phenol (≤ 40 μmol/L) had no consistent effect on the number of erythroid colony-forming bone-marrow cells from Swiss Webster or C57BL/J6 mice (Neun *et al*., 1992) and only inhibited the growth of bone-marrow cells from female C57 BL/6 × DBA/2 mice at millimolar concentrations (Seidel *et al*., 1991).

4.3 Reproductive and reproductive effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Phenol was toxic in cultured rat conceptuses at 10 μmol/L, the lowest concentration tested, and killed all embryos at 200 μmol/L (Chapman *et al*., 1994).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Phenol was mutagenic to *Escherichia coli* B/Sd-4 at highly toxic doses only (survival level, 0.5–1.7%; Demerec *et al.*, 1951), but it did not induce filamentation in the *lon*$^-$ mutant of *Escherichia coli* (Nagel *et al.*, 1982) and was not mutagenic to *Salmonella typhimurium* strains in most studies. In one study, it was weakly mutagenic to *S. typhimurium* TA98 in the presence of an exogenous metabolic system, but only when the assay was performed using a modified medium.

Phenol weakly induced mitotic segregation in *Aspergillus nidulans*.

Phenol did not increase the frequency of sex-linked recessive lethal mutations in *Drosophila melanogaster* following feeding or administration by injection.

Phenol did not induce DNA single-strand breaks in mouse lymphoma L5178Y cells. It was reported in abstracts that phenol induced DNA strand breaks in mouse lymphoma cells, as measured by the alkaline unwinding technique followed by elution through hydroxyapatite (Garberg & Bolcsfoldi, 1985), but that it did not induce strand breaks, as measured by the alkaline elution technique, in rat germ-cell DNA after either single or multiple dose treatments (Skare & Schrotel, 1984).

Phenol induced mutations at the *hprt* locus of Chinese hamster V79 cells in the presence of an exogenous metabolic system from the livers of phenobarbital-induced mice and *tk* locus mutations in mouse lymphoma L5178Y cells in the presence or the absence of an exogenous metabolic activation system. Micronuclei were induced by phenol in Chinese hamster ovary cells in one study and sister chromatid exchanges in mammalian cells were increased in several studies, including three with human lymphocytes.

Phenol was reported to induce DNA oxidative damage in human promyelocytic HL60 cells and to inhibit repair of radiation-induced chromosomal breaks in human leukocytes (Morimoto *et al.*, 1976). However, it only slightly inhibited DNA repair synthesis and DNA replication synthesis in WI-38 human diploid fibroblasts (Poirier *et al.*, 1975).

DNA oxidative damage was not found in bone marrow of mice given a single intraperitoneal injection of phenol. Administration of phenol did not induce micronuclei in bone-marrow cells in three studies; however, micronuclei were induced in the bone marrow of pregnant CD-1 mice after a single oral dose, but micronuclei were not seen in the liver of fetuses. As reported in an abstract, phenol induced micronuclei in male and female mice at doses of 150 and 200 mg/kg bw (Sofuni *et al.*, 1986). In one study, FISH probes for centromeres were used to demonstrate that the micronuclei in the bone-marrow cells of mice injected three times intraperitoneally with 160 mg phenol/kg bw were the result of chromosomal breakage and not aneuploidy. This result substantiates a similar finding reported as an abstract [details not given] (Lowe *et al.*, 1987). Inhibition of topoisomerase I *in vitro* was not found and inhibition of topoisomerase II *in vitro* was observed only if a peroxidase/hydrogen peroxide system was added to the reaction mixture. Covalent binding to DNA was not observed in rat Zymbal glands after in-vivo exposure. In Chinese hamster cells *in vitro*, phenol did not inhibit intercellular communication in two studies, but in a third study, inhibited intercellular communication in CYP1A1-, CY1A2- and CYP2B1-transfected cell lines as well as in the parental line.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Phenol is a basic feedstock for the production of phenolic resins, bisphenol A, caprolactam, chlorophenols and several alkylphenols and xylenols. Phenol is also used in

Table 1. Genetic and related effects of phenol

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	9140[c]	Contruvo *et al*. (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	282	Florin *et al*. (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	NT	2000	Kinoshita *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	250	Pool & Lin (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	800	Haworth *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1500	Kazmer *et al*. (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	9140[c]	Contruvo *et al*. (1977)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	282	Florin *et al*. (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	NT	50	Gilbert *et al*. (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	250	Pool & Lin (1982)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	800	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	9140[c]	Cortruvo *et al*. (1977)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	282	Florin *et al*. (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	250	Pool & Lin (1982)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	800	Haworth *et al*. (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	9140[c]	Cortruvo *et al*. (1977)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	25	Gilbert *et al*. (1980)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	250	Pool & Lin (1982)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	800	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	9140[c]	Cortruvo *et al*. (1977)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	282	Florin *et al*. (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	(+)	2350	Gocke *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250	Pool & Lin (1982)
SAS, *Salmonella typhimurium* TA1536, reverse mutation	–	–	9140[c]	Cortruvo *et al*. (1977)
ANN, *Aspergillus nidulans*, aneuploidy	(+)	NT	1412	Crebelli *et al*. (1987)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
VFS, *Vicia faba*, sister chromatid exchange	+	NT	10000	Zhang *et al*. (1991)
PLS, *Hordeum vulgare*, sister chromatid exchange	+	NT	10000	Zhang *et al*. (1991)
PLS, *Secale cereale*, sister chromatid exchange	+	NT	10000	Zhang *et al*. (1991)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		20000 µg/mL[d]	Sturtevant (1952)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		4700 ppm feed	Gocke *et al*. (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		5250 µg/mL inj	Woodruff *et al*. (1985)
DIA, DNA strand breaks/cross-links, mouse lymphoma L5178YS cells *in vitro*	–	NT	94	Pellack-Walker & Blumer (1986)
G9H, Gene mutation, Chinese hamster V79 cells, *hprt* locus *in vitro*	NT	+	250	Paschin & Bahitova (1982)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	?	(+)	300	McGregor *et al*. (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	5	Wangenheim & Bolcsfoldi (1988)
SIM, Sister chromatid exchange, mouse spleen cells *in vitro*	+	NT	10000	Zhang *et al*. (1991)
MIA, Micronucleus test, Chinese hamster ovary CHO cells *in vitro*	(+)	(+)	175	Miller *et al*. (1995)
DIH, DNA oxidative damage, human promyelocytic HL-60 cells *in vitro*	+	NT	9.4	Kolachana *et al*. (1993)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	(+)	NT	94	Morimoto & Wolff (1980)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	+	282	Morimoto *et al*. (1983)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.5	Erexson *et al*. (1985)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	NT	188	Jansson *et al*. (1986)
DVA, DNA oxidative damage, B6C3F$_1$ mouse bone-marrow cells *in vivo*	–		75 ip × 1	Kolachana *et al*. (1993)
MVM, Micronucleus test, NMRI mouse bone-marrow cells *in vivo*	–		188 ip × 2 d	Gocke *et al*. (1981)
MVM, Micronucleus test, male CD-1 mouse bone-marrow cells *in vivo*	–		250 po × 1	Gad-El Karim *et al*. (1986)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVM, Micronucleus test, pregnant CD-1 mouse bone-marrow cells *in vivo*	+		265 po × 1	Ciranni *et al*. (1988)
MVM, Micronucleus test, CD-1 mouse bone-marrow cells *in vivo*	–		160 ip × 1	Barale *et al*. (1990)
MVM, Micronucleus resulting from chromosomal breakage, male CD-1 mouse bone marrow *in vivo*	+[e]		160 ip × 3 d	Chen & Eastmond (1995a)
AVA, Aneuploidy, male CD-1 mouse bone marrow *in vivo*	–[e]		160 ip × 3 d	Chen & Eastmond (1995a)
BID, Binding (covalent) to DNA, cultured rat Zymbal gland cells *in vitro*	+	NT	750	Reddy *et al*. (1990)
BVD, Binding (covalent) to DNA, female Sprague-Dawley rat Zymbal glands, liver, spleen and bone marrow *in vivo*	–		75 po × 4 d	Reddy *et al*. (1990)
ICR, Inhibition of intercellular communication, V79 Chinese hamster cells	–	NT	NG	Chen *et al*. (1984)
ICR, Inhibition of intercellular communication, V79 Chinese hamster cells	–	NT	400	Malcolm *et al*. (1985)
ICR, Inhibition of intercellular communication, V79 Chinese hamster cells	+	NT	103	Vang *et al*. (1993)
Inhibition of topoisomerase I activity *in vitro*	–	NT	94	Chen & Eastmond (1995b)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Inhibition of topoisomerase II activity *in vitro*	–[f]	NT	47	Chen & Eastmond (1995b)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested; ?, inconclusive
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw /day; NG, not given; inj, injection; ip, intraperitoneal; po, oral
[c] 4.1% of this dose was ozonated before testing
[d] Vaginal douche
[e] The origin of the bone-marrow micronuclei was determined by a multicolour FISH assay using mouse major and satellite probes. Results showed that micronuclei are a result of chromosome breakage and not loss of entire chromosome.
[f] Inhibitory effects were seen following bioactivation using a peroxidase/hydrogen peroxide system.

disinfectants and antiseptics. Occupational exposure to phenol has been reported during its production and use, as well as in the use of phenolic resins in the wood products industry. It has also been detected in automotive exhaust and tobacco smoke.

5.2 Human carcinogenicity data

A study of Finnish woodworkers found a high risk of lung cancer among those exposed to phenol, although the excess risk was stronger in short-term than in long-term workers. This result was not replicated in three other studies which reported results on phenol and lung cancer, although two of them had very low statistical power. In the three studies reporting associations with multiple cancer sites, a few elevated risks were reported, but not at any cancer site in two or more studies. The pattern of results fails to demonstrate a risk of cancer due to phenol exposure.

5.3 Animal carcinogenicity data

Phenol was tested for carcinogenicity by oral administration in rats in one study and in mice in one study. An increased incidence of leukaemia was reported in male rats treated with the lower dose but not in high-dose rats or in mice or female rats. Phenol was a promoter of mouse skin carcinogenesis in two-stage protocols.

5.4 Other relevant data

Phenol is well absorbed from the gastrointestinal tract and through the skin of animals and humans. It is metabolized principally by conjugation (by sulfation and glucuronidation) with a minor oxidation pathway leading to quinone-related reactive intermediates which bind covalently to protein and are detoxified by conjugation with glutathione. Topically applied phenol is a skin irritant and systemic toxicity is seen in liver and kidney after topical and oral dosing.

After in-vivo administration, phenol induced micronuclei in mice and chromosomal aberrations in rats. It also caused oxidative DNA damage in mice, and it bound covalently to rat DNA. In cultured mammalian cells, phenol caused mutations, sister chromatid exchanges and micronuclei. It bound to cellular protein (but not to DNA) and inhibited intercellular communication. It did not induce recessive lethal mutations in *Drosophila melanogaster* and had only a weak effect in inducing segregation in *Aspergillus nidulans*. Phenol was not mutagenic in bacteria.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of phenol.

There is *inadequate evidence* in experimental animals for the carcinogenicity of phenol.

Overall evaluation

Phenol is *not classifiable as to its carcinogenicity to humans (Group 3)*.

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POLYCHLOROPHENOLS AND THEIR SODIUM SALTS

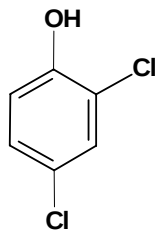
Data were last reviewed in IARC (1979, 1986, 1991) and the compounds were classified in *IARC Monographs* Supplement 7 (1987a).

1. Exposure Data

1.1 Chemical and physical data

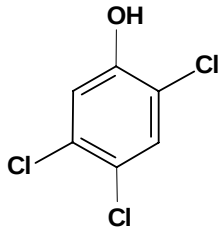
1.1.1 *Nomenclature, structural and molecular formulae and relative molecular masses*

Chem. Abstr. Serv. Reg. No.: 120-83-2
Chem. Abstr. Name: 2,4-Dichlorophenol
IUPAC Systematic Name: 2,4-Dichlorophenol
Synonym: 2,4-Dichlorophenic acid



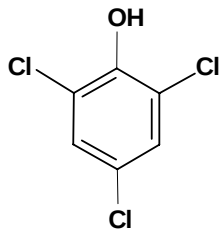
$C_6H_4Cl_2O$ Relative molecular mass: 163.00

Chem. Abstr. Serv. Reg. No.: 95-95-4
Chem. Abstr. Name: 2,4,5-Trichlorophenol
IUPAC Systematic Name: 2,4,5-Trichlorophenol
Synonym: TCP



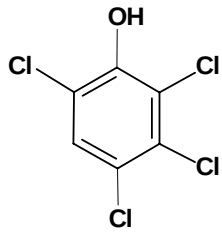
$C_6H_3Cl_3O$ Relative molecular mass: 197.46

Chem. Abstr. Serv. Reg. No.: 88-06-2
Chem. Abstr. Name: 2,4,6-Trichlorophenol
IUPAC Systematic Name: 2,4,6-Trichlorophenol



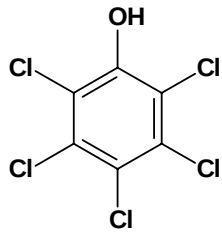
$C_6H_3Cl_3O$ Relative molecular mass: 197.46

Chem. Abstr. Serv. Reg. No.: 58-90-2
Chem. Abstr. Name: 2,3,4,6-Tetrachlorophenol
IUPAC Systematic Name: 2,3,4,6-Tetrachlorophenol



$C_6H_2Cl_4O$ Relative molecular mass: 231.89

Chem. Abstr. Serv. Reg. No.: 87-86-5
Chem. Abstr. Name: Pentachlorophenol
IUPAC Systematic Name: Pentachlorophenol
Synonyms: Chlorophenasic acid; PCP



C_6HCl_5O Relative molecular mass: 266.34

1.1.2 *Chemical and physical properties of the pure substances*

2,4-Dichlorophenol

(*a*) *Description*: Needle-like crystals (Budavari, 1996)
(*b*) *Boiling-point*: 210°C (Lide, 1997)
(*c*) *Melting-point*: 45°C (Lide, 1997)

(*d*) *Solubility*: Slightly soluble in water; soluble in benzene, carbon tetrachloride, diethyl ether and ethanol (Lewis, 1993; Lide, 1997)
(*e*) *Vapour pressure*: 10 Pa at 25°C; relative vapour density (air = 1), 5.62 (United States National Library of Medicine, 1997)
(*f*) *Flash-point*: 113°C (Lewis, 1993)
(*g*) *Conversion factor*: $mg/m^3 = 6.7 \times ppm$

2,4,5-Trichlorophenol

(*a*) *Description*: Colourless needles with a strong phenolic odour (Budavari, 1996)
(*b*) *Boiling-point*: 247°C (Lide, 1997)
(*c*) *Melting-point*: 69°C (Lide, 1997)
(*d*) *Solubility*: Slightly soluble in water; very soluble in acetone, benzene, diethyl ether and ethanol (Lewis, 1993; Lide, 1997)
(*e*) *Vapour pressure*: 2.9 Pa at 25°C (United States National Library of Medicine, 1997)
(*f*) *Conversion factor*: $mg/m^3 = 8.1 \times ppm$

2,4,6-Trichlorophenol

(*a*) *Description*: Colourless crystals with a strong phenolic odour (Budavari, 1996)
(*b*) *Boiling-point*: 246°C (Lide, 1997)
(*c*) *Melting-point*: 69°C (Lide, 1997)
(*d*) *Solubility*: Slightly soluble in water; soluble in acetone, acetic acid, diethyl ether and ethanol (Lewis, 1993; Lide, 1997)
(*e*) *Vapour pressure*: 133 Pa at 76.5°C (United States National Library of Medicine, 1997)
(*f*) *Conversion factor*: $mg/m^3 = 8.1 \times ppm$

2,3,4,6-Tetrachlorophenol

(*a*) *Description*: Brown flakes with a strong odour (Lewis, 1993)
(*b*) *Boiling-point*: 164°C (23 mm Hg) (Lewis, 1993)
(*c*) *Melting-point*: 70°C (Lide, 1997)
(*d*) *Solubility*: Insoluble in water; soluble in acetone, benzene, chloroform, diethyl ether and ethanol (Lewis, 1993; Lide, 1997)
(*e*) *Vapour pressure*: 8 kPa at 190°C (Verschueren, 1996)
(*f*) *Conversion factor*: $mg/m^3 = 9.5 \times ppm$

Pentachlorophenol

(*a*) *Description*: Needle-like crystals (Budavari, 1996)
(*b*) *Boiling-point*: 310°C (decomposes) (Lide, 1997)
(*c*) *Melting-point*: 174°C (Lide, 1997)
(*d*) *Solubility*: Slightly soluble in water; soluble in benzene; very soluble in diethyl ether and ethanol (Lide, 1997)

(*e*) *Vapour pressure*: 0.02 Pa at 20°C; relative vapour density (air = 1), 9.20 (Verschueren, 1996)

(*f*) *Conversion factor*: $mg/m^3 = 10.9 \times ppm$

1.2 Production and use

Production volumes for pentachlorophenol in the United States for the mid-1980s were reported as (thousand tonnes): 1983, 20.4; 1984, 19; 1985, 17.2; and 1986, 14.5 (Agency for Toxic Substances and Disease Registry, 1994). The volume for 1996, the last full year for which data are available, was 9.1 thousand tonnes. There is no known current European production of pentachlorophenol (Norman, 1998). Production data were not available for the other chlorophenols.

Information available in 1995 indicated that 2,4-dichlorophenol was produced in seven countries, 2,4,6-trichlorophenol in five, 2,4,5-trichlorophenol only in Japan and pentachlorophenol in six, while production of 2,3,4,6-tetrachlorophenol had been discontinued (Chemical Information Services, 1995).

2,4-Dichlorophenol and 2,4,5-trichlorophenol have been used in the synthesis of phenoxy acid herbicides, including 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). 2,4,5-Trichlorophenol has also been used as a fungicide and a bactericide. 2,4,6-Trichlorophenol has been used as a pesticide. 2,3,4,6-Tetrachlorophenol has been used as a fungicide (Lewis, 1993; Verschueren, 1996). Chlorophenols have also been formulated and used as salts in some applications.

Pentachlorophenol and its salt, sodium pentachlorophenate, are used primarily as wood preservatives on telephone poles, pilings and fence posts. In Europe, pentachlorophenol and its derivatives, sodium pentachlorophenate and pentachlorophenyl laurate are used to control sap stain in green lumber. It is also used in Europe on millwork to prevent the growth of mould and fungi, and as a preservative for waterproof materials (i.e., tarpaulins) that are used in outdoor applications. In the United States, it is used almost entirely for treatment of utility poles (Agency for Toxic Substances and Disease Registry, 1994).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1990–93 CAREX database for 15 countries of the European Union (Kauppinen *et al.*, 1998) and the 1981–83 National Occupational Exposure Survey (NOES) in the United States (NOES, 1997), approximately 45 000 workers in Europe and as many as 27 000 workers in the United States were potentially exposed to pentachlorophenol (see General Remarks). Recent figures give rough estimates of 500 pentachlorophenol-exposed workers in wood treatment facilities in the United States (Norman, 1998). No current data on numbers of workers exposed to other chlorophenols were available. Occupational exposures to chlorophenols have occurred in their production, in the production and use of some phenoxy acid herbicides, in sawmills and other wood-related industries, the textile industry and tanneries. Occupational exposures to penta-

chlorophenol may occur in its production and in its use as a wood preservative. These various occupational circumstances also involve exposure to polychlorinated dibenzodioxins (IARC, 1997).

1.3.2 *Environmental occurrence*

2,4-Dichlorophenol may be released to the environment in effluents from its manufacture and use as a chemical intermediate and from chlorination processes involving water treatment and wood-pulp bleaching. Releases can also occur from various incineration processes, from metabolism of various pesticides in soil or in the use of 2,4-D, in which it is an impurity. It has been detected at low levels in drinking-water, groundwater and ambient water samples (United States National Library of Medicine, 1997).

2,4,5-Trichlorophenol may be released to the environment through its production, use as a pesticide and pesticide intermediate, and use of pesticides in which it is an impurity (i.e. Silvex and 2,4,5-T). It has been detected at low levels in urban air, ambient water, drinking-water and wastewater samples (United States National Library of Medicine, 1997).

2,4,6-Trichlorophenol may enter the environment as emissions from combustion of fossil fuels and incineration of municipal wastes, as well as emissions from its manufacture and use as a pesticide, and in the use of 2,4-D, in which it is an impurity. Significant amounts may result from the chlorination of phenol-containing waters (United States National Library of Medicine, 1997).

In the past, 2,3,4,6-tetrachlorophenol entered the environment primarily in wastewater during its production and use as a wood preservative (United States National Library of Medicine, 1997).

Use of pentachlorophenol as a wood preservative may result in environmental release from treated wood and other materials. It has been detected at low levels in surface water, groundwater, drinking water, soil and urban air samples (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 0.5 mg/m^3 as the 8-h time-weighted threshold limit value, with a skin notation, for occupational exposures to pentachlorophenol in workplace air. Values ranging from 0.05 to 0.5 mg/m^3 have been used as standards or guidelines in other countries (International Labour Office, 1991). The ACGIH has not proposed any occupational exposure limit for 2,4-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol or 2,3,4,6-tetrachlorophenol. Finland and Sweden have an 8-h time-weighted average exposure limit of 0.5 mg/m^3, with a skin notation, for 2,3,4,6-tetrachlorophenol (United States National Library of Medicine, 1997).

The World Health Organization has established an international drinking-water guideline for 2,4,6-trichlorophenol of 200 µg/L and a provisional international drinking-water guideline for pentachlorophenol of 9 µg/L. No international guideline for 2,4-

dichlorophenol, 2,4,5-trichlorophenol or 2,3,4,6-tetrachlorophenol in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

2.1 Case reports

Gilbert *et al*. (1990) identified 182 workers in Hawaii who had been continuously employed for at least three months during 1960–81 in the treatment of wood using various chemicals including pentachlorophenol. A search at the local tumour registry identified two registered cancers in the cohort, both of them colorectal. However, the expected numbers of cases were not calculated.

Cheng *et al*. (1993) analysed mortality in 109 workers who had been employed for one year or longer since 1974 in the pentachlorophenol section of a chemical manufacturing plant in China. During follow-up to 1990, three deaths were recorded, of which one was from lung cancer.

2.2 Studies of occupational populations (see Table 1 for the most relevant studies)

Mortality was reported for a small cohort of 204 workers involved in the manufacture of 2,4,5-T (IARC, 1987b) between 1950 and 1971 (Ott *et al*., 1980) and followed up to 1976, among whom reported exposures included 2,4,5-trichlorophenol. There were five deaths (7.0 expected) among those with one or more years of exposure, including one from cancer (1.3 expected).

Zack and Gaffey (1983) reported the mortality status of 884 white men employed for at least one year between 1955 and 1977 by a chemical plant in Nitro, WV, USA, involved in the production of 2,4,5-trichlorophenol and 2,4,5-T. 4-Aminobiphenyl, a human bladder carcinogen (see IARC, 1982), was produced from 1941 to 1952 in this plant. There were nine cases of bladder cancer, with 0.91 expected; deaths from cancer other than of the bladder were not in excess. One case of liposarcoma was reported among workers assigned to 2,4,5-T operations. Zack and Suskind (1980) reported cancer outcomes of a cohort of 121 males involved in a 1949 accident at the same plant. Follow-up revealed nine cancer deaths between 1949 and 1978, with 9.0 expected. Three of these were lymphatic or haematopoietic in origin (0.9 expected [$p = 0.047$]), and one was a primary dermal fibrous histiocytoma (0.15 expected).

In a cohort study of workers in two Danish chemical plants (Lynge, 1985), potential exposure to 2,4,5-trichlorophenol occurred between 1951 and 1959, when small amounts were produced or purchased to make 2,4,5-T. No overall increase in cancer incidence rate was observed, but there were significantly increased risks of soft-tissue sarcoma and lung cancer in certain subcohorts. [The Working Group noted that 2,4-dichlorophenol is an intermediate in the production of 2,4-D, which was produced by the larger of the two plants.]

Table 1. Industry-based studies and population-based studies of cancer in chlorophenol-exposed groups

Reference	Exposure	Measure of relative risk	Soft-tissue sarcoma		Non-Hodgkin lymphoma	
			Exposed cases	RR (95% CI)	Exposed cases	RR (95% CI)
Kogevinas *et al*. (1997)	Phenoxy acids or chlorophenols	SMR	9	2.0 (0.9–3.8)	34	1.3 (0.9–1.8)
Ramlow *et al.* (1996)	Pentachlorophenol	SMR			3 versus < 2.5 expected	
Mikoczy *et al*. (1994)	Tannery workers	SIR	5	3.2 (1.0–7.4)	4	0.7 (0.2–1.8)
Hertzman *et al*. (1997)	Workers at sawmills using chlorophenols	SMR	6	1.4 (0.6–2.8)	36	1.1 (0.8–1.4)
		SIR	11	1.2 (0.7–1.9)	65	1.2 (0.96–1.5)
Smith *et al*. (1984)	Potential exposure to chlorophenols	OR		1.6 (0.5–5.2)		
Hardell *et al*. (1995)	Chlorophenols (high-grade)	OR	34	3.3 (1.8–6.1)		
Pearce *et al*. (1986)	Potential exposure to chlorophenols	OR			9	1.3 (0.6–2.7)[a]
Hardell *et al*. (1994)	Chlorophenols (low-grade)	OR			19	3.3 (1.6–6.8)
	Chlorophenols (high-grade)				16	9.4 (3.6–25)
Woods *et al*. (1987)	High-exposure chlorophenols	OR		0.93 (0.5–1.8)		0.92 (0.91–1.4)[b]

RR, relative risk; CI, confidence interval; SMR, standardized mortality ratio; OR, odds ratio; SIR, standardized incidence ratio

[a] 90% CIs

[b] Figures as reported. The 95% CI appears incompatible with the point estimate of risk.

Cook *et al*. (1986) examined mortality between 1940 and 1979 among 2189 men involved in the manufacture of 2,4,5-trichlorophenol and 2,4,5-T. There were 298 deaths observed (standardized mortality ratio (SMR), 0.91), including 61 from cancer (SMR, 0.96) and five from non-Hodgkin lymphoma (SMR, 2.4; 95% confidence interval (CI), 0.8–5.6).

Cook *et al*. (1980) observed three cancer deaths (1.6 expected) among 61 male employees involved in an accident at a trichlorophenol-producing plant in Michigan and followed up to the end of 1978. One death was reported to be from a fibrosarcoma.

In the Federal Republic of Germany (Thiess *et al*., 1982), 74 workers were involved in an accident in 1953 in a plant producing 2,4,5-trichlorophenol. Follow-up through 1980 revealed three deaths from stomach cancer, with relative risks of the order of 4–5 depending on the comparison group; there was no excess of cancers at other sites combined.

With the exception of the accident cohort in Germany (Thiess *et al*., 1982), studies have since been incorporated in a multi-centre study coordinated by the International Agency for Research on Cancer, which collated data on 21 863 workers exposed to phenoxy acid herbicides, chlorophenols and polychlorinated dibenzodioxins from 36 cohorts of chemical manufacturers and herbicide sprayers in 12 countries (Kogevinas *et al*., 1997). The design and findings of the study have been reviewed in detail in an earlier monograph (IARC, 1997). Methods of follow-up varied between countries, and included use of national and municipal death registries, examination of plant records, and contact with workers and their families and physicians. The loss to follow-up was 4.4%. Mortality during 1939–92 was compared with that expected from the relevant national rates by the person–years method. In subjects with any exposure to phenoxy acids or chlorophenols there were 4159 deaths from all causes (SMR, 0.97; 95% CI, 0.94–1.00) including 1127 from cancer (SMR, 1.06; 95% CI, 1.00–1.13). Significant increases in mortality were seen for cancer of the larynx (21 deaths; SMR, 1.6; 95% CI, 1.0–2.5), other respiratory organs(12 deaths; SMR, 2.3; 95% CI, 1.2–3.9) and endocrine organs (ICD-9 code 194; 5 deaths; SMR, 3.6; 95% CI, 1.2–8.4). In addition, non-significant excesses were observed for cancer of the lung (380 deaths; SMR, 1.1; 95% CI, 1.0–1.2), cancers of connective and other soft tissues (9 deaths; SMR, 2.0; 95% CI, 0.9–3.8) and non-Hodgkin lymphoma (34 deaths; SMR, 1.3; 95% CI, 0.9–1.8). No analysis was presented for exposure specifically to chlorophenols.

Associations with chlorophenols were, however, analysed in two case–control studies nested within 24 of the 36 cohorts of the IARC study. These compared 11 cases of soft-tissue sarcoma and 32 cases of non-Hodgkin lymphoma with 55 and 158 controls, respectively (Kogevinas *et al.*, 1995). Exposure to chlorophenols, phenoxy acid herbicides, dibenzodioxins and -furans and other agents was assessed by a team of industrial hygienists (Kauppinen *et al*., 1994). Odds ratios for non-Hodgkin lymphoma, not adjusted for exposure to other agents, were 1.3 (95% CI, 0.5–3.1) for any chlorophenol, 2.8 (0.5–17.0) for pentachlorophenol and 1.0 (0.3–3.1) for 2,4-dichlorophenol. No excess risk was found in relation to other chlorophenols, but the number of exposed cases was small. The odds

ratios for high cumulative exposure were 2.7 (0.9–8.0) for any chlorophenol and 4.2 (0.6–29.6) for pentachlorophenol. Only two cases of soft-tissue sarcoma were classified as exposed to chlorophenols (odds ratio, 1.3; 95% CI, 0.2–6.9) and neither was exposed to pentachlorophenol.

Ramlow *et al*. (1996) described a cohort of 770 male workers with potential exposure to pentachlorophenol who were employed by the Dow Chemical Company in the United States during 1937–80. The men were identified from employment records, and their cumulative exposure to pentachlorophenol and dibenzodioxins was classified on the basis of recorded job history and historical industrial hygiene measurements. The mortality of the cohort during 1940–89 was compared with that of the white male population of the United States by a modified life-table method. In addition, internal comparisons between different exposure categories were carried out by a Mantel–Haenszel method with baseline risks derived from 27 435 men employed by the same company during the same period but with no potential exposure to pentachlorophenol or dioxins. Mortality from all causes (229 deaths; SMR, 0.9; 95% CI, 0.8–1.1) and all cancers (50 deaths; SMR, 0.95; 95% CI, 0.7–1.3) was less than expected. Small excesses were observed for cancers of the stomach (4 deaths versus 2.4 expected; SMR, 1.7; 95% CI, 0.5–4.3), larynx (2 versus 0.7; SMR, 2.9; 95% CI, 0.4–10.3) and kidney (3 versus 1.3; SMR, 2.3; 95% CI, 0.5–6.7) and for non-Hodgkin lymphoma and myeloma combined (5 versus 2.5; SMR, 2.5; 95% CI, 0.7–4.7). Of the five observed deaths in the last category, three were from non-Hodgkin lymphoma and two from myeloma. With a lag period of 15 years, mortality from kidney cancer and from non-Hodgkin lymphoma and myeloma tended to increase with cumulative exposure to pentachlorophenol.

Mikoczy *et al*. (1994) studied 2026 workers at three Swedish leather tanneries, who had been employed for at least one year between 1900 and 1989. Chlorophenols had been used at these plants since about 1950 and were in use until 1980. Other potentially hazardous exposures included chromium compounds (IARC, 1990), vegetable tannins, arsenic sulfides (IARC, 1987c), mercury compounds (IARC, 1993), azo and benzidine dyes (IARC, 1987d), formaldehyde (IARC, 1995), solvents and aluminium compounds. Levels of exposure to chlorophenols were not reported, but blood samples from two tanners at one of the plants showed elevated concentrations of polychlorinated dibenzodioxins and dibenzofurans, which sometimes contaminate chlorophenols. Subjects were identified from company records, which were complete from as early as 1930 at one plant and from 1946 and 1966 at the other two, and were followed up through national death and tumour registries until death, emigration or their eightieth birthday. Five cohort members (0.2%) were lost to follow-up. Mortality during 1952–89 was compared with that in the two counties in which the plants were situated, and cancer incidence during 1958–89 with national rates, in each case by the person–years method. Mortality from all causes and from all cancers was close to expectation (SMR, 1.04 and 1.09, respectively). The overall incidence of cancer was somewhat elevated (233 cases observed versus 200 expected; standardized incidence ratio (SIR), 1.16; 95% CI, 1.02–1.32) with excesses of multiple myeloma (6 versus 2.8; SIR, 2.2; 95% CI, 0.8–4.7) and cancers of the lip

(5 versus 2.5; SIR, 2.0; 95% CI, 0.6–4.6), pancreas (9 versus 6.0; SIR, 1.5; 95% CI, 0.7–2.9), nose (2 versus 0.55; SIR, 3.8; 95% CI, 0.5–13.6), lung (20 versus 16.6; SIR, 1.2; 95% CI, 0.8–1.9), breast (20 versus 15.4; SIR, 1.2; 95% CI, 0.8–2.1), cervix (5 versus 3.0; SIR, 1.7; 95% CI, 0.5–3.9), prostate (32 versus 25.0; SIR, 1.3; 95% CI, 0.9–1.8) and soft tissues (5 versus 1.6; SIR, 3.2; 95% CI, 1.0–7.4). However, there were fewer cases of non-Hodgkin lymphoma than expected (4 versus 5.7; SIR, 0.7; 95% CI, 0.2–1.8). No analysis of cancer incidence was reported specifically for exposure to chlorophenols.

Hertzman and colleagues (1997) analysed mortality and cancer incidence among 26 487 men who had been employed at any of 14 sawmills in British Columbia, Canada, for one or more years between 1950 and 1985. Eleven of the mills had used tetra- and pentachlorophenol fungicides from the 1940s until 1989. Urine analyses in 172 employees from a pilot sawmill showed total levels of penta- and tetrachlorophenols ranging from 5 to 1252 μg/L, with a median of 108 μg/L in the summer and 52 μg/L in the fall (Hertzman *et al.*, 1988). Personal cumulative exposures to chlorophenol in the full cohort were classified on the basis of job history. Individual records were linked with the provincial death file and the cancer incidence file, the Canadian mortality database and several other record systems, and mortality during 1950–90 and cancer incidence during 1969–89 were compared with those of the province by the person–years method. Among 23 829 workers at mills using chlorophenols, there were 4539 deaths (SMR, 0.96; 95% CI, 0.94–0.99) including 1155 from cancer (SMR, 1.07; 95% CI, 1.02–1.12), 369 from lung cancer (SMR, 1.10; 95% CI, 1.01–1.20), six from soft-tissue sarcoma (SMR, 1.4; 95% CI, 0.6–2.8), 116 from male genital cancer (SMR, 1.2; 95% CI, 1.0–1.4), 38 from cancer of the kidney (SMR, 1.4; 95% CI, 1.0–1.8), 23 from lymphosarcoma (SMR, 1.5; 95% CI, 1.0–2.1) and 36 from all non-Hodgkin lymphoma (SMR, 1.1; 95% CI, 0.8–1.4). Incidence rates were elevated for all cancers except skin (1498 cases; SIR, 1.05; 95% CI, 1.01–1.10), cancer of the rectum (105 cases; SIR, 1.2; 95% CI, 1.0–1.4), cancer of the lung (344 cases; SIR, 1.11; 95% CI, 1.02–1.22), cancer of the mediastinum (5 cases; SIR, 3.1; 95% CI, 1.2–6.5) and chronic lymphocytic leukaemia (24 cases; SIR, 1.7; 95% CI, 1.2–2.4). There were 11 incident cases of soft-tissue sarcoma (SIR, 1.2; 95% CI, 0.7–1.9). The risk of incident non-Hodgkin lymphoma increased significantly with cumulative exposure to chlorophenols, but this was due in part to a lower than expected incidence in the low-exposure categories. The risks in the five exposure categories from lowest to highest were 0.68 (4 cases), 0.59 (9 cases), 1.04 (11 cases), 1.02 (15 cases) and 1.30 (26 cases).

2.3 Studies in the general population

2.3.1 *Soft-tissue sarcoma*

A New Zealand study of soft-tissue sarcoma (see IARC, 1986) found an odds ratio of 1.6 (90% CI, 0.5–5.2) for potential exposure to chlorophenols for five days or more, more than 10 years before diagnosis (Smith *et al.*, 1984). Work in pelt-treatment departments (where 2,4,6-trichlorophenol had been used) or in tanneries (where pentachlorophenol and

2,4,6-trichlorophenol were used) yielded an odds ratio of 7.2 (6 exposed cases; $p = 0.04$). When meat works and tanneries were contacted, it was found that two of the cases could not have been exposed to chlorophenols and exposure of a third was unlikely, while two could have been exposed to 2,4,6-trichlorophenol and one to pentachlorophenol.

In a case–control study in the north of Sweden, Hardell and Eriksson (1988) identified 55 men aged 25–80 years with histologically confirmed soft-tissue sarcomas that had been diagnosed during 1978–83 and reported to the local cancer registry. By the time of the study, 18 of these men were alive and 37 were dead. They were compared with three control groups: 220 men selected from the National Population Registry and matched to the cases for age and county of residence, 110 men similarly matched who had died during 1978–83 and 190 patients with other cancers who were selected from the Regional Cancer Registry and were of a similar age range to the cases. Exposure to various chemicals including chlorophenols was ascertained by a postal questionnaire sent to subjects or their next of kin, sometimes supplemented by a telephone interview. The overall response rate was 94.6%. No association was found with exposure to chlorophenols [numerical risk estimates were not reported], but the power to detect such a relationship was said to be low.

Eriksson *et al*. (1990) carried out a case–control study of soft-tissue sarcoma in central Sweden. Two hundred and thirty-seven histologically confirmed male cases, aged 25–80 years and diagnosed during 1978–86, were identified from the local cancer registry. The controls (one per case) were selected from population registries and individually matched for age, sex, county of residence and vital status. Exposure to suspected risk factors was ascertained by a questionnaire mailed to the subjects or their next of kin. If answers were incomplete, additional information was obtained by telephone interview. The response rates for cases and controls were 92% and 88%, respectively. With allowance for a latency of five years, high-grade exposure to chlorophenols (i.e., for at least one week continuously or at least one month in total) was reported for 15 cases and three controls (odds ratio, 5.3; 95% CI, 1.7–16.3). For pentachlorophenol specifically, the corresponding odds ratio was 3.9 (95% CI, 1.2–12.9) based on 11 exposed cases. No elevation of risk was found with shorter duration of exposure to chlorophenols.

Data from these two studies and from two earlier investigations (Hardell & Sandström, 1979; Eriksson *et al*., 1981) that have been summarized previously (IARC, 1986) were subsequently incorporated in a meta-analysis (Hardell *et al*., 1995). Risk was significantly elevated in subjects with high-grade exposure to chlorophenols (34 exposed cases; odds ratio, 3.3; 95% CI, 1.8–6.1), but in those with the most prolonged exposure (> 77 days), it was a little higher (odds ratio, 3.4; 95% CI, 1.7–7.8). Twenty-seven cases had high-grade exposure to pentachlorophenol (odds ratio, 2.8; 95% CI, 1.5–5.4). The associations were not specific to any single histological or anatomical subtype of sarcoma.

2.3.2 *Non-Hodgkin lymphoma*

A New Zealand case–control study of non-Hodgkin lymphoma involving 83 cases, 168 controls with other cancer and 228 general population controls, found an odds ratio of 1.3 (90% CI, 0.6–2.7) for potential exposure to chlorophenols when using other cancer

patients as controls, and an odds ratio of 0.9 (90% CI, 0.4–2.4) when using general population controls (Pearce *et al*., 1986). The odds ratio for fencing work, which involves exposure to chemicals such as chromated copper-arsenate as well as pentachlorophenol, was 2.0 (90% CI, 1.3–3.0). The odds ratio for slaughterhouse employment, which involved potential exposure to 2,4,6-trichlorophenol, was 1.8 (90% CI, 1.1–3.1); however, only four of the 19 cases who had worked in a slaughterhouse reported working in the pelt department, where 2,4,6-trichlorophenol was used.

In a re-analysis of data from an earlier case–control study (Hardell *et al*., 1981; see IARC, 1986), Hardell *et al*. (1994) compared 105 men aged 25–84 years who had been admitted to an oncology department in Sweden during 1974–78 and 335 controls from the same community who had been selected from the National Population Register and from a death registry. Information about exposure to chlorophenols and various other chemicals had been obtained through a postal questionnaire completed either by the subjects themselves or, if they had died, by their next of kin, and had been supplemented if necessary by telephone interview. Analysis by the Mantel–Haenszel method, with stratification by age and vital status, indicated associations with both low-grade (odds ratio, 3.3; 95% CI, 1.6–6.8) and high-grade (odds ratio, 9.4; 95% CI, 3.6–25) exposure to chlorophenols. These risk estimates were only slightly reduced in a multivariate analysis that allowed also for exposure to phenoxy acid herbicides, organic solvents, DDT and asbestos. The elevation of risk appeared to apply to all histological subtypes of non-Hodgkin lymphoma.

2.3.3 *Other cancers and multiple sites*

As described in an earlier monograph (IARC, 1986), a case–control study in Sweden found a significant association between nasal and nasopharyngeal cancer and exposure to chlorophenols, independent of exposure to wood dust (Hardell *et al*., 1982). The same group of researchers also reported positive associations with high-grade exposure to chlorophenols in case–control studies of colon cancer (odds ratio, 1.8; 95% CI, 0.6–5.3) and primary liver cancer (odds ratio, 2.2; 95% CI, 0.7–7.3) (Hardell, 1981; Hardell *et al*., 1984).

In a case–control study in the north of Sweden, Hallquist *et al*. (1993) compared 188 men and women aged 20–70 years who had thyroid cancer with age- and sex-matched controls (two per case) selected from a register of the local population. The cases were identified retrospectively from a cancer registry and excluded a proportion of patients (19%) who had died by the time of the study. Exposure to potential risk factors, including chlorophenols, was ascertained by postal questionnaire with a supplementary telephone interview if answers were incomplete. The response rates for the cases and controls were 95% and 90%, respectively. Of the 171 cases analysed, 107 had papillary tumours. Four cases and three controls reported exposure to chlorophenols (odds ratio, 2.8; 95% CI, 0.5–18). [The Working Group noted that the method of statistical analysis was not the most appropriate for individually matched data, but this is unlikely to have produced serious bias.]

Lampi *et al*. (1992) compared cancer registration rates during 1953–86 in each of three adjacent municipalities in southern Finland with those for the region in which these communities were situated. High concentrations of total chlorophenols had been found in tap-water (70–140 μg/L) in one of the municipalities, Kärkölä, and also in groundwater (up to 190 mg/L). These were thought to have originated from a sawmill where a fungicide containing tetrachlorophenol had been used to treat wood. In addition, some of the local population were exposed to chlorophenols occupationally and through consumption of contaminated fish. Overall cancer incidence in Kärkölä was close to that expected, but there was an excess of soft-tissue cancer (incidence rate ratio, 1.6; 95% CI, 0.7–3.5) that was not apparent in the other two municipalities. Rates of nodal non-Hodgkin lymphoma were elevated both in Kärkölä (incidence rate ratio, 2.1; 95% CI, 1.3–3.4) and in the two neighbouring communities.

To explore further the possible role of chlorophenols, 173 residents of the three municipalities in southern Finland who developed lymphoma, leukaemia or cancers of the colon, urinary tract or soft tissues during 1967–86 were compared with 688 controls randomly selected from the same population and individually matched for age (to within two years) and sex (Lampi *et al*., 1992). Information about occupational and residential histories, water supplies and fish consumption was obtained by postal questionnaire, from either the subjects themselves or their next of kin (overall response rate, 88%). Risk of both soft-tissue cancer and non-Hodgkin lymphoma was increased in subjects with reported or inferred probable exposure to polluted drinking-water, the association with non-Hodgkin lymphoma being significant (risk ratio, 3.4; 95% CI, 1.0–12). In addition, three patients with non-Hodgkin lymphoma had consumed contaminated fish (risk ratio, infinity, lower 95% confidence limit, 1.1). Leukaemia was associated with most potential sources of exposure to chlorophenols, although not significantly. Findings for the other tumours were unremarkable.

In a population-based case–control study among men aged 20–79 years in 13 counties of Washington State, United States (Woods *et al*., 1987), the case group comprised 128 patients with soft-tissue sarcoma and 576 with non-Hodgkin lymphoma, who were diagnosed during 1981–84 (79% response rate). The controls were 694 men randomly selected and group-matched to the cases for age and vital status (76% response rate). Living controls were obtained by random-digit dialling and from social security records, while deceased controls were identified from the death certificates of members of the study population who died during the study period from causes other than suicide or homicide. Information about occupational history and exposure to specific chemicals was obtained by interview of the subjects themselves or a proxy. Where reports of exposure to chemicals could be checked by questioning a supervisor or co-worker, agreement was found to be good. Analysis was by the Mantel–Haenszel method and by logistic regression with adjustment for age in 5- or 10-year groups. Neither disease was associated with reported exposure to chlorophenols. For the highest-exposure category, the odds ratios were 0.9 (95% CI, 0.5–1.8) for soft-tissue sarcoma and 0.92 (95% CI, 0.9–1.4) [the Working Group noted that the latter confidence interval appeared incompatible with the

risk estimate given] for non-Hodgkin lymphoma. Risks were elevated for work in some jobs entailing likely exposure to chlorophenols, such as manufacturers of chlorophenols, but not for all potentially exposed jobs combined. Nor did risk increase with duration of exposure or with allowance for latency.

As part of a nested case–control study that is described more fully in the monograph on phenol (see this volume), Kauppinen *et al*. (1993) assessed exposure to chlorophenols in 136 men with respiratory cancer and 408 matched controls from a cohort of Finnish woodworkers. Nine cases were classified as exposed (odds ratio, 0.9; 90% CI, 0.4–1.8), and, after adjustment for smoking habits (when known), the risk estimate was little changed.

In another nested case–control study based on the same cohort, Partanen *et al*. (1993) compared exposure to chlorophenols and other suspected risk factors in four cases of Hodgkin's disease, eight cases of non-Hodgkin lymphoma, 12 cases of leukaemia and 152 matched referents. Exposures were reconstructed through plant- and period-specific job–exposure matrices. Two of the cases were classed as exposed to chlorophenols (odds ratio, 0.9; 95% CI, 0.2–4.5).

3. Studies of Cancer in Experimental Animals

2,4,6-Trichlorophenol was tested for carcinogenicity in one experiment in two strains of mice by oral administration, and 2,4,5- and 2,4,6-trichlorophenols were tested in one experiment by subcutaneous injection in two strains of mice. 2,4,5-Trichlorophenol was also tested in one experiment for promoting activity in female mice. All three experiments were considered to be inadequate (IARC, 1979).

Two different pentachlorophenol formulations were tested for carcinogenicity by oral administration in two separate experiments in mice. A dose-related increase in the incidence of hepatocellular adenomas and carcinomas was observed in males exposed to either formulation and of hepatocellular adenomas in females exposed to one of the formulations. A dose-related increase in the incidence of adrenal phaeochromocytomas was observed in male mice exposed to either formulation, and an increase was also seen in females exposed to one of the formulations at the highest dose. A dose-related increase in the incidence of malignant vascular tumours of the liver and spleen was seen in female mice exposed to either formulation (IARC, 1991).

3.1 Oral administration

3.1.1 *2,4-Dichlorophenol*

Mouse: Groups of 50 male and 50 female B6C3F_1 mice, eight weeks of age, were administered 2,4-dichlorophenol (purity, > 99%) in the diet at concentrations of 0, 5000 and 10 000 mg/kg of diet (ppm) for two years. Mean body weights of high-dose groups of both sexes were reduced. Treatment did not affect survival rates. No increase in the incidence of tumours was found (United States National Toxicology Program, 1989).

Rat: Groups of 23–28 male and 22–29 female Sprague-Dawley rats were administered 2,4-dichlorophenol (purity, 99%) at concentrations of 0, 3, 30 and 300 mg/L (ppm) in the drinking-water starting prenatally for up to 24 months. Three-week-old weanling females were exposed to the same concentrations of 2,4-dichlorophenol through breeding to untreated males at 90 days of age and during lactation. Litter size was reduced at the highest dose. No increase in the incidence of total tumours was found [individual tumour types unspecified] (Exon & Koller, 1985).

Groups of 50 male and 50 female Fischer 344 rats, seven weeks of age, were administered 2,4-dichlorophenol (purity, > 99%) at concentrations of 0, 5000 and 10 000 ppm in the diet (males) and 0, 2500 and 5000 ppm (females) for 104 weeks. Mean body weights of the high-dose groups of both sexes were reduced. No increase in the incidence of tumours was found (United States National Toxicology Program, 1989).

3.1.2 *2,4,6-Trichlorophenol*

Mouse: Groups of 50 male B6C3F_1 mice, six weeks of age, were administered 2,4,6-trichlorophenol (96–97% pure with 17 minor contaminants; chlorinated dibenzo-*para*-dioxins were not determined) in the diet at concentrations of 5000 or 10 000 ppm for 105 weeks. Groups of 50 female B6C3F_1 mice, six weeks of age, received diets containing 10 000 or 20 000 ppm 2,4,6-trichlorophenol for 38 weeks, at which time the concentrations were reduced to 2500 and 5000 ppm because of excessive growth retardation, and the study was continued for a further 67 weeks. Groups of 20 untreated mice of each sex served as controls. Survival of males was 16/20 controls, 44/50 low-dose and 45/50 high-dose mice. Survival of females was 17/20 controls, 44/50 low-dose and 40/50 high-dose mice. Body weights of treated groups were lower than controls during the study. The incidences of hepatocellular adenomas (3/20 controls, 22/49 low-dose and 32/47 high-dose males and 1/20 control, 12/50 low-dose and 17/48 high-dose females) were increased in both sexes. The incidences of hepatocellular carcinomas in males were 1/20 control, 10/49 low-dose and 7/47 high-dose and those in females were 0/20 control, 0/50 low-dose and 7/48 high-dose. In males, combined incidences of hepatocellular adenomas and carcinomas were significantly increased (4/20 controls, 32/49 low-dose, 39/47 high-dose; $p < 0.001$ for each dose group). The combined incidence of hepatocellular adenomas and carcinomas was significantly elevated in the high-dose group of females (1/20 control, 12/50 low-dose, 24/48 high-dose; $p < 0.001$) (United States National Cancer Institute, 1979). [The Working Group noted the impurity of the test substance.]

Groups of 16 male and 16 female A/J mice, six to eight weeks of age, were given 2,4,6-trichlorophenol (reagent grade) by gavage in tricaprylin three times per week for eight weeks at a total dose of 1200 mg/kg bw. No increase in the incidence of lung tumours was found compared with vehicle-treated controls (Stoner *et al.*, 1986).

Rat: Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were administered 2,4,6-trichlorophenol (96–97% pure with 17 minor contaminants; chlorinated dibenzo-*para*-dioxins were not determined) in the diet at concentrations of 5000 or

10 000 ppm for 106 or 107 weeks. Groups of 20 rats of each sex served as controls. Survival of males was 18/20 controls, 35/50 low-dose and 34/50 high-dose and of females was 14/20, 39/50 and 39/50. Body weights of treated rats were lower than those of controls throughout the study. The incidence of monocytic leukaemia was increased in both groups of treated males (3/20 controls, 23/50 low-dose, $p = 0.013$; 29/50 high-dose; $p = 0.002$) (United States National Cancer Institute, 1979). [The Working Group noted the impurity of the test substance.]

3.1.3 *Pentachlorophenol*

Rat: In an experiment not designed as a carcinogenicity study, groups of male and female MRC-Wistar rats [age unspecified] were administered pentachlorophenol (86% pure, containing 2,3,7,8-tetrachlorodibenzo-*para*-dioxin and 2,3,7,8-tetrachlorodibenzofuran) in the diet at 0 (12 male, 13 female) or 500 mg/kg (5 male, 9 female) for 94 weeks. In the group given pentachlorophenol, which had the longest survival, 6/9 female rats ($p < 0.01$) had liver adenomas compared with 0/13 controls (Mirvish *et al*., 1991). [The Working Group noted the low purity of the material tested and the inadequate reporting.]

Groups of 50 male and 50 female Fischer 344/N rats, six weeks of age, were administered diets containing pentachlorophenol (approximately 99% pure) at concentrations of 200, 400 and 600 ppm for 105 weeks. Two further groups of 60 males and 60 females received diets containing 0 (control) or 1000 ppm pentachlorophenol for 12 months followed by control diet. Ten male and 10 female controls and 10 males and 10 females receiving 1000 ppm were killed and evaluated histopathologically at seven months. All groups were evaluated histologically at 106 weeks. Weight gains of groups receiving 400 and 600 ppm were less than those of controls and weight gains of 1000 ppm groups were less than those of controls during treatment but recovered to control levels while on control diet. Survival was 12/50 controls, 16/50 at 200 ppm, 21/50 at 400 ppm, 31/50 at 600 ppm and 27/50 at 1000 ppm in males and 28/50, 33/50, 34/50, 28/50 and 28/50 in females. No significant increase in tumour incidence was observed in rats receiving pentachlorophenol in the diet for two years. In the group receiving 1000 ppm for 12 months, mesotheliomas of the tunica vaginalis occurred in 9/50 females versus 1/50 in controls ($p = 0.014$) (United States National Toxicology Program, 1997).

3.2 Intraperitoneal injection

Mouse: Groups of 16 male and 16 female A/J mice, six to eight weeks of age, were given 2,4,6-trichlorophenol (reagent grade) by intraperitoneal injection three times per week for eight weeks for total doses of 240, 600 or 1200 mg/kg bw. No increase in the incidence of lung tumours was found (Stoner *et al*., 1986).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

The absorption of 2,4-dichloro-, 2,4,5-trichloro-, 2,4,6-trichloro-, 2,3,4,5- and 2,3,4,6-tetrachlorophenol is relatively rapid when they are given orally, dermally or by inhalation. Chlorophenols are almost exclusively metabolized to conjugates which are mainly excreted in the urine. Half-lives are from hours to days, the compounds with higher chlorine content having longer half-lives (IARC, 1986).

Although there are some discrepancies between different studies, the kinetics of pentachlorophenol can be summarized as follows. The half-times for oral absorption, plasma elimination and urinary excretion are of the order of 1.3 h, 10–20 days and 18–20 days, respectively, regardless of exposure level (although also shorter half-lifes have been calculated). The highest concentrations are found in liver, kidney and brain, but wide variations between different organs are found. Pentachlorophenol is metabolized *in vitro* by human liver microsomes to tetrachlorohydroquinone, which has also been found in urine of exposed workers, and to a lesser extent, to pentachlorophenol glucuronide. Blood and urine levels in occupationally exposed people and in people with no known exposure have been extensively measured (IARC, 1991). Pentachlorophenol is a strong inducer of cytochrome P450 enzymes, especially CYP3A, in cultured human hepatoma cells (Dubois *et al*., 1996).

4.1.2 *Experimental systems*

The highest concentrations of studied chlorophenols (2,4-, 2,4,6- and 2,3,4,6-) have been found in kidney, liver and spleen and the lowest concentrations in muscle and brain, either after parenteral administration of these chlorophenols themselves or as metabolites of other organochlorine compounds. Over 80% of the dose is excreted in urine and 5–20% in faeces. Metabolism varies somewhat depending on chlorine content; the low-chlorine substances tend to be excreted as glucuronide and sulfate conjugates; with higher chlorine substitution, excretion of the unchanged substance tends to increase. Formation of chlorinated 1,4-quinones is a minor pathway except for 2,3,5,6-tetrachlorophenol (WHO, 1989).

Absorption of pentachlorophenol is relatively rapid in all species studied, but elimination differs between species and also between sexes. Metabolism occurs through glucuronic acid conjugation and hydrolytic dechlorination to tetrachlorohydroquinone, which is further conjugated. In contrast to rodents, rhesus monkeys eliminate pentachlorophenol in urine unchanged (IARC, 1991).

Five cysteinyl adducts of haemoglobin and albumin have been identified in the blood of rats following administration of pentachlorophenol up to 40 mg/kg. Adducts were formed by reactions with the pentachlorophenol metabolites tetrachloro-1,4-benzoquinone and its semiquinones (Waidyanatha *et al*., 1996).

Detailed toxicokinetic studies have been performed in both rats (Yuan *et al*., 1994) and mice (Reigner *et al*., 1992), comparing intravenous and gavage (and in rat feed) administration of pentachlorophenol. In mice, after either intravenous or oral administration, the elimination half-life was about 5–6 h. Only 8% of the dose (15 mg/kg bw) was excreted unchanged in urine, while 20% was excreted as tetrachlorohydroquinone and its conjugates. Sulfate conjugates represented 90% of the total conjugates of pentachlorophenol and tetrachlorohydroquinone.

4.2 Toxic effects

4.2.1 *Humans*

One case report describes the accidental death of a worker following acute dermal exposure to 'pure' dichlorophenol (Kintz *et al*., 1992). [The purity of the solution was not reported nor were the levels of dioxin impurities reported.] The victim (an adult male) had a seizure within 20 min of the accident and died soon thereafter. Dichlorophenol levels in the blood, urine, bile and stomach contents were 24.3, 5.3, 18.7 and 1.2 mg/L, respectively.

Several cases of acute accidental, suicidal and occupational poisoning due to pentachlorophenol have been reported and reviewed, and the minimal lethal dose of pentachlorophenol in man has been estimated to be 29 mg/kg bw (WHO, 1987). Symptoms of acute poisoning include central nervous system disorders, dyspnoea and hyperpyrexia; the cause of death is cardiac arrest, and poisoning victims usually show marked rigor mortis. Examination *post mortem* shows non-specific organ damage. One case of fatal poisoning was associated with high pentachlorophenol concentrations in bile and kidney (Wood *et al*., 1983).

Occupational exposures to technical-grade pentachlorophenol have resulted in various disorders of the skin and mucous membranes (WHO, 1987). The incidence of chloracne was highest in people who had confirmed direct skin contact (O'Malley *et al*., 1990). Several health and biomonitoring surveys of workers with plasma pentachlorophenol concentrations ranging from nanograms to milligrams per litre showed some minor and often transitory changes in various biochemical, haematological and electrophysiological parameters, but no clinical effect was seen (Klemmer *et al*., 1980; Triebig *et al*., 1981; Zober *et al*., 1981). In addition, no adverse health effects or increased mortality were observed in 88 men employed in wood treatment. These men had worked for 0.33 to 26.3 years and had urinary pentachlorophenol concentrations of 174 ± 342 ppb (μg/kg; standard deviation) versus 35 ± 53 ppb for controls. Although workers were exposed to other wood-treatment chemicals (chromated copper-arsenate and tributyl tin oxide), no difference from controls was observed in urinary concentrations of these chemicals (Gilbert *et al*., 1990).

A study by McConnachie and Zahalsky (1991) reported on 38 individuals from 10 families who were exposed to pentachlorophenol by living in manufacturer-treated log homes. The exposure period lasted from 1.0 to 13.0 years and the serum pentachlorophenol levels of the subjects ranged from 0.01 to 3.4 ppm (mg/L). Altered immune function was

observed, including activated T cells, autoimmunity, functional immunosuppression and B cell dysregulation 0.0–9.0 years after pentachlorophenol exposure. [Control levels of pentachlorophenol were not reported. The controls were not screened for hypertension, smoking or use of alcohol or non-prescription drugs.]

Anecdotal exposure to pentachlorophenol has been associated with aplastic anaemia and/or red-cell aplasia (Roberts, 1983). Thirteen cases of industrial, home and accidental pentachlorophenol exposure in 11 men and two women having aplastic anaemia, pure red cell aplasia and associated disorders were reported. Exposure levels were not known except for one patient, who had concentrations in the serum of 250 ng/mL and in bone marrow of 330 ng/mL (Roberts, 1990).

4.2.2 *Experimental systems*

Repeated-dosing (14-day), subchronic (13-week) and chronic (two-year) toxicity studies of 2,4-dichlorophenol (> 99% pure) were conducted by the United States National Toxicology Program (1989). Male and female Fischer 344/N rats and B6C3F_1 mice were exposed to dichlorophenol in the feed. The repeated dosing study was conducted using five animals per group and dietary levels of 0, 2500, 5000, 10 000, 20 000 or 40 000 mg/kg [ppm] in the feed. In the high-dose group, one male mouse died before the end of the study. In rats and mice, feed consumption was reduced in the 20 000 and 40 000 mg/kg groups. Body weights were reduced at 20 000 mg/kg and higher in rats and at 40 000 mg/kg in mice. Gross pathology at necropsy revealed no treatment-related lesions.

The subchronic study was conducted using the same dietary levels as the repeated-dosing study but groups comprised 10 animals of each sex. All mice in the high-dose group died during the first three weeks. Body weights were reduced in mice at 20 000 mg/kg and in rats at 20 000 and 40 000 mg/kg. Feed consumption was decreased at 20 000 and 40 000 mg/kg in rats and at 10 000 mg/kg and higher in mice. Bone-marrow atrophy in rats and syncytial alteration of hepatocytes in mice were observed at 10 000 mg/kg and higher.

A chronic study was conducted by feeding diets containing 0, 5000 or 10 000 mg/kg to male and female mice and male rats or diets containing 0, 2500 or 5000 mg/kg to female rats (50 animals per sex per group). Body weights relative to controls were lower in the 10 000-mg/kg groups and at both dose levels for female rats. Survival was comparable in all groups. An increased incidence of syncytial alteration of hepatocytes was observed in treated male mice at 5000 and 10 000 mg/kg. No other treatment-related histological change was found.

Female Sprague-Dawley rats (12–14 animals/group) received the following chlorophenols in drinking-water: 2-chlorophenol (98% pure; impurities not reported) at 0, 5, 50 or 500 mg/L, 2,4-dichlorophenol (99% pure) or 2,4,6-trichlorophenol (98% pure) at 0, 3, 30 or 300 mg/L (Exon & Koller, 1985). The chemical was given to rats from three weeks of age throughout breeding (at 90 days of age with untreated males), gestation and lactation. To determine the effect of pre- and postnatal exposure to these chemicals, the offspring were weaned at three weeks of age and continued on treatment for 12–15 weeks (n = 8 per group, selected randomly from each dose group). Red blood-

cell counts, packed-cell volume and haemoglobin were increased in all groups of parental rats treated at the highest doses of 2-chlorophenol and 2,4-dichlorophenol. Other haematological parameters (white blood-cell count, mean corpuscular volume and packed-cell volume) were not affected at any dose level of the three chemicals. Immune response was affected in rats treated with 2,4-dichlorophenol. Cell-mediated immunity (measured as delayed-type hypersensitivity) was decreased at 30 and 300 mg/L, while humoral immunity was enhanced (increased serum antibody production) at 300 mg/L. Macrophage function was not affected. Liver and spleen weights were also increased at 300 mg/L 2,4-dichlorophenol. For 2,4,6-trichlorophenol, liver weight was increased at 30 and 300 mg/L and spleen weight at 300 mg/L.

Data on the acute toxicity of pentachlorophenol given to experimental animals by various routes have been summarized (WHO, 1987).

The oral LD_{50} was 36–177 mg/kg bw in mice (Borzelleca *et al.*, 1985) and 27–175 mg/kg bw in rats (Gaines, 1969). Cutaneous minimal lethal doses ranged from 39 to 170 mg/kg bw in rabbits (Kehoe *et al.*, 1939; Deichmann *et al.*, 1942) to 300 mg/kg bw in rats (Gaines, 1969). The acute toxicities of some known and possible metabolites of pentachlorophenol have also been reported (Borzelleca *et al.*, 1985; Renner *et al.*, 1986).

Symptoms of acute toxicity are similar to those in humans, including hyperpyrexia and neurological and respiratory dysfunction (WHO, 1987). Furthermore, palmitoylpentachlorophenol, which has been isolated from human fat (Ansari *et al.*, 1985), causes selective pancreatic toxicity in rats after single oral doses of 100 mg/kg bw (Ansari *et al.*, 1987).

A number of toxic effects described in acute and short-term toxicity studies have been attributed to impurities present in technical-grade pentachlorophenol preparations. The toxicity of impurities became clear when comparative studies with pure and technical-grade pentachlorophenol products were reported (Johnson *et al.*, 1973; Goldstein *et al.*, 1977; Kimbrough & Linder, 1978). Rats receiving 500 mg/kg technical-grade pentachlorophenol in the diet for eight months had slow growth rates, liver enlargement, porphyria and increased activities of some liver microsomal enzymes (Goldstein *et al.*, 1977); rats fed purified pentachlorophenol at the same dose and for the same period of time showed only a reduction in growth rate and increased liver glucuronyl transferase activity. Analogous results were reported in a similar study (Kimbrough & Linder, 1978). Technical-grade pentachlorophenol, but not the pure compound, caused a porphyria similar to that due to hexachlorobenzene when given orally to rats for several months at increasing doses (Wainstok de Calmanovici & San Martin de Viale, 1980).

Several toxic effects of pentachlorophenol have been explained by the uncoupling effect of pentachlorophenol on oxidative phosphorylation (Ahlborg & Thunberg, 1980). Studies of structure–activity relationships among a series of chlorinated phenols showed that the effect increases with increasing chlorination of the phenol ring (Farquharson *et al.*, 1958). Pentachlorophenol and other chlorophenols inhibited some liver microsomal enzymes (Arrhenius *et al.*, 1977a,b), and pentachlorophenol strongly inhibited sulfotransferase activity in rat and mouse liver cytosol (Boberg *et al.*, 1983). Other

in-vitro assays have shown that the hydrophobicity of pentachlorophenol (log octanol/water partition coefficient = 3.32) correlates with the ability of the compound to bind to plasma proteins in trout (99.39%) and rat (99.52%) (Schmieder & Henry, 1988). However, the toxicity of chlorophenols in V79 Chinese hamster cells was correlated not only with hydrophobicity but also with electron-withdrawing properties of ring substituents (Jansson & Jansson, 1993).

Reduced humoral immunity was observed in mice exposed to technical-grade pentachlorophenol, as well as impairment of T-cell cytolytic activity *in vitro* (Kerkvliet *et al*., 1982a,b). In rats exposed to technical-grade pentachlorophenol, decreased cell-mediated and humoral immunity was demonstrated, while phagocytosis by macrophages and numbers of induced peritoneal macrophages were increased (Exon & Koller, 1983). Polychlorinated dibenzodioxin and -furan contaminants are thought to be the chemical species responsible for the immunotoxicity of technical-grade pentachlorophenol (Kerkvliet *et al*., 1985).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

Two studies on birth outcomes of wives of employees potentially exposed to 2,4,5-trichlorophenol and/or pentachlorophenol did not show any significant association with regard to reproductive events (IARC, 1986).

The effect of pentachlorophenol exposure on reproductive outcome of 398 day-care teachers was evaluated. Exposed teachers (n = 221) came from day-care centres containing chemical-treated wood. A facility was deemed to be contaminated if the concentration of pentachlorophenol in the wood was greater than 100 mg/kg. A positive correlation existed between pentachlorophenol and polychlorinated dibenzodioxin/furan concentrations but not between these chemicals and γ-hexachlorocyclohexane. The median air concentrations of pentachlorophenol, polychlorinated dibenzodioxins/furans and γ-hexachlorocyclohexane in the exposed facilities were 0.25 μg/m^3, 0.5 pg/m^3 and 0.2 μg/m^3, respectively. Women exposed at any time during pregnancy were identified and after correction for lifestyle, 49 exposed and 507 unexposed pregnancies were analysed. Significantly reduced birth weight and length was observed in the offspring from exposed pregnancies. The women were mainly exposed to pentachlorophenol; however, the possible impact of polychlorinated dibenzodioxins/furans and γ-hexachlorocyclohexane on the effects reported is not clear (Karmaus & Wolf, 1995).

4.3.2 *Experimental systems*

Pregnant Fischer 344 rats (34 rats per group) were administered 2,4-dichlorophenol (99.2% pure; no dioxins detected) by gavage at 0, 200, 375 and 750 mg/kg per day on gestation days 6 to 15. Decreased maternal body weight gain and urogenital staining of the fur were observed at all dose levels. Maternal death, alopecia, respiratory rales and porphyrin accumulation in the area of the eyes, nares and mouth were observed at 750 mg/kg per day. Early embryonic death and decreased fetal weight were found at

750 mg/kg per day but were not significant. Delayed ossification of sternebrae and vertebral arches was observed at 750 mg/kg per day. The toxicity of 2,4-dichlorophenol to the embryo and fetus at 750 mg/kg per day may have been secondary to maternal toxicity (Rodwell *et al*., 1989).

Administration of 2,4,6-trichlorophenol (purified by recrystallization; 99% pure; the level of dioxin impurities was not reported) in corn oil by gavage at 0, 100, 500 and 1000 mg/kg per day (five days per week for 11 weeks) to adult male Long-Evans rats did not affect weight gain, organ weights, plasma testosterone or caudal sperm counts. When these males were mated with untreated females, fertility and reproductive performance (including litter size and pup weight) of the males in the trichlorophenol-treated groups were comparable to those of the control group. Female rats were also treated with the same daily dose levels of 2,4,6-trichlorophenol on five days per week for two weeks before mating with control males, then daily throughout pregnancy. At 1000 mg/kg per day, maternal toxicity including alopecia, decreased weight gain before and during pregnancy, lethargy, irregular breathing and, in a few instances, death were observed. 2,4,6-Trichlorophenol did not affect litter size or survival of pups to postnatal day 4. Pup weight was decreased at birth with the doses of 500 and 1000 mg/kg per day. This effect was transient and was not significant when corrected for litter size. Male and female reproductive functions were not affected at any dose level (Blackburn *et al*., 1986).

The developmental toxicity of 2,3,4,6-tetrachlorophenol was evaluated using purified and commercial grades of the compound. The commercial grade contained 73% 2,3,4,6-tetrachlorophenol, 27% pentachlorophenol and ppm levels of various dibenzo-*para*-dioxins and dibenzofurans, whereas the purified tetrachlorophenol was 99.6% pure, with only pentachlorophenol detected as an impurity (0.1%). Pregnant Sprague-Dawley rats were dosed by gavage at 0, 10 and 30 mg/kg per day on days 6–15 of gestation. In a preliminary study, 30 mg/kg per day was established as the maximum tolerated dose for the pregnant dam. These doses had no effect on maternal body weight, number of resorptions, fetal body weight, sex ratio or fetal crown–rump length. Data on litter size was not reported in this study. No maternal toxicity or teratogenicity was observed at any dose level. Delayed ossification of the skull bones occurred at 30 mg/kg/day with both grades of chemical, an effect indicative of fetotoxicity (Schwetz *et al*., 1974a).

Female Sprague-Dawley rats (12–14 animals/group) received the following chemicals in drinking-water: 2-chlorophenol (98% pure; impurities not reported) at 0, 5, 50 or 500 mg/L, 2,4-dichlorophenol (99% pure) or 2,4,6-trichlorophenol (98% pure) at 0, 3, 30 or 300 mg/L. The chemicals were given to rats from three weeks of age throughout breeding (at 90 days of age with untreated males), gestation and parturition. Conception, litter size (live and stillborn pups), birth weight, survival to weaning and weaning weight were recorded. For all three chemicals, litter size was decreased at the highest dose level. For 2-chlorophenol, the number of stillborn pups was increased at 500 mg/L. Whether these effects were secondary to maternal toxicity rather than fetotoxicity is not clear, since maternal parameters (body weight during pregnancy, feed consumption, clinical signs of toxicity) were not reported (Exon & Koller, 1985).

In contrast to these two drinking-water studies, gavage studies, conducted using much higher dose levels, saw no effect of 2,4-dichlorophenol and 2,4,6-trichlorophenol on litter size (Blackburn *et al*., 1986; Rodwell *et al*., 1989). [These conflicting results may be due to the different modes of administration (gavage versus drinking-water) and vehicles (corn oil versus water).]

Purified and commercial grades of pentachlorophenol were administered orally to Sprague-Dawley rats at doses ranging from 3 to 70 mg/kg bw per day (preliminary study) and 5 to 50 mg/kg bw per day (teratology study) at various intervals during days 6–15 of pregnancy. Pentachlorophenol was determined to be embryotoxic and fetotoxic but not teratogenic. The most sensitive period was during early organogenesis. The maximal tolerated dose was determined to be 50 mg/kg per day. The no-observed-effect-level (NOEL) for maternal toxicity was 15 mg/kg per day. The NOEL for fetal effects was 5 mg/kg bw per day for commercial pentachlorophenol. Doses higher than 5 mg/kg bw per day (i.e., ≥ 15 mg/kg per day) induced dose-related maternal and fetal toxicity (e.g., increases in resorptions, subcutaneous oedema, dilated ureters and anomalies of the skull, ribs, vertebrae and sternebrae). Purified pentachlorophenol had slightly greater maternal and fetal toxicity, with a significant increase in delayed ossification of the skull bones but no other effect on embryonal or fetal development (Schwetz *et al.*, 1974b). Ingestion of 3 mg/kg bw per day of a commercially available purified grade of pentachlorophenol had no effect on reproduction, neonatal growth, survival or development (Schwetz *et al.*, 1978).

Charles River CD rats were given a single oral dose of radiolabelled pentachlorophenol (purity, > 99%) equal to 75% of the LD_{50} (60 mg/kg bw) on day 15 of gestation, after having been given the same dose of unlabelled compound (purity not reported) on days 8–13 of gestation. Maternal serum pentachlorophenol levels peaked at approximately 1% of administered dose 8 h after dosing. Placental pentachlorophenol levels peaked at approximately 0.3% of administered dose/g tissue 12 h after dosing (most of this was due to the blood content). Fetal tissue pentachlorophenol levels remained constant at approximately 0.05%, demonstrating negligible transfer of pentachlorophenol to the fetus. Pentachlorophenol administration did not alter the number of resorptions, nor did it significantly affect malformations. Malformations in 3/51 fetuses (one each of exencephaly, macrophthalmia, and taillessness) were noted in the treated group compared with 0/44 in controls (no skeletal malformations were observed). Given the lack of significant pentachlorophenol placental transfer and malformations, the authors concluded that any effect observed in the fetuses was likely to have been indirectly related to the toxicity induced in the maternal rats (Larsen *et al.*, 1975).

In two later studies (Exon & Koller, 1982; Welsh *et al.*, 1987), pentachlorophenol was administered to Sprague-Dawley rats throughout mating and pregnancy. The results confirmed the findings of embryo- and fetotoxicity and lethality, in the absence of maternal toxicity. Adverse effects on the development of the rat conceptus occurred only at maternally toxic dosages.

Pentachlorophenol was reported not to be embryolethal or teratogenic in CD rats given 75 mg/kg bw per day on days 7–18 of gestation (Courtney *et al*., 1976). Sea urchin

eggs exposed to pentachlorophenol (0.2 mg/L medium or above) had delayed development and were malformed (Ozretic & Krajnovic-Ozretic, 1985).

4.4 Genetic and related effects

4.4.1 *Humans*

Four studies have been published in which cytogenetic effects on peripheral lymphocytes were investigated in workers exposed occupationally to chlorophenols.

No significant difference was found between a control group and workers who had been exposed 10 years previously to 2,4,5-trichlorophenol, either as regards chromosomal damage or sister chromatid exchanges (Blank *et al.*, 1983).

With regard to workers exposed to pentachlorophenol, two studies did not show increased incidence of sister chromatid exchanges (Willye *et al*., 1975) or sister chromatid exchanges and chromosomal aberrations (Ziemsen *et al*., 1987). However, increases in the incidence of dicentric chromosomes and acentric fragments were detected by Bauchinger *et al*. (1982), although no increase in sister chromatid exchanges was observed.

4.4.2 *Experimental systems* (see Table 2 for references)

Nineteen chlorophenols have been tested for prophage induction in *Escherichia coli*. Most showed negative, marginally positive or inconclusive activity, with the exception of 2,3,4-, 2,3,6-, 2,4,5-, 2,4,6- and 3,4,5-trichlorophenols, 2,3,4,5-tetrachlorophenol and pentachlorophenol, which were negative or weakly positive in the absence of and positive in the presence of metabolic activation. Various chlorophenols have been tested for mutagenicity in several strains of *Salmonella typhimurium*, with or without exogenous metabolic activation. Only pentachlorophenol showed marginal activity in TA98 with metabolic activation. 3- and 4-Chlorophenols and 2,3,6-, 2,4,5- and 2,4,6-trichlorophenols of unspecified purity appeared to be mutagenic in TA97, TA98, TA100 and/or TA104 strains, usually only in the presence of exogenous metabolic activation.

2,4- and 2,6-Dichlorophenols, 2,4,5-trichlorophenol, 2,3,4,6-tetrachlorophenol and pentachlorophenol, without exogenous metabolic activation, did not cause *hprt* mutations in V79 Chinese hamster cells.

2,4,6-Trichlorophenol demonstrated weak mutagenic activity in the *tk* locus of L5178Y mouse cells without exogenous metabolic activation. On the other hand, it did not induce *hprt* mutations and structural chromosomal aberrations in V79 cells without exogenous metabolic activation, but did induce hyperdiploidy and micronuclei. It was concluded that the genotoxic action of this chemical may primarily result from chromosome malsegregation. However, after detailed examination of the role of incubation and recovery times, it was shown that 2,4,6-trichlorophenol induces chromosomal aberrations in CHO cells with or without activation and in V79 cells without metabolic activation provided sufficient time is allowed for the cells to reach mitosis. It was reported that 2,3,4-, 2,3,6- and 3,4,5-trichlorophenols and 2,3,4,5-, 2,3,4,6- and 2,3,5,6-tetrachlorophenols without metabolic activation were negative or inconclusive for induction of chromosomal aberrations in Chinese hamster lung or ovary cells (with the exception of 2,3,6-trichlorophenol, which was positive

Table 2. Genetic and related effects of chlorophenols

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2-Chlorophenol				
PRB, *Escherichia coli*, prophage λ induction	–	–	500	DeMarini *et al.* (1990)
3-Chlorophenol				
PRB, *Escherichia coli*, prophage λ induction	?	–	10	DeMarini *et al.* (1990)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Strobel & Grummt (1987)
SA4, *Salmonella typhimurium* TA104, reverse mutation	–	(+)	125	Strobel & Grummt (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Strobel & Grummt (1987)
SAS, *Salmonella typhimurium* TA97, reverse mutation	+	+	5	Strobel & Grummt (1987)
4-Chlorophenol				
PRB, *Escherichia coli*, prophage λ induction	–	–	78	DeMarini *et al.* (1990)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	5	Strobel & Grummt (1987)
SA4, *Salmonella typhimurium* TA104, reverse mutation	–	(+)	125	Strobel & Grummt (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	125	Strobel & Grummt (1987)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	+	5	Strobel & Grummt (1987)
2,3-Dichlorophenol				
PRB, *Escherichia coli*, prophage λ induction	–	(+)	13	DeMarini *et al.* (1990)
2,4-Dichlorophenol				
PRB, *Escherichia coli*, prophage λ induction	–	–	39	DeMarini *et al.* (1990)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	167	Haworth *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	167	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	167	Haworth *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	167	Haworth *et al.* (1983)

Table 2 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2,4-Dichlorophenol (contd)				
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	8	Probst *et al.* (1981)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	25	Jansson & Jansson (1986)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	30	US National Toxicology Program (1989)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	6.3	US National Toxicology Program (1989)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	150	US National Toxicology Program (1989)
AIA, Aneuploidy, Chinese hamster lung V79 cells *in vitro*	+	NT	81	Önfelt (1987)
2,5-Dichlorophenol				
PRB, *Escherichia coli*, prophage λ induction	–	(+)	3	DeMarini *et al.* (1990)
2,6-Dichlorophenol				
PRB, *Escherichia coli*, prophage λ induction	–	(+)	13	DeMarini *et al.* (1990)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	500	Jansson & Jansson (1986)
3,4-Dichlorophenol				
PRB, *Escherichia coli*, prophage λ induction	–	–	13	DeMarini *et al.* (1990)
3,5-Dichlorophenol				
PRB, *Escherichia coli*, prophage λ induction	–	(+)	3	DeMarini *et al.* (1990)

Table 2 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2,3,4-Trichlorophenol				
PRB, *Escherichia coli*, prophage λ induction	(+)	+	0.8	DeMarini *et al.* (1990)
CIC, Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	–	–	120	Sofuni *et al.* (1990)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	+	70.2	Sofuni *et al.* (1990)
2,3,5-Trichlorophenol				
PRB, *Escherichia coli*, prophage λ induction	–	?	13	DeMarini *et al.* (1990)
2,3,6-Trichlorophenol				
PRB, *Escherichia coli*, prophage λ induction	–	+	3	DeMarini *et al.* (1990)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	50	Strobel & Grummt (1987)
SA4, *Salmonella typhimurium* TA104, reverse mutation	–	–	125	Strobel & Grummt (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	5	Strobel & Grummt (1987)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	50	Strobel & Grummt (1987)
CIC, Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	?	+	200	Sofuni *et al.* (1990)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	+	175	Sofuni *et al.* (1990)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	NT	400 (commercial)[c]	Armstrong *et al.* (1993)
2,4,5-Trichlorophenol				
PRB, *Escherichia coli*, prophage λ induction	(+)	+	0.8	DeMarini *et al.* (1990)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	25	Rasanen *et al.* (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	25	Nestmann *et al.* (1980)

Table 2 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2,4,5-Trichlorophenol (contd)				
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	33	Haworth *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	–	5	Strobel & Grummt (1987)
SA4, *Salmonella typhimurium* TA104, reverse mutation	–	–	125	Strobel & Grummt (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	25	Rasanen *et al*. (1977)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	25	Nestmann *et al*. (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	33	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	25	Rasanen *et al*. (1977)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	25	Nestmann *et al*. (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	33	Haworth *et al*. (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	25	Nestmann *et al*. (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	25	Rasanen *et al*. (1977)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	25	Nestmann *et al*. (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	33	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5	Strobel & Grummt (1987)
SAS, *Salmonella typhimurium* TA97, reverse mutation	+	+	25	Strobel & Grummt (1987)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	50	Jansson & Jansson (1986)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	NT	140 (commercial)[c]	Armstrong *et al*. (1993)
SLH, Sister chromatid exchange, human lymphocytes *in vivo*	–		NG	Blank *et al*. (1983)
CLH, Chromosomal aberrations, human lymphocytes *in vivo*	–		NG	Blank *et al*. (1983)

Table 2 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2,4,6-Trichlorophenol				
PRB, *Escherichia coli*, prophage λ induction	(+)	+	3	DeMarini *et al.* (1990)
BSD, *Bacillus subtilis rec* strains, differential toxicity	(+)	NT	500 µg/disk	Kinae *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	25	Rasanen *et al*. (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	0.5	Kinae *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	166	Haworth *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	125	Strobel & Grummt (1987)
SA4, *Salmonella typhimurium* TA104, reverse mutation	–	(+)	25	Strobel & Grummt (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	25	Rasanen *et al*. (1977)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	166	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	25	Rasanen *et al*. (1977)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	0.5	Kinae *et al*. (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	166	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	25	Rasanen *et al*. (1977)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	0.5	Kinae *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	166	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	5	Strobel & Grummt (1987)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	+	5	Strobel & Grummt (1987)
SCG, *Saccharomyces cerevisiae* MP1, gene conversion	–	NT	400	Fahrig *et al*. (1978)
SCH, *Saccharomyces cerevisiae* MP1, homozygosis by mitotic recombination or gene conversion	–	NT	400	Fahrig *et al*. (1978)
SCF, *Saccharomyces cerevisiae* MP1, forward mutation	+	NT	400	Fahrig *et al*. (1978)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		10000 inj	Valencia *et al*. (1985)

Table 2 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2,4,6-Trichlorophenol (contd)				
DIA, DNA strand breaks, PM2 DNA *in vitro*	NT	+	NG	Juhl *et al*. (1989)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	100	Jansson & Jansson (1986)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	180	Jansson & Jansson (1992)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	80	McGregor *et al*. (1988)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	500	Galloway *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	500	Galloway *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	–	NT	60	Jansson & Jansson (1992)
CIC, Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	+	NT	400 (commercial)[c]	Armstrong *et al*. (1993)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	NT	500 (repurified)[c]	Armstrong *et al*. (1993)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	+	400 (commercial)[c]	Armstrong *et al*. (1993)
AIA, Aneuploidy, Chinese hamster lung V79 cells *in vitro*	+	NT	100 (commercial)	Armstrong *et al*. (1993)
AIA, Aneuploidy, Chinese hamster lung V79 cells *in vitro*	+	NT	30	Jansson & Jansson (1992)

Table 2 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2,4,6-Trichlorophenol (contd)				
MIA, Micronucleus test, Chinese hamster lung V79 cells *in vitro*	+	NT	30	Jansson & Jansson (1992)
MST, Mouse spot test	+	NT	50	Fahrig *et al*. (1978)
3,4,5-Trichlorophenol				
PRB, *Escherichia coli*, prophage λ induction	–	+	0.8	DeMarini *et al*. (1990)
CIC, Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	–	?	30	Sofuni *et al*. (1990)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	30	Sofuni *et al*. (1990)
2,3,4,5–Tetrachlorophenol				
PRB, *Escherichia coli*, prophage λ induction	–	+	0.8	DeMarini *et al*. (1990)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5	Zeiger *et al*. (1988)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5	Zeiger *et al*. (1988)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5	Zeiger *et al*. (1988)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	5	Zeiger *et al*. (1988)
CIC, Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	–	(+)	60	Sofuni *et al*. (1990)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	+	29.8	Sofuni *et al*. (1990)
2,3,4,6-Tetrachlorophenol				
PRB, *Escherichia coli*, prophage λ induction	–	–	39	DeMarini et al. (1990)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	25	Rasanen *et al*. (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	50	Zeiger *et al*. (1988)

Table 2 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2,3,4,6-Tetrachlorophenol				
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	25	Rasanen *et al*. (1977)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	50	Zeiger *et al*. (1988)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	25	Rasanen *et al*. (1977)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	25	Rasanen *et al*. (1977)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	50	Zeiger *et al*. (1988)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	50	Zeiger *et al*. (1988)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	100	Jansson & Jansson (1986)
CIC, Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	?	(+)	250	Sofuni *et al*. (1990)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	(+)	+	100	Sofuni *et al*. (1990)
2,3,5,6-Tetrachlorophenol				
PRB, *Escherichia coli*, prophage λ induction	?	(+)	25	DeMarini *et al*. (1990)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	50	Zeiger *et al*. (1988)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	50	Zeiger *et al*. (1988)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	50	Zeiger *et al*. (1988)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	17	Zeiger *et al*. (1988)
CIC, Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	–	+	60	Sofuni *et al*. (1990)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	+	175	Sofuni *et al*. (1990)

Table 2 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Pentachlorophenol				
PRB, Prophage PM2 induction, SOS repair test, DNA strand breaks, cross-links or related damage	–	NT	26650	Witte *et al*. (1985)
PRB, *Escherichia coli*, prophage λ induction	(+)	+	13	DeMarini *et al*. (1990)
BSD, *Bacillus subtilis rec* strains, differential toxicity	+	NT	5	Shirasu *et al*. (1976)
BSD, *Bacillus subtilis rec* strains, differential toxicity	–	–	2.2	Matsui *et al*. (1989)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5	Nishimura *et al*. (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	10	Haworth *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5	Nishimura & Oshima (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	10	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	10	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	5	Nishimura *et al*. (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	10	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	5	Nishimura & Oshima (1983)
SCG, *Saccharomyces cerevisiae* D4, gene conversion	+	NT	50	Fahrig (1974)
SCG, *Saccharomyces cerevisiae* MP1, gene conversion	+	NT	400	Fahrig *et al*. (1978)
SCH, *Saccharomyces cerevisiae* MP1, homozygosis by mitotic recombination or gene conversion	–	NT	400	Fahrig *et al*. (1978)
SCF, *Saccharomyces cerevisiae* MP1, forward mutation	+	NT	400	Fahrig *et al*. (1978)
ACC, *Allium cepa*, chromosomal aberrations	–		1.5	Venegas *et al*. (1993)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		1865 feed	Vogel & Chandler (1974)
DMN, *Drosophila melanogaster*, aneuploidy	–		400 ppm feed	Ramel & Magnusson (1979)

Table 2 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Pentachlorophenol (contd)				
DIA, DNA strand breaks, cross-links or related damage, Chinese hamster ovary CHO cells *in vitro*	–	NT	10	Erlich (1990)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	15	Hattula & Knuutinen (1985)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	50	Jansson & Jannson (1986)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	(+)	–	3	Galloway *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	(+)	100	Galloway *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster lung V79 cells *in vitro*	+	+	240	Ishidate (1988)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	NT	90	Ziemsen *et al*. (1987)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	90	Ziemsen *et al*. (1987)
HMM, Host-mediated assay in NMRI mice	–		75 sc × 1	Buselmaier *et al*. (1972)
MST, Spot test, C57BL/6JHan × T mice	(+)		50 ip × 1	Fahrig *et al*. (1978)
MVA, Micronucleus test, amphibian *Caudiverbera caudiverbera* larvae *in vivo*	–		1.5 µg/mL	Venegas *et al*. (1993)
BID, Binding (covalent) to DNA, quail and fetal rat hepatocytes *in vitro*	+	NT	13	Dubois *et al*. (1997)

Table 2 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Pentachlorophenol (contd)				
BID, Binding (covalent) to DNA, human hepatoma HepG2 cells *in vitro*	+	NT	13	Dubois *et al*. (1997)
SPM, Sperm morphology, (C57BL/6×C3H)F_1 mice *in vivo*	–		50 ip × 5	Osterloh *et al*. (1983)

[a] +, positive; (+), weak positive;–, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; inj, injection; ip, intraperitoneal; sc, subcutaneous

[c] With 3 h incubation + 17 h recovery

in the ovary cells). In the presence of metabolic activation, all of the above were clearly or weakly positive in both cell lines, with the exception of 2,3,4-trichlorophenol (negative in the lung cell line) and 3,4,5-trichlorophenol (negative in the ovary cell line).

Pentachlorophenol caused gene conversion and forward mutation in yeast. It did not cause micronucleated erythrocytes in the amphibian *Caudiverbera caudiverbera* or chromosomal aberrations in the root tips of the plant *Allium cepa*. *In vitro*, positive results were reported for the induction of chromosomal aberrations in Chinese hamster lung V79 cells but no effect was observed in human lymphocytes without exogenous metabolic activation. In single studies, sister chromatid exchanges were not induced in human lymphocytes but a weak effect was reported in Chinese hamster ovary CHO cells. Pentachlorophenol gave weakly positive results in a mouse spot test. It did not modify the recombinogenic or mutagenic effects of *N*-ethyl-*N*-nitrosourea in the mouse spot test or sperm morphology *in vivo* in mice. It generated low levels of unidentified DNA adducts, as detected by ^{32}P-postlabelling, upon incubation *in vitro* with primary liver cells of quail (*Coturnix coturnix*) or fetal rat or the human liver cell line HepG2.

In addition to genotoxicity studies with chlorophenols themselves, the corresponding activity of some of their metabolites has also been examined. The major metabolite of pentachlorophenol in mice and rats, tetrachloro-*para*-hydroquinone, induced mutations in the *hprt* locus (but not the ouabain-resistance locus) of V79 Chinese hamster cells (Jansson & Jansson, 1991), covalent damage (including 8-hydroxyguanine) in naked DNA in the presence of Cu(II) (Naito *et al*., 1994), DNA strand breaks and accumulation of p53 in NIH 3T3 cells *in vitro* and transformation of mouse embryo fibroblasts in a two-stage model (Wang *et al*., 1997). The same metabolite, as well as tetrachloro-1,4-benzoquinone and tetrachloro-1,2-benzoquinone, caused DNA strand breaks in V79 Chinese hamster cells (Dahlhaus *et al*., 1996).

A mixture of metabolites obtained after incubation of 2,4,6-trichlorophenol with rat liver S9 mix induced strand breaks in plasmid DNA. The strand breakage was prevented by dimethylsulfoxide or catalase, suggesting that oxygen radicals were responsible (Juhl *et al*., 1989).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposures to chlorophenols and their salts have occurred in their production, in the production of some phenoxy acid herbicides, in the wood industry, the textile industry and tanneries. They have been detected at low levels in ambient air and water.

5.2 Human carcinogenicity data

Mortality and/or cancer incidence has been analysed in several cohort studies of chemical manufacturers, almost all of which have been incorporated within a multicentre international collaborative study, and also in a case–control study nested within this

cohort. Two other cohort studies have focused on leather tanneries in Sweden and sawmills in Canada where chlorophenols were used. In addition, case–control studies have examined the association of chlorophenols with soft-tissue sarcoma (one study in New Zealand, four in Sweden and one in the United States), non-Hodgkin lymphoma (one study in New Zealand, one in Sweden and one in the United States), thyroid cancer (one study in Sweden), nasal and nasopharyngeal cancer (one study in Sweden), colon cancer (one study in Sweden) and liver cancer (one study in Sweden).

These investigations have shown significant associations with several types of cancer, but the most consistent findings have been for soft-tissue sarcoma and non-Hodgkin lymphoma. Although the odds ratios in some case–control studies may have been inflated by recall bias, this cannot explain all of the findings. Nor are they likely to have arisen by chance. It is not possible, however, to exclude a confounding effect of polychlorinated dibenzo-*para*-dioxins which occur as contaminants in chlorophenols.

5.3 Animal carcinogenicity data

2,4-Dichlorophenol was tested in one study in mice and in two studies in rats by oral administration. No increase in the incidence of tumours was found.

2,4,5-Trichlorophenol has not been adequately tested for carcinogenicity.

2,4,6-Trichlorophenol was tested in one study in mice and in one study in rats by oral administration and in one study in mice in a screening test for lung tumours. In mice, it increased the incidences of benign and malignant tumours of the liver and in rats mononuclear cell leukaemia. It did not induce lung adenomas in mice.

No data on the carcinogenicity of tetrachlorophenols in experimental animals were available to the Working Group.

Three different pentachlorophenol formulations were tested for carcinogenicity by oral administration in two experiments in mice and in one study in rats. In mice, a dose-related increase in the incidence of hepatocellular adenomas and carcinomas was observed in males exposed to either formulation and of hepatocellular adenomas in females exposed to one of the formulations. A dose-related increase in the incidence of adrenal phaeochromocytomas was observed in male mice exposed to either formulation, and an increase was also seen in females exposed to one of the formulations at the highest dose. A dose-related increase in the incidence of malignant vascular tumours of the liver and spleen was seen in female mice exposed to either formulation. In rats, no increase in tumours was seen following oral administration of pentachlorophenol for 24 months. However, in rats in the same study receiving a higher concentration for 12 months and held for an additional year, an increased incidence of mesotheliomas of the tunica vaginalis was observed.

5.4 Other relevant data

Chlorophenols are absorbed fairly rapidly, distributed mainly to the kidney and liver and excreted principally via urine; low chlorine-substituted compounds are conjugated with sulfate and glucuronide to a greater extent than the more highly chlorine-substituted compounds. Chlorinated *para*-hydroquinone formation is a minor metabolic pathway

but not for 2,3,5,6-tetrachlorophenol and pentachlorophenol. In rats, the liver is the main target organ. Otherwise, few remarkable effects have been observed.

2,4,6-Trichlorophenol may exhibit weak aneugenic and clastogenic activity. Information on other chlorophenols is inadequate to allow assessment of their genotoxicity.

Pentachlorophenol, after metabolic activation, may exhibit weak clastogenic activity by enhancing oxidative DNA damage.

5.5 Evaluation

There is *limited evidence* in humans for the carcinogenicity of combined exposures to polychlorophenols or to their sodium salts.

There is *evidence suggesting lack of carcinogenicity* of 2,4-dichlorophenol in experimental animals.

There is *inadequate evidence* in experimental animals for the carcinogenicity of 2,4,5-trichlorophenol.

There is *limited evidence* in experimental animals for the carcinogenicity of 2,4,6-trichlorophenol.

There is *sufficient evidence* in experimental animals for the carcinogenicity of pentachlorophenol.

Overall evaluation

Combined exposures to polychlorophenols or to their sodium salts are *possibly carcinogenic to humans (Group 2B)*.

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1,1,2,2-TETRACHLOROETHANE

Data were last reviewed in IARC (1979) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

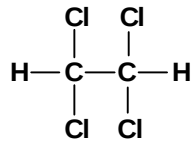
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 79-34-5
Chem. Abstr. Name: 1,1,2,2-Tetrachloroethane
IUPAC Systematic Name: 1,1,2,2-Tetrachloroethane
Synonym: Acetylene tetrachloride

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2H_2Cl_4$ Relative molecular mass: 167.85

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Nonflammable, colourless liquid with a chloroform-like odour (Budavari, 1996)
(*b*) *Boiling-point*: 146.5°C (Lide, 1995)
(*c*) *Melting-point*: –43.8°C (Lide, 1995)
(*d*) *Solubility*: Slightly soluble in water (1 g/350 mL at 25°C); miscible with methanol, ethanol, benzene, diethyl ether, petroleum ether, carbon tetrachloride, chloroform, carbon disulfide, dimethylformamide and oils. Has the highest solvent power of the chlorinated hydrocarbons (Lide, 1995; Budavari, 1996)
(*e*) *Vapour pressure*: 665 Pa at 20°C; relative vapour density (air = 1), 5.79 (Verschueren, 1996)
(*f*) *Conversion factor:* $mg/m^3 = 6.87 \times ppm$

1.2 Production and use

1,1,2,2-Tetrachloroethane is used as a solvent, for cleansing and degreasing metals, in paint removers, varnishes, lacquers, photographic film, resins and waxes, extraction of oils and fats, as an alcohol denaturant, in organic synthesis, in insecticides, as a weed-killer and fumigant and as an intermediate in the manufacture of other chlorinated hydrocarbons (Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

National estimates on exposure were not available.

1.3.2 *Environmental occurrence*

Most 1,1,2,2-tetrachloroethane emissions enter the atmosphere, where it is extremely stable (half-life, > 2 years). It has been detected at low levels in urban air, ambient air, drinking-water, ambient water, groundwater, wastewater and soil samples (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 6.9 mg/m^3 as the threshold limit value for occupational exposures to 1,1,2,2-tetrachloroethane in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for 1,1,2,2-tetrachloroethane in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

The only epidemiological study available evaluated the mortality experience of Second World War army personnel engaged in treating clothing as a defence against gas warfare. In one treatment process, tetrachloroethane was the solvent used for the impregnate. Of the 3859 persons assigned to this process, 1099 whites and 124 blacks had had job duties with probable exposure to the solvent. Among these persons, no significant excess mortality from cancer occurred. Slight excesses were reported for leukaemia (standardized mortality ratio (SMR), 2.7; based on four deaths) and cancer of the genital organs (SMR, 1.6; based on three deaths) (Norman *et al*., 1981).

3. Studies of Cancer in Experimental Animals

1,1,2,2-Tetrachloroethane was tested for carcinogenicity in one experiment in mice and in one in rats by oral administration. In male and female mice, it produced hepato-

cellular carcinomas. Although a few hepatocellular carcinomas were observed in male rats, no significant increase in the incidence of tumours was observed in animals of either sex. The compound was inadequately tested in one experiment in mice by intraperitoneal injection (IARC, 1979).

3.1 Oral administration

Rat: In a rat liver foci assay for tumour-initiating activity, groups of 10 male Osborne-Mendel rats were subjected to a two-thirds partial hepatectomy and, 24 h later, were given 1,1,2,2-tetrachloroethane or corn oil by gavage at the maximum tolerated dose in corn oil. Six days after partial hepatectomy, rats received 0.05% phenobarbital in the diet for seven weeks, then control diets for seven further days, after which they were killed and their livers were examined. The numbers of enzyme-altered foci in the liver were 0.41 ± 0.31 and 0.26 ± 0.19 foci/cm^2 in the test and control (corn oil) groups, respectively. It was concluded that 1,1,2,2-tetrachloroethane did not show initiating activity in this system (Milman *et al*., 1988).

In a promotion study, groups of 10 rats were given an intraperitoneal injection of 30 mg/kg bw *N*-nitrosodiethylamine (NDEA) 24 h after a two-thirds partial hepatectomy. Six days later, the rats received 1,1,2,2-tetrachloroethane in corn oil at the maximum tolerated dose or corn oil by gavage on five days per week for seven weeks. The rats were held for an additional seven days and then killed and the livers were examined. The numbers of enzyme-altered foci were 4.36 ± 0.85 foci/cm^2 in the treated group and 1.77 ± 0.49 foci/cm^2 in the control (corn oil) group, indicating that 1,1,2,2-tetrachloroethane shows promoting activity (Milman *et al*., 1988).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

About 97% of inhaled 1,1,2,2-tetrachloroethane was retained in the lungs 1 h after exposure (IARC, 1979).

4.1.2 *Experimental animals*

The biotransformation of 1,1,2,2-tetrachloroethane was studied in rats and mice using [^{14}C]1,1,2,2-tetrachloroethane. The metabolic disposition study was conducted after oral administration of the unlabelled compound on five days per week for four weeks, followed by a single dose of the radiolabelled compound to simulate conditions of a bioassay for carcinogenicity testing. After oral administration of 0.59 mmol/kg bw (98.5 mg/kg bw) [^{14}C]1,1,2,2-tetrachloroethane to rats and 1.19 mmol/kg bw (198.7 mg/kg bw) to mice, 7% and 9.7% of the administered radioactivity were recovered in the expired air of rats and

mice, respectively. Rats and mice excreted 46% and 30% of the administered radioactivity as metabolites, respectively, mainly in the urine. Some covalent binding of 1,1,2,2-tetrachloroethane metabolites to proteins was noted in this study (Mitoma *et al.*, 1985). The biotransformation of 1,1,2,2-tetrachloroethane is complex; dichloroacetic acid has been identified as the major urinary metabolite, along with trichloroethanol and trichloroacetic acid (Yllner, 1971). The formation of the latter two metabolites was suggested to be due to reductive biotransformation of 1,1,2,2-tetrachloroethane to give trichloroethene, which may be further oxidized to chloral hydrate by cytochrome P450 (Byington & Leibman, 1965). Trichloroethanol and trichloroacetic acid are formed from chloral hydrate by reduction and respiratory oxidation and are major urinary metabolites of trichloroethene (Daniel, 1963). Hydroxylation of 1,1,2,2-tetrachloroethane, yielding dichloroacetyl chloride, is the predominant pathway to dichloroacetic acid (Halpert & Neal, 1981).

The interaction of 1,1,2,2-tetrachloroethane with DNA, RNA and proteins of male Wistar rats and BALB/c mice *in vivo* was measured 22 h after intraperitoneal injection. The covalent binding index to liver DNA was about 500 (Colacci *et al.*, 1987).

Addition of α-naphthoflavone, metyrapone or glutathione to incubations decreased the covalent binding of 1,1,2,2-tetrachloroethane to olfactory and hepatic tissues *in vitro* (Eriksson & Brittebo, 1991).

Incubation of 1,1,2,2-tetrachloroethane with hepatic microsomes and an NADPH-generating system results in the production of chlorinated metabolites, the major ones being mono- and dichloroacetate (Ivanetich & Van Den Honert, 1981).

1,1,2,2-Tetrachloroethane also appears to be metabolized by hepatic nuclear cytochrome P450 (Casciola & Ivanetich, 1984).

Incubation of 1,1,2,2,-tetrachloro[1,2-^{14}C]ethane with a reconstituted monooxygenase system or with intact rat liver microsomes led to the formation of a metabolite capable of binding covalently to proteins and other nucleophiles. The only soluble metabolite detected upon incubation of 1,1,2,2-tetrachloroethane with a reconstituted system was dichloroacetic acid. Pronase digestion of the ^{14}C-labelled microsomal proteins indicated the presence of several derivatized amino acids, which were hydrolysed by alkali to yield dichloroacetic acid. The results are consistent with biotransformation of 1,1,2,2-tetrachloroethane by cytochrome P450 to dichloroacetyl chloride, which can bind covalently to various nucleophiles or hydrolyse to dichloroacetic acid (Halpert, 1982).

4.2 Toxic effects

The toxicity of 1,1,2,2-tetrachloroethane has been reviewed (Luotamo & Riihimäki, 1996).

4.2.1 *Humans*

Numerous deaths due to ingestion, inhalation or cutaneous absorption of 1,1,2,2-tetrachloroethane have been recorded. The solvent affects primarily the central nervous system and the liver and causes polyneuritis and paralysis. Of 380 workers exposed to the solvent, 133 (35%) exhibited tremor and other nervous symptoms. Accidental and

occupational exposure produced liver damage, ranging from severe fatty degeneration to necrosis and acute atrophy, which was frequently fatal, and gastrointestinal disorders; toxic effects were also observed in the haematopoietic system (IARC, 1979).

4.2.2 *Experimental systems*

1,1,2,2-Tetrachloroethane causes central nervous system depression and is highly hepatotoxic in mice and dogs; it produced embryotoxic effects and a low incidence of malformations in mice. A single oral dose (437 mg/kg bw) of 1,1,2,2-tetrachloroethane decreased the activity of some hepatic cytochrome P450-dependent monooxygenases and, to a smaller extent, that of UDP-glucuronosyl transferase (IARC, 1979).

A decrease in monooxygenase, UDP-glucuronosyl transferase, epoxide hydrolase and aminolaevulinic synthetase activity was observed after an intraperitoneal dose (300 or 600 mg/kg bw) to mice (Paolini *et al*., 1992). 1,1,2,2-Tetrachloroethane inactivated a phenobarbital-induced isolated rat hepatic cytochrome P450 isoenzyme but not a β-naphthoflavone-induced isoenzyme in a reconstituted system *in vitro* (Halpert *et al*., 1986). After an intraperitoneal dose of 1,1,2,2-tetrachloroethane to rats, an accentuated spectral signal of conjugated dienes was observed in extracted endoplasmic lipids, which was interpreted as indicating lipid peroxidation; generation of a nitroxide radical was observed in livers from rats treated simultaneously with the electron spin resonance probe compound, *N*-benzylidene-2-methylpropylamine-*N*-oxide (Paolini *et al*., 1992).

When 0.124 mmol/kg bw 1,1,2,2-tetrachloroethane was administered to male Fischer 344/N rats by gavage once daily, all rats died or were moribund by the termination of the experiment at 21 days. When the dose was 0.62 mmol/kg bw per day, liver weight was elevated and cytoplasmic vacuolation of hepatocytes occurred in all rats. No treatment-related effects were observed in the kidney (United States National Toxicology Program, 1996).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Treatment of AB-Jena and DBA mice with 300–400 mg/kg bw 1,1,2,2-tetrachloroethane per day during organogenesis produced embryotoxic effects and a low incidence of malformations (exencephaly, cleft palate, anophthalmia, fused ribs and vertebrae). The effects were related to the dose and period of treatment (IARC, 1979).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

1,1,2,2-Tetrachloroethane induced DNA damage in the *Escherichia coli* differential toxicity assay. It did not induce forward mutation and gave conflicting results for the induction of reverse mutation in *Salmonella typhimurium*. Only one study reported evidence of reverse mutation in strains TA100, TA104, TA98 and TA97, and these tester strains were more sensitive in the presence of an exogenous metabolic activation system. 1,1,2,2-Tetrachloroethane induced gene conversion and mutation in *Saccharomyces cerevisiae* and aneuploidy, without a requirement for exogenous metabolic activation, but not genetic crossing-over, in *Aspergillus nidulans*. It did not increase the frequency of sex-linked recessive lethal mutations in *Drosophila melanogaster*.

1,1,2,2-Tetrachloroethane did not induce chromosomal aberrations in Chinese hamster ovary cells. It induced sister chromatid exchanges in Chinese hamster ovary and mouse BALB/c 3T3 cell cultures and cell transformation in BALB/c 3T3 cells *in vitro*.

Unscheduled DNA synthesis was not induced in hepatocytes of mice given a single gavage treatment of 1,1,2,2-tetrachloroethane. Results from a single study showed that 1,1,2,2-tetrachloroethane bound covalently to DNA in liver, lung, kidney and stomach of rats and mice given a single intraperitoneal injection.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

1,1,2,2-Tetrachloroethane is used as a solvent. It has been detected at low levels in urban and ambient air and in drinking-, ground- and wastewater.

5.2 Human carcinogenicity data

The available epidemiological data are inadequate for evaluation.

5.3 Animal carcinogenicity data

1,1,2,2-Tetrachloroethane was tested in one experiment in mice and in one in rats by oral administration. In mice, it produced hepatocellular carcinomas in males and females. It was inadequately tested by intraperitoneal administration in mice. In one small experiment in rats, no initiating but promoting activity of 1,1,2,2-tetrachloroethane was found.

5.4 Other relevant data

1,1,2,2-Tetrachloroethane bound covalently to DNA but did not induce unscheduled DNA synthesis in mice *in vivo*. It induced sister chromatid exchanges and cell transformation, but not chromosomal aberrations or unscheduled DNA synthesis, in rodent cells *in vitro*. It induced gene conversion and mutation in yeast and aneuploidy, but not genetic crossing-over, in fungus. 1,1,2,2-Tetrachloroethane induced DNA damage and showed some evidence of being mutagenic in bacteria.

Table 1. Genetic and related effects of 1,1,2,2-tetrachloroethane

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ECD, *Escherichia coli* pol A, differential toxicity (spot)	+	NT	16000/disc	Brem *et al*. (1974)
SAF, *Salmonella typhimurium*, forward mutation, arabinose resistance	–	–	150	Roldán-Arjona *et al*. (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2000	Nestmann *et al*. (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Haworth *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	+	125	Strobel & Grummt (1987)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	+	NT	1680/disc	Brem *et al*. (1974)
SA4, *Salmonella typhimurium* TA104, reverse mutation	–	(+)	500	Strobel & Grummt (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	NT	1680/disc	Brem *et al*. (1974)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2000	Nestmann *et al*. (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2000	Nestmann *et al*. (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Haworth *et al*. (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	1680/disc	Brem *et al*. (1974)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2000	Nestmann *et al*. (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2000	Nestmann *et al*. (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	(+)	+	5	Strobel & Grummt (1987)
SAS, *Salmonella typhimurium* TA97, reverse mutation	+	+	5	Strobel & Grummt (1987)
SCG, *Saccharomyces cerevisiae* strain D7, gene conversion, *trp*5 locus	+	NT	875	Callen *et al*. (1980)
SCH, *Saccharomyces cerevisiae* strain D7, homozygosis, *ade*2 locus	+	NT	875	Callen *et al*. (1980)
ANG, *Aspergillus nidulans* strain P1, genetic crossing-over	–	NT	640	Crebelli *et al*. (1988)
SCR, *Saccharomyces cerevisiae* strain D7, reverse mutation, *ilv*1 locus	+	NT	875	Callen *et al*. (1980)
ANN, *Aspergillus nidulans* strain P1, aneuploidy	+	NT	320	Crebelli *et al*. (1988)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		1500 ppm feed	Woodruff *et al*. (1985)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	+	56	Galloway *et al.* (1987)
SIM, Sister chromatid exchange, BALB/c 3T3 cells *in vitro*	+	+	500	Colacci *et al.* (1992)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	–	653	Galloway *et al.* (1987)
TBM, Cell transformation, BALB/c 3T3 mouse cells	–	NT	250	Tu *et al.* (1985)
TBM, Cell transformation, BALB/c 3T3 mouse cells	(+)	+	125	Colacci *et al.* (1990)
TBM, Cell transformation, BALB/c 3T3 mouse cells	NT	+	62.5	Colacci *et al.* (1992)
UVM, Unscheduled DNA synthesis, $B6C3F_1$ mouse hepatocytes *in vivo*	–		1000 po × 1	Mirsalis *et al.* (1989)
BID, DNA binding (covalent), calf thymus DNA *in vitro*	–	+	10	Colacci *et al.* (1987)
BVD, DNA binding, male BALB/c mouse liver, kidney, lung and stomach *in vivo*	+		1.46 ip × 1	Colacci *et al.* (1987)
BVD, DNA binding, male Wistar rat liver, kidney, lung and stomach *in vivo*	+		1.46 ip × 1	Colacci *et al.* (1987)
BVP, Binding to protein, male BALB/c mouse lung, liver, kidney and stomach *in vivo*	+		1.46 ip × 1	Colacci *et al.* (1987)
BVP, Binding to protein, male Wistar rat lung, liver, kidney and stomach *in vivo*	+		1.46 ip × 1	Colacci *et al.* (1987)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; po, oral; ip, intraperitoneal

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of 1,1,2,2-tetrachloroethane.

There is *limited evidence* in experimental animals for the carcinogenicity of 1,1,2,2-tetrachloroethane.

Overall evaluation

1,1,2,2-Tetrachloroethane is *not classifiable as to its carcinogenicity to humans (Group 3)*.

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TOLUENE

Data were last evaluated in IARC (1989a).

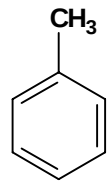
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 108-88-3
Chem. Abstr. Name: Methylbenzene
IUPAC Systematic Name: Toluene
Synonyms: Methylbenzol; phenylmethane

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_7H_8 Relative molecular mass: 92.14

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid with characteristic aromatic hydrocarbon odour (Budavari, 1996)
(*b*) *Boiling-point*: 110.6°C (Lide, 1995)
(*c*) *Melting-point*: –94.9°C (Lide, 1995)
(*d*) *Solubility*: Very slightly soluble in water (515 mg/L at 20°C); soluble in acetone; and miscible with carbon disulfide, chloroform, diethyl ether, ethanol and glacial acetic acid (Budavari, 1996; Verschueren, 1996; Lide, 1997)
(*e*) *Vapour pressure*: 1.3 kPa at 6.4°C; relative vapour density (air = 1), 3.14 (Verschueren, 1996)
(*f*) *Flash point*: 4.4°C, closed cup (Budavari, 1996)
(*g*) *Explosive limits*: Upper, 7.0%; lower, 1.27% by volume in air (American Conference of Governmental Industrial Hygienists, 1992)
(*h*) *Conversion factor*: $mg/m^3 = 3.77 \times ppm$

1.2 Production and use

Production capacities for toluene in western Europe in 1994 were reported as (thousand tonnes): Austria, 4; Belgium, 73; France, 65; Germany, 1185; Italy, 495; the Netherlands, 255; Portugal, 140; Spain, 280 and United Kingdom, 555 (Fabri *et al*., 1996). Production in the United States in 1993 was reported to be 2277 thousand tonnes (United States International Trade Commission, 1994). Information available in 1995 indicated that toluene was produced in 35 countries (Chemical Information Services, 1995).

Toluene is used as a high-octane blending stock in gasoline; as a solvent for paints and coatings, gums, resins, oils, rubber and adhesives; and as an intermediate in the preparation of many chemicals, dyes, pharmaceuticals, detergents and explosives (Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), as many as 2 million workers in the United States were potentially exposed to toluene (see General Remarks). Occupational exposures to toluene may occur in painting, varnishing, various cleaning operations, laboratories, car repair shops and many other workplaces where toluene is produced or used as solvent or intermediate to prepare other chemicals. Extensive occupational exposure data are presented in a previous monograph (IARC, 1989a).

1.3.2 *Environmental occurrence*

Toluene is released into the atmosphere principally from the volatilization of petroleum fuels and toluene-based solvents and thinners and in motor vehicle exhaust. It is also present in emissions from volcanoes, forest fires and crude oil. It has been detected at low levels in surface water, groundwater, drinking-water and soil samples (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 188 mg/m^3 as the 8-h time-weighted average threshold limit value, with a skin notation, for occupational exposures to toluene in workplace air. Values of 100–380 mg/m^3 are used as standards or guidelines in other countries (International Labour Office, 1991).

The World Health Organization has established a provisional international drinking-water guideline for toluene of 700 µg/L (WHO, 1993).

2. Studies of Cancer in Humans

The epidemiological studies are summarized in Table 1.

Table 1. Summary of epidemiological studies on toluene

Author, country	Study type	Comparison	Size	Results[a]	
Svensson *et al.* (1990), Sweden	Cohort of rotogravure printers. Mortality and cancer incidence	Local region rates	1020	Stomach	SMR, 2.7 (1.1–5.6) SIR, 2.3 (0.9–4.8)
				Colorectal	SMR, 2.2 (0.9–4.5) SIR, 1.5 (0.7–2.8)
				Respiratory	SMR, 1.4 (0.7–2.5) SIR, 1.8 (1.0–2.9)
				Leukaemia/lymphoma	SMR, 1.0 (0.2–2.8)
				Leukaemia	SIR, 1.7 (0.3–4.9)
Walker *et al.* (1993), United States	Cohort of shoe manufacture workers. Mortality	National rates	7814	Men	
				Buccal cavity and pharynx	SMR, 0.9 (0.2–2.2)
				Digestive	SMR, 0.9 (0.6–1.3)
				Colon	SMR, 1.3 (0.8–2.1)
				Lung	SMR, 1.6 (1.2–2.0)
				Kidney	SMR, 1.7 (0.6–3.7)
				Lymphoma and haematopoietic	SMR, 0.9 (0.5–1.6)
				Women	
				Colon	SMR, 1.2 (0.8–1.8)
				Lung	SMR, 1.3 (0.9–1.9)
Blair *et al.* (1998), United States	Cohort of aircraft maintenance workers. Mortality. Internal analysis on multiple myeloma, non-Hodgkin lymphoma and breast cancer	Unexposed within cohort	< 14 457 exposed unexposed	Multiple myeloma	
				Men	RR, 0.9 (0.2–4.8)
				Women	RR, 5.0 (1.1–23.1)
				Non-Hodgkin lymphoma	
				Men	RR, 1.0 (0.2–4.2)
				Women	RR, 2.2 (0.4–13.2)
				Breast (women)	RR, 2.0 (0.9–4.2)
				(Other substances also had excess risks.)	

Table 1 (contd)

Author, country	Study type	Comparison	Size	Results[a]	
Austin & Schnatter (1983), United States	Nested case–control study of brain cancer in petrochemical industry	Other deceased workers	21 cases 2 × 80 controls	Brain	OR < 1.0
Wilcosky *et al.* (1984), United States	Nested case–control study of five types of cancer among rubber workers	20% sample of cohort	4–101 per case series, approx. 1300 controls	'Solvent A' Stomach Respiratory Prostate Lymphosarcoma Lympholeukaemia	 OR, 1.4 (NS) [OR, 1.0] OR, 1.0 OR, 2.6 (NS) OR, 2.8 (NS)
Carpenter *et al.* (1988), United States	Nested case–control study of central nervous system in nuclear workers	Living at time of case occurrence	89 cases 356 controls	Central nervous system	OR, 2.0 (NS)
Olsson & Brandt (1980), Sweden	Case–control study of Hodgkin's lymphoma	Neighbourhood controls	25 cases 50 controls	Hodgkin's lymphoma	[Crude OR, 4.0]

Table 1 (contd)

Author, country	Study type	Comparison	Size	Results[a]	
Gérin *et al.* (1998), Canada	Case-control study, many sites	Population controls and cancer controls	99–857 per case series, 1066 controls	Oesophagus	OR, 1.9 (0.9–4.2)
				Stomach	OR, 1.7 (0.6–4.8)
				Colon	OR, 1.8 (0.7–4.4)
				Rectum	OR, 3.2 (1.3–8.0)
				Pancreas	OR, 0.6 (0.2–2.2)
				Lung	OR, 1.1 (0.5–2.7)
				Prostate	OR, 0.4 (0.1–1.4)
				Melanoma	OR, 0.4 (0.1–0.9)
				Non-Hodgkin lymphoma	OR, 0.9 (0.4–1.9)

SMR, standardized mortality ratio; SIR, standardized incidence ratio; RR, relative risk; OR, odds ratio; NS, not significant
Most of the study groups were exposed to many substances in addition to toluene.
[a] Unless otherwise stated, results pertain to males.

2.1 Industry-based studies

Austin and Schnatter (1983) performed a nested case–control study of brain cancer within a cohort of employees at a petrochemical plant in Texas (United States). Twenty-one deceased brain tumour patients and two control groups (80 deceased ex-employees in each) were selected. Job history records were assessed by industrial hygienists for the purpose of assigning potential for exposure to each of 42 substances, one of which was toluene. Results were expressed as percentages of cases and controls exposed. Cases had lower exposure prevalence than controls (36% versus 45–53%) [leading to an apparent approximate odds ratio of 0.6, 95% CI, 0.2–2.2]. [The Working Group had some difficulty understanding the constitution of the control groups.]

In a nested case–control study among rubber workers in the United States (Wilcosky *et al*., 1984), described in more detail in the monograph on dichloromethane (see this volume), one of the substances evaluated was toluene and another was 'solvent A' (a proprietary mixture containing mostly toluene). For toluene itself, the numbers of exposed cases were very low (less than three for each case series). For lung cancer, the odds ratio was 0.6 based on three exposed cases. For lymphatic leukaemia, there were two cases exposed to toluene (odds ratio, 3.0; $p > 05$).There were somewhat higher numbers exposed to 'solvent A', with increased relative risks for stomach cancer (odds ratio, 1.4; $n = 15$), lymphosarcoma (odds ratio, 2.6; $n = 6$) and lymphatic leukaemia (odds ratio, 2.8; $n = 7$). [The Working Group noted that the numbers of cases exposed to pure toluene was small and the odds ratio estimates imprecise. Workers were typically exposed to multiple exposures and positive associations were found for many of the other substances analysed in this study, indicating a lack of specificity in the toluene or 'solvent A' associations].

Carpenter *et al*. (1988) carried out a nested case–control study of cancer of the central nervous system among workers at two nuclear facilities located in Tennessee (United States). They identified 89 cases (72 males and 17 females) who had died between 1943 and 1979. Four controls, living at the time the case was diagnosed, were matched to each case. Job history records were scrutinized by an industrial hygienist to assess potential exposure to each of 26 chemicals or chemical groups. Toluene, xylene (see this volume) and 2-butanone (methyl ethyl ketone) were evaluated as one chemical group; the matched relative risk was 2.0 (95% confidence interval (CI), 0.7–5.5; $n = 28$) in comparison with unexposed workers. Almost all cases had had low exposure, according to the classification used and there was no dose–response trend. The authors stated that the relative risks were adjusted for internal and external exposure to radiation.

Svensson *et al*. (1990) studied a cohort of 1020 Swedish rotogravure printers exposed primarily to toluene and employed for a minimum period of three months in eight plants during 1925–85. Data were available on air levels of toluene since 1943 in one plant and since 1969 in most. Based on these measurements and on present concentrations of toluene in blood and subcutaneous fat, the yearly average air levels in each plant were estimated. They reached a maximum of about 450 ppm [1700 mg/m^3] in the 1940s and 1950s but were only 30 ppm [113 mg/m^3] by the mid-1980s. Exposure to benzene had occurred up to the beginning of the 1960s, but not since then. Records of

employment were combined with these retrospectively estimated plant-specific exposure levels to derive cumulative exposure estimates. The mortality experience of the cohort, during the follow-up period of 1952–86, was compared with that of the geographical region in which the plants were located, and cancer incidence, during the follow-up period of 1958–85, was analogously compared with regional incidence rates. The 'all causes' standardized mortality ratio (SMR) was 1.0 (129 observed deaths). There was no increase in mortality from non-malignant respiratory diseases (SMR, 0.8; 95% CI, 0.3–1.9; *n* = 5). For all cancers combined, there was some overall excess of mortality (SMR, 1.4; 95% CI, 1.0–1.9; *n* = 41) and morbidity (standardized incidence ratio (SIR), 1.3; 95% CI, 1.0–1.6). Among specific cancers, there were no excess risks for urinary cancers or leukaemias, lymphomas and myelomas. There were indications of excess risk for respiratory tract cancer (SMR, 1.4; 95% CI, 0.7–2.5; *n* = 11; SIR, 1.8; 95% CI, 1.0–2.9; *n* = 16) , for stomach cancer (SMR, 2.7; 95% CI, 1.1–5.6; *n* = 7; SIR, 2.3; 95% CI, 0.9–4.8; *n* = 7) and colo-rectal cancer (SMR, 2.2; 95% CI, 0.9–4.5; *n* = 7; SIR, 1.5; 95% CI, 0.7–2.8; *n* = 9) . Restricting analysis to those with at least five years of exposure did not lead to higher relative risk estimates. Further, there was no dose–response relationship with cumulative toluene dose (ppm years). [The Working Group noted that this study population had the 'purest' exposure to toluene of the groups evaluated in this monograph. This study had the best exposure assessment. Although the absence of an excess risk of nonmalignant respiratory disease is reassuring, it was based on very small numbers and thus does not prove that this cohort had 'normal' smoking habits].

Blair *et al.* (1998) updated a cohort mortality study reported by Spirtas *et al*. (1991) on 14 457 workers who had been employed as civilians for at least one year during the interval 1952 to 1956 in an aircraft maintenance facility located in Utah (United States). The study methods are described in the monograph on dichloromethane (see this volume). About 13% of the cohort were deemed to be exposed to toluene (Stewart *et al*., 1991). Using Poisson regression analysis, rate ratios were estimated for each of three types of cancer, multiple myeloma, non-Hodgkin lymphoma and breast cancer. Among toluene-exposed workers, there was an indication of an excess of multiple myeloma among women (RR, 5.0; 95% CI, 1.1–23.1; *n* = 4) but not among men (RR, 0.9; 95% CI, 0.2–4.8; *n* = 2). There was no meaningful excess risk of non-Hodgkin lymphoma among men (RR, 1.0; 95% CI, 0.1–4.2; *n* = 3) or among women (RR, 2.2; 95% CI, 0.4–13.1; *n* = 2). There was a slight excess of breast cancer (RR, 2.0; 95% CI, 0.9–4.2; *n* = 10). [The Working Group noted that the numbers on which these associations were based were very small and that workers typically had multiple exposures.]

Walker *et al*. (1993) conducted a cohort mortality study among 7814 shoe-manufacturing workers (2529 males and 5285 females) from two plants in Ohio (United States) that have been in operation since the 1930s. The workers, men and women, were potentially exposed to solvents and solvent-based adhesives. It was thought that toluene may have been a predominant exposure, but a hygiene survey in 1977–79 showed that, in addition to toluene (10 measurements ranged from 10 ppm to 72 ppm [38–270 mg/m^3]), there were also 2-butanone (methyl ethyl ketone), acetone, hexane and

several other solvents in concentrations as high as or higher than that of toluene. It is not clear whether these substances were present in earlier years. Benzene (IARC, 1987) may have been present as an impurity of toluene. Mortality follow-up was from 1940 to 1982. Relative risk estimates (SMRs) for white workers were derived by comparison with the general population of the United States. Among men, the SMR for all causes of death combined was close to 1.0, as was the SMR for all cancers combined. This cohort had no excess of lymphatic and haematopoietic cancer as a whole (SMR; 0.9; 95% CI, 0.6–1.3; $n = 29$) nor for any subtype. There were excess risks of lung cancer among men (SMR, 1.6; 95% CI, 1.2–2.0; $n = 68$) and among women (SMR, 1.3; 95% CI, 0.9–1.9; $n = 31$). Relative risk of lung cancer did not increase with increasing duration of employment. Mortality from chronic non-malignant respiratory disease was significantly elevated among men (SMR, 1.6; 95% CI, 1.1–2.2) but was less than expected among women (SMR, 0.8; 95% CI, 0.4–1.3), a finding suggesting a possible contribution of smoking to the male mortality from respiratory cancer. Adjustment for the potential effects of smoking by Axelson's (1978) method reduced the relative risk estimate for lung cancer to 1.4 (95% CI, 1.1–1.8). There were slight excess risks for colon cancer among men (SMR, 1.3; 95% CI, 0.8–2.1; $n = 18$) and among women (SMR, 1.2; 95% CI, 0.8–1.8; $n = 28$). Other cancers showed no excess risk. [The Working Group noted that there was sparse information on what substances were historically present in this workplace. The procedure for adjustment of smoking is imperfect and could leave a confounded estimate.]

2.2 Community-based studies

Olsson and Brandt (1980) carried out a hospital-based case–control study of Hodgkin's disease and chemical exposures in Lund, Sweden. Twenty-five consecutive male cases aged 20–65 years were included. Two neighbourhood-matched controls were selected for each case from the Swedish population register. Interviews with study subjects focused on a detailed job history, and in particular on exposure to solvents. Interview data were supplemented with enquiries to employers in some cases. Using a criterion of at least one year of exposure more than 10 years before diagnosis, 12 of the 25 patients with Hodgkin's disease had been exposed occupationally to organic solvents and six of the 50 controls, giving an odds ratio of 6.6 (95% CI, 1.8–23.8). Six of the cases and three of the controls had been exposed to toluene [crude odds ratio, 4.0]. All toluene-exposed cases and controls were also exposed to other solvents. [The Working Group noted the opportunity for information bias, since the interviewer was not blind to disease status or to the study objectives.]

Using data collected in the population-based case–control study of cancer among male residents of Montreal, Canada, described in the monograph on dichloromethane (see this volume), Gérin *et al*. (1998) carried out an analysis focusing on cancer risks in relation to benzene, toluene, xylene (see this volume) and styrene exposure. For these analyses, the control group for each case series consisted of a combination of the 533 population controls with 533 cancer controls selected at random from the pool of eligible cancer controls. Fifteen per cent of the entire study population had been exposed to toluene at

some time (i.e., lifetime exposure prevalence). Among the main occupations in which toluene exposure was deemed in this study to have occurred were painters (except construction), vehicle mechanics and repairers, shoemakers and carpenters. Cumulative exposure indices were created on the basis of duration, concentration, frequency and the degree of certainty in the exposure assessment, and subjects were subdivided into subgroups with low, medium and high cumulative exposure. Logistic regression analyses were carried out, with adjustment for age, ethnic group, income level and smoking status, as well as asbestos and chromium compounds in the analysis of lung cancer. For the following cancer sites, there was little indication of excess risk in relation to exposure to toluene (results are shown for high exposure or for medium/high combined when numbers were too small): pancreas (odds ratio, 0.6; 95% CI, 0.2–2.2; *n* = 3), lung (odds ratio, 1.1; 95% CI, 0.5–2.7; *n* = 12), prostate (odds ratio, 0.4; 95% CI, 0.1–1.4; *n* = 3), urinary bladder (odds ratio, 1.0; 95% CI, 0.4–2.5; *n* = 7), kidney (odds ratio, 1.0; 95% CI, 0.5–2.1; *n* = 8), melanoma (odds ratio, 0.4; 95% CI, 0.1–0.9; *n* = 5) and non-Hodgkin lymphoma (odds ratio, 0.9; 95% CI, 0.4–1.9; *n* = 8). For the following sites, the odds ratios were above 1.5: oesophagus (odds ratio, 1.9; 95% CI, 0.9–4.2; *n* = 9), stomach (odds ratio, 1.7; 95% CI, 0.6–4.8; *n* = 5), colon (odds ratio, 1.8 ; 95% CI, 0.7–4.4; *n* = 9) and rectum (odds ratio, 3.2; 95% CI, 1.3–8.0; *n* = 8). Most workers exposed to toluene were also exposed to benzene, xylene and perhaps other substances. Further analyses of colon cancer and rectal cancer showed that the apparent excesses related to toluene were not attributable to benzene exposure, but the relative contributions of toluene and xylene could not confidently be disentangled.

3. Studies of Cancer in Experimental Animals

Toluene was tested for carcinogenicity in one strain of rats by gavage at one dose level and in one strain of rats by inhalation. These studies were inadequate for evaluation. Toluene was used as a vehicle control in a number of skin-painting studies. Some of these studies were inadequate for evaluation. In others, repeated application of toluene to the skin of mice did not result in an increased incidence of skin tumours (IARC, 1989a).

3.1 Inhalation exposure

3.1.1 *Mouse*

Groups of 60 male and 60 female B6C3F$_1$ mice, 9–10 weeks of age, were administered toluene (purity, > 99%) by whole-body inhalation at concentrations of 0 (controls), 120, 600 or 1200 ppm [0, 450, 2260 or 4520 mg/m^3] for 6.5 h per day on five days per week for 104 weeks. Exposure concentrations were based on the results from 13-week studies in which deaths were observed at concentrations of 2500 ppm [9400 mg/m^3] and higher. Ten females per group were killed after 15 months. Survival was 17/60, 22/60, 16/60 and 19/60 control, low-, mid- and high-dose males and 30/50,

33/50, 24/50 and 32/50 control, low-, mid- and high-dose females, respectively. All animals were necropsied and all major tissues examined histopathologically. No increase in the incidence of any non-neoplastic or neoplastic lesion was observed (United States National Toxicology Program, 1990).

3.1.2 *Rat*

Groups of 60 male and 60 female Fischer 344 rats, six to seven weeks of age, were administered toluene (purity, > 99%) by whole-body inhalation at concentrations of 0 (controls), 600 or 1200 ppm [0, 2260 or 4520 mg/m^3] for 6.5 h per day on five days per week for 103 weeks. Exposure concentrations were based on the results from 15-week studies in which deaths were observed at concentrations of 3000 ppm [11 300 mg/m^3] and significantly decreased body weights occurred at 2500 ppm [9400 mg/m^3]. Ten females per group were killed after 15 months. Mean body weight was generally similar among groups. Survival was 30/50, 28/50 and 22/50 control, low- and high-dose males and 33/50, 35/50 and 30/50 control, low- and high-dose females, respectively. All animals were necropsied and all major tissues examined histopathologically. No increase in tumours was found in either sex (United States National Toxicology Program, 1990).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

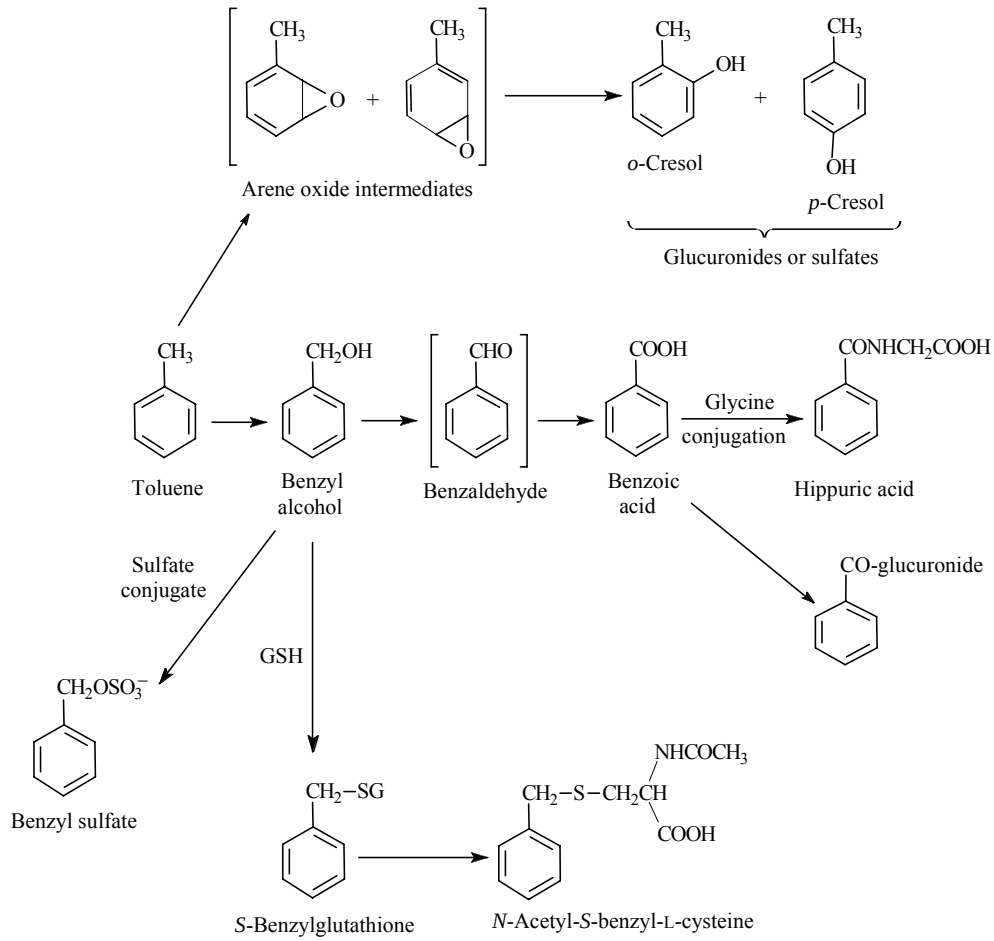
The major metabolic pathway of toluene is to benzyl alcohol, which is oxidized to benzaldehyde and subsequently to benzoic acid (Figure 1). Most of the benzoic acid is converted to hippuric acid, but some is conjugated with UDP-glucuronate to form the acyl-glucuronide. A much smaller fraction of a dose of toluene is converted to *ortho*- and *para*-cresol, which are excreted in urine as the sulfate or glucuronide conjugates.

4.1.1 *Humans*

During inhalation exposure of human volunteers to low levels of toluene (200–300 mg/m^3), approximately 50% of the inhaled toluene was absorbed (Löf *et al*., 1993). Such studies at low toluene exposure are complicated by the presence of toluene from other sources, in blood or in urine (Pierce *et al*., 1996). If the deuterated [$^2H_8$]toluene is used for exposure, this problem is avoided [but an isotope effect may reduce the rate of the metabolism of deuterated toluene compared to normal toluene, possibly by 30–50%]. When toluene is administered orally, it is virtually completely absorbed from the gastrointestinal tract (Baelum *et al*., 1993).

During exposure at 100 ppm [380 mg/m^3], women had a higher toluene concentration in exhaled air than men, both at rest and under a work load of 100 W: a 5 ppm [19 mg/m^3] difference was observed in exhaled levels of approximately 10–20 ppm

Figure 1. Metabolic pathways for toluene



[38–76 mg/m³]. In both sexes, work tended to increase the toluene concentration in exhaled air by up to 5 ppm; however there was very wide interindividual variation (Baelum, 1990). There was a linear correlation between toluene concentration in ambient air (8-h time-weighted average) of workers exposed to 10–300 ppm [38–1180 mg/m³] and the post-shift toluene levels in finger-prick blood or the toluene concentration in end-of-shift expired breath (Foo *et al*., 1988, 1991). A similar correlation was observed between the time-weighted average toluene exposure level during a five-day working week (10–420 mg/m³) and the toluene concentration in subcutaneous adipose tissue (Nise *et al*., 1989); the elimination kinetics of toluene in blood showed a three-phase behaviour, with a very rapid phase ($t_{½}$ approximately 10 min), a slower phase ($t_{½}$ approximately 2 h) and a very slow phase ($t_{½}$ 45–180 h). The latter long half-life may be related to slow release from adipose tissue, which accumulates toluene. A semi-empirical

physiological toxicokinetic model of toluene has been developed by Pierce *et al*. (1996). This model takes into account person-specific characteristics like adipose tissue fraction, blood–air partition coefficient, age, ventilation rate and body weight. The hepatic toluene metabolism parameters were taken from the literature, but 'extrahepatic metabolism', as well as the fraction of cardiac output that perfuses adipose tissue were fitted individually to best describe the data. The data show that systemic toluene clearance is well in excess of hepatic blood flow, indicating extensive extrahepatic metabolism. A high adipose fraction is associated with low blood concentrations of toluene, and simulations show that, 98 h after exposure, the adipose tissue contained more than 97% of the toluene present in the body. In human blood, toluene is distributed between red blood cells and plasma at a ratio of approximately 40:60 (Lam *et al*., 1990).

Another physiological toxicokinetic model (Tardif *et al*., 1993b, 1997) has been used to predict potential interactions between, e.g., toluene, ethylbenzene and *meta*-xylene; the model and experimental data from exposed volunteers indicate that no biologically significant changes in their toxicokinetics will occur if these three solvents are present in the air as a mixture within the permissible concentrations for mixtures (Tardif *et al*., 1997). A model approach also predicted that interactions between dichloromethane and toluene at their current threshold limit values are not relevant for humans (Pelekis & Krishnan, 1997).

The analgesic drugs paracetamol and acetylsalicylic acid at normal clinical doses had no acute effect on toxicokinetics of toluene inhaled at 300 mg/m^3 (Löf *et al*., 1990b); similarly, neither carbohydrate diets nor the consumption of 47 g ethanol as wine on the evening before exposure to 200 mg/m^3 toluene for 2 h had any effect on toluene kinetics (Hjelm *et al*., 1994).

Several authors have pointed out that the urinary excretion of hippurate is a poor indicator of exposure to toluene at 200 ppm [760 mg/m^3] or lower (Jonai & Sato, 1988; Foo *et al*., 1991; Pierce *et al*., 1996). Therefore, data on ethnic differences in hippurate or cresol excretion in urine at these low exposure levels (e.g., Inoue *et al*., 1988) are of doubtful significance. Toluene level in expired air may be a more reliable parameter (Foo *et al*., 1991). Although at the level of the individual, data on urinary hippurate cannot be reliably used to estimate low toluene exposures, they can be used at the group level to establish whether at a certain location the toluene exposure remained below a particular threshold (Lauwerys, 1983).

The first step in toluene metabolism is catalysed by several cytochrome P450 species: human liver microsomes convert toluene mainly to benzyl alcohol (over 90%) as well as to *ortho*- and *para*-cresol (3 and 5%, respectively) (Tassaneeyakul *et al*., 1996; Nakajima *et al*., 1997). The major CYP isoenzyme responsible for oxidation to benzyl alcohol is CYP2E1; diethyldithiocarbamate, a selective and potent CYP2E1 inhibitor, decreased benzyl alcohol formation by more than 75%. Also CYP2B6, CYP2C6, CYP1A2 and CYP1A1 (in decreasing order) are active. *para*-Cresol is formed by CYP2B2 and CYP2E1, while CYP1A2 forms both *ortho*-and *para*-cresol (Nakajima *et al*., 1997). Among 35 surgical human liver samples (23 men and 12 women with either

primary liver tumours or hepatic metastases), there was only a four-fold difference in the rate of oxidation by microsomes. No difference was observed between microsomes from smokers and from non-smokers in formation of benzyl alcohol or *para*-cresol, but the formation of *ortho*-cresol was somewhat increased; alcohol consumption had no measurable effect (Nakajima *et al*., 1997).

Human polymorphisms in several enzymes involved in toluene metabolism are known. In Mongoloid populations, deficiency in the low K_m form of aldehyde dehydrogenase H2 (ALDH2) is common: approximately half of the Japanese population lacks this enzyme. In ALDH2-deficient exposed workers, an increased level of benzyl alcohol was found, but benzaldehyde was not detectable; urinary excretion of hippurate was decreased in the deficient individuals. The CYP1A1 polymorphism, alcohol consumption and smoking were all associated with decreased hippurate excretion, but the interdependence was too complex to allow detailed conclusions on the mechanisms to be drawn (Kawamoto *et al*., 1995).

The toxicokinetics of inhaled toluene have been studied in two groups of healthy volunteers. Löf *et al*. (1990a) exposed six women (26–40 years of age) for 4 h while in a sedentary position to toluene at the Swedish hygienic threshold limit of 3.25 mmol/m^3 (or 300 mg/m^3). Three of the women were rapid hydroxylators and three were slow hydroxylators. Of the inhaled toluene, 51% (range, 48–56) was absorbed, leading to a steady-state blood concentration of 5.0 μM (range, 2.9–9.0) after 90 min. The second, rapid half-life time of elimination ($t_{½\beta}$) was 40 min (range, 25–71 min). Hippurate synthesis was the almost exclusive metabolic pathway, as reflected by its urinary excretion, with *ortho*-cresol excretion 1000-fold lower, in both the rapid and slow hydroxylators. When [$^2H_8$]toluene was used in a similar experiment (Löf *et al*., 1993), three elimination phases with $t_{½}$ of 3 min, 40 min and 740 min were observed. At 4 h after exposure, 65% of the total uptake had been excreted as hippurate; this reached 78% after 20 h. However, in the same period, a more than four-fold higher amount of non-deuterated hippurate was excreted, indicating that at low toluene exposure levels, hippurate cannot be used as an indicator for occupational monitoring (see above). *ortho*-Cresol is not expected to be more reliable for the same reason. This could explain the wide scattering of points when the *ortho*-cresol content in urine was correlated to a presumed toxic effect, urinary excretion of retinol-binding protein, in workers who had been exposed to less than 100 ppm [380 mg/m^3] toluene (Ng *et al*., 1990).

The acute interaction with ethanol was studied by oral administration of toluene as a 2 mg/min infusion for 3 h through a feeding tube into the stomach (Baelum *et al*., 1993). The infusion was chosen such that the exposure level was similar to inhalation of approximately 200 mg/m^3 in combination with light exercise (50 W). Toluene was measured in exhaled air to monitor the toluene concentration in alveolar arterial blood. When ethanol was co-administered orally at a dose of 0.32 g/kg bw, a pronounced increase in the alveolar toluene concentration occurred, from 0.07 (range, 0.00–0.12) without ethanol to 74 (range, 60–93) mg/m^3 with ethanol. The rate of urinary excretion of the hippurate was reduced by ethanol, but otherwise little affected. Excretion of *ortho*-cresol

increased from a total per person of 1.7 (range, 0.6–3.5) μmol without ethanol to 2.9 (range, 2.3–3.7) μmol with ethanol. A very high hepatic extraction ratio of virtually 100% was calculated, but this is probably an overestimate. The results indicate that a single alcoholic drink has a very strong, acute inhibitory effect on the hepatic elimination of toluene. The site of this inhibition has not been identified, but the formation of benzylic alcohol seems to be most affected.

4.1.2 *Experimental systems*

In guinea-pigs, the presence of surfactants (e.g., Triton X-45 or X-100) decreased the skin absorption of toluene (Boman *et al*., 1989). Intermittent skin exposure (for 1 min, every 30 min, repeated eight times) resulted in a blood toluene area-under-the-curve (AUC) of 16% compared to that seen with continuous toluene exposure (Boman *et al*., 1995), with little change in the extent of absorption at each repeated exposure, indicating that the skin did not become more permeable with repeated exposure.

Sullivan and Conolly (1988) compared toluene levels in the blood of Sprague-Dawley rats after inhalation with those seen after subcutaneous or oral administration. They concluded that, at low exposure levels, subcutaneously administered toluene better mimics steady-state levels observed after inhalation exposure, while at high exposures, oral dosage gives satisfactory results. However, orally administered toluene was more rapidly eliminated, presumably because of first-pass oral metabolism.

Tardif *et al*. (1992, 1993a, 1997) have developed a physiologically based toxicokinetic model for toluene in rats (and humans—see Section 4.1.1). They determined the conditions under which interaction between toluene and xylene(s) occurred during inhalation exposure, leading to increased blood concentrations of these solvents, and decreased levels of the hippurates in urine. Similar metabolic interactions have been observed for toluene and benzene in rats (Purcell *et al*., 1990): toluene inhibited benzene metabolism more effectively than the reverse. Tardif *et al*. (1997) also studied the exposure of rats (and humans) to mixtures of toluene, *meta*-xylene and ethylbenzene, using their physiologically based pharmacokinetic model; the mutual inhibition constants for their metabolism were used for simulation of the human situation.

Studies with rat liver microsomes using CYP isoenzyme-specific monoclonal antibodies showed that CYP2E1 and CYP2C11/6 contribute to the oxidation of toluene to benzyl alcohol and *para*-cresol; the 2E1 activity was increased by a one-day fast as well as by ethanol treatment. Phenobarbital and 3-methylcholanthrene treatment reduced the activities of both isoenzymes. CYP2B1/2B contribute to formation of benzyl alcohol, *ortho*- and *para*-cresol, while CYP1A1/1A2 convert toluene to *ortho*-cresol exclusively. Mouse liver microsomes form more *ortho*- and *para*-cresol than those from rats. Effects on the various toluene-metabolizing CYP isoenzymes of sex, age and pregnancy in rat liver have been studied in relation to toluene oxidation. Adult males had higher activities than females, whereas at three weeks of age there was no difference (Nakajima *et al*., 1991, 1992, 1993). Exposure of rats to toluene in air for 6 h (500–4000 ppm [1900–15 200 mg/m^3]) induced the hepatic CYP2E1, CYP2B1/2 and CYP3A1/2, but reduced CYP2C11/6, and had no effect on

CYP1A1/1A2 (Wang *et al*., 1993; see Nakajima & Wang, 1994 for review). Cytochrome activities in the lung of rats, on the other hand, were reduced within 1 h by intraperitoneal toluene exposure (1 g toluene/kg bw) (Furman *et al*., 1991).

Some [*methyl*-^{14}C]toluene becomes covalently bound during incubation with rat liver microsomes (Gut *et al*., 1996).The oxidative metabolism of toluene is induced by phenobarbital (CYP2B1) and benzene (CYP2E1) exposure (Gut *et al*., 1996); phenobarbital also increases covalent binding of toluene, but the nature of this binding has not been determined. At oral and intraperitoneal doses of 100–370 mg/kg toluene, urinary thioether excretion was increased, suggesting that a mercapturate may have been present, but this has not been characterized (van Doorn *et al*., 1980). [Other authors have never mentioned mercapturates as toluene metabolites, although benzyl mercapturic acid has been identified as a metabolite of benzyl alcohol derived from benzyl acetate.]

In isolated rat hepatocytes obtained from acetone- or phenobarbital-treated rats, the metabolism of toluene at low (below 100 µM) or high (100–500 µM) concentration was increased, in particular after phenobarbital treatment. Ethanol (7 and 60 mM) inhibited the overall metabolism of toluene (sum of benzyl alcohol, benzaldehyde, benzoic acid and hippuric acid), leading to accumulation of benzyl alcohol (Smith-Kielland & Ripel, 1993).

When rats were treated with ethanol (2 g ethanol/80 mL liquid diet) or phenobarbital (4 days at 80 mg/kg intraperitoneally) before inhalation exposure to toluene (50–4000 ppm [1900–15 200 mg/m^3]), the urinary excretion of all metabolites (hippurate, acyl glucuronide, benzoate, *ortho*- and *para*-cresol) was increased, in particular after phenobarbital treatment and toluene exposures of about 2000 ppm [7600 mg/m^3] (Wang & Nakajima 1992). In the phenobarbital-treated group, the 4000-ppm exposure became quite toxic, leading to death of several rats. In rats treated only with toluene, the hippurate was by far the major metabolite (over 90%), with the acyl glucuronide appearing at higher toluene concentrations. The contribution of the cresol conjugates was minor.

4.1.3 *Comparison of human and rodent data*

In a general sense, the kinetics and metabolism of toluene in humans, rats and mice are very similar: the hippurate is in all cases by far the major metabolite, while in all species the *ortho*- and *para*-cresols are minor metabolites. To what extent formation of a potentially reactive sulfate conjugate of benzyl alcohol occurs (van Doorn *et al*., 1980; Chidgley *et al*., 1986) is uncertain, mainly because mercapturates formed from toluene have not been characterized. Similarly, whether the covalent binding observed in rat liver microsomes has any toxicological relevance is uncertain.

Although in rats and mice toluene may induce several CYP isoenzymes, exposure in humans is normally too low to be likely to cause such induction; however, toluene sniffers may expose themselves repeatedly to such high concentrations that induction could occur (Nakajima & Wang, 1994).

4.2 Toxic effects

Prolonged contact between toluene and human skin may cause nonallergic contact dermatitis. Human exposure to toluene also causes nervous system symptoms and signs and excessive exposure may cause adverse effects on the kidney and liver. Adverse effects on the nervous system have been observed in experimental animals. In studies of spontaneous abortion, perinatal mortality and congenital malformations in humans, the numbers of cases were small and the mothers had also been exposed to other substances. Embryotoxicity that generally occurs concurrently with maternal toxicity has been seen in some studies in mice and rats but not rabbits (IARC, 1989a).

4.2.1 *Humans*

Increased frequency of subjective symptoms, but no indication of hepatic or renal damage, was observed among 452 toluene-exposed workers, when the actual toluene exposure was 24.7 ± 4.43 ppm [93 ± 17 mg/m^3] (geometric mean ± standard deviation) and toluene represented more than 90% of the airborne solvent vapours (Ukai *et al*., 1993). Similarly, no clinical chemical indication of hepatic damage was observed among 153 workers with exposure to toluene of 1–60 ppm [3.8–230 mg/m^3] during workdays for two to five years (Wang *et al*., 1996).

Several cases of severe metabolic acidosis after recreational toluene sniffing have been described; renal tubule damage has been proposed as the pathogenetic mechanism (Batlle *et al*., 1988; Goodwin, 1988; Pearson *et al*., 1994; Hong *et al*., 1996).

4.2.2 *Experimental systems*

Intraperitoneal injection of male Charles-Foster rats with 0.2 mL of a 5 mmol/L toluene solution on alternate days for 30 days resulted in slight increases of serum aspartate- and alanine aminotransferases, alkaline phosphatase and bilirubin (Rana & Kumar, 1993).

Inhalation exposure to toluene (1000 ppm [3800 mg/m^3], 6 h per day, five days per week for three months) of male Wistar rats had a very slight effect on the hepatic ultrastructure; limited proliferation of smooth endoplasmic reticulum and an increase of lysosomes were observed. Similar findings were observed after six months' exposure to 100 ppm [380 mg/m^3] toluene. The proliferation of smooth endoplasmic reticulum was more prominent after simultaneous exposure to 500 ppm toluene and 500 ppm *meta*-xylene (Rydzyński *et al*., 1992).

Administration of toluene (1 g/kg intraperitoneally) to male CD rats increased the formation of the fluorescent 2′,7′-dichlorofluorescein from the non-fluorescent 2′,7′-dichlorofluorescin by isolated cortical synaptosomes and microsomes, indicating generation of reactive oxygen species, but did not increase the amount of conjugated dienes (Mattia *et al*., 1991).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

In a case–control study (Lindbohm *et al*., 1990), spontaneous abortions were investigated in a cohort of women who had at some time been biologically monitored for exposure to solvents. Data on pregnancies, congenital malformations and spontaneous abortions were collected from national registries and polyclinic archives. Exposure to toluene during pregnancy of cases (women with spontaneous abortion) and controls (normal birth) was assessed by an industrial hygienist based on an extensive questionnaire. The odds ratio for exposure to toluene was slightly elevated; it was higher for 'low' exposure (1.8; 95% CI, 0.7–4.7) than for 'high' exposure (odds ratio, 1.4; 95% CI, 0.4–4.9), and the risk was limited to 'shoe work' (odds ratio, 9.3; 95% CI, 1.0–84.7; 5 cases). 'High/frequent' paternal exposure was also related to spontaneous abortions (odds ratio, 2.3; 95% CI, 1.1–4.7; 28 cases) (Taskinen *et al*., 1989). No relationship between paternal or maternal exposure and congenital malformations was observed. In a similar case–control study on solvent exposure and pregnancy outcome among laboratory assistants (Taskinen *et al*., 1994), the odds ratio for spontaneous abortion was increased among women who were exposed to toluene on at least three days a week during the first trimester of the pregnancy (odds ratio, 4.7; 95% CI, 1.4–15.9; 10 cases). No elevated odds ratio for congenital malformations was observed for any solvent, but the power of the study was limited.

In a study of spontaneous abortions (Ng *et al*., 1992), reproductive and occupational exposure history was obtained from 55 women exposed to toluene (actual mean, 88 ppm [332 mg/m^3]; range, 50–150 ppm [188–565 mg/m^3]) and two control groups (one of which consisted of a 0–25-ppm [0–94 mg/m^3] toluene exposure group). Spontaneous abortion rate was 12.4% among the 50–150-ppm exposed group, 2.9% in the 0–25-ppm exposure group and 4.5% in the control group. [The Working Group noted the low frequency of spontaneous abortions among the controls and the bias-prone method for ascertainment of cases.] Among 20 toluene-exposed rotogravure printers (median actual air toluene concentration, 36 ppm [136 mg/m^3]), plasma follicle stimulating and luteinizing hormone levels were lower than those in 44 unexposed referents (Svensson *et al*., 1992).

Several case series have demonstrated that high exposure to toluene through sniffing during pregnancy induces a syndrome that closely resembles the fetal alcohol syndrome, with pre- and postnatal growth deficiency, microcephaly and developmental delay, typical craniofacial features including micrognathia, small palpebral fissures and ear anomalies (Goodwin, 1988; Hersch, 1989; Arnold *et al*., 1994; Pearson *et al*., 1994).

4.3.2 *Experimental systems*

When pregnant Sprague-Dawley rats were exposed to toluene (6 h per day on days 7 through 17 of gestation), weight suppression of the dams and of the offspring, as well as high fetal mortality and retardation of embryonic growth, but no external, internal or skeletal anomalies, or deterioration of pre- or postweaning behavioural test scores were

observed at an exposure level of 2000 ppm [7540 mg/m^3]. No adverse effects were observed at an exposure level of 600 ppm [2260 mg/m^3] (Ono *et al*., 1995).

Toluene (1.2 g/kg bw per day) given by subcutaneous injection on days 14 through 20 to pregnant Wistar rats caused decreased body weight gain in the pups that persisted into adulthood. No such effect was observed when the same dose was administered on days 8 through 15. No malformations, variations in skeletal development or long-lasting behavioural changes were observed (da Silva *et al*., 1990). Similar reduction in the gain of body and organ weight was observed after administration of 520 mg/kg bw of toluene by gavage to Sprague-Dawley rats on days 6 through 19 of gestation. No effect on the number of implantations, stillbirths or malformations was observed (Gospe *et al*., 1994).

Sprague-Dawley rats exposed to toluene (982 ± 52 ppm [3700 ± 196 mg/m^3], 18 h per day, on seven days per week for 61 days) showed no evidence of histological damage to the testes two weeks or 10 months after cessation of the exposure (Nylén *et al*., 1989). Toluene (the concentration of which decreased during the incubations) did not induce malformations in explanted rat embryos at the highest concentrations tested (0.23–0.09 mg/L), but retarded the growth of the embryos at the lowest concentration tested (0.05–0.02 mg/L) (Brown-Woodman *et al*., 1991).

4.4 Genetic and related effects

4.4.1 *Humans*

Richer *et al*. (1993) exposed five male volunteers to 50 ppm [188.5 mg/m^3] toluene in a controlled exposure chamber for 7 h per day for three days on three occasions at two-week intervals. Blood samples were taken before and after each three-day exposure. No effects upon sister chromatid exchange frequencies were observed.

The frequencies of chromosomal aberrations were measured in peripheral blood lymphocytes of 24 men in Italy (aged 29–60 years) who had been employed for 3–15 years in a rotogravure room in which the annual mean toluene concentrations were 56–277 ppm [210–1040 mg/m^3]. They were compared with data for 24 male, age-matched controls from the general population. No significant difference was observed (Forni *et al.*, 1971). [The Working Group noted that smoking and alcohol drinking habits were not considered.]

An excess of chromosomal aberrations (chromatid and isochromatid breaks) was reported in the lymphocytes of 14 Swedish workers (aged 23–54 [sex unspecified]) exposed to toluene for 1.5–26 years (average level, 100–200 ppm [377–750 mg/m^3]) with occasional excursions to 500–700 ppm [1900–2640 mg/m^3] in a rotogravure printing factory in comparison with 42 healthy, but unmatched unexposed male and female adult controls (Funes-Cravioto *et al.*, 1977). [The Working Group noted that smoking and alcohol drinking habits were not considered, and that the appropriateness of the controls cannot be judged.]

No differences were found in the frequencies of chromosomal aberrations or sister chromatid exchanges in the peripheral blood lymphocytes of 32 men (aged 21–50 years) employed in a rotogravure factory in Finland and exposed to toluene (7–112 ppm

[26–420 mg/m^3]) for 3–35 years in comparison with 15 men (aged 27–62 years) from a research institute. Benzene contamination of the toluene had been checked since 1962 and was always below 0.05%, averaging 0.006% (Mäki-Paakkanen *et al.*, 1980). No increase in the frequency of sister chromatid exchanges was observed in seven workers in the Swedish paint industry who were exposed to various solvents, including more than 100 mg/m^3 toluene, each compared with a control matched by age, sex, place of residence and smoking habits (Haglund *et al.*, 1980; see also IARC, 1989b). [The Working Group noted the small number of workers studied.]

Bauchinger *et al.* (1982) reported increases in the frequencies of sister chromatid exchanges, chromatid breaks, chromatid exchanges and gaps in the peripheral lymphocytes of 20 workers (aged 32–60 years) at a rotogravure plant in the Federal Republic of Germany who had been exposed to toluene (200–300 ppm [750–1130 mg/m^3]) for more than 16 years, compared with 24 matched controls from the same factory. For breaks, exchanges and sister chromatid exchanges per cell ± SE, respectively, the frequencies were: controls, 0.0019 ± 0.0005, 0.004 ± 0.0002, 8.18 ± 0.25; toluene-exposed, 0.0036 ± 0.0002, 0.0015 ± 0.0005, 9.62 ± 0.37. Much of the increase in breaks was due to a single individual and the difference in sister chromatid exchanges was partially due to those who smoked tobacco. For sister chromatid exchanges, grouped according to smoking habits, the results were: (*a*) nonsmokers; controls, 7.75 ± 0.25 (n = 15); toluene-exposed, 8.55 ± 0.27 (n = 8); (*b*) smokers; controls, 8.89 ± 0.41 (n = 9); toluene-exposed, 10.33 ± 0.49 (n = 12). A significant increase in gaps was also found, although this was small: controls, 0.019 ± 0.003; toluene-exposed, 0.0248 ± 0.0024. In an abstract, a synergistic effect of smoking and exposure to toluene on the frequency of sister chromatid exchanges was also reported (Bauchinger *et al.*, 1983). Schmid *et al.* (1985) examined lymphocytes from 27 workers in the same plant who, at the time of blood sampling, had not been exposed to toluene for from four months to five years. In comparison with 26 controls, those who had been exposed within the last two years (n = 13) showed higher numbers of chromatid breaks per 100 cells, whereas those not exposed for more than two years (n = 14) had the same chromatid break frequency as the controls: 0.20 ± 0.05; not exposed to toluene > 2 years, 0.20 ± 0.06; not exposed to toluene < 2 years, 0.39 ± 0.07.

The frequency of chromosomal aberrations in 20 employees exposed mainly to toluene in various printing inks at a rotogravure plant was no different from that in 23 control workers; an increased frequency was observed in smokers in both groups (Pelclová *et al.*, 1987).

In 1990, Pelclová *et al.* extended their analysis of chromosome aberrations in rotogravure printers, carrying out chromosome analysis in peripheral lymphocytes of three groups of workers. There were 42 rotogravure printers (37 smokers, 5 nonsmokers; mean age, 39 years) exposed to rotogravure printing dyes and highly purified toluene at working air concentrations of 104–1170 ppm [390–4380 mg/m^3] for 12 years on average, 28 office and technical employees of the same plant (17 smokers, 11 nonsmokers; mean age, 44 years), more than half of whom worked for 2 h daily in the rotogravure workshop and a control population consisting of 32 employees (17 smokers, 15 nonsmokers; mean

age, 37 years) from a nearby brewery and dairy. Air pollution was stated to be 'high' in this area of the town. Measurements of blood toluene and urinary hippuric acid were made at the end of a work shift. The values (± standard deviation) for the controls, office/technical workers and printers, respectively, were: blood toluene – not measured, 10.3 ± 3.1 and 124.0 ± 63.1 μmol/L; urinary hippuric acid – 6.31 ± 3.41, 12.89 ± 4.64 and 38.28 ± 17.53 mmol/L. Increased incidences of chromatid breaks were observed in the printer and the office/technical groups, while gaps per cell and chromosomal exchanges were increased only in the office/technical group. Chromatid breaks per cell, the most prominent chromosomal damage, in the three groups were: 0.0153 ± 0.0119, 0.0211 ± 0.0143 ($p < 0.01$) and 0.0250 ± 0.0195 ($p < 0.01$), while the frequencies of chromosomal exchanges and gaps were significantly increased only in the office/technical workers: chromosomal exchanges per cell were 0.0013 ± 0.0042, 0.0029 ± 0.0045 ($p < 0.05$) and 0.0007 ± 0.0026; gaps per cell were 0.0288 ± 0.0209, 0.0443 ± 0.0278 ($p < 0.05$) and 0.0371 ± 0.0202. The high incidence of aberrations could be explained by the exposure to toluene, but an influence of rotogravure printing dyes cannot be excluded. Smoking and high air pollution in the urban area were contributing factors in all three groups.

Nise *et al*. (1991) compared the frequencies of chromosomal aberrations and nuclei in lymphocytes of 21 men (aged 30–63 years; 10 smokers, 11 nonsmokers) exposed to toluene for 0.5–37 years during their employment as rotogravure printers and 21 controls (aged 30–63 years [sex not stated]; 13 smokers, 8 nonsmokers). The median time-weighted air level of toluene over a one-week period in 1986 was 150 mg/m^3 for the printers and the median blood concentrations on the day of lymphocyte sampling were: controls ≤ 0.01 μmol/L; toluene-exposed, 1.6 μmol/L (range, 1.0–6.6). Earlier toluene exposures were estimated to be about 800 mg/m^3 in the 1970s and about 1500 mg/m^3 in the 1950s and 1960s (when contaminating benzene exposures would have been about 150 mg/m^3). Lymphocytes were treated with either phytohaemagglutinin (PHA), which stimulates T cells, or pokeweed mitogen (PWM), which stimulates B cells. There was a significant increase in the frequency of micronuclei in PWM-stimulated peripheral blood lymphocytes in the printers, as compared to the controls (2.8‰ versus 1.5‰, $p = 0.03$; all p adjusted for age and smoking). The frequency of small micronuclei (size ratio micronuclei/main nucleus ≤ 0.03) in PWM-stimulated lymphocytes was associated with the exposure (1‰ versus 0.3‰; $p = 0.05$). Furthermore, among the exposed subjects there was an association between blood toluene and small micronuclei (0.17‰ per mmol/L; $p = 0.0005$). Small micronuclei in PHA-treated cultures showed no association with any exposure parameter. However, in the printers, an estimated cumulative exposure index was weakly correlated with the frequency of total micronuclei in PHA-stimulated cells (0.00003‰ per mg/m^3 × year; $p = 0.07$). Among the printers, chromosomal breaks in PHA-stimulated cells were associated with the duration of earlier benzene-contaminated toluene exposure (0.03% per year; $p = 0.01$); benzene contamination was about 10% up to 30 years previously, around 0.5% more recently and falling to < 0.01% at the time of the study.

Popp *et al.* (1992) analysed the frequencies of sister chromatid exchanges and of DNA strand breakage/cross-linking (alkaline elution assay) in a group of 20 women (45 smokers, 16 nonsmokers) working in a shoemaking plant who were exposed to benzene (mean, 4.16 mg/m^3) and toluene (mean, 70.06 mg/m^3) for at least eight years; the results were compared with those from a group of 20 non-exposed women (4 smokers, 16 non-smokers) from the general population. Sister chromatid exchange frequencies were significantly higher, but only marginally so, among the solvent-exposed women compared with all controls: controls, 6.05 ± 1.01; toluene-exposed, 6.55 ± 0.70 ($p < 0.05$, Wilcoxon test); and among the smokers in the control group compared with nonsmokers in the same group. No comment was made upon the higher average sister chromatid exchange frequency in the smoking controls compared with the smoking toluene-exposed group: controls, 7.19 ± 1.43; toluene-exposed, 6.54 ± 0.32. The relative DNA elution rate through polycarbonate filters was significantly increased ($p < 0.001$). The elution rate through polyvinylidene fluoride (HVLP) filters also showed a tendency to increase ($p = 0.052$). The sister chromatid exchange rates of the female workers were significantly correlated ($p < 0.01$) with the relative DNA elution rate through HVLP filters. There was no correlation with the actual benzene and toluene uptake measured by personal air monitoring. Four months after cessation of work, DNA strand breakage decreased significantly ($p < 0.05$) in blood samples of six reinvestigated exposed women.

4.4.2 *Experimental systems* (see Table 2 for references)

The genetic and related effects of toluene have been reviewed (Dean, 1978, 1985; Fishbein, 1988; IARC, 1989a; McGregor, 1994).

When tested in bacteria, toluene did not induce prophage, differential killing or gene mutation. In single studies with *Saccharomyces cerevisiae*, toluene did not induce either gene conversion or gene mutation (WHO, 1985, secondary description).

Toluene did not induce sex-linked recessive lethal mutations or translocations, but did induce sex-chromosome loss and nondisjunction in male *Drosophila melanogaster* and induced mitotic arrest (C-mitosis) in embryos of the grasshopper, *Melanoplus sanguinipes.*

Toluene did not enhance morphological transformation of Syrian hamster embryo cells by the SA7 adenovirus or, as reported in an abstract, disruption of gap-junctional intercellular communication in Chinese hamster V79 cells (Awogi *et al.*, 1986).

Toluene induced DNA single-strand breaks (as measured by alkaline elution) in primary cultures of rat hepatocytes but did not cause DNA damage or repair, as measured by the 'nick-translation' assay, in cultured human fibroblasts. Toluene induced *tk* locus mutations in mouse lymphoma L5178Y cells in one study but not in another which was reported as an abstract (Lebowitz *et al.*, 1979). It did not induce sister chromatid exchanges or chromosomal aberrations in either Chinese hamster ovary cells (WHO, 1985, secondary description) or human lymphocytes *in vitro*. [The Working Group noted that the tests with human lymphocytes were conducted only without an exogenous metabolic system.]

In a single study, toluene induced kinetochore- and centromere-negative micronuclei in human MCL-5 cells that stably express cDNAs encoding human CYP1A2, CYP2A6,

Table 2. Genetic and related effects of toluene

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links	–	–	100	Nakamura *et al*. (1987)
PRB, Prophage induction, *Escherichia coli* WP2s (λ)	–	–	NG	Rossman *et al*. (1991)
ECL, *Escherichia coli pol*A, differential toxicity (liquid suspension test)	–	–	400000	McCarroll *et al*. (1981b)
ERD, *Escherichia coli rec* strains, differential toxicity	–	–	400000	McCarroll *et al*. (1981b)
BSD, *Bacillus subtilis rec* strains, differential toxicity	–	–	127000	McCarroll *et al*. (1981a)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2150	Nestmann *et al*. (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1000	Bos *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500	Spanggord *et al*. (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	167	Haworth *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1000	Connor *et al*. (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2150	Nestmann *et al*. (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1000	Bos *et al*. (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500	Spanggord *et al*. (1982)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	167	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2150	Nestmann *et al*. (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1000	Bos *et al*. (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500	Spanggord *et al*. (1982)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	167	Haworth *et al*. (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2150	Nestmann *et al*. (1980)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1000	Bos *et al*. (1981)

Table 2 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2500	Spanggord *et al*. (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2150	Nestmann *et al*. (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	Bos *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	Spanggord *et al*. (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	167	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	Connor *et al*. (1985)
SAS, *Salmonella typhimurium* UTH8413, reverse mutation	–	–	1000	Connor *et al*. (1985)
SAS, *Salmonella typhimurium* UTH8414, reverse mutation	–	–	1000	Connor *et al.* (1985)
Melanoplus sanguinipes embryo, C-mitosis	+		40 000 ppm inh	Liang *et al*. (1983)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		13 000 ppm feed	Rodriguez Arnaiz & Villalobos-Pietrini (1985b)
DMH, *Drosophila melanogaster*, heritable translocations	–		13 000 ppm feed	Rodriguez Arnaiz & Villalobos-Pietrini (1985b)
DMN, *Drosophila melanogaster*, aneuploidy	+		8700 ppm feed	Rodriguez Arnaiz & Villalobos-Pietrini (1985a)
DIA, DNA strand breaks, rat hepatocytes *in vitro*	+	NT	3	Sina *et al*. (1983)
G5T, Gene mutation, mouse lymphoma L5187Y cells, *tk* locus *in vitro*	+	+	200	McGregor *et al*. (1988)
T7S, Cell transformation, SA7/Syrian hamster embryo cells *in vitro*	–	NT	1000	Casto (1981)
DIH, DNA damage, human diploid fibroblasts *in vitro*	–	NT	276	Snyder & Matheson (1985)

Table 2 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
RIH, DNA repair (nick translation), human diploid fibroblasts *in vitro*	–	NT	276	Snyder & Matheson (1985)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	NT	1500	Gerner-Smidt & Friedrich (1978)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	NT	92	Richer *et al*. (1993)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	1500	Gerner-Smidt & Friedrich (1978)
AIH, Aneuploidy, AHH-1 cells, kinetochore staining *in vitro*	(+)	NT	460	Doherty *et al*. (1996)
AIH, Aneuploidy, MCL-5 cells, kinetochore staining *in vitro*	(+)	NT	460	Doherty *et al*. (1996)
AIH, Aneuploidy, h2E1 cells, kinetochore staining *in vitro*	+	NT	184	Doherty *et al*. (1996)
MIH, Micronucleus test, AHH-1 cells *in vitro*	(+)[c]	NT	460	Doherty *et al*. (1996)
MIH, Micronucleus test, MCL-5 cells *in vitro*	+[c]	NT	9.2	Doherty *et al*. (1996)
MIH, Micronucleus test, h2E1 cells *in vitro*	+[c]	NT	9.2	Doherty *et al*. (1996)
DVA, DNA strand breaks, female BDF_1 mouse blood, bone marrow and liver *in vivo* (comet assay)	–		500 ppm inh 6 h/d 5 d/wk 8 wk	Plappert *et al*. (1994)
MVM, Micronucleus test, CD-1 mouse bone marrow *in vivo*	–		1720 po × 2	Gad-el-Karim *et al*. (1984)
MVM, Micronucleus test, male NMRI mouse bone marrow *in vivo*	+		217 po × 2	Mohtashamipur *et al*. (1985)
MVM, Micronucleus test, CD-1 mouse bone marrow *in vivo*	–		860 po × 1	Gad-el-Karim *et al*. (1986)
MVM, Micronucleus test, male $B6C3F_1$ mouse bone marrow *in vivo*	+		104 ip × 2	Mohtashamipur *et al*. (1987)
MVR, Micronucleus test, male Sprague-Dawley rat bone marrow *in vivo*	(+)		217 ip × 2	Roh *et al*. (1987)

Table 2 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CBA, Chromosomal aberrations, rat bone marrow *in vivo*	+		800 sc × 12	Dobrokhotov (1972)
CBA, Chromosomal aberrations, rat bone marrow *in vivo*	+		1000 sc × 12	Lyapkalo (1973)
CBA, Chromosomal aberrations, male albino rat bone marrow *in vivo*	+		162 ppm inh 4 h/d 5 d/wk 16 wk	Dobrokhotov & Enikeev (1977)
CBA, Chromosomal aberrations, rat bone marrow *in vivo*	–		1.5 ppm inh 4 h/d 5 d/wk 16 wk	Aristov *et al*. (1981)
CBA, Chromosomal aberrations, CD-1 mouse bone marrow *in vivo*	–		1720 po × 2	Gad-el-Karim *et al*. (1984)
CBA, Chromosomal aberrations, male Sprague-Dawley rat bone marrow *in vivo*	+		435 ip × 2	Roh *et al*. (1987)
SPM, Sperm morphology, (CBA × BALB/c) mice *in vivo*	–		900	Topham (1980)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; ip, intraperitoneal; sc, subcutaneous; inh, inhalation

[c] Primarily kinetochore-negative micronuclei (greater percentage stain kinetochore-positive at 2–5 mM doses in MCL-5 and h2E1 cells)

CYP3A4, CYP2E1 and epoxide hydrolase and in h2E1 cells which contain a cDNA for CYP2E1; kinetochore-positive micronuclei were induced only at the highest dose. AHH-1 cells constitutively expressing CYP1A1 showed a small increase in micronucleus frequency.

In the single cell gel electrophoresis assay, no DNA breakage/alkali-labile sites were detected in blood, bone marrow or liver of mice exposed to 500 ppm [1900 mg/m^3] toluene for 6 h per day on five days per week for eight weeks. Toluene was reported to induce chromosomal aberrations in the bone-marrow cells of rats following exposure by inhalation and subcutaneous or intraperitoneal injection but not in that of orally dosed mice or in other single rat studies with exposure by inhalation (Donner *et al.*, 1981, abstract only), oral gavage (Feldt *et al.*, 1985) or intraperitoneal injection (WHO, 1985, secondary description). The frequency of micronucleated bone-marrow cells of rats given intraperitoneal injections was slightly increased, while micronuclei were more frequent in toluene-treated mice after intraperitoneal injection, but not after oral dosing in a different laboratory or after intraperitoneal injection with doses of up to 1000 mg/kg bw (WHO, 1985, secondary description). It was noted that pretreatment of male NMRI mice with inducers (phenobarbital, Aroclor 1254, 3-methylcholanthrene) of cytochrome P450 enhanced the frequency of micronuclei induced by toluene, while simultaneous injections of toluene with inhibitors (metyrapone, α-naphthoflavone) decreased the observed clastogenic activities (Mohtashamipur *et al.*, 1987).

Toluene reduced the number of sister chromatid exchanges induced by benzene when both compounds were administered intraperitoneally to DBA/2 mice (Tice *et al.*, 1982) and greatly reduced the frequency of micronuclei induced by benzene when the two compounds were simultaneously administered orally to CD-1 mice (Gad-El-Karim *et al.*, 1984), intraperitoneally to Sprague-Dawley rats (Roh *et al.*, 1987) or subcutaneously to NMRI mice (Tunek *et al.*, 1982).

As reported in an abstract, oral administration of toluene did not induce dominant lethal effects in random-bred male SHR mice (Feldt *et al.*, 1985).

Toluene did not induce sperm-head abnormalities in mice.

Toluene can activate cyclin-dependent kinase 2 in rat liver epithelial (RLE) and HL60 cells *in vitro* and it also causes hyperphosphorylation of p53 and pRB105 in these cells. These activities are shared with benzene but, unlike benzene, toluene did not increase the p53–DNA site-specific binding in RLE cells (Dees & Travis, 1994; Dees *et al.*, 1996).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Toluene is an industrial chemical produced in high volume, that is used in blending gasoline and as a solvent. Occupational exposure to toluene is extensive and occurs in its production and during the manufacture and use of toluene-containing paints, thinners, cleaning agents, coatings and adhesives. It is commonly detected in ambient air and at low levels in water.

5.2 Human carcinogenicity data

Toluene was mentioned as an exposure in eight studies. Two were community-based case–control studies, one of which involved brain cancer and one involved several types of cancer. Of the six industry-based studies, three were analysed as cohort studies and three were configured as nested case–control studies of one or a few types of cancer. In two of the studies, that of shoe-manufacturing workers in the United States and particularly that of Swedish rotogravure printers, it was believed that toluene was the predominant exposure; in the other studies, there were probably concomitant exposures. Cancers of most sites were not significantly associated with toluene exposure in any study. Stomach cancer mortality was significantly elevated in the Swedish rotogravure printers study, it was slightly, though not significantly, elevated in two other studies, and it was not associated at all in a fourth. Rates of lung cancer were significantly elevated in the cohort of shoe manufacturers and in the Swedish cohort of rotogravure printers, but was not associated at all in two other studies. Colorectal cancer was significantly elevated in the Swedish rotogravure printers study and in the Canadian case–control study, and colon cancer was nonsignificantly elevated in the shoe manufacturers cohort. While results on leukaemias and lymphomas generally showed no association, these were based on small numbers. Considering the multiple exposure circumstances in most studies and the weak consistency of findings, these results are not strong enough to conclude that there is an association.

5.3 Animal carcinogenicity data

Toluene was tested for carcinogenicity by inhalation exposure in one study in mice and in one study in rats. No significant increase in the incidence of tumours was observed. Repeated application of toluene to the skin of mice did not result in an increased incidence of skin tumours.

5.4 Other relevant data

Toluene is mainly converted to benzyl alcohol and excreted as hippurate. Its toxicokinetics in humans have been extensively studied.

Toluene toxicity is most prominent in the central nervous system after acute and chronic exposure. Reproductive toxicity has been observed in exposed humans and rats.

In the more recent cytogenetic studies in occupationally exposed populations, increases in chromosomal aberrations (two studies), micronuclei (one study) and of DNA strand breaks (one study) have been described. These effects have also been observed in rats and mice in some studies and in cultured mammalian cells. DNA adducts have not been detected.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of toluene.

There is *evidence suggesting lack of carcinogenicity* of toluene in experimental animals.

Overall evaluation

Toluene is *not classifiable as to its carcinogenicity to humans (Group 3).*

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TOLUENE DIISOCYANATES

Data were last reviewed in IARC (1986) and the compounds were classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Commercial toluene diisocyanate mixtures

Chem. Abstr. Serv. Reg. No.: 26471-62-5
Chem. Abstr. Name: 1,3-Diisocyanatomethylbenzene
IUPAC Systematic Name: Isocyanic acid, methyl-*meta*-phenylene ester
Synonyms: Diisocyanatotoluene; TDI; toluene diisocyanate

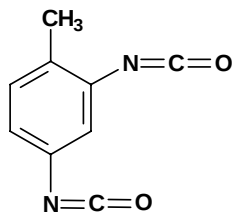
2,4-Toluene diisocyanate

Chem. Abstr. Serv. Reg. No.: 584-84-9
Chem. Abstr. Name: 2,4-Diisocyanato-1-methylbenzene
IUPAC Systematic Name: Isocyanic acid, 4-methyl-*meta*-phenylene ester
Synonyms: 2,4-Diisocyanatotoluene; 2,4-TDI; 2,4-toluene diisocyanate

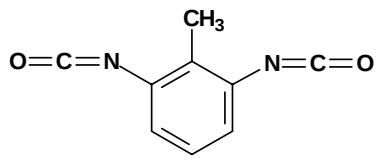
2,6-Toluene diisocyanate

Chem. Abstr. Serv. Reg. No: 91-08-7
Chem. Abstr. Name: 1,3-Diisocyanato-2-methylbenzene
IUPAC Systematic Name: Isocyanic acid, 2-methyl-*meta*-phenylene ester
Synonyms: 2,6-Diisocyanatotoluene; 2,6-TDI; 2,6-toluene diisocyanate

1.1.2 *Structural and molecular formulae and relative molecular mass*



2,4-Toluene diisocyanate



2,6-Toluene diisocyanate

$C_9H_6N_2O_2$ Relative molecular mass: 174.16

1.1.3 *Chemical and physical properties of the pure substances*

(*a*) *Description*: Colourless to pale yellow liquid with pungent odour (United States National Library of Medicine, 1997)
(*b*) *Boiling-point*: 251°C (2,4-isomer) (Lide, 1997)
(*c*) *Melting-point*: 20°C (2,4-isomer); 18°C (2,6-isomer) (Lide, 1997)
(*d*) *Solubility*: 2,4- and 2,6-Toluene diisocyanates decompose in water and are very soluble in acetone and benzene (Lide, 1997)
(*e*) *Vapour pressure*: 1.3 Pa at 20°C (2,4-isomer) (Lewis, 1993)
(*f*) *Flash point*: 132°C (2,4-isomer) (Lewis, 1993)
(*g*) *Conversion factor*: $mg/m^3 = 7.1 \times ppm$

1.2 Production and use

Worldwide production capacities for toluene diisocyanates in 1987 were reported as (thousand tonnes): western hemisphere, 356; eastern Europe, 46; western Europe, 380; and Japan and the Far East, 88 (Ulrich, 1989). Worldwide production capacities in 1993 were reported as (thousand tonnes): North America, 485; Europe, 530; Pacific region, 308; and Latin America, 102.5 (Anon., 1995).

Toluene diisocyanate is commonly produced as a mixture of the 2,4- and 2,6-isomers, that is used as a monomer in the preparation of polyurethane foams, elastomers and coatings, as a cross-linking agent for nylon-6, and as a hardener in polyurethane adhesives and finishes. Polyurethane elastomers made from toluene diisocyanates are used in coated fabrics and clay-pipe seals. Polyurethane coatings made from toluene diisocyanates are used in floor finishes, wood finishes and sealers, and in coatings for aircraft, tank trucks, truck trailers and truck fleets (United States National Library of Medicine, 1997).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 40 000 workers in the United States were potentially exposed to toluene diisocyanates (see General Remarks). Occupational exposures to toluene diisocyanates may occur during their production and in the production of polyurethane foams, elastomers, coatings, adhesives and finishes. Exposure may also occur in the use of some polyurethane products. Data on occupational exposure levels have been presented in a previous monograph (IARC, 1986). More recent exposure levels have been reported in connection with epidemiological (Section 2) and toxicological (Section 4) studies.

1.3.2 *Environmental occurrence*

Toluene diisocyanates may be released to the environment as fugitive emissions and from stack exhaust during the production, transport and use of toluene diisocyanate in the manufacture of polyurethane foam products and coatings. They have been detected at low levels in wastewater samples (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 0.036 mg/m^3 as the 8-h time-weighted average threshold limit value for occupational exposures to 2,4-toluene diisocyanate in workplace air. Similar values have been used as standards or guidelines for 2,4- or 2,6-toluene diisocyanates in several countries. In some other countries, values ranging from 0.04 to 0.14 mg/m^3 for mixed isomers have been used (International Labour Office, 1991).

No international guideline for toluene diisocyanates in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

2.1 Cohort studies

Sorahan and Pope (1993) studied 5824 men and 2465 women who had been employed for at least six months during 1958–79 at 11 factories in England and Wales which made polyurethane foams. Exposures to isocyanates were classified by an occupational hygienist on the basis of recorded job titles. The highest-exposure category comprised jobs in which either the 8-h time-weighted average exposure during 1978–86 was greater than 4 ppb [28.4 μg/m^3] or peak exposures exceeded 10 ppb [71 μg/m^3] on most days. Cohort members were followed up through National Health Service records, and their mortality during 1958–88 and cancer incidence during 1971–86 were compared with national rates by the person–years method. In addition, internal comparisons of risk according to exposure were carried out by Poisson regression analysis, and through a nested case–control study. Overall mortality in the cohort was close to expectation (816 deaths; standardized mortality ratio (SMR), 0.97), as was total mortality from cancer (221 deaths; SMR, 0.9). In men, no notable elevation of mortality was recorded for any specific cancer. In women, significant excesses of deaths were observed for cancers of the pancreas (6 versus 2.2 expected; SMR, 2.7; 95% confidence interval (CI), 1.0–6.0) and lung (16 versus 9.1 expected; SMR, 1.8; 95% CI, 1.0–2.9). However, there was no significant elevation of mortality from these tumours in both sexes combined (pancreas, 14 deaths versus 10.3 expected; lung, 81 deaths versus 81.3 expected), and in the internal analyses, risk was not related to isocyanate exposure. Mortality from rectal cancer was low (5 deaths versus 10.2 expected in men and women combined). In female workers, high rates of pancreatic (standard rate ratio (SRR), 3.2) and lung cancer (SRR, 2.3) were seen, as well as increased incidence of cancers of the larynx (3 cases versus 0.3 expected) and kidney (4 versus 0.9), but all of the cases were classified as having minimal or zero exposure to isocyanates. An earlier survey in the industry had indicated a high prevalence of smoking among female employees, and the authors concluded that this may have contributed to the increased frequency of some cancers in women.

In Sweden, a cohort study was carried out at nine factories manufacturing polyurethane foam that incorporated toluene diisocyanates or methylenediphenyl diiso-

cyanate (see this volume) (Hagmar *et al*., 1993a). Exposures to airborne isocyanates had been monitored at all of the plants since 1965. Time-weighted average concentrations of toluene diisocyanates had generally been below 100 μg/m^3, and those of methylenediphenyl diisocyanate below 10 μg/m^3, but with peaks up to 3 mg/m^3 and 0.35 mg/m^3, respectively. Other potential exposures included freons, silicone oils and waxes, amine accelerators, ethanolamine, methylene-bis-(2-chloroaniline) (MOCA) (see IARC, 1993), triethylamine, triethylene diamine, styrene (see IARC, 1994) and various other organic solvents. The cohort comprised 4154 workers who were employed during 1958–87 at a time when personnel records were complete, and who had worked for at least one year by 1987. The vital status of all subjects at 31 December 1987 was ascertained, and information about those who had died and about incident cancers was obtained from Statistics Sweden and the National Tumour Registry. Rates of death and cancer incidence were compared with those in the national population by the person–years method. There were fewer deaths in total than expected (130 deaths; SMR, 0.8; 95% CI, 0.7–0.9); mortality from cancer (33 deaths; SMR, 0.8; 95% CI, 0.5–1.1) and overall cancer incidence (72 cases; standardized incidence ratio (SIR), 0.8; 95% CI, 0.6–1.0) were also below expectation. Among the subset of subjects classified as exposed to toluene diisocyanates or methylenediphenyl diisocyanate, there were 39 incident cancers (45.8 expected) including five cases of rectal cancer (1.8 expected) and no cases of lung cancer (4.0 expected). With allowance for a minimum latency of 10 years, the SIR for rectal cancer was 3.2 (3 cases).

A nested case–control study was carried out in an expanded cohort of 7023 men and women from the same factories (Hagmar *et al*., 1993b). The subjects had worked during 1958–87, but unlike in the cohort study, no minimum period of employment was specified. Each of 119 subjects with a cancer registered during 1959–87 was matched with three controls of the same sex and age (to within six years), who were under follow-up at the time the cancer occurred. Because of missing information, the final analysis was based on 114 cases and 313 referents. Exposures were rated by an occupational hygienist who was unaware of subjects' disease status, and risks were estimated by conditional logistic regression. No association was found between exposure to isocyanates and overall cancer incidence (odds ratio, 0.9; 90% CI, 0.6–1.3). Nor was there any association with rectal cancer. Among subjects with high exposure there was a non-significant increase in prostate cancer (4 cases; odds ratio, 2.7; 95% CI, 0.4–18.1).

Schnorr *et al*. (1996) studied 2717 male and 1893 female employees from four polyurethane foam plants in the United States, all of whom had worked for at least three months during 1958–84 in a department or job in which exposure to toluene diisocyanates occurred. Airborne concentrations of toluene diisocyanates had been greater than 0.2 mg/m^3 at one of the plants during 1965–69, but personal monitoring in 1984–85 at the three plants which were still then operating indicated 4-h time-weighted average exposures below 0.04 mg/m^3. Other potential exposures included dichloromethane (see this volume) , aliphatic amines, nitrogen dioxide, acrolein (see IARC, 1995) and acrylonitrile (see this volume). The cohort was followed through the National Death Index,

social security and internal revenue records, and state bureaux of motor vehicles; vital status was determined for 96.9% of subjects at 31 December 1993. Their mortality was compared with that of the national population by the person–years method, with adjustment for sex, race, age and calendar period. Mortality from all causes was close to expectation (316 deaths; SMR, 0.95; 95% CI, 0.85–1.1) as was mortality from all cancers (71 deaths; SMR, 1.0; 95% CI, 0.8–1.3) and from lung cancer (20 deaths; SMR, 1.0; 95% CI, 0.6–1.6). There were small excesses of deaths from rectal cancer (3 versus 1.1 expected) and Hodgkin's disease (2 versus 0.9), but these were not significant, and there was no tendency for risk of cancer mortality to rise with increasing duration of exposure.

2.2 Case–control study

In the Montreal case–control study carried out by Siemiatycki (1991) (see monograph on dichloromethane in this volume), the investigators estimated the associations between 293 workplace substances and several types of cancer. Isocyanates were one of the substances, and it was stated that the most common form in this study was toluene diisocyanates. The main occupations to which isocyanate exposure was attributed in this study were motor vehicle refinishers, motor vehicle mechanics and foundry workers. Only 0.8% of the study subjects had ever been exposed to isocyanates. For most types of cancer examined (oesophagus, stomach, colon, rectum, pancreas, prostate, bladder, kidney, skin melanoma, lymphoma), there was no indication of an excess risk due to isocyanates. For lung cancer, in the population subgroup of French Canadians (the majority ethnic group in this region), based on 10 cases exposed at any level, the odds ratio was 2.2 (90% CI, 0.9–5.3). [The interpretation of the null results has to take into account the small numbers and presumably low exposure levels. Workers had multiple exposures.]

3. Studies of Cancer in Experimental Animals

Commercial mixtures of 2,4- and 2,6-toluene diisocyanates administered by gavage induced a dose-related increase in the incidence of subcutaneous fibromas and fibrosarcomas (combined) in male rats, together with an increase in the incidence of pancreatic acinar-cell adenomas in male rats and of pancreatic islet-cell adenomas, neoplastic nodules of the liver and mammary gland fibroadenomas in female rats. In female mice, dose-related increases in the combined incidence of haemangiomas and haemangiosarcomas and of hepatocellular adenomas were observed after gavage administration. No treatment-related tumour was observed after exposure of mice or rats to commercial toluene diisocyanates by inhalation, although the results of the study with rats have not been reported fully (IARC, 1986).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

Toluene diisocyanates are reactive molecules that combine readily with nucleophiles, and as such have a propensity to react with proteins at the site of application to animals, in other tissues and with plasma (Kennedy *et al*., 1994). They are hydrolysed in aqueous media to the corresponding diamines, which can react with unchanged toluene diisocyanates to form polymeric ureas (Chadwick & Cleveland, 1981; Ulrich, 1983).

The major metabolites of toluene diisocyanates in both animals and humans are toluene diamines and their acetylated products (Rosenberg & Savolainen, 1985; Bartels *et al.*, 1993; Lind *et al*., 1996).

4.1.1 *Humans*

The toxicokinetics of 2,4- and 2,6-toluenediisocyanates in 11 chronically exposed workers at two flexible foam polyurethane production plants have been reported. The toluene diisocyanate concentrations in air varied between 0.4 and 4 μg/m^3 in one plant and in the other between 10 and 120 μg/m^3. In one of the plants, the plasma 2,4-toluene diamine levels were 0.4–1 ng/mL before a 4–5-week holiday and 0.2–0.5 ng/mL afterwards. The corresponding plasma levels of 2,6-toluene diamine were 2–6 and 0.5–2 ng/mL, respectively. In the other plant, the plasma 2,4-toluene diamine concentrations were 2–23 ng/mL before the holiday and 0.5–6 ng/mL afterwards and those of 2,6-toluene diamine were 7–24 ng/mL before and 3–6 ng/mL afterwards. The plasma concentrations of 2,4-toluene diamine were 2–24 ng/mL before a 12-day holiday, and 1–14 ng/mL afterwards. The corresponding values for plasma 2,6-toluene diamine were 12–29 and 8–17 ng/mL, respectively. The urinary elimination rates for 2,4-toluene diamine before the holiday were 0.04–0.54 and 0.02–0.18 μg/h afterwards. The corresponding values for 2,6-toluene diamine were 0.18–0.76 μg/h before and 0.09–0.27 μg/h after the holiday. The half-life in urine ranged from 5.8 to 11 days for 2,4- and 2,6-toluene diamines. The differences in exposure were reflected by the plasma toluene diamine concentrations. The mean half-life in plasma was 21 (range, 14–34) days for 2,4-toluene diamine and 21 (16–26) days for 2,6-toluene diamine. The study showed that the half-life in plasma of chronically exposed workers for 2,4- and 2,6-toluene diamine was twice as long as for volunteers with short-term exposure. An indication of a two-phase elimination pattern in urine was found. The first phase was related to the more recent exposure and the second, much slower one was probably related to release of toluene diamines in urine from toluene diisocyanate adducts in the body (Lind *et al.*, 1996).

The average air concentration of toluene diisocyanates at a toluene diisocyanate flexible foam plant was 29.8 μg/m^3 (12.5–19.9; n = 12). The highest exposure measured was approximately 3 mg/m^3 toluene diisocyanates. 2,4- and 2,6-Toluene diamine levels in urine and in plasma from four exposed workers and one volunteer were determined

after strong acid hydrolysis. The plasma toluene diamine concentrations among the workers were 1–38 g/L and 7–24 μg/L for 2,4- and 2,6-toluene diamine, respectively. The individual plasma levels among the workers over the three-day periods varied from 7 to 73%. For a volunteer, plasma concentration reached a maximum about 24 hours after the last exposure. The half-time of plasma toluene diamines for the volunteer was about 10 days. The urine levels varied greatly with time and exposure. High levels were found during or shortly after the exposure (Tinnerberg *et al.*, 1997).

4.1.2 *Experimental systems*

Timchalk *et al*. (1994) examined the route-dependent metabolism of [^{14}C]toluene 2,4-diisocyanate and [^{14}C]toluene 2,4-diamine in Fischer 344 rats. Forty-eight hours after an oral dose of 60 mg [^{14}C]toluene 2,4-diisocyanate/kg bw, 81%, 8% and 4% of the radioactivity was found in the faeces, urine and tissue/carcass/gastrointestinal tract contents, respectively. Markedly different results were obtained following inhalation exposure of rats to 2 ppm [14.2 mg/m^3] [^{14}C]toluene 2,4-diisocyanate for 2 h. Forty-eight hours after exposure, 47%, 15% and 34% of the recovered radioactivity was in the faeces, urine and tissue/carcass/gastrointestinal tract contents, respectively.

In comparative studies, [^{14}C]toluene 2,4-diamine, the hydrolysis product of [^{14}C]-toluene 2,4-diisocyanate, was administered to rats at doses of 3 mg/kg bw (orally or intravenously) and 60 mg/kg bw orally. After 48 h, the distribution of radioactivity was similar in all cases (urine, 64–72%; faeces, 20–31%; and tissue/carcass/gastrointestinal tract, 2–5%). Comparison of the toluene 2,4-diisocyanate inhalation group with the oral toluene 2,4-diisocyanate and toluene 2,4-diamine treatment groups indicated that a larger percentage of the inhaled radioactivity was in the tissues/carcass and that excretion of radioactivity into the urine was slower following toluene 2,4-diisocyanate inhalation.

Following inhalation or oral exposure to [^{14}C]toluene 2,4-diisocyanate, about 90% and 65% of the quantitated urinary metabolites were acid-labile conjugates. In contrast, only 16–39% of the urinary metabolites were conjugated following oral administration of [^{14}C]toluene 2,4-diamine.

Inhalation exposure to toluene 2,4-diisocyanate results primarily in the formation of acid-labile conjugates, with little or no toluene 2,4-diamine being formed. This suggests that the disposition of inhaled toluene 2,4-diisocyanate is quite different from that of orally administered toluene 2,4-diisocyanate or of intravenously or orally administered toluene 2,4-diamine.

4.2 Toxic effects

The toxicity of toluene diisocyanates has been reviewed (WHO, 1987).

4.2.1 *Humans*

Toluene diisocyanates are potent respiratory irritants and sensitizers, even at low airborne concentrations. Chronic bronchitis, chronic restrictive pulmonary disease and

hypersensitivity pneumonitis have also been described among toluene diisocyanate-exposed people (IARC, 1986).

A follow-up study (Pisati *et al*., 1993) of patients with toluene diisocyanate-induced asthma suggested that a short period of exposure and a short duration of symptoms before diagnosis, followed by complete cessation of exposure, are likely to lead to improvement of the symptoms and lung function. A decrease only of the exposure led to deterioration of lung function, and long exposure and duration of symptoms were unfavourable prognostically.

No deterioration of lung function, but an increased frequency of respiratory symptoms were observed in a follow-up study among non-sensitized workers with a mean exposure to toluene diisocyanates of 3 ppb [21.3 μg/m^3] (Omae *et al*., 1992a). This study also suggested that among workers with a mean exposure of 8 ppb [57 μg/m^3], peak exposures to 30 ppb [213 μg/m^3] and above were associated with a loss of ventilatory function among employees not sensitized to toluene diisocyanates (Omae *et al*., 1992b).

4.2.2 *Experimental systems*

Inhalation exposure to toluene diisocyanates is irritating to the eyes and respiratory tract, and induced chronic rhinitis, interstitial pneumonia and catarrhal bronchitis after long-term exposure. Respiratory sensitization to toluene diisocyanate developed in guinea-pigs after inhalation but also after dermal exposure (IARC, 1986).

Toluene diisocyanates induced respiratory epithelial inflammation, metaplasia and necrosis in mice at the lowest concentration tested (0.71 mg/m^3) after the shortest exposure period studied (6 h per day for four days). The reaction became more severe when the exposure period was extended to 9 or 14 days. No effects were observed in the olfactory epithelium, trachea or lungs (Zissu, 1995)

In-vitro tracheal hyperreactivity to carbachol was induced in mice by cutaneous application of toluene diisocyanates (isomeric composition not indicated), followed by nasal toluene diisocyanate challenge; this was not accompanied by an elevation of toluene diisocyanate-specific IgE. The reaction could be transferred to naive recipient mice by transfusion of lymphoid cells from sensitized mice (Scheerens *et al*., 1996).

Inhalation exposure of guinea-pigs to toluene diisocyanates (3 h per day on five consecutive days) led to sensitization (antibody formation, pulmonary reactiveness to toluene diisocyanate-albumin conjugate), at exposure levels ≥ 0.14 mg/m^3 (Huang *et al*., 1993).

When guinea-pigs were sensitized to toluene diisocyanates by daily instillations for one week on the nasal mucosa and further exposed nasally once a week for four weeks, pulmonary alveolitis, characterized by infiltration of mononuclear cells and eosinophils, was observed. Vasculitis was not found, and fibrosis was negligible, but small non-necrotizing granulomas, containing epithelioid histiocytes, multinucleated giant cells, lymphocytes and eosinophils were also observed. The histological picture was thus reminiscent of the hypersensitivity pneumonitis described in humans after exposure to toluene diisocyanates (Yamada *et al*., 1995).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

When female Wistar rats were exposed by inhalation to toluene diisocyanates (nominal concentrations 1, 3 or 9 mg/m^3, 6 h per day) on days 6 through 15 of gestation, a slight increase of asymmetric sternebrae was observed at the highest dose, but no adverse effect on maternal weight gain, number of corpora lutea, implantation sites, pre- and postimplantation loss, fetal or placental weight, gross and visceral anomalies or degree of ossification was detected (Buschmann *et al*., 1996).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Unless otherwise indicated, studies were carried out with an 80/20 mixture of 2,4/2,6-toluene diisocyanates. In one of two studies, toluene diisocyanate induced mutations in *Salmonella typhimurium* strains TA100, TA1538, and TA98 in the presence of an exogenous metabolic activation system only. It induced sex-linked recessive lethal mutations in *Drosophila* in a single study.

Toluene diisocyanate did not induce unscheduled DNA synthesis in rat primary hepatocytes. 2,4-Toluene diisocyanate induced mutations in mouse lymphoma L5178Y cells at the *tk* locus in the presence of exogenous metabolic activation and increased the frequency of sister chromatid exchanges but not chromosomal aberrations in Chinese hamster ovary cells. 2,6-Toluene diisocyanate induced gene mutations in L5178Y cells in the presence of an exogenous metabolic activation system and induced sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cell cultures.

In human lymphocyte cultures prepared from a male donor, toluene diisocyanate induced DNA single-strand breaks and chromosomal aberrations, but not sister chromatid exchanges.

Micronuclei were not induced in erythrocytes of mice or rats exposed to atmospheric concentrations of 1.1 mg/m^3 toluene diisocyanate for 6 h per day on five days per week for four weeks.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Toluene diisocyanates are industrial chemicals produced in large volumes. Exposure to toluene diisocyanates may occur during their production and in the processing and handling of polyurethane foams.

Table 1. Genetic and related effects of toluene diisocyanates

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	NG	Andersen *et al*. (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	–	1250	Anderson & Styles (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	NT	–	1250	Anderson & Styles (1978)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Andersen *et al*. (1980)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	+	NG	Andersen *et al*. (1980)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	NT	–	1250	Anderson & Styles (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	NG	Andersen *et al*. (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	NT	–	1250	Anderson & Styles (1978)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		15000 ppm feed	Foureman *et al*. (1994)
URP, Unscheduled DNA synthesis, rat primary hepatocytes, *in vitro*	–	NT	50	Shaddock *et al*. (1990)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*[c]	?	+	75	McGregor *et al*. (1991)
GST, Gene mutation, mouse lymphama L5178Y cells, *tk* locus *in vitro*[d]	–	+	25	McGregor *et al.* (1991)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*[c]	+	–	300	Gulati *et al*. (1989)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*[d]	+	–	50	Gulati *et al*. (1989)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*[c]	–	–	1000	Gulati *et al*. (1989)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*[d]	+	–	600	Gulati *et al*. (1989)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
DIH, DNA single-strand breaks, human lymphocytes *in vitro*[e]	+	NT	2400	Marczynski *et al*. (1992)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*[e]	–	–	90	Mäki-Paakkanen & Norppa (1987)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*[e]	(+)	+	45	Mäki-Paakkanen & Norppa (1987)
MVM, Micronucleus test, CD-1 mouse erythrocytes *in vivo*	–		1.1 mg/m^3 inh 6 h/d, 5 d/wk, 4 wk	Loeser (1983)
MVR, Micronucleus test, Sprague-Dawley CD rat erythrocytes *in vivo*	–		1.1 mg/m^3 inh 6 h/d, 5 d/wk, 4 wk	Loeser (1983)

[a] +, positive; (+), weakly positive; –, negative; ?, inconclusive; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; NG, not given; inh, inhalation. All data are from tests using an 80:20 mixture of 2,4-toluene diisocyanate:2,6-toluene diisocyanate unless otherwise indicated.

[c] Test using 2,4-toluene diisocyanate

[d] Test using 2,6-toluene diisocyanate

[e] Results are from cultures of peripheral blood lymphocytes obtained from one donor for each study.

5.2 Human carcinogenicity data

The risk of cancer associated with occupational exposure to isocyanates has been examined in three industrial cohort studies and in a population-based case–control study of several types of cancer. No strong association or consistent pattern has emerged.

5.3 Experimental data

Commercial mixtures of 2,4- and 2,6-toluene diisocyanates were tested for carcinogenicity in mice and rats by gavage and by inhalation exposure. Administration by gavage induced a dose-related increase in the incidence of subcutaneous fibromas and fibrosarcomas (combined) in male rats, together with an increase in the incidence of pancreatic acinar-cell adenomas in male rats and in pancreatic islet-cell adenomas, neoplastic nodules of the liver and mammary gland fibroadenomas in female rats. In female mice, dose-related increases in the combined incidence of haemangiomas and haemangiosarcomas and of hepatocellular adenomas were observed; no treatment-related tumour was seen in male mice, possibly due to poor survival. No treatment-related tumour was observed after exposure of mice or rats to commercial toluene diisocyanate by inhalation, although the results of the study with rats have not been reported fully.

5.4 Other relevant data

Toluene diisocyanates are metabolized to toluene diamines in humans and rats. Toluene diisocyanates are irritants and respiratory sensitizers in humans and rats.

Toluene diisocyanate did not induce micronuclei in mammalian erythrocytes *in vivo*. It induced DNA damage and chromosomal aberrations but not sister chromatid exchanges in human lymphocytes *in vitro*. It induced gene mutation and sister chromatid exchanges but not DNA damage or chromosomal aberrations in rodent cells *in vitro*. It induced sex-linked mutations in *Drosophila* and in some experiments was mutagenic in bacteria. The presence of an exogenous metabolic activation system led to inconsistent results, sometimes enhancing and at other times eliminating the genotoxic effects of toluene diisocyanate.

5.5 Evaluation

There is *inadequate evidence* for the carcinogenicity of toluene diisocyanates in humans.

There is *sufficient evidence* for the carcinogenicity of toluene diisocyanates in experimental animals.

Overall evaluation

Toluene diisocyanates are *possibly carcinogenic to humans (Group 2B)*.

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1,1,1-TRICHLOROETHANE

Data were last reviewed in IARC (1979) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

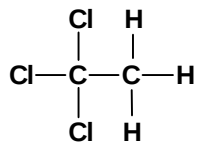
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 71-55-6
Chem. Abstr. Name: 1,1,1-Trichloroethane
IUPAC Systematic Name: 1,1,1-Trichloroethane
Synonyms: Chloroethene; methyl chloroform

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2H_3Cl_3$ Relative molecular mass: 133.40

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid (Lewis, 1993)
(*b*) *Boiling-point*: 74°C (Lide, 1995)
(*c*) *Melting-point*: –30.4°C (Lide, 1995)
(*d*) *Solubility*: Slightly soluble in water (0.07 g/100 mL at 20°C (Verschueren, 1996)); soluble in acetone, benzene, carbon tetrachloride, methanol, ethanol and diethyl ether (American Conference of Governmental Industrial Hygienists, 1992; Lewis, 1993; Budavari, 1996)
(*e*) *Vapour pressure*: 13.3 kPa at 20°C; relative vapour density (air = 1), 4.6 (Verschueren, 1996)
(*f*) *Explosive limits*: Upper, 16%; lower, 7% by volume (American Conference of Governmental Industrial Hygienists, 1992)
(*g*) *Conversion factor*: $mg/m^3 = 5.46 \times ppm$

1.2 Production and use

Total world demand for 1,1,1-trichloroethane in 1987 was 578 thousand tonnes; demand in the United States in 1990 was 280 thousand tonnes. In 1989, production capacity in the United States was estimated to be 470 thousand tonnes and production capacity outside the United States was estimated to be approximately 454 thousand tonnes. All non-essential emissive uses of 1,1,1-trichloroethane will be phased out by the year 2000 (Snedecor, 1993). Production in the United States in 1993 was reported to be 205 246 tonnes (United States International Trade Commission, 1994).

1,1,1-Trichloroethane is used as a solvent for adhesives, in metal degreasing and in the manufacture of vinylidene chloride. Other applications include its use in pesticides, textile processing, cutting fluids, aerosols, lubricants, cutting oil formulations, drain cleaners, shoe polishes, spot cleaners, printing inks and stain repellents (American Conference of Governmental Industrial Hygienists, 1992; WHO, 1992; Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

No national estimates of exposure were available to the Working Group.

1.3.2 *Environmental occurrence*

1,1,1-Trichloroethane is likely to enter the environment from air emissions or in wastewater from its production and use in vapour degreasing, metal cleaning and other applications. It can also enter the environment in leachates and volatile emissions from landfills. It has been detected at low levels in wastewater, groundwater, drinking-water, ambient water, ambient air, and urban air samples (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 1910 mg/m^3 as the threshold limit value for occupational exposures to 1,1,1-trichloroethane in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

The World Health Organization has established a provisional international drinking water guideline for 1,1,1-trichloroethane of 2000 µg/L (WHO, 1993).

2. Studies of Cancer in Humans

2.1 Cohort study

In Finland, a cohort of 2050 male and 1924 female workers biologically monitored for occupational exposure to trichloroethylene (see IARC, 1995a), tetrachloroethylene (see IARC, 1995b) and 1,1,1-trichloroethane was followed up for cancer incidence during 1967–92. The Finnish population was used for estimating expected numbers of cases. In

the whole cohort, observed/expected numbers of incident cases (all sites) were 112/98 in male and 125/130 in women. Among workers exposed to 1,1,1-trichloroethane, seventeen incident cancers were seen (standardized incidence ratio (SIR), 1.6; 95% confidence interval (CI), 0.9–2.5). Ratios for which the 95% confidence interval included unity were related to cancer of the central nervous system (3 cases; SIR, 6.1) and multiple myeloma (2 cases; SIR, 16) (Anttila *et al*., 1995).

2.2 Case–control studies

A population-based case–control study on brain cancer was carried out in some areas in the United States with petroleum refining and chemical manufacturing industries (i.e., activities suspected of being associated with brain cancer) and is described in detail in the monograph on dichloromethane (see this volume). Probability, intensity, duration and calendar time of life-long individual exposures to each of six chlorinated aliphatic hydrocarbons, including 1,1,1-trichloroethane, were assessed through an ad-hoc job–exposure matrix. Whereas risk excesses of some consistency were associated with exposure to other chlorinated aliphatic hydrocarbons, exposure to 1,1,1-trichloroethane showed little indication of an association with brain cancer (Heineman *et al*., 1994).

In the Montreal case–control study carried out by Siemiatycki (1991) (for details, see the monograph on dichloromethane in this volume), the investigators estimated the associations between 293 workplace substances and several types of cancer. 1,1,1-Trichloroethane was one of the substances evaluated. About 1% of the study subjects had ever been exposed to 1,1,1-trichloroethane. Among the main occupations to which 1,1,1-trichloroethane exposure was attributed in this population were electricians, industrial equipment mechanics and rail transport equipment mechanics. For most types of cancer examined (oesophagus, stomach, colon, rectum, pancreas, prostate, bladder, skin melanoma, lymphoma), there was no indication of an excess risk due to 1,1,1-trichloroethane. For lung cancer in the French Canadians (the major ethnic group in this region) based on seven cases exposed at any level, the odds ratio was 3.5 (90% CI, 1.0–12.0). For kidney cancer among the whole population, based on four cases exposed at any level, the odds ratio was 2.4 (90% CI, 1.0–6.0). [The interpretation of the positive results has to take into account the multiple testing context. Workers had multiple exposures.]

3. Studies of Cancer in Experimental Animals

1,1,1-Trichloroethane was tested for carcinogenicity in one experiment in mice and in one in rats by oral administration and in one experiment by inhalation exposure in rats. Although a few liver tumours were observed in male mice, these experiments were considered to be inadequate for evaluation (IARC, 1979).

3.1 Oral administration

Rat: A group of 40 male and 40 female Sprague-Dawley rats, seven weeks of age, was given 500 mg/kg bw technical-grade 1,1,1-trichloroethane (maximum levels of stabilizers and impurities: 1,4-dioxane, 3.8%; 1,2-epoxybutane, 0.47%; nitromethane, 0.27%; *N*-methylpyrrole, < 1 ppm; chloroform, 100 ppm; carbon tetrachloride, 250 ppm; 1,1-dichloroethane, 426 ppm; 1,2-dichloroethane, 2300 ppm; 1,2,3-trichloroethane, 41.8 ppm; 1,1-dichloroethylene, 398 ppm; *trans*-1,2-dichloroethylene, 50 ppm; trichloroethylene, 200 ppm; tetrachloroethylene, 475 ppm) dissolved in olive oil by gavage once a day on four to five days per week for 104 weeks. A group of 50 males and 50 females treated with olive oil alone served as controls. After the end of the treatment period, animals were held until spontaneous death. The experiment lasted for 141 weeks. A complete autopsy was carried out on each animal and histopathological examinations were performed on almost all organs and any other organ with pathological lesions. An increased incidence of leukaemia/lymphoma was found in treated males and females [no statistical analysis given]. The incidences of leukaemia/lymphoma were 3/50 control males, 9/40 treated males, 1/50 control females and 4/40 treated females (Maltoni *et al*., 1986). [The Working Group noted that survival and body weight are indicated only in graphs; survival at 112 weeks of age was about 30% and 50% for control and treated males and about 35% and 55% for control and treated females, respectively; no noteworthy difference in body weight was observed between control and treated animals.]

3.2 Inhalation

3.2.1 *Mouse*

Groups of 50 male and 50 female B6C3F_1 mice, five to six weeks of age, were exposed to target concentrations of 0 (controls), 150, 500 or 1500 ppm [0, 820, 2700 or 8200 mg/m^3] production-grade 1,1,1-trichloroethane (94% (by volume) 1,1,1-trichloroethane, 5% stabilizers (butylene oxide, *tert*-amyl alcohol, methyl butynol and nitromethane), 1% minor impurities) for 6 h per day on five days per week for 24 months (total of 516 exposure days). Time-weighted average measured exposure levels were: 151 ± 2, 502 ± 5 or 1505 ± 11 ppm. Complete gross examination was performed, and almost all organs and any grossly observed lesions suggestive of a tumour were examined histologically. There was no difference in survival between exposed mice and controls. [Survival was indicated only in graphs and was about 40–80% in males and 50–70% in females in all groups.] The body weights of treated male and female mice were similar to those of controls. A significant increasing trend was observed for combined incidences of benign tumours (adenoma and cystadenoma) of the lachrymal Harderian glands in females (3/50 control, 1/50 low-dose, 2/50 mid-dose and 7/50 high-dose; $p = 0.05$ linear trend by one-sided Cochran-Armitage test). The incidence of benign Harderian gland tumours in this study was within the normal variability at this institute (mean control incidence in females, 6.9%; range, 4–12%). In males, no significant change in the incidence of any tumour was observed (Quast *et al*., 1988).

3.2.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, four to six weeks of age, were exposed to target concentrations of 0 (controls), 150, 500 or 1500 ppm [0, 820, 2700 or 8200 mg/m^3] production-grade 1,1,1-trichloroethane (94% (by volume) 1,1,1-trichloroethane, 5% stabilizers (butylene oxide, *tert*-amyl alcohol, methyl butynol and nitromethane), 1% minor impurities) for 6 h per day on five days per week for 24 months (total of 516 exposure days). Time-weighted average measured exposure levels were: 151 ± 2, 502 ± 5 or 1505 ± 11 ppm. Complete gross examination was performed, and almost all organs and any grossly observed lesions suggestive of a tumour were examined histologically. There was no difference in survival between exposed rats and controls. [Survival was indicated only in graphs and was about 50–70% in males and 40–60% in females in all groups.] A significant decrease in body weight was observed in high-dose females. No significant increase was seen in the incidence of any tumour in males or females (Quast *et al*., 1988).

3.3 Multistage protocols and preneoplastic lesions

Rat: In an initiation study, a group of 10 male Osborne-Mendel rats, weighing 180–230 g, was subjected to a two-thirds partial hepatectomy and, 24 h later, was given a single dose of 3000 mg/kg bw 1,1,1-trichloroethane (purity, 97–99%) (maximum tolerated dose) in corn oil by gavage. Similar groups of animals were treated with 2 mL/kg bw corn oil alone (vehicle controls) or 30 mg/kg bw *N*-nitrosodiethylamine (NDEA; positive controls) followed by a two-thirds partial hepatectomy. Starting six days after partial hepatectomy, the rats received 500 mg phenobarbital/kg of diet (0.05% w/w) for seven weeks, then control diet for seven more days, after which time they were killed and the livers examined histologically for γ-glutamyltranspeptidase (γ-GT)-positive foci. There was no significant increase in the number of total γ-GT-positive foci (none and 0.27 ± 0.19/cm^2 in the 1,1,1-trichloroethane group and vehicle controls, respectively). NDEA increased the number of γ-GT-positive foci (4.04 ± 1.47) (Milman *et al*., 1988).

In a promotion study, groups of 10 male Osborne-Mendel rats (weighing 180–230 g) were given a single intraperitoneal injection of 30 mg/kg bw NDEA 24 h after a two-thirds partial hepatectomy. Starting six days later, the rats received daily 2000 mg/kg bw 1,1,1-trichloroethane (purity, 97–99%) (two-thirds of the maximum tolerated dose) in corn oil by gavage on five days per week for seven weeks. Control rats received corn oil alone during the promotion phase. After the promotion phase, rats were held for seven additional days, after which they were killed and the liver examined histologically for γ-GT-positive foci. There was no significant difference in the number of total γ-GT-positive foci between the 1,1,1-trichloroethane group and controls (2.16 ± 1.16 and 1.62 ± 0.33/cm^2, respectively) (Milman *et al*., 1988).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

1,1,1-Trichloroethane is rapidly taken up by humans after inhalation exposure. Experimental data collected in human subjects indicate that absorption of 1,1,1-trichloroethane is nearly complete following a single breath exposure (Morgan *et al.*, 1972), and that a steady-state lung retention of 25–30% in humans is achieved within 1–3 hours of continuous exposure (Monster, 1979; Nolan *et al.*, 1984). Steady-state blood levels are approximately 5–6 times that of alveolar air (Åstrand *et al.*, 1973; Monster, 1979) and increase with increasing air concentration, increasing alveolar ventilation and cardiac output (Åstrand *et al.*, 1973). The percentage uptake of inhaled 1,1,1-trichloroethane decreased rapidly from approximately 95% at the beginning of a four-hour exposure to 30% at the end (Monster, 1979).

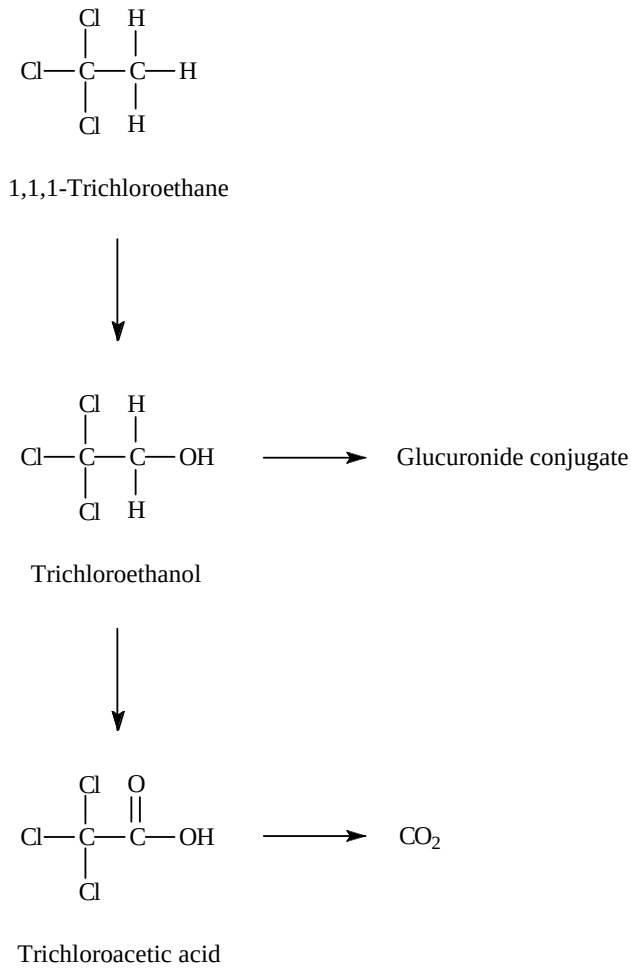
The absorption of 1,1,1-trichloroethane by the skin in humans has been shown to be dependent on the duration of exposure and the area of skin exposed (Fukabori *et al.*, 1977; Riihimaki & Pfaffli, 1978; Stewart & Dodd, 1964). 1,1,1-Trichloroethane vapours are absorbed through exposed skin to some extent, although absorption through the respiratory tract is expected to predominate during whole-body exposure to vapours. A quantitative examination of the relative magnitudes of percutaneous and respiratory absorption indicated that a whole-body exposure to 600 ppm [3280 mg/m^3] 1,1,1-trichloroethane for over 3.5 hours was equivalent to an inhalation exposure of only 0.6 ppm [3.3 mg/m^3] over the same time period (Riihimaki & Pfaffli, 1978).

After cessation of inhalation exposure, 1,1,1-trichloroethane is rapidly eliminated from the blood; 60–80% is eliminated within two hours after exposure and more than 95–99% within 50 hours (Åstrand *et al.*, 1973; Monster, 1979; Nolan *et al.*, 1984).

Blood concentrations of 1,1,1-trichloroethane in humans following dermal exposure are dependent on the duration of exposure. A two-hour exposure once a day resulted in higher blood levels than one-hour exposures twice a day (Fukabori *et al.*, 1977). At the end of a whole-body dermal exposure to 600 ppm [3280 mg/m^3] 1,1,1-trichloroethane vapour for 3.5 hours, the blood concentration of 1,1,1-trichloroethane reached a maximum of approximately 0.09 mg/L (Riihimaki & Pfaffli, 1978). This level quickly dropped after exposure ceased. In comparison, the steady-state blood concentration of 1,1,1-trichloroethane during inhalation exposure to 325 ppm [1770 mg/m^3] for four hours was approximately 4 mg/L (Åstrand *et al.*, 1973) and during exposure to 350 ppm [1910 mg/m^3] for six hours was approximately 2 mg/L (Nolan *et al.*, 1984).

Metabolism appears to play a relatively minor role in the overall disposition of absorbed 1,1,1-trichloroethane in humans. Less than 10% of the absorbed dose is metabolized; a large fraction is excreted unchanged in exhaled air, regardless of the route of exposure. The major metabolites of 1,1,1-trichloroethane are water-soluble

Figure 1. Biotransformation of 1,1,1-trichloroethane



trichloroethanol and its glucuronide conjugate, trichloroacetic acid and carbon dioxide (Figure 1).

The total amount of trichloroethanol and trichloroacetic acid excreted in urine accounts for 77% of the predicted amount of metabolized 1,1,1-trichloroethane. Excretion of trichloroethanol and trichloroacetic acid in urine is slow in relation to exhalation of 1,1,1-trichloroethane and these metabolites may accumulate with repeated exposure (Nolan *et al.*, 1984). The kinetics of elimination of 1,1,1-trichloroethane from blood into exhaled air are exponential. Elimination half-times for the initial, intermediate and terminal phases have been estimated at 1–9 hours, 6–20 hours and > 26 hours (Monster, 1979; Nolan *et al.*, 1984). Half-times for elimination from blood have been estimated to be 10–27 hours for trichloroethanol and 70–85 hours for trichloroacetic acid (Monster,

1979; Nolan *et al.*, 1984). Daily occupational exposure to 1,1,1-trichloroethane has been shown to result in a progressive increase in levels of urinary metabolites. Levels decline over the weekend, after exposure ceases (Seki *et al.*, 1975).

4.1.2 *Experimental animals*

1,1,1-Trichloroethane is rapidly absorbed by experimental animals after inhalation exposure. The initial uptake is governed by tissue loading and metabolism. Because 1,1,1-trichloroethane is poorly metabolized, absorption is expected to be lower after a steady state is reached (Dallas *et al.*, 1989).

The relative concentrations of 1,1,1-trichloroethane in the blood of experimental animals correlate with the levels found in humans (Carlson, 1981; Eben & Kimmerle, 1974; McEwen & Vernot, 1974; Schumann *et al.*, 1982b) after comparable exposure regimens.

1,1,1-Trichloroethane inhaled by animals distributes primarily into fat, liver and, to a lesser extent, kidney and brain, and is rapidly cleared after cessation of exposure (Holmberg *et al.*, 1977; Savolainen *et al.*, 1977; Schumann *et al.*, 1982a; Takahara, 1986). A linear relationship between exposure concentration and tissue concentration was found (Holmberg *et al.*, 1977).

The concentration of 1,1,1-trichloroethane in blood was determined in rats after one gavage dose in water (Reitz *et al.*, 1988). The blood level of 1,1,1-trichloroethane peaked approximately five minutes after the dose was given and then quickly decreased following exposure, being negligible after two hours.

Metabolism has been shown to be saturable in animals over a range of exposure levels of 150–1500 ppm [820–8200 mg/m^3] (Schumann *et al.*, 1982a); thus, as the exposure level and absorbed dose increase, metabolism will contribute less to overall elimination of 1,1,1-trichloroethane.

The data on 1,1,1-trichloroethane metabolism by animals are consistent with the human data. Approximately 90% of the inhaled dose is excreted unchanged in expired air, while the remainder is eliminated as CO_2 in expired air and as trichloroethanol and trichloroacetic acid in the urine (Ikeda & Ohtsuji, 1972; Eben & Kimmerle, 1974; Schumann *et al.*, 1982a,b; Koizumi *et al.*, 1984). A similar pattern of metabolism and subsequent excretion occurred in acutely and chronically exposed mice; the majority of 1,1,1-trichloroethane was excreted unchanged in the expired air and a small percentage was metabolized.

Metabolism following oral exposure is similar to metabolism following inhalation exposure. Reitz *et al.* (1988) found that approximately 3% of a dose ingested in drinking water by rats was metabolized and excreted as CO_2 in expired air or as metabolites in urine. Mice metabolized 1,1,1-trichloroethane more extensively than rats. This is consistent with the metabolic differences between rats and mice following inhalation exposure (Schumann *et al.*, 1982a), implying that mice may be the more sensitive species to effects of 1,1,1-trichloroethane that are based on biotransformation.

The pattern of excretion of 1,1,1-trichloroethane in animals is similar to that of humans. In rats exposed to 1,1,1-trichloroethane in the drinking water for eight hours (total dose of 116 mg/kg bw), the primary route of excretion was rapid elimination in expired air; only 3% of the ingested dose was metabolized (Reitz *et al.*, 1988). Virtually all of the ingested 1,1,1-trichloroethane was excreted within 30 hours after exposure.

Rapid elimination of 1,1,1-trichloroethane from blood after dermal exposure has been demonstrated in guinea-pigs (Jakobson *et al.*, 1982).

4.1.3 *Comparison of animals and humans*

In attempting to correlate the human and animal data, Nolan *et al.* (1984) validated a physiologically based pharmacokinetic model for 1,1,1-trichloroethane. The model predicted greater absorption, blood levels and metabolism of 1,1,1-trichloroethane in rodents than in humans. On the basis of toxicokinetic data, rats were suggested to be a better model than mice to evaluate potential health effects in humans.

The blood levels of 1,1,1-trichloroethane in human subjects were lower following exposure to 350 ppm [1910 mg/m^3] (approximately 2 mg/L) (Nolan *et al.*, 1984) than those found in rats and mice following exposure to 150 ppm [820 mg/m^3] (9.6 mg/L and 12.6 mg/L, respectively) (Schumann *et al.*, 1982b). The species differences between humans and rats are probably the result of a lower 1,1,1-trichloroethane blood:air partition coefficient and greater adipose tissue volume in humans (Dallas *et al.*, 1989).

4.2 Toxic effects

The toxicity of 1,1,1-trichloroethane has been reviewed (WHO, 1992; Agency for Toxic Substances and Disease Registry, 1995).

4.2.1 *Humans*

At least 30 fatalities have been associated with exposure to 1,1,1-trichloroethane, mostly due to deliberate inhalation or to accidental occupational exposure. Death was due to suffocation, the lungs showing acute oedema and congestion. Exposure to 1,1,1-trichloroethane impairs psychophysiological functions (IARC, 1979).

In a cross-sectional study of workers exposed to 1,1,1-trichloroethane in two textile mills (for 149/151, duration of exposure more than 12 months, for 135/151, estimated current exposure level (50–250 ppm [273–1365 mg/m^3])), no differences in the reported symptoms, electrocardiograms or laboratory examinations pertaining to liver function were observed (Kramer *et al.*, 1978). Case reports describing hepatic damage after exposure to 1,1,1-trichloroethane have been published (Cohen & Frank, 1994).

In an experimental inhalation exposure study at either stable or fluctuating exposure levels (time-weighted average, 200 ppm [1090 mg/m^3]), with or without 10-min peaks of exposure of 400 ppm [2180 mg/m^3]) combined with physical exercise, increased body sway but no change in visually evoked potentials or electroencephalography was observed in young healthy male volunteer participants (Laine *et al.*, 1996).

Case reports have been published on sensory neuropathies induced by exposure to 1,1,1-trichloroethane (House *et al.*, 1996).

4.2.2 *Experimental systems*

1,1,1-Trichloroethane causes central nervous system depression in rats and liver damage has been reported only after exposure to nearly lethal doses. Continuous inhalation exposure for 14 weeks caused hepatotoxicity in mice (IARC, 1979). The very limited hepatic toxicity was substantiated in a long-term carcinogenicity study (United States National Cancer Institute, 1977), in which no gross or histopathological evidence of 1,1,1-trichloroethane-induced damage was observed in Osborne-Mendel rats (time-weighted average dosage 750 or 1500 mg/kg bw/day, five days per week for 78 weeks by gavage) or in B6C3F_1 mice (2807 or 5615 mg/kg bw/day for 78 weeks by gavage), although markedly shortened survival was noted at both dose levels in rats of both sexes and in female mice.

Similarly, in another long-term carcinogenicity study (Quast *et al.*, 1988), very slight microscopic hepatotoxic changes were observed in rats of both sexes at 6, 12 and 18 months, but no more at 24 months after exposure to 1500 ppm [8190 mg/m^3] 1,1,1-trichloroethane for 6 h per day on five days per week for two years. No toxic changes were observed in mice.

Administration of 1,1,1-trichloroethane to male Fischer 344/N rats (82.7 or 165.4 mg/kg bw) once daily for 21 days induced a slight increase in the relative liver weight, but no microscopic hepatic damage (United States National Toxicology Program, 1996).

A small but significant elevation of serum sorbitol dehydrogenase activity was observed in female Sprague-Dawley rats 18 h after an intraperitoneal dose of 1,1,1-trichloroethane of 909 mg/kg bw (1/8 of the LD_{50}), but not at a dose level of 455 mg/kg bw (Lundberg *et al.*, 1986). After a single intragastric dose of 667 mg/kg bw 1,1,1-trichloroethane, a small increase in glutamic pyruvic transaminase but not sorbitol dehydrogenase or glutamate dehydrogenase activities was observed in female Wistar rats (Liangfu & Tianju, 1992).

When 82.7 or 165.4 mg/kg bw 1,1,1-trichloroethane was administered to male Fischer 344/N rats by gavage once daily for 21 days, a decrease in the total urine output and an increase in the urinary alanine aminotransferase activity were observed at the high dose. However, no sign of hyaline nephropathy, or any other microscopic effect on the kidney, was observed (United States National Toxicology Program, 1996).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

When female Long-Evans rats were exposed to 1,1,1-trichloroethane by inhalation (11 470 ± 1100 mg/m^3 for 6 h per day) for two weeks before mating and through day 20 of gestation (York *et al.*, 1982), no maternal toxicity was observed, and the only sign of

fetotoxicity was decreased fetal weight in the groups exposed during gestation only. Increased incidence of skeletal and soft tissue variations was observed in fetuses from the group exposed both before mating and during gestation (but not in groups exposed only during either of the periods alone). No teratogenic effects or effects on behaviour, as measured by the open field, running wheel activity or amphetamine challenge tests, or on pup survival were observed.

In a two-generation reproduction study (Lane *et al*., 1982), ICR Swiss mice were continuously administered 1,1,1-trichloroethane in the drinking-water (580, 1750 or 5830 mg/L with the aim of producing daily doses of 100, 300 or 1000 mg/kg bw) starting five weeks before the mating of the F_0 generation. No treatment-related effects on fertility, gestation, viability, pup survival, weight gain or terata were observed.

In a reproduction study in CD rats, male and female breeders were exposed to 1,1,1-trichloroethane in drinking water (3, 10, or 30 mg/L) for 14 days before cohabitation and during the cohabitation. Sperm-positive females remained on the same regimen during pregnancy and lactation until postnatal day 21. No significant changes in reproductive competence, teratogenic effects or postnatal growth or development changes were noted, with the exception of a slight increase in mortality from implantation to postnatal day 1, caused by a high mortality in one litter (George *et al*., 1989).

When pregnant CD-1 mice were exposed to 2000 ppm [10 900 mg/m^3] 1,1,1-trichloroethane for 17 h on days 12 through 17 of gestation, no effect on the pregnancy outcome was observed (Jones *et al*., 1996). However, pups from treated dams gained less weight, exhibited delays in developmental landmarks and acquisition of the righting reflex, had poorer performance on tests of motor coordination and exhibited delays in negative geotaxis than sham or untreated pups. The findings were similar when the dams were exposed to 8000 ppm [43 700 mg/m^3] for 3 h per day on days 12 through 17 of gestation.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

1,1,1-Trichloroethane did not induced SOS response in the *umu* test using *Salmonella typhimurium* strain TA1535/pSK1002 but did induce mutations in *S. typhimurium* strains TA100 and TA1535 in the presence or absence of exogenous metabolic activation. It induced reverse mutations in *Escherichia coli* in the presence of exogenous metabolic activation in one of three studies. It did not induce DNA damage, gene conversion, mutation or aneuploidy in *Saccharomyces cerevisiae.* It did not induce genetic crossing-over or aneuploidy in *Aspergillus nidulans*, mutation in *Tradescantia* or sex-linked recessive lethal mutation in *Drosophila melanogaster*.

In one study, 1,1,1-trichloroethane bound to calf thymus DNA and microsomal RNA and protein when incubated in the presence of rat or mouse liver microsomes.

Table 1. Genetic and related effects of 1,1,1-trichloroethane

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
PRB, *Prophage*, induction, SOS response, strand-breaks or cross-links	–	–	666	Nakamura *et al*. (1987)
SAF, *Salmonella typhimurium*, forward mutation	NT	–	1000	Skopek *et al*. (1981)
SAF, *Salmonella typhimurium*, forward mutation (Ara test)	–	–	375	Roldán-Arjona *et al*. (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	70	Simmon *et al*. (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	NG, vapour	Nestmann *et al*. (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	(+)	144	Gocke *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	Haworth *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	150	Nestmann *et al*. (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1000	Falck *et al*. (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	266×10^3 mg/m^3	Shimada *et al*. (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	–	500	Strobel & Grummt (1987)
SA4, *Salmonella typhimurium* TA104, reverse mutation	–	+	5	Strobel & Grummt (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	NG, vapour	Nestmann *et al*. (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Gatehouse (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	144	Gocke *et al*. (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	Richold & Jones (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	Haworth *et al*. (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	80	Nestmann *et al*. (1984)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1000	Falck *et al*. (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	266	Shimada *et al*. (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1000	Nestmann *et al*. (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Gatehouse (1981)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Richold & Jones (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1000	Falck *et al*. (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1000	Nestmann *et al*. (1980)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	5000	Richold & Jones (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1000	Falck *et al*. (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	Nestmann *et al*. (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	134	Norpoth *et al*. (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Gatehouse (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	Falck *et al*. (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	5	Strobel & Grummt (1987)
SAS, *Salmonella typhimurium* TA97, reverse mutation	+	+	5	Strobel & Grummt (1987)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	NT	+	268	Norpoth *et al*. (1980)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	–	–	1000	Gatehouse (1981)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	–	–	1000	Falck *et al*. (1985)
SSD, *Saccharomyces cerevisiae*, differential toxicity	–	–	750	Sharp & Parry (1981a)
SCG, *Saccharomyces cerevisiae* D4, gene conversion	–	–	125	Jagannath *et al*. (1981)
SCG, *Saccharomyces cerevisiae* JD1, gene conversion	–	–	750	Sharp & Parry (1981b)
SCG, *Saccharomyces cerevisiae* D7, gene conversion	NT	–	2600	Zimmermann & Scheel (1981)
ANG, *Aspergillus nidulans*, strain P1 genetic crossing-over	–	NT	1300	Crebelli *et al*. (1988)
SCR, *Saccharomyces cerevisiae* XV185-14C, reverse mutation	–	–	1488	Mehta & von Borstel (1981)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SCN, *Saccharomyces cerevisiae* D6, aneuploidy	–	–	500	Parry & Sharp (1981)
SCN, *Saccharomyces cerevisiae* D61.M, aneuploidy	–	NT	6000	Whittaker *et al*. (1990)
ANN, *Aspergillus nidulans* strain P1, aneuploidy	–	NT	1300	Crebelli *et al*. (1988)
TSM, *Tradescantia* species, mutation	–	NT	27.5×10^3 mg/m^3	Schairer & Sautkulis (1982)
DMX, *Drosophila melanogaster*, Basc strain, sex-linked recessive lethal mutations	–		3335 µg/mL feed	Gocke *et al*. (1981)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	133	Shimada *et al*. (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	?	NG	Tennant *et al*. (1986)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	–	680	Mitchell *et al*. (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	?	536	Myhr & Caspary (1988)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	NT	–	10	Perry & Thomson (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	?	?	1000	Galloway *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	–	160	Galloway *et al*. (1987)
TBM, Cell transformation, BALB/c-3T3 mouse cells	+	NT	4	Tu *et al*. (1985)
TRR, Cell transformation, Fischer rat embryo cells,	+	NT	13	Price *et al*. (1978)
T7S, Cell transformation, SA7/Syrian hamster embryo cells	+	NT	11×10^3 mg/m^3	Hatch *et al*. (1983)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
MVM, Micronucleus test, NMRI mouse bone marrow *in vivo*	–		2000 ip × 2	Gocke *et al*. (1981)
MVM, Micronucleus test, $B6C3F_1$ mouse bone marrow *in vivo*	–		67 ip × 2	Salamone *et al*. (1981)
MVM, Micronucleus test, CD-1 mouse bone marrow *in vivo*	–		43 ip × 2	Tsuchimoto & Matter (1981)
BID, Binding (covalent) to calf thymus DNA *in vitro*	NT	+	7.6	Turina *et al*. (1986)
BIP, Binding(covalent) to RNA or protein *in vitro*	NT	+	7.6	Turina *et al*. (1986)
BVD, Binding (covalent) to DNA, male Wistar rat and BALB/c mouse liver, kidney, lung and stomach *in vivo*	(+)		1.2 ip × 1	Turina *et al*. (1986)
BVP, Binding (covalent) to RNA or protein, male Wistar rat and BALB/c mouse liver, kidney, lung and stomach *in vivo*	+		1.2 ip × 1	Turina *et al*. (1986)
SPM, Sperm morphology, mice *in vivo*	–		1340 ip × 5	Topham (1980)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; ip, intraperitoneal

1,1,1-Trichloroethane did not induce unscheduled DNA synthesis in rat primary hepatocytes. It showed inconclusive evidence of gene mutation at the *tk* locus in mouse lymphoma L5178Y cells in the presence of an exogenous metabolic activation system. Results for induction of sister chromatid exchanges were also inconclusive. 1,1,1-Trichloroethane increased the frequency of chromosomal aberrations in Chinese hamster ovary cell cultures and induced morphological transformation in BALB/c 3T3 and in Fischer rat and virally-enhanced Syrian hamster embryo cells *in vitro*.

1,1,1-Trichloroethane bound to DNA, RNA and protein in liver, lung, kidney and stomach of mice and rats given a single intraperitoneal injection but did not induce micronuclei in mouse bone marrow following two injections, or abnormal sperm morphology in mice given five daily intraperitoneal injections.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

1,1,1-Trichloroethane is a solvent. It has been detected in waste-, ground-, drinking- and ambient water as well as in ambient and urban air.

5.2 Human carcinogenicity data

An increased risk for central nervous system and multiple myeloma was reported from a cohort study of workers exposed to 1,1,1-trichloroethane in Finland. These findings were not confirmed by two case–control studies carried out in the United States and Canada, while an increased risk for cancer of the lung and kidney was shown in the Canadian study.

5.3 Animal carcinogenicity data

1,1,1-Trichloroethane was tested for carcinogenicity by oral administration in rats in two experiments and in mice in one experiment. Although leukaemia was seen in both sexes of rats in one study and a few liver tumours occurred in male mice, the results of these studies were considered to be inadequate for evaluation. 1,1,1-Trichloroethane was tested by inhalation in rats in two experiments and in mice in one experiment. No chemically related increase in tumour incidence was observed in either rats or mice in one adequate study. Another inhalation study was considered to be inadequate.

In a multistage study for γ-glutamyltranspeptidase (γ-GT)-positive foci in the liver of male rats, neither single administration of 1,1,1-trichloroethane by gavage after a two-thirds partial hepatectomy followed by treatment with phenobarbital (initiation study) nor repeated administration of 1,1,1-trichloroethane by gavage after a two-thirds partial hepatectomy and initiation with *N*-nitrosodiethylamine (promotion study) increased the number of γ-GT-positive foci.

5.4 Other relevant data

Absorption of 1,1,1-trichloroethane vapour is mainly through the respiratory tract. It is rapidly eliminated from blood. Metabolism plays a minor role in this process, more than 90% being eliminated unchanged, both in exposed people and rodents. The main metabolites are trichloroethanol, trichloroacetic acid and carbon dioxide.

1,1,1-Trichloroethane is neurotoxic and hepatotoxic, following exceptionally high exposure concentrations of people and also in rodents. No structural damage has been reported in reproductive toxicity studies in rats and mice, but delayed development, particularly of neurological attributes, has been reported in one study with mice.

1,1,1-Trichloroethane covalently bound to DNA, RNA and protein in mice and rats but did not induce micronuclei or abnormal sperm head morphology in mice *in vivo*. It induced chromosomal aberrations and cell transformation in mammalian cell cultures and it showed inconclusive evidence of sister chromatid exchange induction. It did not induce unscheduled DNA synthesis or gene mutation in mammalian cells *in vitro*. 1,1,1-Trichloroethane did not cause mutation in plants or sex-linked mutation in *Drosophila*. It did not induce DNA damage, gene conversion, mutation or aneuploidy in yeast or genetic crossing-over or aneuploidy in fungi, but it was mutagenic to some bacterial strains.

5.5 Evaluation

There is *inadequate evidence* for the carcinogenicity of 1,1,1-trichloroethane in humans.

There is *inadequate evidence* for the carcinogenicity of 1,1,1-trichloroethane in experimental animals.

Overall evaluation

1,1,1-Trichloroethane is *not classifiable as to its carcinogenicity to humans (Group 3).*

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TRIS(2,3-DIBROMOPROPYL) PHOSPHATE

Data were last reviewed in IARC (1979) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

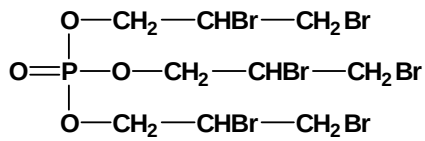
Chem. Abstr. Serv. Reg. No.: 126-72-7

Chem. Abstr. Name: 2,3-Dibromo-1-propanol phosphate (3:1)

IUPAC Systematic Name: 2,3-Dibromo-1-propanol phosphate

Synonyms: Phosphoric acid, tris(2,3-dibromopropyl) ester; Tris

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_9H_{15}Br_6O_4P$ Relative molecular mass: 697.61

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Viscous liquid (Budavari, 1996)

(*b*) *Boiling-point*: 390°C (WHO, 1995)

(*c*) *Melting-point*: 5.5°C (WHO, 1995)

(*d*) *Solubility*: Slightly soluble in water (0.8 mg/L at 24°C); miscible with carbon tetrachloride, chloroform and dichloromethane (Verschueren, 1996; United States National Library of Medicine, 1997)

(*e*) *Vapour pressure*: 0.03 Pa at 25°C (WHO, 1995)

(*f*) *Octanol/water partition coefficient (P)*: log *P*, 3.02 (WHO, 1995)

(*g*) *Conversion factor*: $mg/m^3 = 28.54 \times ppm$

1.2 Production and use

Production of tris(2,3-dibromopropyl) phosphate in the United States in 1975 was estimated to be between 4100 and 5400 tonnes. There are no reports of current production anywhere other than for research purposes (WHO, 1995).

Tris(2,3-dibromopropyl) phosphate has been used as a flame retardant for plastics and in synthetic textiles and fibres, which have been fabricated into children's clothing (Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

Occupational exposures to tris(2,3-dibromopropyl) phosphate may have occurred during its production in the textile and polyurethane foam industries (IARC, 1979).

1.3.2 *Environmental occurrence*

Environmental release in the past has been shown to result from textile finishing plants and laundering of the finished product (United States National Library of Medicine, 1997).

Tris(2,3-dibromopropyl) phosphate was found in the air and soil in the United States in the 1970s. None was found in samples taken from various water and soil sources in Japan at this time. General population exposures may have occurred from the use of clothing treated with the compound (WHO, 1995).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not proposed any occupational exposure limit for tris(2,3-dibromopropyl) phosphate. Finland, Sweden and France have a carcinogen notation (United States National Library of Medicine, 1997).

No international guideline for tris(2,3-dibromopropyl) phosphate in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

In a cohort mortality study in the United States, a group of 628 male workers was classified as exposed to tris(2,3-dibromopropyl) phosphate either on a 'routine' or 'non-routine' basis; 36 deaths occurred in this group (35 expected), seven of which were due to cancer compared with 6.6 that would have been expected (Wong *et al.*, 1984).

3. Studies of Cancer in Experimental Animals

Tris(2,3-dibromopropyl) phosphate was tested for carcinogenicity in one experiment in mice and in one in rats by oral administration and in one experiment in female mice by skin application. In mice, following oral administration, it produced benign and malignant tumours of the forestomach and lung in animals of both sexes, benign and malignant liver tumours in females and benign and malignant tumours of the kidney

(of the tubule cells) in males. In rats, it produced benign and malignant tumours of the kidney (of the tubule cells) in males and benign kidney tumours (of the tubule cells) in females. After skin application to female mice, it produced tumours of the skin, lung, forestomach and oral cavity (IARC, 1979).

3.1 Oral administration

Rat: A group of 50 male Fischer 344 rats, four weeks of age, was administered 100 mg/kg bw tris(2,3-dibromopropyl) phosphate [purity unspecified] dissolved in vegetable oil by gavage on five days per week for four weeks. After four weeks, the group was divided into three subgroups of 20, 15 and 15 rats. The first group received no further exposure; in the second subgroup, tris(2,3-dibromopropyl) phosphate administration was continued for 48 weeks; the third subgroup received vegetable oil (vehicle) alone for the remainder of the experiment. Two control groups consisted of 27 rats treated with vegetable oil (vehicle) alone and seven rats which received no treatment. The study was terminated at 52 weeks. Rats were killed at various time intervals to study the reversibility of tris(2,3-dibromopropyl) phosphate-induced lesions. In the rats treated for 52 weeks with tris(2,3-dibromopropyl) phosphate, one developed a kidney adenocarcinoma and 3/5 rats surviving at 52 weeks had adenomas of the descending colon (Reznik *et al*., 1981). [The Working Group noted the short duration of the experiment.]

3.2 Carcinogenicity of metabolites

3.2.1 *2,3-Dibromo-1-propanol*

Mouse: Groups of 50 male and 50 female $B6C3F_1$ mice, eight weeks of age, were administered skin applications of 0, 88 or 177 mg/kg bw 2,3-dibromo-1-propanol (98% pure) in 95% ethanol on five days per week for 36–39 weeks (males) or 39–42 weeks (females). The study was terminated at 36–39 weeks (males) and 39–42 weeks (females) because sera from sentinel mice housed in the same room as the study animals were found to be positive for antibodies to lymphocytic choriomeningitis virus. As shown in Table 1, increased incidences of skin papillomas, forestomach papillomas and forestomach carcinomas were observed in both sexes. Hepatocellular adenomas were seen in 1/50 control, 2/50 low-dose and 9/50 high-dose ($p < 0.05$) male mice; no data for liver were reported in females (Eustis *et al*., 1995).

Rat: Groups of 50 male and 50 female Fischer 344/N rats, eight weeks of age, were administered skin applications of 0, 188 or 375 mg/kg bw 2,3-dibromo-1-propanol (98% pure) in 95% ethanol on five days per week for 48–51 weeks (males) or 52–55 weeks (females). The study was terminated at 48–51 weeks for males and 52–55 weeks for females because of reduced survival of the high-dose groups and because sentinel mice housed in the same room as the rats tested positive for lymphocytic choriomeningitis virus. As shown in Table 2, there were increased incidences of skin neoplasms (all types), squamous-cell carcinomas of the skin, basal-cell tumours [not further specified] of the skin, squamous-cell carcinomas of the oral mucosa, squamous-cell papillomas of the oesophagus, squamous-cell papillomas of the forestomach, adenocarcinomas of the

Table 1. Increased tumour incidences in mice administered 2,3-dibromo-1-propanol by skin application

Tumour type	Controls		Low dose		High dose	
	Males	Females	Males	Females	Males	Females
Skin papillomas	0/50	0/50	3/50	1/50	9/50**	5/50*
Forestomach papillomas	0/50	0/50	12/50**	12/49**	20/49**	17/50**
Forestomach carcinomas	0/50	0/50	2/50	7/49**	1/49	6/50*

*$p < 0.05$
**$p < 0.01$
From Eustis *et al.* (1995)

Table 2. Increased tumour incidences in rats administered 2,3-dibromo-1-propanol by skin application

Tumour type	Controls		Low dose		High dose	
	Males	Females	Males	Females	Males	Females
Skin (all types)	1/50	0/50	22/50**	3/50	33/50**	18/50**
Skin, squamous-cell carcinomas	0/50	0/50	5/50*	0/50	8/50**	1/50
Skin, basal cell tumours	0/50	0/50	13/50**	3/50	21/50**	12/50**
Oral mucosa, squamous-cell carcinomas	0/50	0/50	16/50**	15/50**	25/50**	27/50**
Oesophagus, squamous-cell papillomas	0/50	0/50	19/50**	9/50	33/50**	38/50
Stomach, squamous-cell papillomas	0/50	1/50	1/50	3/50	17/50**	23/50**
Small intestine, adenocarcinomas	0/50	0/50	8/50**	3/50	11/50**	4/50
Large intestine, adenomatous polyps	1/50	0/50	13/50**	12/50**	29/50**	37/50**
Nasal mucosa, adenomas	0/50	0/50	48/50**	44/50**	48/50**	49/50**
Zymbal gland, adenocarcinomas	0/50	1/50	8/50**	2/50	29/50**	19/50**
Liver, carcinomas	0/50	0/50	1/50	2/50	3/50	6/50*

*$p < 0.05$
**$p < 0.01$
From Eustis *et al.* (1995)

small intestine, adenomatous polyps of the large intestine, adenomas of the nasal mucosa, Zymbal gland adenocarcinomas and liver carcinomas (Eustis *et al*., 1995).

3.2.2 *Bis(2,3-dibromopropyl) phosphate*

Rat: Groups of 40 male and 40 female Wistar rats, five weeks of age, were administered the magnesium salt of bis(2,3-dibromopropyl) phosphate [purity not specified] mixed in the diet at concentrations of 0 (control), 80 (low-dose), 400 (mid-dose) or 2000 (high-dose) mg/kg diet (ppm) for 24 months. Oesophageal papillomas were observed in 0/40 control, 0/40 low-dose, 6/40 mid-dose ($p < 0.05$) and 2/40 high-dose males and in 0/40 control, 0/40 low-dose, 0/40 mid-dose and 6/40 high-dose ($p < 0.05$) females. Papillomas of the forestomach were seen in 0/40 control, 0/40 low-dose, 8/40 mid-dose ($p < 0.05$) and 17/40 high-dose ($p < 0.01$) males and in 0/40 control, 0/40 low-dose, 4/40 mid-dose and 20/40 high-dose ($p < 0.01$) females. Adenocarcinomas of the small intestine were observed in 0/40 control, 0/40 low-dose, 2/40 mid-dose and 14/40 high-dose ($p < 0.01$) males and in 0/40 control, 0/40 low-dose, 0/40 mid-dose and 9/40 high-dose ($p < 0.01$) females. Hepatocellular carcinomas were observed in 0/40 control, 1/40 low-dose, 7/40 mid-dose ($p < 0.05$) and 24/40 high-dose ($p < 0.01$) female rats (Takada *et al*., 1991).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

The excretion balance and tissue distribution of radiolabelled tris(2,3-dibromopropyl) phosphate in rats were examined by Lynn *et al*. (1980, 1982) and Nomeir and Matthews (1983). After intravenous administration of 1.76 mg/rat, Lynn *et al*. (1980) recovered 57% of the dose in the urine in five days and identified the diester bis(2,3-dibromopropyl) phosphate as a minor urinary metabolite (7.8% of urinary ^{14}C). In further work, Lynn *et al*. (1982) recovered a total of 86% of the dose in the excreta (58% urine, 9% faeces, 19% as expired $^{14}CO_2$) with a further 9% in the carcass. Bile-duct-cannulated rats excreted 34% of the dose in the bile in 24 h, 20% being eliminated in the first hour after dosing. No unchanged tris(2,3-dibromopropyl) phosphate was detected in the urine, but dibromopropanol was present in addition to the diester previously reported. On high-performance liquid chromatography, numerous ^{14}C peaks remained unidentified.

Nomeir and Matthews (1983) compared the disposition of tris(2,3-dibromo[1-^{14}C]-propyl) phosphate given intravenously and orally to rats at a dose of 1.4 mg/kg bw (2 μmol/kg bw). Absorption from the gastrointestinal tract was extensive and rapid, the

tissue levels of ^{14}C being essentially identical after both routes of administration. After 24 h, 24% of an oral dose and 17% of an intravenous dose were present in urine and 11% (oral dose), 7% (intravenous dose) in faeces, with 21% (oral dose) and 26% (intravenous dose) present in seven tissues examined. A further 20% was recovered as exhaled $^{14}CO_2$ after intravenous dosing; no data were reported on exhalation after oral dosing. The tissue distribution of ^{14}C was widespread, with an elimination half-life of 60 h from most tissues and 91 h from liver and kidney.

Lynn *et al.* (1982) detected three major ^{14}C-containing compounds in plasma and the pattern was dominated by bis(2,3-dibromopropyl) phosphate as early as 5 min after intravenous dosing. 2,3-Dibromopropanol was also detected up to 8 h after dosing. The elimination of bis(2,3-dibromopropyl) phosphate was biphasic, with half-lives of 6 and 36 h and it was detected up to five days after dosing.

Tissue distribution was examined at five time points, with separate determinations of total ^{14}C, tris(2,3-dibromopropyl) phosphate and bis(2,3,-dibromopropyl) phosphate. The results confirmed the rapid disappearance of tris(2,3-dibromopropyl) phosphate, this being detected only at 5 and 30 min. Bis(2,3-dibromopropyl) phosphate was the major component in blood, lung, muscle and fat and had a long elimination period. At five days after dosing, there was significant retention of ^{14}C in the kidney, this comprising various polar components with some bis(2,3-dibromopropyl) phosphate also detected. The extensive biliary excretion of tris(2,3-dibromopropyl) phosphate-related radioactivity and low faecal elimination of the radiolabel indicate that enterohepatic circulation contributes to the retention of ^{14}C in the body (Lynn *et al.*, 1982).

In addition to the previously reported bis(2,3-dibromopropyl) phosphate and 2,3-dibromopropanol, Nomeir and Matthews (1983) characterized four additional metabolites by mass spectrometry, that arose from further hydrolysis and dehydrobromination of the 2,3-dibromopropane moiety, namely 2-bromo-2-propenyl-2,3-dibromopropyl phosphate, bis(2-bromo-2-propenyl) phosphate, 2,3-dibromopropyl phosphate and 2-bromo-2-propenyl phosphate. All six metabolites were found in 24-h urine and 3-h bile, with bis(2-bromo-2-propenyl) phosphate and 2-bromo-2-propenyl phosphate predominating in urine, while bis(2,3-dibromopropyl) phosphate and 2-bromo-2-propenyl-2,3-dibromopropyl phosphate were the major metabolites identified in bile; 67% of urinary and 47% of biliary ^{14}C were accounted for by a variety of unidentified metabolites.

In vitro, liver microsomes from rat, mouse, hamster and guinea-pig all activate tris(2,3-dibromopropyl) phosphate resulting in covalent binding to protein. This binding was cytochrome P450-dependent and was inhibited by glutathione. *In vivo* in rats, the kidney was the principal target organ for covalent binding to protein and, at high doses, to DNA. This binding was enhanced by pretreatment with polychlorinated biphenyls but not sodium phenobarbital and was partly prevented by cobalt chloride ($CoCl_2$) pretreatment. There was much less binding to liver protein and DNA and minimal binding to muscle (Søderlund *et al.*, 1981, 1982a).

Nelson *et al.* (1984) and Søderlund *et al.* (1984) identified the proximate mutagenic metabolite of tris(2,3-dibromopropyl) phosphate as 2-bromoacrolein and used stable

isotope techniques in microsomal incubations to show that it is formed by cytochrome P450-dependent oxidative debromination at C-3 of one of the 2,3-dibromopropyl groups followed by β-elimination to break the phosphoester bond. Søderlund *et al.* (1984) also showed the evolution of bromide ion release in microsomes in a glutathione-dependent reaction.

These studies have been extended by Pearson *et al.* (1993a,b), who showed that a number of metabolites contribute to protein binding in addition to 2-bromoacrolein. The major metabolic pathway leading to protein binding is C-2 oxidation of the 2,3-dibromopropyl groups, giving a reactive α-bromoketone which might either alkylate proteins directly or be hydrolysed to bis(2,3,-dibromopropyl) phosphate and an α-bromo-α′-hydroxyketone which could mediate the alkylation of protein.

4.2 Toxic effects

4.2.1 *Humans*

In a cohort of 3579 white male chemical workers with potential exposures to brominated compounds including tris(2,3-dibromopropyl) phosphate, no significant overall or cause-specific mortality excess was detected (Wong *et al*., 1984).

4.2.2 *Experimental systems*

Tris(2,3-dibromopropyl) phosphate caused extensive acute renal tubule necrosis at doses of 175 mg/kg bw and higher in male rats. Treatment also resulted in hepatotoxicity, but this effect was less pronounced and occurred at higher doses (Søderlund *et al*., 1980). Administration of radioactively labelled tris(2,3-dibromopropyl) phosphate to rats as a single intraperitoneal dose of 250 mg/kg bw led to pronounced binding of radioactivity to kidney but not to liver protein 9 h later (Dybing *et al*., 1980). Morales and Matthews (1980) and Lynn *et al*. (1982) also showed that the kidney accumulated the highest rate of radioactivity after injection of [^{14}C]tris(2,3-dibromopropyl) phosphate, compared with other organs. Dybing and Søderlund (1980) treated rats intraperitoneally with 250 mg/kg bw unlabelled tris(2,3-dibromopropyl) phosphate and determined parameters of kidney and liver toxicity 24 h later. The treatment resulted in increased plasma urea and creatinine levels. Kluwe *et al*. (1981) reported that tris(2,3-dibromopropyl) phosphate treatment of rodents resulted in decreased non-protein sulfhydryl content in the liver, but not in the kidney as the major target organ.

In young male Fischer 344 rats treated with 100 mg/kg bw tris(2,3-dibromopropyl) phosphate by gavage, severe tubular nephrosis was observed, starting from the corticomedullar junction and spreading to the peripheral cortex (Reznik *et al*., 1981). A single intraperitoneal dose of 154 mg/kg bw tris(2,3-dibromopropyl) phosphate given to male Sprague-Dawley rats caused cortical damage, significant increases in serum creatinine level and a depression of *para*-aminohippurate uptake in cortical slices (Elliott *et al*., 1982).

Cunningham *et al*. (1993, 1994) fed male Fischer 344 rats a diet containing 0, 50 or 100 ppm (mg/kg) tris(2,3-dibromopropyl) phosphate for 14 days. In the kidney, the

treatment induced significant cell proliferation that was localized in the renal outer medulla region. The proliferation rates of the inner medulla, the cortex and the liver were not increased.

In mice, hamsters and guinea-pigs, no clear evidence of renal damage was found at doses of 500–1000 mg/kg bw tris(2,3-dibromopropyl) phosphate (Søderlund *et al*., 1982a), which were clearly nephrotoxic to rats. Analysis of protein binding of radiolabelled tris(2,3-dibromopropyl) phosphate showed that binding to kidney protein also was much higher in rats than in the other species investigated.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

In a study by Seabaugh *et al*. (1981), pregnant Sprague-Dawley rats received 0, 5, 25 or 125 mg/kg bw tris(2,3-dibromopropyl) phosphate by gavage on days 6–15 of gestation. Weight gain during gestation was significantly decreased in the animals treated with 125 mg/kg bw per day, but no other compound-related toxic or teratogenic effect was observed.

4.4 Genetic and related effects

The genotoxicity of tris(2,3-dibromopropyl)phosphate has been reviewed (van Beerendonk *et al*., 1994a).

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 3 for references)

Tris(2,3-dibromopropyl) phosphate is mutagenic in *Salmonella typhimurium* in the presence of a metabolic activation system and in V79 Chinese hamster lung cells. With or without metabolic activation, it produces sister chromatid exchanges in the latter system and morphological transformation in C3H 10T½ and Syrian hamster embryo cells. It binds covalently to proteins and DNA, and causes DNA single strand breaks in mammalian cells *in vitro* and *in vivo*. It is mutagenic (in somatic and germ cells), clastogenic and recombinogenic in *Drosophila melanogaster* and induces bone-marrow micronuclei in mice and hamsters, liver micronuclei in rats and gene mutations in mouse kidney *in vivo*.

4.4.3 *Mechanistic aspects*

Mechanistic and metabolic studies have suggested that the genotoxicity of tris(2,3-dibromopropyl) phosphate may be mediated by its conversion to reactive metabolites, the most important of which may be 2-bromoacrolein (Nelson *et al*., 1984; Søderlund *et al*.,

Table 3. Genetic and related effects of tris(2,3-dibromopropyl) phosphate

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS repair activity, *Salmonella typhimurium* TA1535/pSK1002 *umu* test	NT	+	14	Shimada *et al*., 1989
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	5	Søderlund *et al*. (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	56	Salamone & Katz (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	+	9	Lynn *et al*. (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	+	35	Søderlund *et al*. (1982b)
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	+	35	Holme *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	+	35	Søderlund *et al*. (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	+	9	Lynn *et al*. (1982)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	+	9	Zeiger *et al*. (1982)
DMM, *Drosophila melanogaster*, somatic mutation (and recombination)	+		1.74 µg/mL feed	Vogel & Nivard (1993)
DMM, *Drosophila melanogaster*, somatic mutation (and recombination)	+		87 µg/mL feed	van Beerendonk *et al*. (1994b)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		13960 µg/mL feed	van Beerendonk *et al*. (1994b)
DMN, *Drosophila melanogaster*, aneuploidy	+		13960 µg/mL feed	van Beerendonk *et al*. (1994b)
DIA, DNA strand breaks, rat hepatoma cell line (Reuber) *in vitro*	–	NT	35	Gordon *et al*. (1985)
DIA, DNA strand breaks, male Wistar rat liver and testicular cells *in vitro*	+	NT	3.5	Søderlund *et al*. (1992)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	150	Sala *et al*. (1982)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	NT	+	14	Holme *et al*. (1983)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	NT	+	14	Søderlund *et al*. (1985)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	+	17.2	Sala *et al*. (1982)
TCM, Cell transformation, C3H 10T½ mouse cells	–[c]	–[c]	80	Sala *et al*. (1982)
TCM, Cell transformation, C3H 10T½ mouse cells	+	NT	2	Schechtman *et al*. (1987)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	+	+	25	Sala *et al*. (1982)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	7	Gordon *et al*. (1985)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	7	Søderlund *et al*. (1985)
DVA, DNA single-strand breaks, male Wistar rat (various organs) cells *in vivo*	+		244 ip × 1	Søderlund *et al*. (1992)
DVA, DNA strand breaks, male Wistar rat kidney *in vivo*	+		25 ip × 1	Pearson *et al*. (1993b)
GVA, Gene mutation, *lac*I Big Blue® mouse kidney cells *in vivo*	+		600 po × 4	de Boer *et al*. (1996)
MVM, Micronucleus test, B6C3F_1 mouse bone-marrow cells *in vivo*	+		1020 ip × 2	Salamone & Katz (1981)
MVR, Micronucleus test, Wistar rat hepatocytes *in vivo*	+		174.5 (ph) ip × 1	van Beerendonk *et al*. (1994a)
MVC, Micronucleus test, Chinese hamster bone marrow *in vivo*	+		400 ip × 1	Sala *et al*. (1982)
BID, Binding (covalent) to DNA, Wistar rat liver and kidney *in vitro*	NT	+	350	Søderlund *et al*. (1981)
BIP, Binding (covalent) to microsomal proteins, Wistar rat liver and kidney *in vitro*	NT	+	43.5	Søderlund *et al*. (1981)
BVD, Binding (covalent) to DNA, male Wistar rat kidney and liver *in vivo*	+		250 ip × 1	Søderlund *et al*. (1981)
BVP, Binding (covalent) to proteins, male Wistar rat kidney and liver *in vivo*	+		50 ip × 1	Søderlund *et al*. (1981)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BVP, Binding (covalent) to proteins, male Wistar rat kidney, liver and testes *in vivo*	+		250 ip × 1	Pearson *et al*. (1993b)
SPM, Sperm morphology, B6C3F$_1$ mice *in vivo*	+		817 ip × 5	Salamone & Katz (1981)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; ip, intraperitoneal; ph, partial hepatectomy; po, oral
[c] Positive only when 12-*O*-tetradecanoylphorbol 13-acetate (0.1 μg/mL) was added to the media for three days following the first 24 h of treatment.

1984). 2-Bromoacrolein and 2,3-dibromopropanal are mutagenic in *Salmonella typhimurium* TA100, with or without metabolic activation, cause single-strand breaks in DNA of a rat hepatoma cell line and morphological transformation of Syrian hamster embryo cells (Gordon *et al*., 1985). Furthermore, 2-bromoacrolein forms adducts with DNA which block DNA replication *in vitro*. It also induces DNA–protein cross-links in *Drosophila melanogaster* (van Beerendonk, 1992, 1994c).

An equimolar dose of the metabolite bis(2,3-dibromopropyl) phosphate was markedly more nephrotoxic and led also to damage of the descending loop of Henle. Intraperitoneal injection of bis(2,3-dibromopropyl) phosphate to Sprague-Dawley rats resulted in necrosis of the renal cortex, which was less severe in female than in male rats (Elliott *et al*., 1983). Renal dysfunction, as indexed by serum creatinine level and in-vitro renal cortical uptake of *para*-aminohippurate and *N*-methylnicotinamide, was similar in males and females. Evidence for the role of bis(2,3-dibromopropyl) phosphate and mono(2,3-dibromopropyl) phosphate as nephrotoxic metabolites of tris(2,3-dibromopropyl) phosphate was provided by Lynn *et al*. (1982), Søderlund *et al*. (1982b) and Fukuoka *et al*. (1988). In isolated proximal tubule cells from rat kidney, 100 μM bis(2,3-dibromopropyl) phosphate inhibited the uptake of α-methylglucose, a parameter that the authors used to assess cytotoxicity (Boogard *et al*., 1989).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

During the 1970s, tris(2,3-dibromopropyl) phosphate was produced in low volumes, with occupational exposure likely to have occurred in its production and use in the textile industry. It does not appear to have been produced since then. The primary exposure to the general population appears to have been through wearing clothing treated with the chemical.

5.2 Human carcinogenicity data

A small cohort study of workers exposed to tris(2,3-dibromopropyl) phosphate was uninformative.

5.3 Animal carcinogenicity data

Tris(2,3-dibromopropyl) phosphate was tested for carcinogenicity in mice and rats by oral administration. In mice, it produced benign and malignant tumours of the forestomach and lung in animals of each sex, benign and malignant liver tumours in females and benign and malignant tumours of the kidney in males. In rats, it produced benign and malignant tumours of the kidney in males and benign kidney tumours in females. In a study of limited duration in male rats, benign tumours of the colon were reported. After skin application to female mice, it produced tumours of the skin, lung, forestomach and oral cavity.

A metabolite of tris(2,3-dibromopropyl) phosphate, bis(2,3-dibromopropyl) phosphate, was tested for carcinogenicity in rats by oral administration and another metabolite, 2,3-dibromo-1-propanol, was tested in mice and rats by skin application. They produced a variety of tumours, including skin, forestomach and hepatocellular tumours, in mice and rats and tumours of the oesophagus, intestine, nasal mucosa and Zymbal glands in rats.

5.4 Other relevant data

Tris(2,3-dibromopropyl) phosphate and its metabolites bis(2,3-dibromopropyl)-phosphate and mono(2,3-dibromopropyl) phosphate are nephrotoxic in rodents.

Tris(2,3-dibromopropyl) phosphate is mutagenic in bacteria and causes genetic damage in cultured mammalian cells, *Drosophila melanogaster* and mice, probably via metabolism to a number of intermediates of which 2-bromoacrolein may be particularly important.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of tris(2,3-dibromopropyl) phosphate.

There is *sufficient evidence* in experimental animals for the carcinogenicity of tris(2,3-dibromopropyl) phosphate.

Overall evaluation

Tris(2,3-dibromopropyl)phosphate *is probably carcinogenic to humans (Group 2A)*.

In making the overall evaluation, the Working Group took into consideration that tris(2,3-dibromopropyl) phosphate is consistently active in a wide range of mammalian in-vivo and in-vitro test systems.

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VINYL BROMIDE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

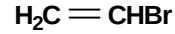
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 593-60-2
Chem. Abstr. Name: Bromoethene
IUPAC Systematic Name: Bromoethylene

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_2H_3Br Relative molecular mass: 106.96

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless gas with a characteristic pungent odour; colourless liquid under pressure (American Conference of Governmental Industrial Hygienists, 1992)
(*b*) *Boiling-point*: 15.8°C (Lide, 1997)
(*c*) *Melting-point*: –137.8°C (Lide, 1997)
(*d*) *Density*: 1.522 at 20°C (Lide, 1997)
(*e*) *Solubility*: Insoluble in water; soluble in acetone, benzene, chloroform and ethanol; very soluble in diethyl ether (American Conference of Governmental Industrial Hygienists, 1992; Lide, 1997)
(*f*) *Vapour pressure*: 119 kPa at 20°C; relative vapour density, 3.7 (American Conference of Governmental Industrial Hygienists, 1992)
(*g*) *Explosive limits*: Upper, 15%; lower, 9% by volume (United States National Library of Medicine, 1998a)
(*h*) *Conversion factor*: $mg/m^3 = 4.37 \times ppm$

1.2 Production and use

Information available in 1995 indicated that vinyl bromide was produced in three countries (Germany, Japan and the United States) (Chemical Information Services, Inc., 1995).

Vinyl bromide has been used as an intermediate in organic synthesis and in the manufacture of polymers, copolymers, flame retardants, pharmaceuticals and fumigants (American Conference of Governmental Industrial Hygienists, 1992).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997; United States National Library of Medicine, 1998b), approximately 1822 workers in the United States were potentially exposed to vinyl bromide (see General Remarks).

1.3.2 *Environmental occurrence*

Vinyl bromide may form in air as a degradation product of 1,2-dibromoethane. It may also be released to the environment from facilities which manufacture or use vinyl bromide as a flame retardant for acrylic fibres. Vinyl bromide has been qualitatively identified in ambient air samples (United States National Library of Medicine, 1998a)

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 22 mg/m^3 as the 8-h time-weighted average threshold limit value, with an animal carcinogen notation, for occupational exposures to vinyl bromide in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for vinyl bromide in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Vinyl bromide was tested for carcinogenicity in female mice by skin application and by subcutaneous injection, and in rats by inhalation exposure. In the inhalation study in rats, there was a dose-related increase in the incidence of liver angiosarcomas and Zymbal gland carcinomas; an increased incidence of liver neoplastic nodules and hepatocellular carcinoma was also noted. In the limited studies in mice by skin application and subcutaneous administration, no local tumour was observed (IARC, 1986).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

Vinyl bromide is readily absorbed upon inhalation by rats and showed an 11-fold accumulation within the rats compared with the concentration in gaseous phase. Metabolism is saturable at exposure concentrations greater than 250 mg/m^3. Following inhalation of vinyl bromide by rats, rabbits and monkeys, plasma levels of nonvolatile bromide increased with exposure duration, and more rapidly in phenobarbital-pretreated rats.

A volatile alkylating metabolite was formed in a mouse-liver microsomal system. The primary metabolite formed *in vitro* by mixed function oxidases is 2-bromoethylene oxide, which rearranges to 2-bromoacetaldehyde.

In rats, the conversion of vinyl bromide to reactive metabolites occurs primarily in hepatocytes. Irreversible binding of such metabolites to proteins and RNA has been established both with rat-liver microsomes *in vitro* and in rats in *vivo*. They can also alkylate the cytochrome P450 prosthetic group of phenobarbital-treated rat-liver microsomes. Exposure of rats to vinyl bromide causes a decrease in hepatic cytochrome P450 (IARC, 1986).

4.2 Toxic effects

4.2.1 *Humans*

Vinyl bromide inhalation is reported to cause loss of consciousness. It is a skin and eye irritant and causes a 'frost-bite' type of burn (IARC, 1986).

4.2.2 *Experimental systems*

Subacute inhalation studies performed with rats, rabbits and monkeys showed no significant haematological, gross pathological or histopathological change. Vinyl bromide is far less hepatotoxic than vinyl chloride in rats. However, its hepatotoxicity is enhanced in rats pretreated with polychlorinated biphenyls, as demonstrated by enzymatic and histological signs of liver damage. Like other halogenated compounds transformed to reactive metabolites, vinyl bromide alters rat intermediary metabolism, leading to acetone exhalation (IARC, 1986).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects (see Table 1 for references)

Vinyl bromide is mutagenic to *Salmonella typhimurium* and induced somatic mutations in *Drosophila melanogaster*. It is considered that vinyl bromide reacts with DNA to form various etheno-adducts which are the same as those formed by vinyl chloride (Bolt *et al.*, 1986).

Table 1. Genetic and related effects of vinyl bromide

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SAF, *Salmonella typhimurium* BA13/BAL13, forward mutation, arabinoside resistance	+	+	15190	Roldán-Arjona *et al*. (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	1% in air	Lijinsky & Andrews (1980)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	+	+	0.2% in air	Bartsch *et al*. (1979)
DMM, *Drosophila melanogaster*, somatic mutation (*white/white*$^{+}$)	+		4000 ppm in air	Vogel & Nivard (1993)
DMM, *Drosophila melanogaster*, somatic mutation (*white/white*$^{+}$)	+		2000 ppm in air	Rodriguez-Arnaiz *et al*. (1993)

[a] +, positive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Occupational exposure may occur during the production of vinyl bromide and its polymers.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Vinyl bromide was tested in female mice by skin application and by subcutaneous injection, and in rats by inhalation exposure. In the inhalation study in rats, there was a dose-related increase in the incidence of liver angiosarcomas and Zymbal gland carcinomas; an increased incidence of liver neoplastic nodules and hepatocellular carcinoma was also noted.

5.4 Other relevant data

Vinyl bromide was mutagenic to *Salmonella typhimurium* and *Drosophila melanogaster*.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of vinyl bromide were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of vinyl bromide.

Overall evaluation

Vinyl bromide is *probably carcinogenic to humans (Group 2A)*.

In making the overall evaluation, the Working Group took into consideration that all available studies showed a consistently parallel response between vinyl bromide and vinyl chloride. In addition, both vinyl chloride and vinyl bromide are activated via a P450-dependent pathway to their corresponding epoxides. For both vinyl chloride and vinyl bromide, the covalent binding of these compounds to DNA forms the respective etheno adducts. The weight of positive evidence for both compounds was also noted among the studies for genotoxicity, although the number and variety of tests for vinyl bromide were fewer.

6. References

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Part Three
Compounds Not Reviewed in Plenary Sessions

Part Three A
Extensive New Data Requiring New Summaries

1,3-DICHLOROPROPENE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

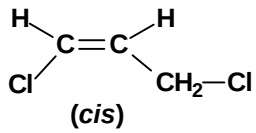
1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 542-75-6

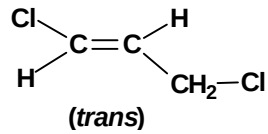
Chem. Abstr. Name: 1,3-Dichloro-1-propene

IUPAC Systematic Name: 1,3-Dichloropropene

1.1.2 *Structural and molecular formulae and relative molecular mass*



(*cis*)



(*trans*)

$C_3H_4Cl_2$ Relative molecular mass: 110.97

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Liquid with chloroform-like odour (Budavari, 1996)

(*b*) *Boiling-point*: 104°C (*cis*), 112°C (*trans*) (Verschueren, 1996)

(*c*) *Solubility*: Insoluble in water; soluble in acetone and toluene (Lewis, 1993)

(*d*) *Vapour pressure*: 5720 Pa at 25°C (*cis*), 4522 Pa at 25°C (*trans*); relative vapour density (air = 1), 3.83 (Verschueren, 1996)

(*e*) *Flash-point*: 35°C, open cup (Lewis, 1993)

(*f*) *Conversion factor*: $mg/m^3 = 4.54 \times ppm$

1.2 Use

1,3-Dichloropropene is used in organic synthesis and as a soil fumigant (Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 2200 workers in the United States were potentially exposed to 1,3-dichloro-

propene (see General Remarks). Occupational exposures may occur in its manufacture and use in organic synthesis and as a soil fumigant.

1.3.2 *Environmental occurrence*

1,3-Dichloropropene is released into the air and in wastewater during its production and use as a soil fumigant and chemical intermediate. 1,3-Dichloropropene may also leach into groundwater. Considerable variation in the amounts of 1,3-dichloropropene lost by volatilization and degradation can be expected depending on the method of application, soil type, moisture and temperature. It has been detected in low levels in ambient air and drinking-water (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 4.5 mg/m^3 as the threshold limit value for occupational exposures to 1,3-dichloropropene in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

The World Health Organization has established an international drinking-water guideline for 1,3-dichloropropene of 20 μg/L (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Technical-grade 1,3-dichloropropene (containing 1.0% epichlorohydrin (see this volume)) was tested for carcinogenicity by gavage in one experiment in mice and in one experiment in rats. In mice, it produced dose-related increases in the incidence of benign and/or malignant tumours of the urinary bladder, lung and forestomach. In male rats, it produced dose-related increases in the incidence of benign and malignant tumours of the forestomach and benign liver tumours; in female rats, it produced benign tumours of the forestomach. In one experiment, by subcutaneous administration in female mice, the *cis*-isomer produced malignant tumours at the injection site. In a two-stage skin application study in mice, the *cis*-isomer was not active as an initiator. A study in mice in which *cis*-1,3-dichloropropene was applied three times per week to the skin of mice for up to 85 weeks was inconclusive (IARC, 1986; Yang *et al*., 1986).

3.1 Inhalation exposure

3.1.1 *Mouse*

Mouse: Groups of 50 male and 50 female B6C3F$_1$ mice, 5–6 weeks of age, were exposed to technical-grade 1,3-dichloropropene (*cis*-isomer, 49.5%; *trans*-isomer, 42.6%;

0.7% 1,2-dichloropropane with epoxidized soya bean as a stabilizer) by inhalation at concentrations of 0, 5, 20 or 60 ppm [0, 23, 91 or 272 mg/m^3] for 6 h per day on five days per week for 24 months. Ten mice of each sex per group were killed at six and 12 months. At the end of the study, necropsy and histopathological examination of all organs were performed. No sign of toxicity was recorded. Approximately 90% of male and 80–96% of female mice survived until the end of the study. Exposure-related histopathological alterations were observed in nasal tissues of male and female mice exposed to 60 ppm for 24 months, but not for six or 12 months. The alterations were characterized by hypertrophy and hyperplasia of the respiratory epithelium and degeneration of the olfactory epithelium. Hyperplastic changes also occurred in the epithelium of the urinary bladder. In male mice, there was a significant increase in the incidence of bronchioalveolar adenomas at the highest dose: 9/50 in controls compared with 6/50, 13/50 and 22/50 in the groups exposed to 5, 20 and 60 ppm, respectively (Lomax *et al*., 1989).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, 6–8 weeks of age were exposed to 1,3-dichloropropene (as described above). Hyperplastic and degenerative changes occurred to the nasal cavities as well as hyperplastic changes in the urinary bladder. No increase in tumour incidence was observed (Lomax *et al.*, 1989).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Isomers of *N*-acetyl-*S*-(3-chloroprop-2-enyl)-L-cysteine have been detected in urine samples of 1,3-dichloropropene applicators (Osterloh *et al*., 1984; Van Welie *et al*., 1989).

4.1.2 *Experimental systems*

In rats, 1,3-dichloropropene is absorbed rapidly, after either inhalation or oral administration, and is eliminated principally by metabolism (glutathione conjugation) within 24–48 h. The major urinary metabolite of *cis*-1,3-dichloropropene in rats is the mercapturic acid *N*-acetyl-*S*-(*cis*-3-chloroprop-2-enyl)-L-cysteine. Glutathione conjugation *in vitro* in rat liver cytosol of the *cis*-isomer occurs four to five times faster than that of the *trans*-isomer. 1,3-Dichloropropene is an alkylating agent and the *trans*-isomer is less reactive than the *cis*-isomer (IARC, 1986).

At higher inhalation exposure levels in rats, absorption mechanisms become saturated as a result of compromised respiration, while saturation of metabolism also occurs, presumably as result of limitations on glutathione *S*-transferase activity. Plasma elimination of 1,3-dichloropropene is biphasic, the half-life of the slower phase being roughly

25 to 45 min (Stott & Kastl, 1986). The plasma elimination half-life of the glutathione conjugate of 1,3-dichloropropene is about 17 h, irrespective of the inhalation exposure concentration (Fisher & Kilgore, 1989).

Both *cis*- and *trans*-isomers of 1,3-dichloropropene are conjugated with glutathione and excreted as mercapturic acids (55% *cis*-1,3-dichloropropene and 45% *trans*-1,3-dichloropropene in 24 h) in Wistar rats after intraperitoneal administration (Onkenhout *al*., 1986).

1,3-Dichloropropene was given in doses of 0, 25, 50 and 75 mg/kg bw intraperitoneally to male Fischer 344 rats. Excretion of the metabolite *N*-acetyl-*S*-(*cis*-3-chloroprop-2-enyl)-L-cysteine increased in a dose-dependent manner from 0 to 50 mg/kg 1,3-dichloropropene, but no further increase was seen at the 75-mg/kg dose, suggesting that, at higher doses, the metabolism pathway may be saturated or impaired (Osterloh & Xiwen, 1990).

Metabolic activation of 1,3-dichloropropene, as suggested by the use of specific inhibitors of metabolism in the *Salmonella typhimurium* gene mutation assay, proceeds via an hydrolytic-oxidative pathway; the first step of which is hydrolysis to chloroallyl alcohol, which is then oxidized to chloroacrolein (Neudecker & Henschler, 1986).

4.1.3 *Comparison of human and rodent data*

The principal metabolic pathway to mercapturic acids is presumably similar in humans and rodents. Because no data on kinetics or metabolic activation in humans are available, no quantitative comparison can be made.

4.2 Toxic effects

4.2.1 *Humans*

A 27-year-old previously healthy male worker who accidentally drank a solution containing 1,3-dichloropropene (mixture of *cis*- and *trans*-isomers) developed gastrointestinal distress, adult respiratory distress syndrome, haematological and hepatorenal functional impairment, and died 40 h after ingestion (Hernandez *et al*., 1994).

4.2.2 *Experimental systems*

Male and female Fischer 344 rats and B6C3F_1 mice were exposed to 0, 10, 30, 90 or 150 ppm [0, 45, 136, 409 or 681 mg/m^3] technical grade 1,3-dichloropropene (*cis*, 48.6%; *trans*, 42.3%) vapours for 6 h per day on five days per week for 13 weeks (Stott *et al*., 1988). At the end of the exposure, slight degeneration of the nasal olfactory epithelium and mild hyperplasia of the nasal respiratory epithelium were observed in rats exposed to 150 ppm 1,3-dichloropropene. All male and female Fischer 344 rats exposed to 90 or 150 ppm and two of the 10 rats exposed to 30 ppm 1,3-dichloropropene exhibited minimally detectable hyperplasia of the respiratory epithelium. Exposure to 90 or 150 ppm 1,3-dichloropropene also produced diffuse, moderate hyperplasia of the olfactory epithelium in female B6C3F_1 mice.

Male and female Fischer 344 rats and B6C3F_1 mice were exposed by inhalation to 0, 5, 20 or 60 ppm [0, 23, 91 or 272 mg/m^3] 1,3-dichloropropene (*cis*, 49.5%; *trans*, 42.6%)

for 6 h per day on five days per week for up to two years. Significant morphological alterations in the nasal tissues of rats exposed to 60 ppm and mice exposed to 20 or 60 ppm 1,3-dichloropropene were found at the end of the study (Lomax *et al.*, 1989).

Treatment with buthionine sulfoximine (0.2 M in 0.58% NaCl, 4 mL/kg bw) (to inhibit glutathione synthesis) 4 h before dosing with 1,3-dichloropropene or with diethyl maleate (3.1 M in corn oil, 0.4 mL/kg bw) (to deplete glutathione) or corn oil itself 1 h before dosing with 1,3-dichloropropene at 50 and 75 mg/kg bw resulted in elevations of *N*-acetylglucosaminidase excretion. In contrast, treatment with aminooxyacetic acid (0.125 M in 0.85% NaCl, 4 mL/kg bw) (which inhibits β-lyase activity) 1 h before 1,3-dichloropropene injection prevented the 1,3-dichloropropene-induced release of *N*-acetylglucosaminidase from the renal tubule. These results suggest that the nephrotoxic effects of 1,3-dichloropropene may be mediated through the mercapturic acid metabolites in the kidney, rather than glutathione depletion (Osterloh & Xiwen, 1990).

1,3-Dichloropropene was administered to male and female Fischer 344 rats and B6C3F_1 mice for 13 weeks (0, 5, 15, 50 or 100 mg/kg bw per day to rats or 0, 15, 50, 100 or 175 mg/kg bw per day to mice) in the diet by mixing a microencapsulated formulation of 1,3-dichloropropene into animal feed (microencapsulated in a 80/20% starch/sucrose matrix; 1,3-dichloropropene consisted of 50.7% *cis*-, 45.1% *trans*-isomers). There was a decrease in the body weights of male and female rats ingesting more than 5 and 15 mg/kg bw per day, respectively, and a decrease in body weights of mice in all treatment groups relative to controls. A low degree of basal cell hyperplasia in the nonglandular portion of the stomach of male and female rats exposed to more than 15 mg/kg bw per day was also observed, but the severity of the damage was somewhat diminished after a four-week recovery period during which the rats were not exposed to 1,3-dichloropropene. The authors established a no-observed-adverse-effect level for rats as 5 mg/kg bw per day, and a no-observed-adverse-effect levels in mice as 15 mg/kg bw per day (Haut *et al.*, 1996).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

In a study of 64 men employed in the production of chlorinated compounds [time-weighted average exposures, < 1 ppm [4.5 mg/m^3] 1,3-dichloropropene, 3.1 mg/m^3 allyl chloride and 3.8 mg/m^3 epichlorohydrin], sperm counts and percentages of normal sperm were similar in the study group and among 63 controls. The volunteer participation rate for the study group was 64% (IARC, 1986).

4.3.2 *Experimental systems*

Pregnant Fischer 344 rats and New Zealand White rabbits were exposed by inhalation to 0, 20, 60 or 120 ppm [0, 91, 272 or 545 mg/m^3 air] 1,3-dichloropropene (47.7% *cis*, 42.4% *trans*) for 6 h per day during gestation days 6–15 (rats) or 6–18 (rabbits). Dose-dependent decreases in maternal weight gain and food consumption were observed in rats at all doses of 1,3-dichloropropene, and in rabbits at 60 and 120 ppm. In spite of

maternal toxicity, no evidence of teratogenetic or embryotoxic response was observed in rats or rabbits at any of the doses tested (Hanley *et al*., 1987).

Breslin *et al*. (1989) conducted a two-generation reproduction study in Fischer 344 rats with 1,3-dichloropropene (92% technical product). Male and female Fischer 344 rats were exposed by inhalation to 0, 10, 30 or 90 ppm [0, 45, 136 or 409 mg/m^3] 1,3-dichloropropene for 6 h per day on five days per week for two generations. The parental F_0 and F_1 generations were each bred twice. Parental effects (decreased body weights, histopathological effects on nasal mucosa) occurred only at the highest concentration of 1,3-dichloropropene. No adverse effect on reproductive parameters or neonatal growth or survival was found in either of the F_1 or F_2 litters, even at the 90 ppm dose.

Male and female Wistar rats were exposed by inhalation to concentrations of 0, 10, 30 or 90 ppm (v/v) of a mixture of vapours containing 28.1% *cis*-1,3-dichloropropene, 25.6% *trans*-1,3-dichloropropene, 25.6% 1,2-dichloropropane, 20.7% other chemicals (D-D) for 6 h per day on five days per week for 10 weeks. Treated males were paired with untreated virgin females, and treated females were paired with untreated males. Exposure to the vapours produced no adverse effect on the libido, fertility or morphology of the reproductive tracts of rats of either sex (Linnett *et al*., 1988).

Relative testicular weights of Fischer 344 male rats exposed by inhalation to 90 and 150 ppm [409 and 681 mg/m^3] 1,3-dichloropropene (*cis*, 48.6%; *trans*, 42.3%) for 6 h per day on five days per week for 13 weeks were increased and thymus weights of female rats exposed to 150 ppm 1,3-dichloropropene were decreased relative to controls (Stott *et al*., 1988). Changes in testicular or thymic weights at the lower doses of 10 and 30 ppm were not observed.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

1,3-Dichloropropene was mutagenic in *Salmonella typhimurium*, with or without metabolic activation. In one study, the *cis*-isomer was reported not to be mutagenic to *Salmonella typhimurium* TA100 without metabolic activation, and to become active only after exposure to oxygen and consequent generation of autooxidation products. Glutathione was shown to efficiently inhibit the mutagenicity in *Salmonella* of *cis*- and *trans*-1,3-dichloropropene both with and without metabolic activation. There was no difference in the *Salmonella* mutagenicity between *cis*- and *trans*-1,3-dichloropropene.

In one study, a mixture of *cis*- and *trans*-1,3-dichloropropene gave positive results for induction of mutations in *Drosophila melanogaster*.

A mixture of *cis*- and *trans*-1,3-dichloropropene caused sister chromatid exchanges in human lymphocytes *in vitro* as well as in other cultured mammalian cells. It also induced unscheduled DNA synthesis and DNA strand breaks. However, it was negative for induction of chromosomal aberrations in Chinese hamster ovary CHO cells.

Table 1. Genetic and related effects of 1,3-dichloropropene

Test system	Results		Dose (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
***trans*-1,3-Dichloropropene**				
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	10	De Lorenzo *et al*. (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	10	Creedy *et al.* (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	NG	Neudecker & Henschler (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	10	De Lorenzo *et al*. (1977)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	122	Neudecker *et al.* (1977)
SAS, *Salmonella typhimurium* TA1978, reverse mutation	+	+	25	De Lorenzo *et al*. (1977)
***cis*-1,3-Dichloropropene**				
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	10	De Lorenzo *et al*. (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	5	Creedy *et al.* (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	NG	Neudecker & Henschler (1986)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	20	Watson *et al*. (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	10	De Lorenzo *et al*. (1977)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	122	Neudecker *et al.* (1977)
SAS, *Salmonella typhimurium* TA1978, reverse mutation	+	+	25	De Lorenzo *et al*. (1977)
Mixture of *trans*- + *cis*-1,3-dichloropropene				
PRB, SOS-Chromotest, DNA damage in *Escherichia coli* PQ37	+	NT	365	Von der Hude *et al*. (1988)
SAF, *Salmonella typhimurium* TA98, forward mutation (rifampicin resistance)	+	NT	200	Vithayathil *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	55	Stolzenberg & Hine (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	17	Haworth *et al*. (1983)

Table 1 (contd)

Test system	Results		Dose (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation (1,3-dichloropropene purified by chromatography)	–	NT	500	Talcott & King (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	NG	Talcott & King (1984)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	17	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	(+)	–	50	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	NT	200	Vithayathil *et al*. (1983)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		5750 ppm feed	Valencia *et al*. (1985)
DMH, *Drosophila melanogaster*, heritable translocations	–		5750 ppm feed	Valencia *et al*. (1985)
DIA, DNA fragmentation, Chinese hamster lung V79 cells *in vitro*	+	NT	200	Martelli *et al*. (1993)
DIA, DNA fragmentation, rat primary hepatocytes *in vitro*	+	NT	20	Martelli *et al*. (1993)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	NT	35	Martelli *et al*. (1993)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	–	11	von der Hude *et al*. (1987)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	30	Loveday *et al*. (1989)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	100	Loveday *et al*. (1989)
DIH, DNA fragmentation, human hepatocytes *in vitro*	+	NT	35	Martelli *et al*. (1983)
UIH, Unscheduled DNA synthesis, human hepatocytes *in vitro*	+	NT	35	Martelli *et al*. (1983)
SHL, Sister chromatid-exchange, human lymphocytes *in vitro*	+	+	11	Kevokordes *et al*. (1996)
DVA, DNA fragmentation, rat liver, kidney and gastric mucosa *in vivo*	+		62.5 ip × 1	Ghia *et al*. (1993)

Table 1 (contd)

Test system	Results		Dose (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
URP, Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		125 po × 1	Ghia *et al*. (1993)
MVR, Micronucleus test, rat bone-marrow, spleen and liver cells *in vivo*	–		125 po × 1	Ghia *et al*. (1993)
MVM, Micronucleus test, NMRI mice bone-marrow cells *in vivo*	+		187 po × 1	Kevekordes *et al*. (1996)
SPF, Sperm morphology, (C57BL/6 × C3H)F_1 mice *in vivo*	–		75 ip × 5	Osterloh *et al*. (1983)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; po, oral; ip, intraperitoneal

A mixture of *cis*- and *trans*-1,3-dichloropropene has given contradictory results in the mouse micronucleus assay. In the most recent study, it was positive in female (but not in male) NMRI mice. On the other hand, it did not induce micronuclei in the bone marrow, spleen or liver of partially hepatectomized rats, nor did it cause unscheduled DNA synthesis in rat hepatocytes *in vivo*.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

1,3-Dichloropropene is used in organic synthesis and as a soil fumigant. It can be released into the air and waste water and can occur to some extent in ground water.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Technical-grade 1,3-dichloropropene (containing 1.0% epichlorohydrin), when given by gavage, produced tumours of the urinary bladder, lung and forestomach in mice and of the liver and forestomach in rats. Inhalation exposure produced an increase in the incidence of bronchioalveolar adenomas in mice. No increase in tumours was seen in rats. After subcutaneous administration to mice, the *cis*-isomer produced malignant tumours at the site of injection.

5.4 Other relevant data

The principle metabolic pathway of 1,3-dichloropropene is conjugation with glutathione and elimination as mercapturic acids. Enzymatic conjugation with glutathione and non-enzymatic alkylation proceed more rapidly with the *cis*-isomer than with the *trans*-isomer. At the concentrations used in rodent carcinogenicity studies by inhalation, significant morphological alterations in the nasal tissues were observed. No teratogenic or embryotoxic effects were observed in rats and rabbits exposed by inhalation to the mixed isomers.

1,3-Dichloropropene induces micronuclei in the bone marrow of female mice, as well as sister chromatid exchanges and DNA damage in cultured mammalian cells. It is mutagenic to bacteria.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of 1,3-dichloropropene were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of mixed isomers of 1,3-dichloropropene (technical grade).

Overall evaluation

1,3-Dichloropropene (technical-grade) is *possibly carcinogenic to humans (Group 2B)*.

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1,2-DIMETHYLHYDRAZINE

Data were last reviewed in IARC (1974) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

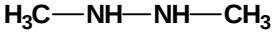
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 540-73-8
Chem. Abstr. Name: 1,2-Dimethylhydrazine
IUPAC Systematic Name: 1,2-Dimethylhydrazine
Synonyms: DMH; hydrazomethane

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2H_8N_2$ Relative molecular mass: 60.10

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Flammable, hygroscopic liquid. Fumes in air and gradually turns yellow. Characteristic ammonia-like odour of aliphatic hydrazines (Budavari, 1996)
(*b*) *Boiling-point*: 81°C (Lide, 1995)
(*c*) *Melting-point*: –9°C (United States National Library of Medicine, 1997)
(*d*) *Solubility*: Miscible with water with much evolution of heat. Also miscible with ethanol, diethyl ether, dimethylformamide and other hydrocarbons (Budavari, 1996)
(*e*) *Vapour pressure*: 9 kPa at 24.5°C (United States National Library of Medicine, 1997)
(*f*) *Conversion factor*: $mg/m^3 = 2.46 \times ppm$

1.2 Production and use

There are no known commercial uses for 1,2-dimethylhydrazine other than as a research chemical (United States National Library of Medicine, 1997).

1.3 Occurrence

1.3.1 *Occupational exposure*

Occupational exposures to 1,2-dimethylhydrazine may occur in the laboratory.

1.3.2 *Environmental occurrence*

The limited production and use of 1,2-dimethylhydrazine as a research chemical may result in its release to the environment in small quantities through various waste streams (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not proposed any occupational exposure limit for 1,2-dimethylhydrazine in workplace air. Sweden and Switzerland have 8-h time-weighted average threshold limit values of 0.2 mg/m^3 and 1.2 mg/m^3, respectively, for exposure in workplace air, with a skin notation (International Labour Office, 1991).

No international guideline for 1,2-dimethylhydrazine in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

1,2-Dimethylhydrazine was tested for carcinogenicity in mice, rats and hamsters following oral and subcutaneous or intramuscular administration, producing tumours at various sites (IARC, 1974).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female Swiss albino mice, six weeks of age, received 1,2-dimethylhydrazine continuously in the drinking-water for life at dose levels of 0.15, 0.3, 0.6, 1.25, 2.5, 5, 10 or 20 mg/L (ppm). A positive dose–response relationship for the incidence of vascular tumours at various sites (muscle, liver, pararenal tissues) and an inverse relationship for the latency period were observed. In males and females combined, tumour incidences were: 1, 6, 9, 23, 61, 91, 95 and 79% at the above dose levels, respectively. The incidence of blood vessel tumours in controls was 1% in males and 3% in females. Tumours were angiomas and angiosarcomas; the percentage of the latter correlated positively with dose. Thus, at the four highest dose levels, almost all tumours were angiosarcomas, while at the low doses, 30–50% of all tumours were angiomas. In

addition, some animals developed lung tumours, but no association was observed between the dose of 1,2-dimethylhydrazine and lung tumour incidence (Toth & Patil, 1982).

Groups of 16 male and 16 female strain A/J mice received 1,2-dimethylhydrazine in the drinking-water at a dose of 240 mg/kg bw, the maximum tolerated dose, for eight weeks. All animals were killed 24 weeks after the initiation of the bioassay. All male and female mice surviving to the end of the experiment developed lung adenomas with an average of 13.6 ± 7.4 adenomas per mouse in males ($p < 0.001$ compared with untreated controls) and 29.4 ± 12.7 in females ($p < 0.001$ compared with untreated controls). The corresponding figures in untreated controls were 38% of males with 0.47 ± 0.63 adenomas per mouse and 25% of females with 0.29 ± 0.56 adenomas per mouse (Stoner *et al*., 1986).

3.1.2 *Rat*

A group of 28 male Fischer rats, seven weeks of age, was treated by gavage with a single dose of 35 mg/kg bw 1,2-dimethylhydrazine hydrochloride dissolved in 0.1 M sodium acetate buffer. The animals were killed 1.5 years after treatment and 22/28 (78.6%) treated rats had colon epithelial tumours [detailed histological description not given]. One of the rats with colon tumours also developed ‘a small intestinal tumour’ and another had a tumour of the Zymbal gland (Schiller *et al*., 1980).

Male and female rats of three inbred strains (DA, HS and AS2, as described by Festing & Staats, 1973), approximately 35–45 days of age, were treated at weekly intervals with 10 doses of 30 mg/kg bw 1,2-dimethylhydrazine hydrochloride in saline by gavage. Controls received saline alone. At intervals of 4, 9, 15 and 25 weeks after the first dose, three DA and three HS rats treated with 1,2-dimethylhydrazine were killed together with one control rat of each strain. The remaining surviving animals were killed 30 weeks after the first dose. Two DA and two HS rats died during the course of the experiment and all the others between weeks 20 and 30. All AS2 rats receiving 30 mg/kg bw 1,2-dimethylhydrazine died between day 6 and 59 after the first dose (profuse diarrhoea and rhinorrhoea developed within the first four days) and survival was only marginally improved by subcutaneous administration of this dose of 1,2-dimethylhydrazine. Consequently, a lower dose was used (10 mg/kg bw) and 12/20 AS2 rats survived to 30 weeks. Fifteen or more weeks after the end of 1,2-dimethylhydrazine treatment, 27.5% of treated DA rats developed diarrhoea, 10% rectal bleeding and 7.5% abdominal swelling. The incidence of large bowel tumours was 10/10 and 13/13 in male and female treated DA rats, with 6.8 tumours per rat in males and 2.8 tumours per rat in females. The incidence was somewhat lower in male HS rats (7/9) and even lower in female HS rats (5/14). The small bowel was affected less frequently than the large bowel ($p < 0.05$), particularly with regard to tumour multiplicity (see Table 1). Histologically, the intestinal tumours were classified as adenocarcinomas. In addition, one hepatocellular carcinoma, seven cholangiomas and three liver angiosarcomas and two Zymbal gland tumours developed in 12 AS2 rats [sex unspecified] treated with 10 mg/kg bw

Table 1. Incidence of intestinal tumours in DA, HS and AS2 rats 30 weeks after the beginning of 1,2-dimethylhydrazine treatment by gavage

	DA		HS		AS2	
	Males	Females	Males	Females	Males	Females
Rats with large bowel tumours	10/10	13/13	7/9	5/14	8/10	2/2
No. of tumours per rat	6.8	2.8	1.4	0.4	2.3	3.5
Rats with small bowel tumours	7/10	1/13	2/9	2/14	7/10	2/2
No. of tumours per rat	0.8	0.08	0.2	0.1	1.6	1.0

From Teague *et al.* (1981)

1,2-dimethylhydrazine. In DA rats, 7/21 males and 0/19 females developed Zymbal gland tumours, with invasion into the bone in all but one case (Teague *et al.*, 1981).

In a study of the relationship between colon tumours and the gut-associated lymphoid tissue, 1,2-dimethylhydrazine hydrochloride dissolved in saline was administered by gavage to outbred male weanling Sprague-Dawley rats starting at seven weeks of age. In experiment 1, 20 rats received five weekly doses of 15 mg/kg bw 1,2-dimethylhydrazine with either 5% mixed fat or 24% corn oil in the diet and were killed after the last treatment. No intestinal tumours were found. In experiment 2, four groups of 10 rats received five weekly doses of 65 mg/kg bw 1,2-dimethylhydrazine and were fed diets containing different types and levels of fat (5% mixed fat, 24% beef tallow, 24% corn oil or 25% Crisco). The 33 surviving animals were killed four months after the first dose. There was no difference in the incidence of colon tumours between diet groups. A total of 49 colon adenocarcinomas developed in the 33 rats, 71% of which were polypoid and 29% sessile. Fifty per cent of the sessile tumours and none of the polypoid tumours were associated with colonic lymphoid aggregates ($p < 0.001$). In experiment 3, four groups of 60 rats were fed different diets and received 15 mg/kg bw 1,2-dimethylhydrazine once a week for five weeks. The animals were killed when moribund or when they showed clinical signs of intestinal tumours. A total of 165 colon tumours developed in 159 rats, 38% of which were polypoid and 62% sessile. As in experiment 2, a highly significant association between colonic tumours and the presence of lymphoid aggregates in a given segment of the colon was found only for sessile adenocarcinomas (28% versus 0 in polypoid adenocarcinomas) (Nauss *et al.*, 1984). [The Working Group noted that only the total number of colon tumours but not the number of rats with tumours was indicated.]

In a study designed to investigate the role of caloric restriction, 38 male Fischer 344 rats (50 days of age) were given 30 mg/kg bw 1,2-dimethylhydrazine hydrochloride dissolved in saline by gavage once a week for six weeks. One week after the final dose, the rats were randomized into groups of 19 and fed one of two semi-purified diets. One group was fed *ad libitum*, with 30 g of food given to each rat. Rats in the calorically restricted group were given 60% of the weight of food that the rats fed *ad libitum* had

consumed the day before. Rats had an average weight of 257 g one week after the final dose of 1,2-dimethylhydrazine. Rats fed *ad libitum* had a final mean weight of 372 ± 6 g. The calorically restricted rats lost weight during the first four weeks and then reached a plateau with slight weight loss to a final weight of 216 ± 4 g ($p < 0.001$). The animals were killed after 28 weeks of feeding the semi-purified diets. All 19 rats (100%) fed *ad libitum* developed tumours of the colon versus 10/19 (53%) of the calorically restricted rats; the incidence of invasive carcinomas was 11% and 6%, respectively ($p < 0.05$). Extra-colonic tumours (those of the duodenum and ear canal) were seen in 32% and 11% ($p < 0.05$) of rats fed *ad libitum* and calorically restricted rats, respectively (Klurfeld *et al.*, 1987).

3.2 Subcutaneous, intraperitoneal, intramuscular, intrarectal or intraplacental administration

3.2.1 *Mouse*

A group of 60 female CF_1 mice received six weekly subcutaneous injections of 20 mg/kg bw 1,2-dimethylhydrazine dihydrochloride. Forty-three survivors were killed 45 weeks after the onset of the experiment and 'over 83%' of them (i.e., 36 mice) developed visible colonic neoplasms with 2.3 tumours per animal. The 3-cm segment of the colon above the anus contained 61% of all neoplasms. Thirteen of the 43 surviving 1,2-dimethylhydrazine-treated mice demonstrated some degree of carpeting, i.e. uncountable numbers of tumours. Histologically, the tumours showed the full spectrum of neoplastic lesions from morphologically benign polyps to adenocarcinomas (Deschner & Long, 1977).

Female mice of eight different strains were treated with 25 weekly subcutaneous injections of 8 mg/kg bw 1,2-dimethylhydrazine (calculated as base). Survivors were killed 50 weeks after the beginning of treatment. Colon tumours developed in all strains, with the highest incidence in BALB/c mice (93.3%) and the lowest incidence in C3HA mice (30.9%) (Table 2). Likewise, tumours of the anal region and clitoral glands developed in mice of all strains with incidence varying from 24% (F_1 hybrid and C57BL/6) to 63.3% (BALB/c strain). Sarcoma of the uterus developed in about 40% in C3H and CBA mice, 20.7% in F_1 hybrids, 7.7% in AKR mice, 2.7% in C57BL/6 mice, but no such tumours in BALB/c, DBA/2 and C3HA mice. Ovarian lesions (mainly big haemorrhagic cysts) were most frequent in BALB/c, DBA/2 and C3HA mice (40.7, 62.9 and 85.7, respectively) and rather rare in C3H and CBA mice (6% and 3.7%, respectively) (see Table 2). None of the above tumours was found in untreated mice of the same strains (Turusov *et al.*, 1982).

Groups of female CBA mice or male and female (C57BL × CBA)F_1 mice were given 1,2-dimethylhydrazine subcutaneously, intraperitoneally or by gavage daily, weekly or once every two weeks. When female CBA mice were given 1,2-dimethylhydrazine weekly subcutaneously, intraperitoneally or by gavage (groups 1, 2 and 3) at a dose of 8 mg/kg bw (base) for 25 weeks, survival was the same, as was the incidence of tumours in the intestine, uterus or anal region. The incidence of liver haemangioendothelioma

Table 2. Incidence (%) of tumours at various sites and ovarian lesions in eight strains of mice treated with 1,2-dimethylhydrazine (8 mg/kg bw for 25 weeks) subcutaneously

	C3H	CBA	(CBA×C57BL/6J)F_1	C57BL/6J	BALB/c	DBA/2	C3HA	AKR
Effective no.[a]	16	29	29	37	30	27	42	39
Colon tumours	75	70.4	79.3	59.4	93.3	55.5	30.9	53.8
Anal region/clitoral gland tumours	50	59.2	24.1	24.3	63.3	25.9	59.5	38.5
Uterine sarcomas	37.5	40.7	20.7	2.7	–	–	–	7.7
Ovarian lesions	6.3	3.7	17.2	35.1	46.7	62.9	85.7	10.2
Liver haemangio-endotheliomas	6.3	18.4	37.9	5.4	23.3	22.2	14.3	20.5

From Turusov *et al.* (1982)

[a] Number of mice surviving 20 weeks after the beginning of treatment with 1,2-dimethylhydrazine

was highest after intraperitoneal administration (13/39 versus 7/34 and 5/27 after oral gavage or subcutaneous administration, respectively). Groups 4, 5 and 6 received the same dose by subcutaneous administration either every two weeks (16 mg/kg bw as a single treatment), weekly (8 mg/kg bw) or daily (1.14 mg/kg bw) for 30 weeks. In the daily treatment group (group 6), all mice died by the end of 1,2-dimethylhydrazine treatment, with ascites and cirrhotic livers which were firm and reduced in size. No such liver damage was observed in groups 4 and 5. Tumour incidence was somewhat higher in group 5 (weekly treatment) than in group 4 (bi-weekly treatment). A comparison with group 6 (daily injections) is not possible because of the short lifespan. Nevertheless, the incidence of liver haemangioendotheliomas in group 6 was similar to that in group 5 (5/28 versus 8/29). Groups 7 and 8 received subcutaneous daily or weekly treatments at a total dose one-third that of groups 5 and 6 (for 10 weeks instead of 30 weeks). Consequently, the lifespan in both groups was similar (no considerable cirrhosis of the liver developed after daily treatment). The incidence of colon tumours was much lower in group 8 (3/69), receiving daily treatments, than in group 7 (12/36) that received weekly treatments ($p < 0.001$) and the same effect was observed with tumours of the anal region (Table 3). Daily treatment resulted in a much higher incidence of renal adenomas (43/69 versus 8/36 in groups 8 and 7, respectively) and liver haemangioendotheliomas (29/69 and 4/36, respectively) as compared to weekly treatment, with no change in the incidence of liver adenoma, lung adenoma and uterine sarcoma. When 1,2-dimethylhydrazine was given in the drinking-water (groups 12 and 13), survival was eight to nine weeks shorter than after administration of the same total dose by subcutaneous injection (groups 10 and 11). In spite of this, the incidence of vascular tumours of the renal capsule in males (group 13) was significantly higher (16/20) than that in males receiving subcutaneous injections (9/20; $p < 0.001$). The decrease in groups 12 and 13 (daily treatment) of the incidence of tumours of the colon and anal region (see Table 3) is still real when allowance is made for shorter survival. Thus, the negative correlation between the incidence of vascular and intestinal tumours is determined not by the route of 1,2-dimethylhydrazine administration but by the size of a single dose, with a predominance of vascular tumours at low single doses and that of intestinal tumours at high single doses (Turusov *et al.*, 1983).

Male mice of six different strains were treated with 15 weekly subcutaneous injections of 8 mg/kg bw 1,2-dimethylhydrazine (base) and were killed 42–43 weeks after the beginning of treatment. Tumour incidence is given in Table 4. The incidence of colon tumours varied from 3–4% (C3H and C3HA strain) to 74% in C57BL mice. The incidence of kidney adenomas ranged from 13 and 14% in C3H and C3HA mice to 79% in CBA mice. Angiosarcomas of the renal capsule developed in almost all mice of the CBA strain (97%) and only in 4% and 7% in C57BL and C3HA mice (Table 4) (Turusov *et al.*, 1985).

Groups of 16 male and 16 female strain A/J mice received intraperitoneal injections of 48, 120 or 240 mg/kg bw 1,2-dimethylhydrazine three times per week for eight weeks, at which time all animals were killed. Lung adenomas were found in 63%, 63% and 94% of male mice at the three dose levels used, with average numbers of adenomas per mouse of

Table 3. Route of administration and dose fractionation in 1,2-dimethylhydrazine carcinogenesis in mice

Group	Strain	Sex	Treatment			Effective no. of mice	Mice with tumours of the									
			Route	Frequency	Duration (wks)		Colon	Anal region	Kidney (adenoma)	Liver		Uterus (sarcoma)	Lung	Blood vessels		
										Adenoma	HAE			Renal capsule	Ovary	Other sites
1	CBA	F	gavage	Weekly	25	34	23	14	–	–	7	16	2	–	–	–
2	CBA	F	i.p.	Weekly	25	39	24	22	–	–	13	11	–	3	2	–
3	CBA	F	s.c.	Weekly	25	27	19	16	–	2	5	11	–	–	–	–
4	CBA	F	s.c.	Bi-weekly	30	28	17	17	–	–	9	12	–	–	–	–
5	CBA	F	s.c.	Weekly	30	29	21	23	–	–	8	14	–	–	2	–
6	CBA	F	s.c.	Daily	30	28	–	6	–	–	5	–	–	–	–	–
7	CBA	F	s.c.	Weekly	10	36	12	21	8	16	4	6	7	–	–	–
8	CBA	F	s.c.	Daily	10	69	3	17	43	28	29	12	15	–	4	–
9	CBA	F	Control	–	–	40	–	–	–	1	–	–	–	–	–	–
10	F_1	F	s.c.	Weekly	25	33	24	10	6	3	16	19	3	1	5	2
11	F_1	M	s.c.	Weekly	20	48	41	14	21	11	6	–	3	9	–	4
12	F_1	F	d.w.	Continuous	25	19	–	–	9	–	11	2	1	4	3	6
13	F_1	M	d.w.	Continuous	20	19	–	–	2	–	7	–	1	16	–	5
14	F_1	F	–	–	–	20	–	–	–	–	–	–	–	–	–	–
15	F_1	M	–	–	–	19	–	–	–	–	–	–	4	–	–	–

From Turusov *et al*. (1983)
HAE, haemangioendothelioma; wks, weeks; i.p., intraperitoneal; s.c., subcutaneous; d.w., drinking-water

Table 4. Strain differences in tumour incidences in mice that received 15 weekly subcutaneous injections of 8 mg/kg bw 1,2-dimethylhydrazine base

Strain	Effective no. of mice	% Animals with				
		Renal capsule angiosarcomas	Renal adenomas	Colon tumours	Liver haemangioendotheliomas	Tumours of anal region
C3H	31	35	13	3	13	13
CBA	33	97	79	33	15	18
(CBA×C57BL)F_1	47	36	30	54	9	21
C57BL	47	4	23	74	0	32
BALB/c	23	13	43	26	9	48
C3HA	28	7	14	4	0	14

From Turusov *et al.* (1985)

0.81 ± 0.98, 1.06 ± 1.12 and 4.25 ± 2.21, respectively. The figures for females were 56%, 69% and 100% with average numbers of adenomas per mouse of 0.75 ± 0.86, 1.81 ± 1.87 and 10.63 ± 5.69. Corresponding figures for controls were 7% and 0.07 ± 0.27 in males and 47% and 0.60 ± 0.83 in females. The difference from controls was significant ($p < 0.05$ to 0.001) in all groups except females given the lowest dose (Stoner *et al.*, 1986).

Groups of male and female CBA mice received 8, 16 or 32 subcutaneous injections of 8 mg/kg bw 1,2-dimethylhydrazine (calculated as base) and were allowed to live until their natural death or were killed when moribund. The incidence of tumours is presented in Table 5. Clear-cut dose–response relationships were observed in the incidence of colon tumours in females and, in part, in males; vascular kidney tumours (renal capsule angiosarcomas) in males; and liver haemangioendotheliomas, uterine sarcomas and ovarian lesions in females. Decreased tumour incidence at some sites (colon, anal region, liver adenomas) at the highest dose in males was due to the early mortality from vascular kidney tumours. In the same study, there were additional groups of mice of similar size receiving the same number of injections of deuterium-labelled 1,2-dimethylhydrazine. The influence of the deuterium-labelling on tumour development at various sites is shown in Table 6 (Turusov *et al.*, 1988).

In another experiment, 37–40 mice per group were given one, two or four injections of 8 mg/kg bw 1,2-dimethylhydrazine (calculated as base) and the mice lived until their natural death. The incidence of epithelial kidney tumours was 5.3%, 5.0%, 55.3% and 64.9% after 0, 1, 2 and 4 injections, respectively. All kidney tumours in the control group or after one injection of 1,2-dimethylhydrazine were small incidental adenomas, while half of the kidney tumours after two or four injections were fatal (Turusov *et al.*, 1990). One male receiving four injections of 1,2-dimethylhydrazine developed a nephroblastoma, a tumour which is exceedingly rare in mice (Turusov, 1992).

Table 5. Incidence of tumours in CBA mice that received 8, 16 or 32 weekly subcutaneous injections of 8 mg/kg (base) bw 1,2-dimethylhydrazine

No. of injections	Sex	Effective no. of mice	Kidney tumours		Colon tumours (%)	Tumours of anal region[a] (%)	Tumours of the liver		Uterine sarcomas (%)	Lesions[b] of ovary (%)
			Vascular (%)	Epithelial (%)			Haemangio-endotheliomas (%)	Adeno-mas (%)		
0	F	44	0	0	0	0	0	27.3	2.3	6.8
8	F	29	3.4	17.2	20.7	44.3	24.1	13.8	31.0	0
16	F	25	0	16.0	44.0	44.0	24.0	12.0	60.0	12.0
32	F	25	4.0	16.0	56.0	44.0	36.0	4.0	52.0	20.0
0	M	48	0	4.2	0	0	0	64.6		
8	M	20	45.0	75.0	40.0	15.0	30.0	20.0		
16	M	20	75.0	55.0	65.0	55.0	30.0	20.0		
32	M	20	85.0	40.0	25.0	15.0	15.0	10.0		

From Turusov *et al.* (1988)

[a] Clitoral or preputial gland

[b] Haemorrhagic cysts or angiomas

Table 6. Combined relative risks (odds ratios)[a] of various tumours in CBA mice that received 1,2-dimethylhydrazine versus those that received 1,2-di-[$^2H_3$]methylhydrazine

Tumour type	Females	Males
Vascular renal tumours	1.90	0.48
Epithelial renal tumours	0.79	1.07
Tumours of the anal region	2.50[b]	2.60[b]
Colon tumours	1.63	5.85[c]
Liver haemangioendotheliomas	0.77	1.45
Hepatomas	0.94	0.27[c]
Uterine sarcomas	1.54	
Ovarian angiomas	0.95	
Lung adenomas	0.59	

From Turusov *et al.* (1988)
[a] Relative risk was calculated by using the one-tailed exact method for the combination of 2×2 tables.
[b] Relative risk differed significantly from 1, $p < 0.05$
[c] Relative risk differed significantly from 1, $p < 0.01$

3.2.2 *Rat*

Subcutaneous administration

Groups of BD IX rats [sex not specified] received a single subcutaneous injection of 40 mg/kg bw 1,2-dimethylhydrazine at the age of one day (11 rats), 10 days (7 rats) or 30 days (13 rats). The rats lived until their natural death. Among 11 rats treated at one day of age, one developed a colon carcinoma, with no dysplasia or hyperplasia in other rats. Among rats treated at the age of 10 days, 3/7 developed 4 colon carcinomas. One rat had a dysplasia and another had a hyperplasia. Four of 13 rats treated at the age of 30 days developed 13 carcinomas and three rats developed carcinomas of the small bowel. Six rats had dysplastic and four rats hyperplastic colon lesions. One rat had a dysplastic lesion and one a hyperplastic lesion of the small bowel (Martin *et al.*, 1974).

Groups of seven-week-old (12 conventional and 12 germ-free) female Fischer rats received 20 weekly subcutaneous injections of 10 mg/kg bw 1,2-dimethylhydrazine and were autopsied one week after the last injection. Another group of 24 conventional and 18 germ-free rats received the same treatment but were killed 20 weeks after the last injection. None of the germ-free rats that were autopsied after the last injection of 1,2-dimethylhydrazine had developed colon tumours, while 2/12 conventional rats showed adenocarcinoma. At 40 weeks, all colon tumours in germ-free rats were adenomas, whereas in conventional rats, four out of a total of six colon tumours were adenocarcinomas (Table 7) (Reddy *et al.*, 1974).

Groups of 15 conventional and 24 germ-free female Fischer rats, 50 days of age, were given 20 weekly subcutaneous injections of 20 mg/kg bw 1,2-dimethylhydrazine

Table 7. Incidence of colon tumours in germ-free and conventional rats treated with 10 mg/kg bw 1,2-dimethylhydrazine subcutaneously

Status	Study duration (weeks)	No. of animals with colon tumours	Tumour	
			No. of adeno-carcinomas	No. of adenomas
Germ-free[a]	20	0/12	0	0
Conventional[a]	20	2/12	2	0
Germ-free[b]	40	2/18	0	2
Conventional[b]	40	6/24	4	2

From Reddy *et al.* (1974)

[a] Animals received subcutaneous injections of 10 mg/kg bw weekly for 20 weeks and then were autopsied.

[b] Animals received subcutaneous injections of 10 mg/kg bw weekly for 20 weeks and were autopsied 20 weeks after last injection (40 weeks total).

dihydrochloride. The rats were killed 15 weeks after the last injection. 1,2-Dimethylhydrazine induced squamous-cell carcinomas of the ear duct, mesenchymal tumours of the kidney, adenomas and adenocarcinomas of the small intestine (mainly in the duodenum) in conventional rats but not in germ-free animals. The multiplicity of colon tumours was much higher in conventional rats (Table 8) (Reddy *et al.*, 1975).

A total of 25 male and 16 female Wistar-Furth (WF) rats, 10 weeks of age, were divided into two groups [number of rats per group not specified], one of which was treated with 1,2-dimethylhydrazine and the other served as untreated control. Nine male and 16 female of Long-Evans (LE) rats of the same age were also given subcutaneous doses of 20 mg/kg bw 1,2-dimethylhydrazine weekly for 20 weeks. Three to six rats of each sex were killed at the 10th, 15th and 25th week after the last injection. The total incidence of intestinal tumours was 70% (12/17) in WF rats and 100% (17/17) in LE rats. The number of intestinal tumours per rat was 1.5 (26/17) for all tumours, 1.2 for carcinomas in WF rats and 5.7 (97/17) and 2.7, respectively, in LE rats; metastatic deposits were found in 3/17 WF rats and 8/17 LE rats. Autoradiographic study did not reveal any difference between the two strains of either the treated group or non-treated controls in relation to the number of [^{3}H]thymidine-labelled cells or of mitotic cells in the mucosa of the descending colon (Takizawa *et al.*, 1978). [The Working Group noted the small number of animals and the lack of information on distribution by sex.]

A group of 15 male Fischer 344 rats, 11 weeks old, received 20 weekly subcutaneous injections of 10 mg/kg bw 1,2-dimethylhydrazine dihydrochloride. The rats were killed 44 weeks after the first injection. A second group of 28 male Fischer rats received 20 weekly subcutaneous injections of 20 mg/kg bw and the rats were killed 31 weeks after the first injection. Five animals of Group 1 developed large bowel adenocarcinomas (1.2 tumour/colon-tumour-bearing rat or 0.4 tumour per rat in the whole group). Twenty-two

Table 8. Tumour incidence in germ-free and conventional rats treated with 20 mg/kg bw 1,2-dimethylhydrazine for 20 weeks

Status	No. of rats	No. of animals with tumours				Multiplicity of colon tumours (per rat)		
		Ear canal	Kidney	Small intestine	Colon	Total tumours	Adenocarcinoma	Adenoma
Germ-free	24	0	0	0	5	$0.2 \pm 0.1^{a,b}$	0.1 ± 0.1^{b}	0.1 ± 0.1^{b}
Conventional	15	13	3	12	14	2.1 ± 0.4	1.2 ± 0.2	0.9 ± 0.3

From Reddy *et al.* (1975)
[a] Mean ± S.E.
[b] Significantly different from conventional group: $p < 0.01$

animals in Group 2 developed large bowel tumours (total 54 or 2.5 tumours per colon-tumour-bearing rat or 1.9 per rat in the group). There were four small bowel adenocarcinomas in Group 1 and 12 small bowel adenocarcinomas in Group 2. One rat (6%) in Group 1 and 21 rats (75%) in Group 2 developed squamous-cell carcinomas of the ear canal (Hagihara *et al.*, 1980).

A group of 60 female Wistar rats, 15 weeks of age, received two subcutaneous injections of 120 mg/kg bw 1,2-dimethylhydrazine (calculated as base) at 10-day intervals. Twenty-three animals were killed 30 weeks after the first injection. Thirteen of 23 animals exhibited tumours of the colon and one a tumour of the small intestine. Twenty-one (91%) of 23 rats developed renal tumours; in 10 rats, they were bilateral and in six rats, there was more than one tumour per affected kidney. All neoplasms were diagnosed as mesenchymal kidney tumours (Sunter & Senior, 1983).

A group of 40 weanling outbred female Wistar rats received a single subcutaneous injection of 200 mg/kg bw 1,2-dimethylhydrazine. Four rats died within four days after the injection. Twenty-five of 36 rats that survived for more than four days and were killed within one year after 1,2-dimethylhydrazine administration developed renal tumours, nine of them bilateral. Pulmonary metastases were found in one animal. Twenty-five (73%) of 34 tumours examined histologically were diagnosed as nephroblastoma-like tumours similar to human Wilms' tumour, three were mesenchymal tumours, three were adenomas, two were adenocarcinomas and one was unclassifiable (Sadrudin *et al.*, 1985).

Five groups of 34 male weanling Sprague-Dawley rats received either fibre-free basal diet or the basal diet uniformly diluted by the addition of a citrus pectin, guar gum or cellulose, so that the final diet consisted of 10% (by weight) fibre source and 90% basal diet. Oat bran, containing 26% fibre, was added into the diet of the fourth experimental group which thus was fed only a 5.2% fibre diet. Twenty-four rats in each diet group received 12 weekly subcutaneous injections of 20 mg/kg bw 1,2-dimethylhydrazine. The control rats were killed at 35 weeks and all carcinogen-treated rats were killed 30 weeks after the start of the study (only four rats did not survive until this time). The percentage of rats developing large bowel tumours was higher in all groups fed diets supplemented with fibre, but the increase was only significant in the group given guar gum (62.5% versus 33.4% in the fibre-free group, $p < 0.05$). There was a four-fold increase in the percentage of rats with proximal colonic adenocarcinomas in the groups fed oat bran, pectin and guar gum as compared to the fibre-free controls ($p < 0.025$) [data presented graphically] (Jacobs & Lupton, 1986).

Three groups of male Sprague-Dawley rats, weighing approximately 200 g, received subcutaneous injections of 21 mg/kg bw 1,2-dimethylhydrazine either as a single treatment (109 rats) or as weekly injections for three months (97 rats) or as weekly injections for six months (100 rats). Among rats receiving a single treatment, seven developed colonic carcinomas which all originated in the areas of lymphoid patches, this being considered by the authors as a proof of carcinoma development *de novo*. No adenomas were observed in this group. After weekly treatment for three months, 54 colonic tumours developed (4 adenomas and 50 adenocarcinomas). In 23 of the latter (46%), the

adenocarcinoma was seen to originate in a pre-existing adenoma and in three (6%) from discrete lymphoid patches. In the 100 rats treated for six months, 65 colonic tumours developed (8 adenomas and 57 adenocarcinomas). In 25 of 57 adenocarcinomas (43.9%), these were seen to originate from a pre-existing adenoma and in 2 (3.5%) from discrete lymphoid patches. In the remaining 30 adenocarcinomas of this group and in 21 adenocarcinomas of the three-month 1,2-dimethylhydrazine-treatment group, the origin of invasive growth could not be established (Rubio *et al.*, 1986).

Groups of male and female outbred SPF rats of the Riph:Wist stock were given subcutaneous injections of 1,2-dimethylhydrazine dihydrochloride starting from the first day of life up to 5, 10, 15 or 20 days after birth. The total dose of 1,2-dimethylhydrazine ranged from 0.36 to 2.16 mg per animal. [The interval between injections is not indicated, but probably these were daily treatments]. The animals were killed when moribund and the maximum survival was one year. No malignant tumour was found in control rats (5 males and 6 females). Hepatocellular carcinomas and kidney (mesenchymal) tumours developed in all groups of rats treated with 1,2-dimethylhydrazine. Thus in the group treated from day 1 to day 15, five of six survivors had liver carcinoma and all six rats had kidney mesenchymal tumours. The numbers of rats at risk in various groups varied from 1 to 11. Of a total of 73 rats, 32 developed malignant liver tumours and 50 developed mesenchymal kidney tumours, 22 of them being bilateral tumours (Sykora *et al.*, 1986).

Eight groups of 10–20 weanling female Wistar rats received subcutaneous injections of 1,2-dimethylhydrazine according to various protocols. All rats were killed when moribund and the experiment was terminated 27 months after the first injection. In Group A receiving 10 weekly injections of 15 mg/kg bw 1,2-dimethylhydrazine, 7 out of 14 rats examined developed intestinal carcinomas (3 colonic and 4 small intestinal carcinomas), 10 kidney fibrosarcomas, 3 hepatocarcinomas and 3 ear duct carcinomas. In Group B, given quarterly injections of 15 mg/kg bw 1,2-dimethylhydrazine, five out of seven rats examined developed intestinal carcinomas and one kidney fibrosarcoma, two hepatocarcinomas, one ear duct carcinoma and two mammary carcinomas were also seen. In Group C that received 27 weekly injections of 1.5 mg/kg bw 1,2-dimethylhydrazine, 1/16 rats had a colonic carcinoma and two rats had hepatocholangioma. Fifteen rats did not show any colonic lesion. In Group D, that received a single injection of 40 mg/kg bw 1,2-dimethylhydrazine, 1/7 rats had a colonic carcinoma. Twelve epithelial hyperplasias and 51 dysplasias were also found. In Group E, receiving eight quarterly injections of 5 mg/kg bw 1,2-dimethylhydrazine, 2/17 rats had colonic carcinomas; two dysplasias were also found. In Group F, that received a single injection of 20 mg/kg bw, 3/9 rats had colonic carcinomas, one of these rats having three carcinomas. Seventeen epithelial hyperplasias and twenty dysplasias were also found. In group G, receiving eight quarterly injections of 1 mg/kg bw 1,2-dimethylhydrazine, no carcinoma developed in 10 rats. No histologically detectable lesions were found in 19 control rats (Decaens *et al.*, 1989).

Eighteen Sprague-Dawley rats [sex not specified] received 19 weekly subcutaneous injections of 21 mg/kg bw 1,2-dimethylhydrazine and were killed 24 and 26 weeks after

the first injection. Twenty-three colon tumours and 10 tumours of the small bowel were observed. In addition, 15 tumours of the auditory canal were found in 10 rats: four squamous carcinomas, 10 papillomas and a pseudoepitheliomatous hyperplasia (Viñas-Salas *et al.*, 1992).

Methylazoxymethanol acetate

Groups of 10 male and female (combined) Sprague-Dawley rats, 40 days of age, susceptible to 1,2-dimethylhydrazine-induced colon tumours and a similar group of Lobund Wistar rats resistant to such induction received 10 weekly subcutaneous injections of 30 mg/kg bw methylazoxymethanol acetate, a metabolite of 1,2-dimethylhydrazine. All surviving rats were killed 20 weeks after the first injection. The carcinogenic effect in the Sprague-Dawley rats was much stronger than in Lobund Wistar rats (see Table 9). The authors concluded that the difference in susceptibility to colon tumour induction between the strains is partially due to inability to metabolize 1,2-dimethylhydrazine but also to some other endogenous factors (Pollard & Zedeck, 1978).

Table 9. Effect of methylazoxymethanol acetate on Sprague-Dawley and Lobund Wistar rats

Rat	No. of rats with tumours/no. of rats examined	No. of tumours in:			Total no. of tumours	No. of tumours/ rat
		Rectum	Colon	Duodenum		
Lobund Wistar	7/7[a]	2	11	9	22	3.1
Sprague-Dawley	10/10	4	84	116	204	20.4

From Pollard & Zedeck (1978)

[a] Three rats died soon after the onset of the experiment.

Intramuscular administration

A group of 25 male Fischer 344 rats, eight weeks of age, received weekly intramuscular injections of 20 mg/kg bw 1,2-dimethylhydrazine for 20 weeks. Rats were killed eight months after the beginning of treatment. Twenty-two (88%) rats developed large bowel tumours and 22 (88%) developed tumours of the small bowel. The average number of tumours per rat was 3.5. The tumours occurred most frequently in the descending colon followed by the transverse colon. A similar group of 98 rats received implantations of large bowel mucosa into the glandular stomach followed by the same treatment with 1,2-dimethylhydrazine as above. Forty-seven of 60 rats with successful grafts in the stomach developed tumours in the grafted colorectal tissue. Most successful were the grafts from the transverse and descending colon (100% and 93%, respectively) and the least successful were those from the proximal ascending colon (33%); grafts from the ascending colon (62%) and rectum (76%) had intermediate values. The percentage of poorly differentiated tumours was much higher in the colorectal tissue grafted from any

site of the large bowel compared to the intrinsic frequency, with an especially large increase in the grafts from the rectum. In both groups treated with 1,2-dimethylhydrazine, ear duct tumours were observed, their total incidence being 37% (Nakagawa *et al.*, 1992).

Intrarectal administration

Groups of 28 conventional and 28 germ-free Fischer rats received weekly intrarectal injections of 20 mg/kg bw 1,2-dimethylhydrazine for 20 weeks. The rats were killed 15 weeks after the last 1,2-dimethylhydrazine injection. A much higher incidence of squamous-cell carcinomas of the ear canal, mesenchymal kidney tumours, carcinomas of the small intestine and colon was observed in conventional rats than in germ-free rats (Table 10). (Reddy *et al.*, 1976).

3.2.3 *Hamster*

A group of 40 male Syrian golden hamsters received weekly intramuscular injections of 43 mg/kg bw 1,2-dimethylhydrazine dihydrochloride (total dose, 142.2 mg/kg bw). A group of 12 hamsters served as controls. All animals were observed until natural death. Fifteen treated animals died of toxicity before the end of dosing. Among the 25 surviving animals, nine developed gastrointestinal tumours (one stomach, one adenocarcinoma of the duodenum, four adenocarcinomas of the ileum and three adenocarcinomas of the colon). Five animals developed hepatocellular carcinomas (Osswald & Krüger, 1969).

3.2.4 *Monkey*

1,2-Dimethylhydrazine was administered subcutaneously to nine *Macaca fascicularis* monkeys (6 males and 3 females) at doses of 16 mg/kg bw, three times per month for up to two years. The experiment was terminated 275 weeks after the first injection, when three surviving monkeys were killed under ether. The total doses of 1,2-dimethylhydrazine received over the entire period ranged from 198 to 7143 mg. Tumours were found in three monkeys: two males developed tumours in the colon and one female in the uterus. Colon tumours were observed 34 and 47 weeks after the beginning of treatment, when the total doses of 1,2-dimethylhydrazine were 1080 mg (528 mg/kg bw) and 3696 mg (400 mg/kg bw), respectively. The death of these animals was preceded by loss of weight, absence of appetite and diarrhoea. Infiltration in the abdomen was palpated. The tumours in both cases were located in the ascending colon. Histologically, the colon tumours had the structure of adenocarcinomas, in one case with complexes of tumour cells in lymphatic vessels. No metastases were found. No intestinal tumours were found in the untreated monkeys. A female that received a total dose of 3648 mg (608 mg/kg bw) 1,2-dimethylhydrazine died of uterine bleeding 55 weeks after the onset of the experiment. At necropsy, a tumour of the corpus uteri was found; the tumour was a fibromyoma with very rare mitoses (Beniashvili *et al.*, 1992).

Table 10. Tumour incidence in germ-free and conventional Fischer rats treated intrarectally with 1,2-dimethylhydrazine

Status	No. of rats	No. of animals with tumours				Multiplicity of colon tumours (per rat)[a]		
		Ear canal	Kidney	Small intestine	Colon	Total tumours	Adeno-carcinoma	Adenoma
Germ-free	28	2	0	3	12	1.0[b] ± 0.1	0.50 ± 0.05	0.50 ± 0.06
Conventional	28	14	10	10	24	2.1 ± 0.2	1.1 ± 0.1	1.0 ± 0.1

From Reddy *et al.* (1976)

[a] Mean ± SE

[b] Significantly different from conventional group, $p < 0.05$

3.3 Transplacental exposure

Six female and three male *Macaca fascicularis* monkeys received 16 mg/kg bw 1,2-dimethylhydrazine by subcutaneous injection twice a month for 12 months. Males and females were kept together. Six female monkeys became pregnant while receiving 1,2-dimethylhydrazine; the deliveries took place 182, 237, 248, 305, 312 and 327 days after the first dose of carcinogen. The total amount of 1,2-dimethylhydrazine varied from 527 to 725 mg; 9 or 10 doses were given. Kept with their mothers during the rearing period, the three male and three female offspring were not subjected to any intervention, whereas the mothers continued to receive 1,2-dimethylhydrazine. Development of the offspring was normal, and all the animals were followed for over seven years. Two offspring died and postmortem examination revealed kidney tumours in both. Of these, monkey No. 1 was a 19-month old female; the amount of 1,2-dimethylhydrazine received by the mother was 177 mg before fertilization, 527 mg during the pregnancy and 710 mg during the neonatal and early development period. The cause of death was bilateral pneumonia. Histologically, the tumour consisted of mesenchymal and epithelial components, the latter resembling embryonal glomeruli. No tumour at any other site was found. Monkey No. 2 was a 14-month-old female. The mother received 560 mg 1,2-dimethylhydrazine before fertilization, 725 mg during embryogenesis and 640 mg during lactation. The monkey died of respiratory failure. Postmortem examination revealed a dense tumour in the cortex of the right kidney and nodes in both lungs. Histologically, the kidney tumour had a variable structure with mesenchymal and epithelial components. The epithelial component contained structures resembling embryonal glomeruli and tubule areas. Pulmonary metastases had the same structure. In both cases, a diagnosis of nephroblastoma was established (Beniashvili, 1989).

3.4 Administration with modifying agents

3.4.1 *Mouse*

A group of 30 female CBA mice (susceptible to 1,2-dimethylhydrazine-induction of uterine sarcomas), 2–3 months of age, received 10 weekly subcutaneous injections of 8 mg/kg bw 1,2-dimethylhydrazine base (group 1). In three other groups, oestradiol dipropionate dissolved in olive oil was administered subcutaneously one day before each injection of 1,2-dimethylhydrazine, for a total of 10, 20 or 25 treatments. A fifth group received 25 injections of oestradiol propionate without 1,2-dimethylhydrazine. Mice were killed 50 weeks after the first treatment. The incidence of uterine sarcomas was 1/30, 7/28, 17/26 and 19/26 in groups 1, 2, 3 and 4, respectively. The difference between group 1 (1,2-dimethylhydrazine alone) and the groups in which 1,2-dimethylhydrazine was combined with oestradiol propionate was significant ($p < 0.05$ to $p < 0.001$). There was a negative dose–response relationship in the time of the observation of the first uterine sarcoma at necropsy (Table 11). No uterine tumours were observed in group 5, which received oestradiol propionate alone (Turusov *et al.*, 1980).

Two groups of 36 and 40 female C3HA mice (a strain resistant to 1,2-dimethylhydrazine induction of uterine sarcomas), 2–3 months of age, received 20 weekly sub-

Table 11. Incidence of uterine sarcomas in CBA mice given 1,2-dimethylhydrazine combined with oestradiol dipropionate subcutaneously 50 weeks after the start of experiment

Group	Treatment	Effective no. of mice	Mice with uterine sarcomas		*p* value compared with group 1	Time (weeks) of observation of the first uterine sarcoma
			No.	%		
1	DMH	30	1	3.3		49
2	DMH + EP 10 weeks	28	7	25.0	0.05	40
3	DMH + EP 20 weeks	26	17	65.4	0.001	17
4	DMH + EP 25 weeks	26	19	73.0	0.001	18
5	EP 25 weeks	27	0	0		

From Turusov *et al.* (1980)
DMH, 1,2-dimethylhydrazine; EP, oestradiol dipropionate

cutaneous injections of 8 mg/kg bw 1,2-dimethylhydrazine (calculated as base). One group received subcutaneous injections of 10 µg/mouse oestradiol dipropionate dissolved in olive oil each time one day before 1,2-dimethylhydrazine administration and weekly for 10 weeks after 1,2-dimethylhydrazine treatment was stopped. The incidence of uterine sarcomas was 0/36 after administration of 1,2-dimethylhydrazine alone (group 1) and 11/40 in the group with 1,2-dimethylhydrazine plus oestradiol propionate (group 2) ($p = 0.008$). Haemorrhagic ovarian lesions were found in 20/34 animals in group 1 and 0/31 in group 2; tumours of the clitoral gland were found in 23/36 and 7/37 ($p = 0.026$) and colon tumours in 3/29 and 12/27 ($p = 0.057$), respectively. One colon polyp (6%) was found in a group receiving oestradiol propionate alone. No uterine or colon tumours or ovarian lesions were found in 29 controls or in 28 mice receiving oestradiol propionate alone (Turusov *et al.*, 1994).

Male and female newborn CBA mice received a single subcutaneous injection of 0.5 mg testosterone propionate in olive oil. At the age of two months, 30 male and 37 female neonatally androgenized and 30 male and 27 female control mice started receiving weekly subcutaneous injections of 8 mg/kg bw 1,2-dimethylhydrazine (total, 20 injections). All the surviving females were killed 30 weeks after the beginning of 1,2-dimethylhydrazine treatment and the surviving males at 34 weeks. There were also 30 androgenized and 20 intact females and 29 androgenized and 25 intact males that served as non-1,2-dimethylhydrazine-treated controls. By the end of the experiment, 27 females had palpable uterine tumours (up to 2 cm) out of 28 androgenized females treated with 1,2-dimethylhydrazine. Two out of 22 intact females treated with 1,2-dimethylhydrazine developed small (up to 0.5 cm) uterine sarcomas detected in mice killed at the end of the experiment ($p < 0.0001$). The incidence of renal capsule angiosarcomas (22/28) and colon tumours (20/28) was significantly higher in neonatally androgenized males treated

with 1,2-dimethylhydrazine than in control males treated with 1,2-dimethylhydrazine (7/28, $p < 0.0002$; and 9/28, $p < 0.005$, respectively) (Smirnova & Turusov, 1988).

A group of 90 male CD-1 mice, 40 days of age, received 20 weekly subcutaneous injections of 20 mg/kg 1,2-dimethylhydrazine dihydrochloride alone (group 1) or in combination with epidermal growth factor (EGF) (group 2). EGF was injected subcutaneously at a dose of 5 μg in 0.25 mL water on alternate days, through weeks 20 to 22. Each mouse received a total of 35 μg EGF. Mice were killed at 30 weeks. Colon tumours were found in 13/20 (mean, 2.25 ± 0.54) mice of group 1 (1,2-dimethylhydrazine alone) versus 18/24 (mean, 2.64 ± 0.65) of group 2 (1,2-dimethylhydrazine plus EGF) ($p > 0.05$). Anal tumours were present in 2/20 (mean, 0.1 ± 0.07) mice of group 1 versus 8/24 (mean 0.33 ± 0.1) ($p < 0.05$) mice of group 2 (Kingnorth *et al.*, 1985).

Groups of male CD-1 mice, 35 days of age, received six weekly subcutaneous injections of 15 mg/kg bw 1,2-dimethylhydrazine. Colitis was produced by seven weekly rectal instillations of 10 mM formyl-norleucylphenylalanine (FNLP) dissolved in dimethylsulfoxide after the mice had been anaesthetized by intramuscular injections of ketamine. Three days after the last injection of 1,2-dimethylhydrazine, all animals received the first of seven enemas of FNLP. Surviving mice were killed 21 weeks after the last injection of 1,2-dimethylhydrazine. At this time, 18/40 mice in the 1,2-dimethylhydrazine plus FNLP group, 5/39 mice receiving 1,2-dimethylhydrazine plus control enema and 6/15 mice receiving FNLP alone had died, either from 1,2-dimethylhydrazine-induced hepatotoxicity, colonic distension or perforation or from anaesthetic complications. In 5/22 animals receiving 1,2-dimethylhydrazine plus FNLP and 1/34 mice receiving 1,2-dimethylhydrazine and control enema ($p = 0.025$), adenocarcinomas developed in the descending colon. None of the mice developed multiple tumours and no tumours occurred in mice receiving FNLP alone. Colitis was confirmed histologically (Chester *et al.*, 1989).

3.4.2 *Rat*

Groups of 12, 7, 5, 8 and 16 male Fischer 34 rats [age unspecified] received a single intraperitoneal injection of 0, 25, 50, 75 or 100 mg/kg bw 1,2-dimethylhydrazine, respectively. Twelve hours after 1,2-dimethylhydrazine treatment, the rats were subjected to either partial hepatectomy or sham hepatectomy. After a two-week period, all the animals were placed on a basal diet containing 0.02% 2-acetylaminofluorene (2-AAF) for two weeks. One week after beginning the 2-AAF diet, the rats were given a single intraperitoneal injection of 2 mg/kg bw carbon tetrachloride in corn oil. After 14 days on the 2-AAF diet, the rats were placed on basal diet for an extra week and were then killed. Liver sections were stained for γ-glutamyltranspeptidase (γ-GT) histochemically. The numbers of γ-GT-positive foci per cm^2 in the liver were: 1, 2, 6, 10 and 19 in the 0-, 25-, 50-, 75- and 100-mg/kg bw groups, respectively [numbers taken from graphs]. The difference between the rats subjected to partial hepatectomy and those with sham hepatectomy in the number of γ-GT-positive foci at the 100 mg/kg bw dose of 1,2-dimethylhydrazine was highly significant ($p < 0.001$): 19.3 ± 2.6, 3.6 ± 0.8 and

0.8 ± 0.2 in rats receiving 100 mg/kg bw 1,2-dimethylhydrazine and subjected to partial hepatectomy, rats receiving 1,2-dimethylhydrazine and subjected to sham hepatectomy and rats that received a vehicle control and were subjected to partial hepatectomy, respectively (Ying *et al*., 1979).

In another study, 3-aminobenzamide (an inhibitor of poly(ADP-ribose)polymerase) enhanced the induction of γ-GT-positive foci produced in the liver by 1,2-dimethylhydrazine in Wistar rats but not in Fischer rats. Rats were administered a single intraperitoneal injection of 100 mg/kg bw 1,2-dimethylhydrazine hydrochloride dissolved in 0.4 mM EDTA followed 4 h later by an intraperitoneal injection of either 600 mg/kg bw 3-aminobenzamide in dimethylsulfoxide or dimethylsulfoxide alone. Two weeks later, the rats were placed on a diet containing 0.02% 2-AAF for two weeks; in the middle of this period, a single intragastric dose of carbon tetrachloride was administered. The rats were killed five weeks after 1,2-dimethylhydrazine administration. The number of γ-GT positive foci per cm^2, their size and the area occupied by foci were significantly higher in Wistar rats treated with 1,2-dimethylhydrazine and 3-aminobenzamide than in those given only 1,2-dimethylhydrazine and dimethylsulfoxide. This effect was not seen in similarly treated Fischer rats (see Table 12) (Denda *et al*., 1988).

Twenty-four male Sprague-Dawley rats were given eight weekly subcutaneous injections of 1,2-dimethylhydrazine at a dose of 9.5 mg/kg bw (calculated as base). Rats were subdivided into three groups of eight rats per group and were kept on a diet containing either 0, 5 or 15% cellulose. Rats were killed two weeks after the last injection of 1,2-dimethylhydrazine. Three other groups of rats were kept on the same diets but not treated with 1,2-dimethylhydrazine. The animals of control and 1,2-dimethylhydrazine-treated

Table 12. Effect of 3-aminobenzamide on the induction by 100 mg/kg bw 1,2-dimethylhydrazine of γ-GT positive foci in Fischer 344 and Wistar rats

Strain of rat	Treatment	No. of rats	γ-GT-positive foci		
			No./cm^2	Size of foci (mm^2)	Area occupied by foci (%)
Fischer 344	DMH + ABA	9	8.1 ± 2.3	0.52 ± 0.55	4.2 ± 2.2
	DMH + DMSO	9	6.8 ± 1.4	0.39 ± 0.14	2.7 ± 1.2
Wistar	DMH + ABA	10	5.8 ± 2.9[a]	0.13 ± 0.12	0.8 ± 0.7[b]
	DMH + DMSO	10	1.3 ± 1.0	0.8 ± 0.05	0.1 ± 0.1

From Denda *et al.* (1988)

[a] $p < 0.001$ compared to rats treated with DMH + DMSO

[b] $p < 0.01$ compared to rats treated with DMH + DMSO

γ-GT, γ-glutamyltranspeptidase; ABA, 3-aminobenzamide; DMH, 1,2-dimethylhydrazine; DMSO, dimethylsulfoxide

groups were maintained on the same caloric intake. 1,2-Dimethylhydrazine induced a significant increase in the mitotic activity as measured by the number of metaphase figures per crypt. The dietary cellulose caused a significant suppression of the 1,2-dimethylhydrazine-induced increase in crypt mitotic activity. Numbers of metaphase figures per crypt in the control rats were as follows: 3.45, 3.20 and 2.71 in rats given diet with 0%, 5% and 15% cellulose, respectively. The corresponding figures for 1,2-dimethylhydrazine-treated rats were 5.46, 3.56 and 3.63, respectively (Cameron *et al.*, 1989).

Male Sprague-Dawley rats, seven weeks of age, received eight weekly subcutaneous injections of 12 mg/kg bw 1,2-dimethylhydrazine (calculated as base). At week 9, pairs of rats (with or without 1,2-dimethylhydrazine treatment) were subdivided into groups and placed on seven different diets for 32 weeks. There was no significant difference in the mean total number of aberrant crypt foci per rat between animals which did or did not develop an adenocarcinoma in the descending colon, suggesting that this marker is not by itself a reliable predictor of the colon adenocarcinoma incidence (Table 13) (Hardman *et al.*, 1991).

Four groups of 30–32 male Sprague-Dawley rats each received subcutaneous injections of 12 mg/kg bw 1,2-dimethylhydrazine (calculated as base) weekly for eight weeks (defined as the initiation stage of the experiment). During the initiation period, all rats received standard food with 5% cellulose and 5% corn oil. The experimental diets were started one week after the last injection of 1,2-dimethylhydrazine. The rats were killed during week 32; 3 hours before scheduled sacrifice, each rat was given an intraperitoneal injection of 1 mg/kg bw colchicine. Neither a diet containing 10% pectin and

Table 13. Incidence of adenocarcinomas of the descending colon and the mean number of aberrant crypt foci in rats given subcutaneous injections of 1,2-dimethylhydrazine and different diets

Dietary group	Adenocarcinomas per rat	Mean no. ± SE of aberrant crypt foci per rat	
		With adenocarcinoma	Without adenocarcinoma
0% fibre	0.47	50.67 ± 7.5	50.13 ± 7.7
5% guar gum	0.33	42.20 ± 10.8	42.56 ± 6.5
10% guar gum	0.33	39.80 ± 6.4	33.77 ± 7.6
5% guar gum/5% pectin	0.37	38.00 ± 9.6	39.88 ± 6.6
10% pectin/5% corn oil	0.23[a]	22.43 ± 5.5	37.71 ± 4.6
10% pectin/10% corn oil	0.37	29.10 ± 7.3	29.06 ± 5.8
10% pectin/20% corn oil	0.06[a]	19.00 ± 19.00	31.32 ± 4.9

From Hardman *et al.* (1991)

[a] Significantly less than in rats on the 0% fibre diet

5% corn oil nor a diet containing 10% pectin and 20% corn oil suppressed the incidence of adenocarcinomas in the ascending colon, but both dietary modifications suppressed the incidence of adenocarcinomas in the descending colon. The decrease in adenocarcinoma incidence, due to addition of pectin, was attributed to a significant ($p < 0.05$) decrease in the incidence of adenocarcinomas in the area of the aggregates of lymphoid nodules, while the incidence of adenocarcinomas in locations distant from aggregates of lymphoid nodules was not significantly altered. The reduction in the risk for colon cancer was not necessarily accompanied by suppression of cell proliferation in the colonic crypts. The ability of a diet containing 10% or 20% corn oil to suppress cell proliferation was altered by the location of the crypt in the colon and by 1,2-dimethylhydrazine treatment (Hardman & Cameron, 1995).

3.5 Genetic studies

3.5.1 *Mouse*

The 100% susceptible ICR/Ha strain and the resistant C57BL/Ha strain were used in a genetic analysis of colon tumour induction by 1,2-dimethylhydrazine. Starting at 12 to 14 weeks of age, all mice received 22 subcutaneous weekly injections of 15 mg/kg bw 1,2-dimethylhydrazine. The mice were then observed up to 44 weeks. The percentages of colon tumours developing in the parental and hybrid mice are summarized in Table 14.

Table 14. 1,2-Dimethylhydrazine-induced colon tumour incidence in two inbred mouse strains and four types of hybrid showing inheritance of a major dominant susceptibility gene

Genotype	No. with tumour/ no. tested	% with tumour	
		Observed	Expected
ICR (M and F)	60/60	100	100
C57BL (M and F)	0/90	0	0
F_1 (M and F)	68/68	100	100
BCS[a] (M and F)	42/42	100	100
F_2 (M and F)	94/120	78	75[c]
BCR[b] (M and F)	46/117	39	50[c]
BCR (males only)	27/57	47	50[c]
BCR (females only)	19/60	32	50

From Evans *et al.* (1977)

[a] Susceptible backcross ($F_1 \times$ ICR)

[b] Resistant backcross ($F_1 \times$ C57BL)

[c] χ^2 test for goodness of titre to 1-gene expectation: F_2, $\chi^2 = 0.70$, $p > 0.4$; male BCR, $\chi^2 = 0.16$, $p > 0.6$. The combined BCR data fall somewhat short of the expected tumour incidence because of the incomplete penetrance in BCR females.

The 100% incidence in the large bowel of F_1 and susceptible backcross hybrids indicated dominance of the ICR-derived susceptibility to carcinogenesis by 1,2-dimethylhydrazine. Findings in the F_2 and resistant backcross hybrids are mutually supportive and in agreement with the respective 75% and 50% tumour incidences expected if a single dominant susceptibility gene is inherited from the ICR grandparent (Evans *et al.*, 1977).

Hybrid crosses were bred from a highly sensitive strain SWR/J mice and AKR/J mice highly resistant to 1,2-dimethylhydrazine carcinogenesis. 1,2-Dimethylhydrazine was injected subcutaneously at a dose of 20 mg/kg bw weekly with a total of 10 injections. Mice were killed 27 weeks after the first injection of 1,2-dimethylhydrazine. The colon tumour incidence in the hybrids and backcrosses is given in Table 15. The interpretation of results by the authors was that susceptibility to 1,2-dimethylhydrazine-induced tumorigenesis is not inherited as a dominant trait and that the kinetic properties of the epithelial cells are not linked to 1,2-dimethylhydrazine susceptibility (Deschner *et al.*, 1988).

Table 15. Incidence of 1,2-dimethylhydrazine-induced colonic tumours 27 weeks after the first injection

Cross	No. of mice	No. (%) of tumour-bearing mice
F_1 (SWR/J × AKR/J)	71	31 (43.7)
F_2	98	52 (52.0)
Backcross, susceptible	73	72 (98.6)
Backcross, resistant	68	5 (7.3)

From Deschner *et al.* (1988)

Groups of A/J and C57BL/6J strains, their F_1 progeny and 23 recombinant inbred strains, at eight weeks of age, received 20 weekly subcutaneous injections of 15 mg/kg bw 1,2-dimethylhydrazine. The animals were killed six weeks after the last 1,2-dimethylhydrazine injection. The analysis of colon tumour incidence led to the conclusion that there are several or many mechanisms in colon carcinogenesis and each of them requires several if not many genes for its functioning (Fleiszer *et al.*, 1988).

Inbred 1,2-dimethylhydrazine-susceptible ICR/Ha female mice were mated to inbred C57BL/6Ha males to produce 18 F_1 progeny (12 males and 6 females). These mice were backcrossed in both orientations to the resistant C57BL/6Ha strain, producing 126 (ICR/Ha × C57BL/6Ha)F_1 × C57BL/6Ha progeny. At the age of eight weeks, mice were given 25 weekly subcutaneous injections of 1,2-dimethylhydrazine at a dose of 20 mg/kg bw. The animals were killed one week after the last injection. Tumours were found in 40/122 progeny from a backcross to the resistant strain. Progeny animals were examined for segregation of 177 genetic markers distributed at intervals of 5–30 cM on all mouse chromosomes. Multiple loci were shown to contribute to the phenotype, with significant linkage to a novel locus, Ccs1, between D12Mit5 and D12Mit6 on mouse chromosome 12. Comparative maps suggested, in the authors' opinion, that the human homologue of Ccs1 is near FOS on human chromosome 14q (Jacoby *et al.*, 1994).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Human colon microsomal cytochrome P450 enzymes can demethylate 1,2-dimethylhydrazine, yielding formaldehyde, and activities in the descending colon are always higher than in the ascending or transverse colon (Newaz *et al.*, 1983). *N*-Demethylation was also detected in microsomes from a human colon tumour line, adenocarcinoma LS 174T cells. The activity was inducible by phenobarbital and/or hydrocortisone.

4.1.2 *Experimental systems*

1,2-Dimethylhydrazine is well absorbed from the colon of the rat, as shown by an in-vivo perfusion technique (Meshkinpour *et al.*, 1985). The absorption was enhanced significantly by bile acids and by hydroxy-fatty acids; fatty acids had no significant effect.

The tissue distribution and covalent binding of ^{3}H was examined in rats given [^{3}H]1,2-dimethylhydrazine according to various dose schedules (Pozharisski *et al.*, 1977). There was widespread covalent binding to protein, DNA and RNA of liver, kidney, duodenum, ileum and colon and this was greater when a large dose was given weekly than when the same dose was divided and given daily. However, while daily dosing resulted in a loss of cytochrome P450 in hepatic microsomes to 47% of control levels, weekly dosing had no effect.

1,2-Dimethylhydrazine is metabolized by a sequence of oxidation steps, first dehydrogenation to azomethane, *N*-oxidation of this to azoxymethane and finally a *C*-oxidation to methylazoxymethanol (Fiala, 1975, 1977). This last metabolite decomposes to give the highly reactive methyldiazonium ion to which the carcinogenicity of the compound has been attributed. The sequential nature of these oxidation steps has been shown in the isolated perfused rat liver (Wolter & Frank, 1982). Fiala (1977) showed that the *C*-oxidation of azoxymethane to methylazoxymethanol is catalysed by hepatic microsomes, while Schoental (1973) found that methylazoxymethanol was converted to the corresponding aldehyde by an NAD-dependent dehydrogenase.

In addition to this pathway of metabolism and activation, methyl radical intermediates may also be involved in the toxicity and metabolism of 1,2-dimethylhydrazine catalysed by haemoglobin, peroxidases and cytochrome P450 (Augusto *et al.*, 1985).

A further pathway of 1,2-dimethylhydrazine metabolism is *N*-demethylation, yielding monomethylhydrazine and formaldehyde. This can be catalysed by the mitochondrial enzyme monoamine oxidase (Coomes & Prough, 1983) and, most probably, by microsomal cytochrome P450 (Fiala, 1977; Hietanen *et al.*, 1986).

In cultured colon epithelial cells from conventional and gnotobiotic Sprague-Dawley rats, some 50% of 1,2-dimethylhydrazine was present in the medium as azoxymethane (minor) and methylazoxymethanol (major) and 50% was unchanged. Release of volatile

metabolites (presumably azomethane) was a very minor pathway. Although production of the soluble metabolites was unaltered, both DNA binding and levels of volatile metabolites were appreciably higher in cells from gnotobiotic rats (Glauert & Bennink, 1983).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Subcutaneous administration of radioactively labelled 1,2-dimethylhydrazine to albino rats resulted in a high degree of DNA, RNA and protein methylation in the intestinal mucosa, liver and kidney (Pozharisski *et al*., 1975). A regenerative response of the colonic mucosa in CF_1 mice was seen two to four days after a single subcutaneous injection of 20 mg/kg bw 1,2-dimethylhydrazine (Deschner, 1978). After repeated injections, focal atypias and an expansion of the proliferative compartment in the mid and upper portions of the crypts were noted. Wargovich *et al*. (1983a) described an initial abrupt reduction in colonic DNA synthesis followed by regenerative increases in proliferation in 1,2-dimethylhydrazine-treated C57BL/6 and CF_1 mice. Following intraperitoneal injection of 60 mg/kg bw and 200 mg/kg bw 1,2-dimethylhydrazine in female HalCR mice, DNA synthesis was transiently inhibited in the descending and ascending colon and, to a lower degree, in a number of other tissues (Koval, 1984). The sensitivity of two mouse strains to long-term 1,2-dimethylhydrazine exposure was found to be related to the indigenous proliferative characteristics of the distal colonic mucosa (Glickman *et al*., 1987).

In the vilus–crypt axis of the descending colon of 1,2-dimethylhydrazine-treated female Wistar rats, mucosal regeneration and preneoplastic alterations were reported by Delapierre *et al*. (1981). In male Wistar rats, subcutaneous treatment with 25 mg/kg bw 1,2-dimethylhydrazine twice weekly for two months and once a week thereafter for up to six months led to a significant increase in the number of epithelial cells in the villi and crypts and in the mitotic pool along the small intestine (Altmann & Snow, 1984). Similar proliferative responses to 1,2-dimethylhydrazine were observed between the proximal and distal colon of male Sprague-Dawley rats (McGarrity *et al*., 1988). Intragastric treatment with 1,2-dimethylhydrazine (25, 50 or 100 mg/kg bw), given twice four days apart, led to a dose-dependent increase in the number of aberrant crypts in the colon of Wistar rats (Bilbin *et al*., 1992). Jacobs and Lupton (1986) reported that cellulose failed to protect against an increased frequency of proximal colonic adenocarcinomas induced by 1,2-dimethylhydrazine administered to Sprague-Dawley rats. In another study, increasing dietary levels of cellulose suppressed significantly the increase in mitotic activity in the colonic crypts of male Sprague-Dawley rats treated with 1,2-dimethylhydrazine (Cameron *et al*., 1989). Food deprivation was reported to increase apoptotic cell counts in rat descending colonic and rectal crypts after subcutaneous injection of 100 mg/kg bw 1,2-dimethylhydrazine (Ishizuka *et al*., 1994).

Mayer *et al*. (1987) reported enlarged mucus-rich crypts with marked hypercellularity appearing very early in the colonic mucosa of male Sprague-Dawley rats given 16 weekly subcutaneous injections of 15 mg/kg bw 1,2-dimethylhydrazine. Hyperbasophilic crypts lacking mucus production were observed later and showed a loss of glucose-6-phosphatase, but marked increases in glucose-6-phosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase. Increased γ-glutamyltranspeptidase activity was observed throughout both the proximal and distal colon of 1,2-dimethylhydrazine-treated female Sprague-Dawley rats before the appearance of macroscopic tumours (Traynor *et al*., 1988). Caderni *et al*. (1995) found that sulfomucin-producing cells usually found in normal distal colon were progressively reduced in aberrant crypts of 1,2-dimethylhydrazine-treated female Sprague-Dawley rats, whereas the number of cells containing sialomucins was increased. However, no correlation was found between the number of aberrant crypt foci and the presence of tumours, as was also observed in male Sprague-Dawley rats (Hardman *et al*., 1991).

In rat liver, 1,2-dimethylhydrazine had no consistent effect on the relative focal volume of γ-glutamyltranspeptidase-positive preneoplastic foci (Denda *et al*., 1988). Locniskar *et al*. (1985) treated Brown-Norway and Fischer rats with 150 mg/kg bw 1,2-dimethylhydrazine by gavage five times over a three-week period. Five months after the final treatment, isolated splenic, colonic intraperithelial lymphocytes and lamina propria lymphocytes from the Brown-Norway strain exhibited low natural killer cell activity and reduced splenic T-lymphocyte proliferation in response to autologous non-T lymphocytes. Furthermore, colonic lamina propria lymphocyte proliferation was low, and Brown-Norway rats had a low incidence of 1,2-dimethylhydrazine-induced colonic neoplasms (7%) in comparison with Fischer rats, which had more effective splenic and colonic intraperithelial lymphocyte natural killer cell activity, enhanced splenic autologous mixed lymphocyte response, enhanced colonic lamina propria lymphocyte proliferation and a higher incidence of colon tumours (20%).

An association between intestinal carcinoma and the occurrence of intestinal lymphoid patches was found in 1,2-dimethylhydrazine-treated male Sprague-Dawley rats (Martin *et al*., 1986).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Keller *et al*. (1984) investigated the embryotoxicity and teratogenicity of 1,2-dimethylhydrazine in pregnant Fischer 344 rats. In the high-dose group treated intraperitoneally with 10 mg/kg bw 1,2-dimethylhydrazine per day on days 6–15 of gestation, maternal weight gain during the early part of gestation and mean fetal weights were significantly reduced. The number of implants and viable fetuses per litter were also lower than those in controls, and the number of malformations (retained testicle, anophthalmia or

severe microphthalmia, unfused ossification centres of vertebrae and sternebrae) was slightly increased. At a daily dose of 5 mg/kg bw, these effects were not observed.

4.4 Genetic and related effects

4.4.1 *Humans*

Autrup *et al.* (1980a) examined the metabolism and DNA binding of [^{14}C]1,2-dimethylhydrazine in cultured human colon explants from 120 patients. The method used did not distinguish between the covalent binding of reactive metabolites and incorporation of label into DNA: the former accounted for 60–80% of the DNA binding observed. There was a 100-fold variation in the DNA binding among the 120 people studied (mean, 940 pmol/10 mg DNA; range, 50–5600). In further work, Autrup *et al.* (1980b) characterized the alkylated DNA bases produced by cultured human colon explants. Of the total of 39 alkylated bases per 10^6 nucleotides (range 3.7–167), 46% were *N*7-methylguanine, 9% were *N*3-methyladenine and 23% were *O*^{6}-methylguanine. This pattern of alkylation was essentially identical in the various anatomical segments of the colon examined, although the extent varied.

4.4.2 *Experimental systems* (see Table 16 for references)

There is conflicting evidence concerning the mutagenicity of 1,2-dimethylhydrazine to bacteria. In a single study, it induced recombination in *Saccharomyces cerevisiae*. *In vitro*, 1,2-dimethylhydrazine formed DNA adducts in human bronchial cells, provoked unscheduled DNA synthesis in rat hepatocytes and induced gene mutation in mammalian cells. It gave positive results in rodents in microbial host-mediated assays. 1,2-Dimethylhydrazine induced micronucleus formation, gene mutation, nuclear aberrations and DNA strand breaks and formed DNA adducts in rodents *in vivo*.

Autrup *et al.* (1980b) examined the pattern of DNA alkylation produced in rat colon explants in culture. Total DNA binding was 1353 ± 32 pmol/10 mg DNA, of the same order as in human colon (620 pmol ± 642/10 mg DNA, range < 100–3270). Some 40% of this represented phosphate-bound radioactivity and the pattern of alkylation (*N*7-methylguanine > *O*^{6}-methylguanine >> *N*3-methyladenine) was very similar to that in human tissue, the *O*^{6}-methylguanine/*N*7-methylguanine ratio being 0.54 in rat and 0.49 in human.

Mouse uterine tumours were examined for genetic alterations in the *p53* tumour suppressor gene (Trukhanova *et al.*, 1998). Fourteen uterine sarcomas, including three primary and seven transplanted malignant fibrous histiocytomas, three stromal sarcomas and one undifferentiated sarcoma, from 1,2-dimethylhydrazine-treated mice were first screened by immunohistochemistry for *p53* missense mutations, followed by single strand conformation polymorphism analysis and DNA sequencing for the identification of point mutations. There was 100% correlation between p53 protein immunopositivity and subsequent detection of *p53* mutations in malignant fibrous histiocytomas had a characteristic *p53* G:C→A:T transition mutation, consistent with *O*^{6}-methylguanine mispairing with thymine.

Table 16. Genetic and related effects of 1,2-dimethylhydrazine

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1500	Von Wright & Tikkanen (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	(+)	570	Parodi *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	+	3	Malaveille *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	150	Wilpart *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	300	Matsushita *et al.* (1993)
SA2, *Salmonella typhimurium* TA102, reverse mutation	+	–	NG	Matsushita *et al.* (1993)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	+	+	3	Malaveille *et al.* (1983)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	–	–	NG	Wilpart *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2255	Parodi *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	NT	+	3	Malaveille *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2255	Parodi *et al.* (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2255	Parodi *et al.* (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	NG	Wilpart *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2255	Parodi *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Wilpart *et al.* (1983)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	NT	120	Von Wright & Tikkanen (1980)
SCH, *Saccharomyces cerevisiae* (RS112), homozygosis by mitotic recombination or gene conversion	+	NT	1000	Schiestl *et al.* (1989)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	NT	60	Mori *et al.* (1988)
UIA, Unscheduled DNA synthesis, mouse hepatocytes *in vitro*	+	NT	60	Mori *et al.* (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	6	Rogers & Back (1981)
G51, Gene mutation, mouse lymphoma L5178Y cells, ouabain resistance, thioguanine resistance, cytosine arabinoside resistance *in vitro*	–	NT	300	Rogers & Back (1981)

Table 16 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BFA, Bile from Sprague-Dawley rats, *Salmonella typhimurium* G46 and TA100 mutagenicity *in vivo*	–		60 sc × 1	Moriya *et al.* (1979)
HMM, Host-mediated assay, *Salmonella typhimurium* TA1950 in NMRI mice *in vivo*	+		100 po × 1	Von Wright & Tikkanen (1980)
HMM, Host-mediated assay, *Salmonella typhimurium* G46 in ICR mice *in vivo*	+		7.5 sc × 1	Moriya *et al.* (1979)
HMM, Host-mediated assay, *Escherichia coli* 343/113 in Swiss albino mice *in vivo*	+		60 ip × 1	Kerklaan *et al.* (1986)
DVA, DNA strand breaks, BALB/c mouse liver, colon, stomach, lung, kidney *in vivo*	+		12.5 po or sc × 1	Brambilla *et al.* (1978)
DVA, DNA fragmentation, Swiss albino mouse liver, lung *in vivo*	+		14 ip × 5	Parodi *et al.* (1981)
DVA, DNA strand breaks, AKR/J, DBA/2, CD1, C57BL/6N, SWR/J, B6D2F_1 mouse liver, kidney, colon *in vivo*	+		50 ip × 1	Bolognesi & Boffa (1986)
DVA, DNA strand breaks, Sprague-Dawley rat liver *in vivo*	+		0.45 po × 2	Kitchin & Brown (1994)
DVA, DNA strand breaks, Sprague-Dawley rat liver *in vivo*	+		1 po × 2	Kitchin & Brown (1996)
RVA, DNA repair exclusive of unscheduled DNA synthesis, Fischer 344 rat hepatocytes and liver nonparenchymal cells *in vivo*	+		2 drink, 4 wk	Bedell *et al.* (1982)
RVA, DNA repair exclusive of unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	+		20 sc × 1	Richardson *et al.* (1985)
RVA, DNA repair exclusive of unscheduled DNA synthesis, rat liver cells *in vivo*	+		1.7 sc × 1	O'Toole *et al.* (1993)
UPR, Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	+		20 po × 1	Mirsalis *et al.* (1982)
UVR, Unscheduled DNA synthesis, Fischer 344 rat kidney cells *in vivo*	–		50 ip × 1	Tyson & Mirsalis (1985)
GVA, Gene mutation, (C57BL/6J × SWR F_1) mouse intestine, *Dlb-1* locus *in vivo*	+		20 sc × 10	Winton *et al.* (1990)

Table 16 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
GVA, Gene mutation, (C57BL/6J × SWR F_1) mouse intestine, *Dlb-1* locus *in vivo*	–		30 ip × 1	Tao & Heddle (1994)
GVA, Gene mutation, (C57BL/6J × SWR F_1) mouse intestine, *Dlb-1* locus *in vivo*	(+)		10 ip × 10	Tao & Heddle (1994)
GVA, Gene mutation, CD-1 mouse liver cells, *p53* intron 6 *in vivo*	+		20 po × 2	Jenkins *et al.* (1997)
SVA, Sister chromatid exchange, C57BL/6J mouse colonic epithelial cells *in vivo*	+		10 ip × 1	Couch *et al.* (1986)
SVA, Sister chromatid exchange, C57BL/6J mouse bone-marrow cells *in vivo*	–		40 ip × 1	Couch *et al.* (1986)
MVM, Micronucleus test, C57BL/6J mouse colonic epithelial cells *in vivo*	+		15 ip × 1	Goldberg *et al.* (1983)
MVM, Micronucleus test, C57BL/6J mouse bone-marrow cells *in vivo*	–		45 ip × 1	Goldberg *et al.* (1983)
MVM, Micronucleus test, CBA mouse bone-marrow cells *in vivo*	+		10 po × 1	Ashby & Mirkova (1987)
MVM, Micronucleus test, $CCBF_1$, CBA, C57BL/6J mouse bone marrow *in vivo*	+		50 po × 1	Albanese *et al.* (1988)
MVM, Micronucleus test, CBA mouse bone marrow *in vivo*	+		25 po × 1	Morrison & Ashby (1995)
MVR, Micronucleus test, Alderley Park rat bone marrow *in vivo*	–		80 po × 1	Albanese *et al.* (1988)
BID, Binding (covalent) to DNA and proteins, human bronchial cells *in vitro*	+	NT	77	Harris *et al.* (1977)
BVD, Binding (covalent) to DNA, various BD-VI rat tissues *in vivo*	+		300 sc	Likhachev *et al.* (1977)
BVD, Binding (covalent) to DNA, Fischer 344 rat hepatocytes and liver nonparenchymal cells *in vivo*	+		1.7 drink, 4 wk	Bedell *et al.* (1982)
BVD, Binding (covalent) to DNA, Fischer 344 rat hepatocytes and liver nonparenchymal cells *in vivo*	+		1.7 drink, 4 wk	Lewis & Swenberg (1983)
BVD, Binding (covalent) to DNA, ICR/Ha mouse colon *in vivo*	+		20 ip × 1	James & Autrup (1983)
BVD, Binding (covalent) to DNA, Fischer 344 rat hepatocytes *in vivo*	+		20 sc × 1	Richardson *et al.* (1985)

Table 16 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BVD, Binding (covalent) to DNA, Wistar rat intestine and liver *in vivo*	+		20 sc × 1	Tacchi-Bedford *et al.* (1988)
BVD, Binding (covalent) to DNA, Wistar or Sprague-Dawley rat intestine and liver *in vivo*	+		300 ip × 1	Netto *et al.* (1992)
BVD, Binding (covalent) to DNA, Fischer 344 rat liver *in vivo*	+		1.7 sc × 1	O'Toole *et al.* (1993)
Colonic nuclear aberration assay in C57BL/6J mice *in vivo*	+		12 po × 1	Wargovich *et al.* (1983)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; sc, subcutaneously; ip, intraperitoneally; po, oral

4.4.3 *Mechanistic considerations*

The conflicting set of results from bacterial mutagenicity tests is probably due to a lack of specific metabolic enzymes in microsomal preparations which were present in intact hepatocytes.

1,2-Dimethylhydrazine forms DNA adducts in various rodent tissues (including the colon and liver) after its metabolic activation *in vivo*. Adducts include *N*7-methylguanine, O^6-methylguanine and O^4-methylthymidine. In one study *C*8-methylguanine was also identified in DNA of liver and colon from rats treated with 1,2-dimethylhydrazine (Netto *et al.*, 1992). Of these adducts, O^6-methylguanine and O^4-methylthymidine are considered to be miscoding bases, since they can act as adenine and cytosine respectively without distortion of the DNA helix during DNA replication (Lawley, 1984). The formation, persistence and repair of such adducts in various tissues may influence the probability of mutation and carcinogenesis and may help to explain the organ-specific carcinogenicity of 1,2-dimethylhydrazine (Zedeck, 1984; Pegg, 1990).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

1,2-Dimethylhydrazine is believed to be used only as a laboratory chemical. No information on potential human exposure is available.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

1,2-Dimethylhydrazine was studied for carcinogenicity in many experiments in rats and mice, mainly by subcutaneous, infrequently by oral and rarely by other routes of administration.

Whatever the route of administration, 1,2-dimethylhydrazine, if given at an appropriate dosage, produced in mice and rats a high incidence of adenomas and adenocarcinomas of the colon and, to a lesser extent, of the small bowel. When given with drinking water or by gavage at low single doses, it produced a high incidence of vascular tumours.

In some experiments in rats, it produced ear duct papillomas and carcinomas, hepatocarcinomas, kidney adenomas, carcinomas and fibrosarcomas. When given to rats at very high single doses, it produced high incidences of nephroblastomas.

In some strains of mice, it produced a high incidence of hormone-dependent angiosarcomas of the kidney capsule (males only), uterine sarcomas or vascular tumours and tumour-like lesions of the ovary.

5.4 Other relevant data

1,2-Dimethylhydrazine is readily absorbed. It can be *N*-demethylated, yielding formaldehyde, and can be oxidized through several steps to yield methylazoxymethanol. It binds covalently to protein, DNA and RNA in many mammalian tissues. The colon of rats is a target organ for 1,2-dimethylhydrazine toxicity, where it can produce aberrant crypts. In developmental studies, it is embryo- and feto-toxic in rats.

1,2-Dimethylhydrazine formed DNA adducts and induced gene mutations, DNA breaks and micronuclei *in vitro* and *in vivo* in rodents. *In vitro* it formed DNA adducts and induced unscheduled DNA synthesis and gene mutations in mammalian cells. Conflicting evidence has been obtained for its genotoxicity in bacteria.

Although the activating pathway has not been clarified in detail, there is good evidence that human tissues, cells and subcellular preparations can activate 1,2-dimethylhydrazine in a similar manner to the corresponding rodent models.

1,2-Dimethylhydrazine requires bioactivation to become mutagenic and alkylates DNA in several species *in vivo*. It is not genotoxic in bacteria, but it is mutagenic for various endpoints in virtually all somatic test systems examined *in vitro* and *in vivo*.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of 1,2-dimethylhydrazine were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,2-dimethylhydrazine.

Overall evaluation

1,2-Dimethylhydrazine is *probably carcinogenic to humans (Group 2A)*.

In making the overall evaluation, the Working Group took into account that 1,2-dimethylhydrazine is consistently mutagenic in a wide range of test systems and gives rise to a similar pattern of DNA damage in human and animal tissues *in vitro*.

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HYDRAZINE

Data were reviewed in IARC (1974) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 302-01-2

Chem. Abstr. Name: Hydrazine

IUPAC Systematic Name: Hydrazine

1.1.2 *Structural and molecular formulae and relative molecular mass*

$H_2N—NH_2$

H_4N_2 Relative molecular mass: 32.05

1.1.3 *Chemical and physical properties of the pure substance*

(from American Conference of Governmental Industrial Hygienists (1992) unless otherwise noted)

(*a*) *Description*: Colourless, fuming, oily liquid with an ammonia-like odour

(*b*) *Boiling-point*: 113.5°C

(*c*) *Melting-point*: 2°C

(*d*) *Solubility*: Miscible with water and methyl, ethyl, propyl and isobutyl alcohols; insoluble in chloroform and diethyl ether

(*e*) *Vapour pressure*: 1.4 kPa at 20°C

(*f*) *Flash-point*: 37.8°C, closed cup

(*g*) *Explosive limits*: Upper, 100%; lower, 4.7% by volume in air

(*h*) *Conversion factor*: $mg/m^3 = 1.31 \times ppm$

1.2 Production and use

The world production capacity for hydrazine in 1981 was estimated to be about 36 thousand tonnes, not including countries with planned economies at that time (WHO, 1987). Production capacity estimates for hydrazine hydrate in 1988 were 25 thousand tonnes in the United States, 10 thousand tonnes in Germany, 10 thousand tonnes in France, 5 thousand tonnes in Japan and 3 thousand tonnes in the United Kingdom (Schirmann, 1989). Production capacity estimates for hydrazine solutions in 1992 were 16 500 tonnes in the United States, 6400 tonnes in Germany, 6100 tonnes in France,

6600 tonnes in Japan, 3600 tonnes in Korea, 3500 tonnes in Russia and 1400 in the People's Republic of China (Schiessl, 1995).

The principal applications of hydrazine solutions include chemical blowing agents, 40%; agricultural pesticides, 25%; and water treatment, 20%. The remaining 15% is used in a variety of fields including pharmaceuticals, explosives, polymers and polymer additives, antioxidants, metal reductants, hydrogenation of organic groups, photography, xerography and dyes (Schiessl, 1995). Anhydrous hydrazine is used as a component of high-energy fuels and rocket propellants (Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

The National Occupational Exposure Survey (NOES, 1997) estimated that 59 147 workers (including 2840 females) in the United States were potentially exposed to hydrazine between 1981 and 1982.

National estimates on exposure were not available from other countries.

1.3.2 *Environmental occurrence*

Production of hydrazine and its use as a chemical intermediate, reducing agent, rocket fuel and boiler-water treatment agent may result in its release to the environment through various waste streams. Hydrazine is also naturally produced by *Azotobacter agile* during nitrogen fixation. It has been detected at low levels in wastewater samples (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 0.013 mg/m^3 as the threshold limit value for occupational exposures to hydrazine in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for hydrazine in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

Choroidal melanoma was observed in one man who had been exposed to hydrazine for six years. A study of men engaged in hydrazine manufacture comprised 423 men, with 64% ascertainment of vital status. None of the five cancers reported (three gastric, one prostatic and one neurogenic) occurred in the group with the highest exposure. A follow-up of this cohort extended the observations to 1982. Mortality from all causes was not elevated (49 observed, 61.5 expected) and the only excess was two lung cancer cases within the highest-exposure category, with a relative risk of 1.2 (95% confidence interval, 0.2–4.5) (IARC, 1987).

A cohort of 427 men who worked at a hydrazine plant in the United Kingdom for at least six months between 1945 and 1971 was followed up until 1992 (Morris *et al*., 1995). Follow-up was complete for 95%. Based on job history records, 78 of the workers were classified as having been exposed to high levels of hydrazine (estimated at about 1–10 ppm [1.3–13 mg/m^3]) and the remaining 375 to moderate or low exposure (< 1 ppm). There were 2145 person–years of follow-up in the latter group. Among the whole group, no increase was observed for all-cause mortality (86 deaths, standardized mortality ratio (SMR), 0.8), or for mortality from lung cancer (8 deaths; SMR, 0.7), cancer of the digestive tract (9 deaths; SMR, 1.0) or other cancers (8 deaths; SMR, 0.8), after comparison with the rates for England and Wales. Restricting attention to the high-risk group, the SMR for all-cause mortality was 0.7 (20 deaths) and that for lung cancer was 1.1 (3 deaths). No deaths from cancer of the digestive tract were observed. The SMR for other cancers was 0.8 (2 deaths). None of the SMRs was significantly different from 1.0. Of the three lung cancer cases in the high-exposure group, two occurred in workers with less than two years of occupational exposure to hydrazine.

3. Studies of Cancer in Experimental Animals

Hydrazine has been tested in mice by oral administration, producing liver and mammary tumours and lung tumours in both P and F_1 generations; after intraperitoneal administration to mice, it produced lung tumours, leukaemias and sarcomas. After oral administration to rats, it produced lung and liver tumours. When tested by inhalation, it produced benign and malignant nasal tumours in rats, benign nasal polyps, a few colon tumours and thyroid adenomas in hamsters, and a slight increase in the incidence of lung adenomas in mice (IARC, 1987).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female NMRI mice, five to six weeks of age, were given hydrazine in the drinking-water at concentrations of 0, 2, 10 and 50 mg/L (ppm) for two years. The highest dose (50 ppm) was toxic, producing severely reduced weight gain and a lower survival; 10 ppm was the maximum tolerated dose (moderate body weight decrease). No increase in the incidence of tumours at any site or at any dose was observed (Steinhoff *et al*., 1990).

3.1.2 *Rat*

Groups of 50 male and 50 female specific pathogen-free bred Wistar rats, six weeks of age, were given hydrazine in the drinking-water at concentrations of 0, 2, 10 and 50 mg/L (ppm) for 24 months. The concentration of 2 ppm was tolerated with little toxicity; 10 ppm proved to be the maximum tolerated dose and 50 ppm was clearly toxic, producing severely decreased body weight gain. An increase in tumour incidence was

observed in the liver: no tumour in the controls (0/100 both sexes combined); two tumours (2%) (1 hepatocellular adenoma, 1 haemangioma) in the 2-ppm group; three tumours (3%) (1 hepatocellular adenoma and 1 carcinoma, 1 cholangioma) in the 10-ppm group; and 14 tumours (14.6%) (8 hepatocellular adenomas, 3 carcinomas and 3 cholangiomas) in the 50-ppm group. In historical controls, the incidence of liver-cell tumours was 9/652 (1.4%) (Steinhoff & Mohr, 1988).

3.1.3 *Hamster*

Syrian hamsters were given hydrazine sulfate in the drinking-water at concentrations of 170, 340 and 510 mg/L (ppm) for two years (average doses, 4.6, 8.3 and 10 mg/kg bw hydrazine (free base)). Hepatocellular carcinomas were observed in hamsters treated with the highest dose of hydrazine sulfate after 78 weeks of exposure; the incidence of hepatocellular carcinomas in the three treated groups was 0/31 at 170 ppm, 4/34 at 340 ppm and 11/34 at 510 ppm (Bosan *et al*., 1987).

3.2 Inhalation exposure

Rat: Groups of 100 male and 100 female Fischer 344 rats were exposed to 0 (control), 75 and 750 ppm [0, 98 and 980 mg/m^3] hydrazine (purity, 98.8%) by inhalation for 1 h once or once per week for 10 weeks. Animals were killed 24–30 months after exposure. In the 750-ppm hydrazine-treated group, polypoid adenomas were found in 4/99 males and 6/95 females. In addition, one nasal squamous-cell carcinoma and four cases of hyperplasia were noted in males and one case of hyperplasia in females (Latendresse *et al*., 1995). [The Working Group noted that data were not presented for control tumour incidences, although the incidence of nasal adenomas in both sexes and that of nasal hyperplasia in males were significant.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

(a) In-vivo pharmacokinetics, tissue distribution and metabolism

Kaneo *et al*. (1984) examined the tissue distribution of [^{15}N]hydrazine in rats after a subcutaneous dose of 10 mg/kg bw by gas chromatography/mass spectrometry, using stable isotope internal standards. Maximal tissue levels of hydrazine were seen 30 min after dosing and it was eliminated from the liver, kidney, lung and plasma with half-lives of 3.3, 2.7, 3.0 and 2.3 h, respectively. At 8 h, levels in the kidney were notably higher

than those in other tissues. The levels of acetylhydrazine in the kidney were much higher than those in other tissues and peaked at 1 h, while the highest concentrations in other tissues occurred between 1 and 4 h after dosing. Only trace amounts of diacetylhydrazine were detected in the tissues. Within 48 h, a total of 30% of the dose was recovered in the urine, 24% as hydrazine and 3% each as acetyl- and diacetylhydrazine. The partial reversibility of hydrazine acetylation was shown after the administration of equimolar doses of acetylhydrazine. Tissue levels of hydrazine were between one quarter and one half of those of acetylhydrazine, while 6% of the dose was recovered in the urine as hydrazine compared with 19% as acetylhydrazine.

Preece *et al*. (1992) examined the influence of dose upon the disposition of hydrazine in rats using oral doses of 3, 9, 27 and 81 mg/kg bw. The plasma and liver areas under the curve (AUCs) for hydrazine increased linearly with doses of up to 27 mg/kg bw but were lower than expected at 81 mg/kg bw. At 3 and 9 mg/kg bw, plasma and liver levels were equivalent but, at higher doses, there was more compound in the plasma. At 24 h after dosing, the plasma:liver ratio was 4.4 at 60 mg/kg bw and 5.7 at 80 mg/kg bw. The urinary recovery of hydrazine and acetylhydrazine fell with increasing dose, from 38 to 17% of a dose for hydrazine and from 5 to 1% for acetylhydrazine. The extent of acetylation decreased, the hydrazine:acetylhydrazine ratio declining from 0.125 to 0.061.

(*b*) *In-vitro metabolic studies*

Hydrazine is metabolized by rat liver microsomal enzymes to unknown products, ultimately yielding molecular nitrogen (Timbrell *et al*., 1982; Jenner & Timbrell, 1995). This was dependent upon oxygen and NADPH and was increased by NADH in the presence of NADPH. Hydrazine metabolism was 20–70% lower in human microsomes prepared from three individuals compared with rats. Hydrazine is also metabolized by rat liver mitochondria, but the monoamine oxidase inhibitors clorgyline and pargyline do not significantly decrease this activity (Jenner & Timbrell, 1995).

(*c*) *Metabolic mechanisms of toxicity*

Studies with isolated rat hepatocytes have indicated that at least three CYP isoenzymes (2E1, 2B1 and 1A1/2) are involved in the detoxication of hydrazine, as inducers of these isoenzymes all reduce its cytotoxicity. Pretreatment of rats with diethyldithiocarbamate increased the cytotoxicity of hydrazine, this being associated with marked inhibition of CYP activities (Delaney & Timbrell, 1995).

Adult male Sprague-Dawley rats were treated with 0.9% saline vehicle (a single intraperitoneal dose) or hydrazine (100 mg/kg bw intraperitoneally), after which the CYP2E1 mRNA and protein levels were monitored by Northern and immunoblot analyses, respectively, and glutathione *S*-transferase-α (GST-α) Ya and Yc subunit levels were determined by immunoblot analysis. Hydrazine treatment produced an approximately 464% increase in renal CYP2E1 protein, but hepatic levels of CYP2E1 and of GST-α Ya and Yc subunits were not significantly altered. The observed increases in renal CYP2E1 protein levels were not accompanied by concomitant increases in

CYP2E1 mRNA, suggesting a post-transcriptional mechanism for the increase in renal CYP2E1 protein (Runge-Morris *et al*., 1996).

4.2 Toxic effects

Toxic responses to hydrazine exposure have been reviewed (WHO, 1987).

4.2.1 *Humans*

One fatal poisoning was reported of a man who had handled hydrazine (hydrazine hydrate) once a week for an unknown number of hours over a period of six months. In simulated conditions, only 0.071 mg hydrazine/m^3 was measured, but probably skin exposure had also occurred. The man experienced conjunctivitis, tremor and lethargy after each exposure. Following the last exposure, he developed fever, diarrhoea and vomited. In hospital, six days later, many disorders were noted: conjunctivitis, stomatitis, arrhythmia, upper abdominal pain, enlarged abdomen, icterus, a tender and palpable liver, black faeces, incoherence and oliguria. X-ray examinations showed pleural effusion and lung shadowing. Laboratory findings comprised elevated blood bilirubin and creatinine levels, and protein and red blood cells in urine. Treatments administered included haemodialysis and B vitamins, which brought only temporary relief. The man died 21 days after the last exposure. Autopsy revealed pneumonia, severe renal tubular necrosis and nephritis and mild hepatocellular damage (Sotaniemi *et al*., 1971).

In two individuals working in gold plating, contact dermatitis against hydrazine was found (Wrangsjö & Mårtensson, 1986).

4.2.2 *Experimental systems*

Megamitochondria were induced in male Wistar rats placed for three or seven days on a diet containing 1% hydrazine (Wakabayashi *et al*., 1987). From biochemical analysis, the authors concluded that the formation of megamitochondria was due to fusion of adjacent mitochondria rather than to mitochondrial swelling.

In four-week-old male Wistar rats, 1% hydrazine in the diet resulted in the formation of megamitochondria, increased lipid peroxidation and decreased levels of reduced glutathione in the liver (Adachi *et al*., 1995).

Timbrell *et al*. (1996) reported that much higher hydrazine concentrations were required in rat hepatocyte cultures in comparison to plasma concentrations in male Sprague-Dawley rats to elicit the following hepatic/hepatocellular effects: lactate dehydrogenase leakage, ATP and GST depletion, increase in citrulline level, protein synthesis inhibition, taurine leakage and triglyceride accumulation.

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Hydrazine was mutagenic to yeast and bacteria and induced DNA damage in bacteria. Hydrazine induced somatic mutations in *Drosophila*. It induced DNA strand breaks in rat hepatocytes and unscheduled DNA synthesis in mouse hepatocytes *in vitro*. Conflicting results were obtained for induction of gene mutations in mouse lymphoma L5178Y cells. It did not induce chromosomal aberrations in rat liver cell line *in vitro* but did induce sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells. In single studies *in vivo* in mice, hydrazine induced DNA strand breaks in liver and lungs. It did not induce sister chromatid exchanges in bone marrow or liver of mouse treated *in vivo* in one study. It weakly induced micronuclei in bone-marrow cells of mice treated *in vivo* in one of three studies. Hydrazine induced the formation of DNA adducts *in vitro* and of *N*7-methylguanine and O^6-methylguanine in liver of mice, rats and hamsters treated *in vivo*. In in-vivo studies with mice, hydrazine did not induce dominant lethal mutations in a single study or sperm abnormalities in two studies.

In the experiment described in Section 3.1.3, in which Syrian hamsters were given hydrazine sulfate in the drinking-water for two years, the levels of methylation of DNA guanine in liver, kidney and lung were measured. Both *N*7- and O^6-methylguanine were readily detectable at six months of exposure, but only trace amounts of these bases were detected after 12 months of exposure; these bases were again detected in liver DNA at exposure times of 18 and 24 months (Bosan *et al*., 1987).

4.4.3 *Mechanistic considerations*

Administration of hydrazine to rodents results in the formation of *N*7-methylguanine and O^6-methylguanine in liver DNA. Co-administration of L-[*methyl*-^{14}C]methionine or [^{14}C]formate with the hydrazine led to labelling of the methylguanines, suggesting involvement of the one-carbon pool in the methylation process (Quintero-Ruiz *et al.*, 1981). It has been proposed that the methylation mechanism involves reaction of hydrazine with endogenous formaldehyde to yield formaldehyde hydrazone, which could be metabolized to the potent methylating agent diazomethane. In experiments using postmitochondrial (S9), microsomal, cytosolic or mitochondrial cell fractions from rat liver *in vitro*, methylation of DNA guanine occurred, S9 being the most active fraction. Neither the P450 monooxygenase nor flavin monooxygenase systems appeared to be important in hydrazine/formaldehyde-induced methylation of DNA. However, sodium azide, cyanamide and carbon monoxide all inhibited S9-supported DNA methylation. Bovine liver catalase, a haem-containing cytochrome, readily transformed hydrazine/formaldehyde to a methylating agent. The data support the proposal that formaldehyde hydrazone is the condensation product of hydrazine and formaldehyde, which is rapidly transformed in various liver cell fractions to a DNA-methylating agent (Lambert & Shank, 1988).

Table 1. Genetic and related effects of hydrazine

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage induction in lysogenic *Escherichia coli*	(+)	NT	5	Heinemann (1971)
PRB, Prophage induction in *Escherichia coli*	+	NT	320	von Wright (1981)
PRB, Prophage induction in *Haemophilus influenzae*	–	NT	6420	Balganesh & Setlow (1984)
ERD, *Escherichia coli* WP2, WP67 *uvrApolA*, CM871 *uvrArecAlexA*, differential toxicity	+	–	20	Green (1981)
SAF, *Salmonella typhimurium*, forward mutation, 8-azaguanine resistance	NT	+	100	Skopek *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	+	10	Anderson & Styles (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Baker & Bonin (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	25	Brooks & Dean (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Garner *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation, fluctuation test	–	+	10	Hubbard *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1000	MacDonald (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	NG	Martire *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	750	Nagao & Takahashi (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	320	Parodi *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	10	Richold & Jones (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1000	Rowland & Severn (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	–	NG	Simmon & Shepherd (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	250	Wade *et al.* (1981)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	+	NT	500	Tosk *et al.* (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	NT	+	1200	Herbold & Buselmaier (1976)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	NT	+	1250	Anderson & Styles (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	75	Cheh *et al.* (1980)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	45	Baker & Bonin (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	250	Martire *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	250	Garner *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	120	Parodi *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	0.01	Richold & Jones (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	250	Rowland & Severn (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	250	Simmon & Shepherd (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	250	Wade *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	NT	–	12000	Herbold & Buselmaier (1976)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Baker & Bonin (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Garner *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1000	MacDonald (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1000	Martire *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Nagao & Takahashi (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	320	Parodi *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	10	Richold & Jones (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1000	Rowland & Severn (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1000	Simmon & Shepherd (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	NT	–	12000	Herbold & Buselmaier (1976)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	NT	–	1250	Anderson & Styles (1978)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	500	Baker & Bonin (1981)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	320	Parodi *et al*. (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	10	Richold & Jones (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1000	Rowland & Severn (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1000	Simmon & Shepherd (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	NT	+	10	Anderson & Styles (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Baker & Bonin (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Garner *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation, fluctuation test	–	–	500	Hubbard *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	MacDonald (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	Martire *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Nagao & Takahashi (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	320	Parodi *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	10	Richold & Jones (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	Rowland & Severn (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	Simmon & Shepherd (1981)
SAS, *Salmonella typhimurium* G46, reverse mutation	NT	+	120	Herbold & Buselmaier (1976)
SAS, *Salmonella typhimurium* TA92, reverse mutation	–	–	1000	Brooks & Dean (1981)
ECK, *Escherichia coli* K-12/343/113, forward or reverse mutation	+	+	200	Mohn *et al.* (1981)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	+	NT	16	von Wright & Tikkanen (1980a)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	+	NT	16	von Wright & Tikkanen (1980b)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation, fluctuation test	+	+	1	Gatehouse (1981)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	+	50	Matsushima *et al.* (1981)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	+	+	48	Noda *et al.* (1986)
EC2, *Escherichia coli* WP2, reverse mutation	+	NT	16	von Wright & Tikkanen (1980b)
EC2, *Escherichia coli* WP2 B/r, reverse mutation	–	+	110	Matsushima *et al.* (1981)
ECR, *Escherichia coli* CM871 *lexArecAuvrA*, reverse mutation	+	NT	16	von Wright & Tikkanen (1980b)
ECR, *Escherichia coli* WP2 *uvr*A pKM101, reverse mutation	+	+	11	Matsushima *et al.* (1981)
SCH, *Saccharomyces cerevisiae JD1*, homozygosis by mitotic gene conversion	+	+	10	Sharp & Parry (1981)
SCH, *Saccharomyces cerevisiae D7*, homozygosis by mitotic gene conversion	+	+	385 ppm	Zimmermann & Scheel (1981)
SCF, *Saccharomyces cerevisiae* rad2-1, forward mutation	+	NT	6420	Lemontt (1978)
SCF, *Saccharomyces cerevisiae* XY597 strains, forward mutation	+	NT	6420	McDougall & Lemontt (1979)
SCR, *Saccharomyces cerevisiae* XV 185-14C, reverse mutation,	+	NT	133	Mehta & von Borstel (1981)
SZF, *Schizosaccharomyces pombe*, forward mutation	+	+	0.5	Loprieno (1981)
SCN, *Saccharomyces cerevisiae* D6, mitotic aneuploidy,	+	+	50	Parry & Sharp (1981)
DMM, *Drosophila melanogaster*, somatic mutation (and recombination)	+		321 feed	Jain *et al.* (1969)
DMM, *Drosophila melanogaster*, somatic mutation (and recombination)	+		321 feed	Shukla (1972)
DMM, *Drosophila melanogaster*, somatic mutation (and recombination)	+		642 feed	Vijaykumar & Jain (1979)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		2500 feed	Yoon *et al.* (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	(+)		321 feed	Shukla (1972)
DIA, DNA strand breaks, rat hepatocytes *in vitro*	+	NT	96	Sina *et al.* (1983)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
URP, Unscheduled DNA synthesis, ACTIN rat primary hepatocytes *in vitro*	–	NT	32	*Mori* et al. *(1988)*
UIA, Unscheduled DNA synthesis, C3H/HeN mouse primary hepatocytes *in vitro*	+	NT	3.2	*Mori* et al. *(1988)*
GCO, Gene mutation, Chinese hamster ovary CHO cells, five loci *in vitro*	–	–	2000	Carver *et al.* (1981)
GCO, Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	–	–	500	Hsie *et al.* (1981)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	NT	852	Amacher *et al.* (1980)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	3.2	Rogers & Back (1981)
G51, Gene mutation, mouse lymphoma L5178Y cells, ouabain, thioguanine or cytosine arabinoside resistance *in vitro*	–	NT	160	Rogers & Back (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	NT	8	MacRae & Stich (1979)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	1167	Natarajan & van Kesteren-van Leeuwen (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	100	Perry & Thomson (1981)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	+	158	Natarajan & van Kesteren-van Leeuwen (1981)
CIR, Chromosomal aberrations, rat liver cell line (RL_1) *in vitro*	–	NT	100	Dean (1981)
GIH, Gene mutation, human fibroblasts, diphtheria toxin resistance (HF Dip^r), *in vitro*	–	+	200	Gupta & Goldstein (1981)
HMM, Repair assay in *Escherichia coli* K12/*uvrB/recA* in male NMRI mice *in vivo*	–		840 po	Hellmér & Bolcsfoldi (1992)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DVA, DNA strand breaks in Swiss albino mouse liver and lung *in vivo*	+		33 ip × 5	Parodi *et al.* (1981)
MST, Mouse spot test, (T×HT)F_1 mice *in utero*	+		80 ip	Neuhäuser-Klaus & Chauhan (1987)
SVA, Sister chromatid exchange, CBA/J mouse bone marrow *in vivo*	–		100 ip × 1	Paika *et al.* (1981)
SVA, Sister chromatid exchange, CBA/J mouse liver *in vivo*	–		100 ip × 1	Paika *et al.* (1981)
MVM, Micronucleus test, ICR mouse bone marrow *in vivo*	–		44 ip × 2	Kirkhart (1981)
MVM, Micronucleus test, B6C3F_1 mouse bone marrow *in vivo*	+		70 ip × 2	Salamone *et al.* (1981)
MVM, Micronucleus test, CDI mouse bone marrow *in vivo*	–		44 ip × 2	Tsuchimoto & Matter (1981)
DLM, Dominant lethal test, ICR/Ha Swiss mice *in vivo*	–		52 ip × 1	Epstein *et al.* (1972)
DNA strand scission, ϕ×174 RF DNA *in vitro* [with haemolysate]	NT	+	96	Runge-Morris *et al.* (1994)
BID, DNA-adduct formation in M13mp18 viral DNA *in vitro*	+	NT	NG	Premaratne *et al.* (1995)
BVD, Formation of *N*7-methylguanine and O^6-methylguanine in male Fischer 344 rat liver DNA *in vivo*	+		60 po × 1	Barrows & Shank (1981)
BVD, Formation of *N*7-methylguanine and O^6-methylguanine in male Fischer 344 and male Sprague-Dawley rat liver DNA *in vivo*	+		45 po × 1	Becker *et al.* (1981)
BVD, Formation of *N*7-methylguanine in male Fischer 344 and male Sprague-Dawley rat liver DNA *in vivo*	+		3 po × 4	Becker *et al.* (1981)
BVD, Formation of *N*7-methylguanine in CBA mouse liver DNA *in vivo*	+		64 ip × 1	Quintero-Ruiz *et al.* (1981)
BVD, Formation of *N*7-methylguanine and O^6-methylguanine in male Syrian golden hamster liver DNA *in vivo*	+		90 po × 1	Bosan *et al.* (1986)
BVD, Formation of *N*7-methylguanine and O^6-methylguanine in male Syrian golden hamster liver DNA *in vivo*	+		1.12 dw 6 m	Bosan *et al.* (1987)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BVD, Formation of *N*7-methylguanine and O^6-methylguanine in neonatal Sprague-Dawley rat liver DNA *in vivo*	+		25 sc × 1	Leakakos & Shank (1994)
BVD, Formation of *N*7-methylguanine and O^6-methylguanine in Wistar rat liver DNA *in vivo*	+		0.2 po × 1	van Delft *et al.* (1997)
BVP, Formation of *N*7-methylguanine in CBA mouse liver RNA *in vivo*	+		64 ip × 1	Quintero-Ruiz *et al.* (1981)
SPM, Sperm morphology (CBA × BALB/c)F_1 mice *in vivo*	–		50 ip × 5	Topham (1981)
SPM, Sperm morphology, B6C3F_1/CRL mice *in vivo*	–		400 ip × 5	Wyrobek *et al.* (1981)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; ip, intraperitoneal; po, oral; dw, drinking-water; sc, subcutaneous

Rats exposed to single doses (gavage) of hydrazine in the range 0.01–10 mg/kg bw showed an increase in *N*7- and O^6-methylguanine above background levels in liver DNA only after doses greater than 0.1–0.2 mg/kg bw, doses which were still non-toxic or only weakly toxic (van Delft *et al*., 1997).

5. Summary of Data Reported and Evaluation[1]

5.1 Exposure data

Exposure to hydrazine may occur in its production, and in the production of chemical blowing agents, agricultural chemicals and in water treatment. It has been detected at low levels in wastewater.

5.2 Human carcinogenicity data

The cancer risk of men exposed to hydrazine was investigated in two small cohort studies. In neither of these studies was an elevated risk observed for all cancers combined or for any specific cancer type.

5.3 Animal carcinogenicity data

Hydrazine was tested for carcinogenicity by oral administration to mice in several experiments, producing mammary and lung tumours. When tested by oral administration or inhalation exposure in rats, it produced lung, liver and nasal tumours and a few colon tumours. In hamsters, it produced liver tumours and thyroid adenomas following oral or inhalation exposure.

5.4 Other relevant data

Following subcutaneous administration of hydrazine to rats, maximum tissue concentrations were reached in about 30 min. Most urinary elimination was as unchanged hydrazine, with acetylhydrazine being the main metabolite but a minor elimination product. Tissue retention was longest in kidney, mainly due to the presence of acetylhydrazine. Hydrazine is metabolized and detoxified by at least three microsomal cytochrome P450 isoenzymes in rat liver (CYP2E1, CYP2B1 and CYP1A1/2), ultimately yielding molecular nitrogen.

Human exposure to hydrazine has resulted in severe effects upon the central nervous system, liver and kidneys. In rats, hydrazine is hepatotoxic, causing accumulation of triglycerides, inhibition of protein synthesis and the formation of macromitochondria.

Hydrazine induces gene mutations in bacteria, yeast and *Drosophila* and in-vivo treatment of mice, rats and Syrian hamsters results in the formation of *N*7-methylguanine and O^6-methylguanine in liver DNA.

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[1] Summary (but not the evaluation) prepared by the Secretariat after the meeting.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of hydrazine.

There is *sufficient evidence* in experimental animals for the carcinogenicity of hydrazine.

Overall evaluation

Hydrazine is *possibly carcinogenic to humans (Group 2B).*

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ISOPRENE

Data were last evaluated in IARC (1994).

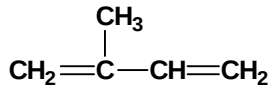
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 78-79-5
Chem. Abstr. Name: 2-Methyl-1,3-butadiene
IUPAC Systematic Name: Isoprene
Synonym: Isopentadiene

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_5H_8 Relative molecular mass: 68.12

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless, volatile liquid (Budavari, 1996)
(*b*) *Boiling-point*: 34.0°C (Lide, 1997)
(*c*) *Melting-point*: –149.5°C (Lide, 1997)
(*d*) *Solubility*: Practically insoluble in water; miscible with ethanol and diethyl ether (Budavari, 1996)
(*e*) *Vapour pressure*: 66 kPa at 20°C; relative vapour density (air = 1), 2.35 (Verschueren, 1996)
(*f*) *Flash-point*: –48°C (Lewis, 1993)
(*g*) *Conversion factor:* $mg/m^3 = 2.79 \times ppm$

1.2 Production and use

Production of isoprene in the United States in 1993 was reported to be 276 841 tonnes (United States International Trade Commission, 1994). Production capacities for isoprene in 1987 were estimated to be (thousand tonnes): United States, 199; the Netherlands, 25; Republic of South Africa, 45; Japan, 105; and the former Soviet Union, about 800 (Weitz & Loser, 1989). In 1992, isoprene monomer reportedly was produced in Brazil, the Netherlands, Japan, Romania, countries of the former Soviet Union and the United States (Lybarger, 1995).

Almost all isoprene produced is used in the preparation of polymers and copolymers. cis-Polyisoprene, primarily for vehicle tyres, is the largest application, with styrene–isoprene–styrene (SIS) block polymers being a rapidly growing secondary application. Butyl rubber is a significant third application. World demand for isoprene for monomer use in 1992 was (thousand tonnes): polyisoprene, 827; SIS, 95; butyl rubber, 25; and other uses, 10 (Weitz & Loser, 1989; Lybarger, 1995).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES) in the United States (NOES, 1997), approximately 4000 workers in the United States were potentially exposed to isoprene (see General Remarks). Occupational exposures to isoprene occur mainly in the production of the monomer and of synthetic rubbers.

1.3.2 *Environmental occurrence*

Isoprene occurs in the environment as emissions from vegetation, particularly from deciduous forests, and as a by-product in the production of ethylene by naphtha cracking. In the United States, the total emission rate of isoprene from deciduous forests has been estimated at 4.9 tonnes per year, with greatest emissions in the summer. The global annual emission of isoprene in 1988 was estimated to be 285 000 thousand tonnes. Isoprene is produced endogenously in humans. It has also been found in tobacco smoke, gasoline, turbine and automobile exhaust, and in emissions from wood pulping, biomass combustion and rubber abrasion (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not proposed any occupational exposure limits for isoprene in workplace air. Poland has an 8-h time-weighted average threshold limit value of 100 mg/m^3 and Russia has a short-term exposure limit of 40 mg/m^3 for exposure in workplace air (International Labour Office, 1991)

No international guideline for isoprene in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Isoprene was tested for carcinogenicity in male mice and in male rats by inhalation exposure in one-year studies. In mice, exposure to isoprene resulted in increased incidence of benign and malignant tumours of the lung, liver and forestomach and of Harderian gland adenomas. The study by inhalation in rats was inadequate for an assessment of carcinogenicity (IARC, 1994).

3.1 Inhalation

3.1.1 *Mouse*

Groups of 50 male B6C3F$_1$ mice [age unspecified] were exposed to isoprene by whole-body inhalation at concentrations of 0, 10, 70, 140, 280, 700 or 2200 ppm [0, 28, 200, 400, 800, 2000 or 6160 mg/m^3] for 4 or 8 h per day on five days per week for 20, 40 or 80 weeks, followed by holding periods until termination of the experiment at 96 or 104 weeks. The isoprene was > 99% pure, containing less than 1% limonene and less than 100 ppm *tert*-butyl catechol. Similar groups of female mice were exposed to 0, 10 or 70 ppm for 80 weeks and held until 104 weeks. All animals received a complete gross necropsy and major tissues and organs were examined histologically. Survival of groups exposed to 280 ppm for 80 weeks or to higher concentrations was less than that of controls and less than 50%. As shown in Table 1, increases in tumour incidence were found in the lung, liver, heart, spleen and Harderian gland in males. In female mice, increased incidence of Harderian gland adenomas (2/49 controls, 3/49 10-ppm and 8/49 ($p < 005$) 70-ppm) and pituitary adenomas (1/49 controls, 6/46 10-ppm and 9/49 ($p < 0.05$) 70-ppm) was seen at the high dose (Cox *et al.*, 1996; Placke *et al*., 1996).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344/N rats, six weeks of age, were exposed to isoprene (> 99% pure) by whole-body inhalation at concentrations 0, 220, 700 or 7000 ppm [0, 614, 1953 or 19 530 mg/m^3] for 6 h per day on five days per week for 104 weeks. Animals received a complete gross examination and major tissues and organs were evaluated histologically. Survival of exposed rats was similar to that of controls (males: control, 18/50; low-dose, 16/50; mid-dose, 15/50; high-dose, 15/50; females: 29/50, 30/50, 28/50, 27/50), and weight gain was not affected by the exposures. Exposed males and females had increased incidences of mammary fibroadenomas (males: control, 2/50; low-dose, 4/50; mid-dose, 6/50; high-dose, 21/50; females, 19/50, 35/50, 32/50, 32/50). The incidence of renal tubule adenomas was increased in treated males (2/50, 4/50, 8/50, 15/50). Some animals also had a renal carcinoma. Treated males also had increased incidence of interstitial-cell adenomas of the testis (33/50, 37/50, 44/50, 48/50) (United States National Toxicology Program, 1997).

Table 1. Summary of isoprene exposure-related neoplasms in male B6C3F$_1$ mice

Group	1	2	3	4	5	6	7	8	9	10	11	12
ppm/weeks	0/80	10/80	70/40	70/80	140/40	280/20	280/80	700/80	2200/20	2200/80	2200/40	2200/80
ppm × weeks[a]	0	800	2800	5600	5600	5600	22 400	5600	22 000	77 000	88 000	176 000
Alveolar/bronchiolar												
Adenoma	11/50	16/50	8/50	4/50	10/50	16/50	13/50	23/50*	14/50	15/50	29/49*	30/50*
Carcinoma	0	1	0	2	1	3	1	7*	2	3	3	7*
Hepatocellular												
Adenoma	11/50	12/50	14/49	15/50	22/50*	18/49	24/50*	27/48*	22/50*	21/50*	28/47*	30/50*
Carcinoma	9	6	11	9	10	12	16	17	12	15	18*	16
Harderian gland												
Adenoma	4/47	4/49	13/48	9/50	12/50*	16/49*	17/50*	26/49*	19/49*	28/50*	31/49*	35/50*
Carcinoma	0	0	0	0	2	3	1	3	1	2	0	2
Haemangiosarcoma												
Heart	0/49	0/50	0/49	0/50	0/50	0/50	2/50	1/50	4/50	1/50	1/49	1/50
Spleen	1/49	3/48	1/47	2/50	3/50	2/47	1/50	2/48	2/48	2/50	0/47	1/49
Histiocytic sarcoma[b]	0/50	2/50	2/50	2/50	1/59	8/50*	4/50	2/50	5/50*	7/50*	7/50*	2/50

From Placke *et al*. (1996) and Cox *et al*. (1996)

[a] Groups 9 and 10 were exposed for 4 h per day instead of 8 h per day

[b] Found in kidney, lung, liver, lymph nodes, bone marrow and spleen

* Incidence significantly greater than in the control group ($p < 0.05$, Fisher's exact test).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Isoprene is formed endogenously at the rate of 0.15 μmol/kg per hour in man. No further data were available to the Working Group (IARC, 1994).

4.1.2 *Experimental systems*

Isoprene is formed endogenously at the rate of 1.9 μmol/kg per hour in both rats and mice. 1,2-Epoxy-2-methyl-3-butene (80%) and 3,4-epoxy-2-methyl-1-butene (20%) are two major metabolites in mouse liver microsomes. The 3,4-epoxide can be further metabolized to isoprene diepoxide. Both rats and mice exhibited saturation kinetics when exposed to isoprene at concentrations above 300 ppm [837 mg/m^3]. The maximal rate of metabolism *in vivo* is more than three times greater in mice than in rats (IARC, 1994).

The most important enzyme converting isoprene to both monoepoxides and also to the diepoxide is CYP2E1, as shown by both recombinant expressed enzymes and in human liver microsomes (Bogaards *et al*., 1996).

Both rat and mouse liver microsomes are able to catalyse the formation of isoprene monoepoxides (Bogaards *et al.*, 1996).

4.1.3 *Comparison of human and rodent data*

The intrinsic rates of formation of monoepoxides in human, rat and mouse liver microsomes are roughly similar, when epoxide hydrolase is inhibited, whereas the amount of monoepoxides at the end of incubation is two and even 15 times higher in mouse liver microsomes than in rat and human liver microsomes, respectively (Bogaards *et al*., 1996). Thus, differences in epoxide hydrolase activity between different species may be of importance for toxicological outcomes.

A physiological toxicokinetic model has been developed for inhaled isoprene in mouse, rat and humans, taking into account published or assumed kinetic parameters (Filser *et al*., 1996). On the basis of this model, at human exposure conditions (up to 50 ppm [140 mg/m^3]), rates of metabolism are about 14 times faster in mice and about eight times faster in rats than in humans.

4.2 Toxic effects

4.2.1 *Humans*

Increasing duration of employment of isoprene rubber production workers showed a correlation with the prevalence and degree of various effects (IARC, 1994). Effects noted in these workers were subtrophic and atrophic processes in the upper respiratory tract, catarrhal inflammation, and degeneration of the olfactory tract.

4.2.2 *Experimental systems*

Inhalation exposure to isoprene (0, 438, 875, 1750, 3500 or 7000 ppm [1222–19 530 mg/m^3]) for 6 h per day on five days per week for two weeks did not affect survival, body weight gain, clinical signs or haematological parameters in male or female Fischer 344 rats. However, both male and female B6C3F$_1$ mice exposed under identical conditions had reduced erythrocyte numbers, haemoglobin concentrations and volume of packed erythrocytes, without increases in reticulocytes and polychromatic erythrocytes. In addition, both male and female mice exhibited forestomach epithelial hyperplasia at exposures ≥ 438 ppm. Male B6C3F$_1$ mice exposed to ≥ 1750 ppm had degeneration of the olfactory epithelium, and 7000 ppm isoprene caused reduced body weight gain and atrophy of the thymus and testis in these mice (Melnick *et al*., 1990).

Male Fischer 344 rats and B6C3F$_1$ mice were exposed by inhalation to 0, 70, 220, 700, 2200 or 7000 ppm [195–19 530 mg/m^3] isoprene for 6 h per day on five days per week for 26 weeks and were monitored for a further 26 weeks post-exposure. All exposure doses induced neoplastic and proliferative lesions in the liver, lung, Harderian gland and forestomach of mice. Exposure to 7000 ppm reduced survival in mice. There was an increase in altered hepatocellular foci and incidence of forestomach hyperplasia at doses ≥ 700 ppm [1950 mg/m^3]. At the end of the 26-week recovery period, alveolar epithelial hyperplasia was increased in the 700 ppm and higher-dose groups, alveolar/bronchiolar neoplasms were increased in the ≥ 2200-ppm and 7000-ppm groups and spinal cord degeneration was evident in all dose groups. Rats exposed to ≥ 700 ppm isoprene developed interstitial-cell hyperplasia of the testis (Melnick *et al*., 1994, 1996).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Sprague-Dawley rats and Swiss CD-1 mice were exposed by inhalation to 0, 280, 1400 or 7000 ppm [800–19 530 mg/m^3] isoprene for 6 h per day on seven days per week on gestational days 6–19 (rats) or 6–17 (mice) (IARC, 1994). There was no adverse effect on rat dams or other reproductive index at any dose level. The only fetal malformation observed in rats was reduced ossification of the vertebral centra, which occurred at 7000 ppm isoprene. In mice, there was reduced fetal body weight at all dose levels and decreased maternal weight gain in the 7000-ppm group. Also in the 7000-ppm group, there was an increased incidence of supernumerary ribs but no increase in fetal malformations.

B6C3F$_1$ mice were exposed by inhalation to 0, 70, 220, 700, 2200 or 7000 ppm [200–19 530 mg/m^3] isoprene for 6 h per day on five days per week for 13 weeks. Effects observed in the males were reduction of testicular weight by 35% in the animals exposed to 7000 ppm, seminiferous tubular atrophy in 20% of animals studied, reduced epididymal weights, lower spermatid headcounts and concentrations, and reduced sperm

motility in the 700- and 7000-ppm groups. Female mice in the 7000-ppm group had significantly longer average oestrous cycles (Melnick *et al*., 1994).

Isoprene [7.34 mmol/kg bw by intraperitoneal injection] affected ovarian follicles in 21-day-old B6C3F$_1$ mice. Small (primordial) follicle counts were reduced by 76 ± 5%; while growing (primary to pre-antral) follicle counts were reduced by 46% ± 8% when compared with the respective sesame seed oil controls (Doerr *et al*., 1995).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 2 for references)

Isoprene did not induce mutations in bacteria or, in single studies, either sister chromatid exchangess or chromosomal aberrations in cultures of Chinese hamster ovary cells. An inhalation study with mice demonstrated that isoprene could induce sister chromatid exchanges and micronuclei in bone-marrow cells. No increase in the incidence of chromosomal aberrations was observed in the same study.

Isoprene can be metabolized by mouse liver microsomes to oxirane intermediates, the main metabolite being 1,2-epoxy-2-methyl-3-butene. Neither this metabolite nor 3,4-epoxy-2-methyl-1-butene was mutagenic to *Salmonella typhimurium* TA100 or TA98 when tested up to lethal concentrations (30 mM), whereas another possible minor metabolite, 2-methyl-1,2,3,4-diepoxybutane was mutagenic in *S. typhimurium* TA100. The diepoxide had a high rate of nicotinamide alkylation (Gervasi *et al*., 1985). Isoprene has been shown to bind covalently to haemoglobin in rat and mice *in vivo*.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to isoprene occurs in the production of the monomer and in the production of synthetic rubbers. Isoprene occurs in the environment due to emissions from vegetation and the production of ethylene by naphtha cracking.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Isoprene was tested for carcinogenicity in mice and rats by inhalation exposure. In two studies in mice, exposure to isoprene resulted in increased combined incidences of benign and malignant tumours of the lung and liver and of Harderian gland adenomas. In one study, haemangiosarcomas of the heart and spleen and histiocytic sarcomas were also found in male mice, as well as increased incidences of pituitary adenomas and Harderian

Table 2. Genetic and related effects of isoprene

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	680	de Meester *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	Mortelmans *et al*. (1986)
SA3, *Salmonella typhimurium* TA1530, reverse mutation			680	de Meester *et al*. (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	680	de Meester *et al*. (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	Mortelmans *et al*. (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Mortelmans *et al*. (1986)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	680	de Meester *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	680	de Meester *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Mortelmans *et al*. (1986)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	5000	US National Toxicology Program (1995)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	5000	US National Toxicology Program (1995)
SVA, Sister chromatid exchange, B6C3F_1 mouse bone-marrow cells *in vivo*	+		430 inh 6 h/d × 12	Tice *et al*. (1988)
MVM, Micronucleus test, B6C3F_1 mouse bone-marrow cells *in vivo*	+		430 inh 6 h/d × 12	Tice *et al*. (1988)
CBA, Chromosomal aberrations, B6C3F_1 mouse bone-marrow cells *in vivo*	–		6900 inh 6 h/d × 12	Tice *et al*. (1988)
BVP, Binding (covalent) male B6C3F_1 mouse haemoglobin *in vivo*	+		2 ip × 1	Sun *et al*. (1989)
BVP, Binding (covalent) male Sprague-Dawley rat haemoglobin *in vivo*	+		2 ip × 1	Sun *et al*. (1989)
BVP, Binding (covalent) B6C3F_1 mouse haemoglobin *in vivo*	+		60 inh 6 h	Bond *et al*. (1991)

[a] +, positive;–, negative

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw /day; inh, inhalation; ip, intraperitoneal

gland adenomas in female mice. In one adequate study with rats, increased incidences were observed for benign neoplasms in the mammary gland in males and females and in the kidney and testis in males.

5.4 Other relevant data

Both rats and mice exhibited saturation kinetics when exposed to concentrations above 300 ppm [840 mg/m^3]. The maximal rate of metabolism *in vivo*, which occurs via monoepoxides and diepoxide and subsequent epoxide hydration, is more than three times greater in mice than in rats. In-vitro studies and a physiological toxicokinetic model suggest that the rates of metabolism in humans is lower.

At high inhalation exposures, proliferative lesions in olfactory epithelium and lung were observed. Forestomach epithelial hyperplasia was detected at lower exposure levels in rats and mice. Adverse effects in reproductive organs of male and female mice were detected after high inhalation doses.

Isoprene did not induce mutations in bacteria or sister chromatid exchanges or chromosomal aberrations in animal cells *in vitro*. Isoprene induced sister chromatid exchanges and micronuclei in bone-marrow cells after inhalation exposure of mice.

Isoprene binds covalently to haemoglobin *in vivo.*

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of isoprene were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of isoprene.

Overall evaluation

Isoprene is *possibly carcinogenic to humans (Group 2B)*.

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ISOPROPANOL

Data were last reviewed in IARC (1977) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

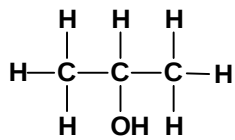
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Reg. No.: 67-63-0

Systematic name: 2-Propanol

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_3H_8O Relative molecular mass: 60.09

1.1.3 *Physical properties* (for details, see IARC, 1977)

(*a*) *Boiling-point*: 82°C

(*b*) *Melting-point*: –89.5°C

(*c*) *Conversion factor*: $mg/m^3 = 2.46 \times ppm$

1.2 Production and use

Isopropanol is manufactured in the United States by the indirect hydration of propylene in processes which may involve the use of concentrated or dilute sulfuric acid, whereas, in European countries and Japan, a direct hydration process is used in which propylene reacts with water in the presence of a catalyst. It is used mainly for the production of acetone, but also as a solvent and in the manufacture of other chemicals and in pharmaceutical and cosmetic formulations (IARC, 1977).

1.3 Occurrence

1.3.1 *Occupational exposure*

Occupational exposure to isopropanol may occur in polypropylene production plants (IARC, 1977).

1.3.2 *Environmental occurrence*

Isopropanol has been detected in trace quantities in some samples of drinking-water in the United States and as a constitutent of tar-water resulting from the distillation of shale tar. It has also been detected in the volatile fractions of grapefruit essence oil, roasted filbert nuts, lime essence, Reunion geranium oil, *Pinus densiflora* logs and milk products (IARC, 1977).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 983 mg/m^3 as the threshold limit value for occupational exposures to isopropanol in workplace air. Similar values have been used as standards or guidelines in many countries, except in Denmark (490 mg/m^3) and Sweden (350 mg/m^3) (International Labour Office, 1991).

No international guideline for isopropanol in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

An increased incidence of cancer of the paranasal sinuses was observed in workers at factories where isopropyl alcohol was manufactured by the strong-acid process (IARC, 1987). The risk for laryngeal cancer may also have been elevated in these workers. It is unclear whether the cancer risk was due to the presence of diisopropyl sulfate, which is an intermediate in the process, to isopropyl oils, which are formed as by-products, or to other factors, such as sulfuric acid.

In the Montreal case–control study carried out by Siemiatycki (1991; see the monograph on dichloromethane in this volume), the investigators estimated the associations between 293 workplace substances and several types of cancer. Isopropanol was one of the substances. About 4% of the study subjects had ever been exposed to isopropanol. Among the main occupations to which isopropanol exposure was attributed in this study were fire fighters, machinists and electricians. For most types of cancer examined (oesophagus, stomach, colon, rectum, pancreas, prostate, bladder, kidney, skin melanoma, lymphoma), there was no indication of an excess risk due to isopropanol. For lung cancer, based on 16 cases exposed at the 'substantial' level, the odds ratio was 1.4 (90% confidence interval, 0.8–2.7). [The interpretation of the null results has to take into account the small numbers and presumed low levels of exposure.]

3. Studies of Cancer in Experimental Animals

Isopropanol has been tested in mice by skin application, inhalation exposure and subcutaneous injection. These studies were inadequate for evaluation (IARC, 1977).

3.1 Inhalation exposure

3.1.1 *Mouse*

Groups of 55 male and 55 female CD-1 mice, approximately seven weeks old, were exposed by inhalation to target concentrations of 0 (control), 500, 2500 and 5000 ppm [0, 1225, 6125 and 12 250 mg/m^3] isopropanol vapour (purity, 99.9%) for 6 h per day on five days per week for 78 weeks. The mean actual concentrations were 504 ± 14, 2509 ± 58 and 5037 ± 115 ppm, respectively. The highest concentration (5000 ppm) was selected as a result of the mortality observed at 10 000 ppm in a previous nine-day inhalation study and the toxic effects observed at 5000 ppm in a previous 13-week study. Animals were killed immediately after the last exposure and a complete autopsy was carried out on each animal. No adverse effect on weight gain was observed among treated and control animals. Almost all organs were examined histologically in the high-dose and control groups. Histological evaluations of the kidneys, testes and gross lesions were performed for the mid- and low-dose groups. No difference in mean survival time was noted for any of the exposure groups. [Mortality and mean survival time were indicated only in bar graphs.] No increased incidence of neoplastic lesions was noted for either sex of mice from any exposure group, but no data were presented (Burleigh-Flayer *et al*., 1997). [The Working Group noted the limited duration of the study.]

3.1.2 *Rat*

Groups of 65 male and 65 female Fischer 344 rats, approximately seven weeks old, were exposed by inhalation to target concentrations of 0 (control), 500, 2500 and 5000 ppm [0, 1225, 6125 and 12 250 mg/m^3] isopropanol vapour (purity, 99.9%) for 6 h per day on five days per week for 104 weeks. The mean actual concentrations were 504 ± 14, 2509 ± 58 and 5037 ± 115 ppm, respectively. The highest concentration (5000 ppm) was selected as a result of the mortality observed at 10 000 ppm in a previous nine-day inhalation study and the toxic effects observed at 5000 ppm in a previous 13-week study. Animals were killed immediately after the last exposure and a complete autopsy was carried out on each animal. Almost all organs were examined histologically in the high-dose and control groups. Histological evaluations of the kidneys, testes and gross lesions were performed for the mid- and low-dose groups. Survival was poor in male rats but was adequate in females. Increased mortality (100% versus 82% for controls) and decreased mean survival time (577 versus 631 days for controls; $p < 0.01$ by life-table analysis) were noted for high-dose male rats. No difference in mean survival time was noted for female rats. [The mortality and mean survival time in other groups were indicated only in bar graphs.] Chronic renal disease was attributed as the main cause of death for male and female rats in the high-dose groups and was also considered to account for much of the mortality observed in mid-dose males. Extensive data were presented on clinical and microscopic renal pathology, but no tumour data were presented. The main cause of death for male controls was mononuclear-cell leukaemia. Concentration-related increases in interstitial-cell adenoma of the testes were observed in male rats found dead or moribund during the study (57.5% of control, 72.2% of low-dose, 84.7% of mid-dose and 93.8% of

high-dose animals) as well as for all animals in the study (64.9% of control, 77.3% of low-dose, 86.7% of mid-dose and 94.7% of high-dose animals) [effective number of animals and statistical significance not indicated]. No increased incidence of neoplastic lesions was observed for female rats from any exposure group but no data were presented (Burleigh-Flayer *et al*., 1997). [The Working Group noted the poor survival in male rats, most marked in the high-dose groups.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

The metabolism and pharmacokinetics of isopropanol have been reviewed (Dhillon & Von Burg, 1995).

4.1.1 *Humans*

Although there are a number of publications on the pharmacokinetics and disposition of isopropanol in humans, most of these are case reports of accidental or deliberate self-administration rather than systematic studies. However, a range of important features of the behaviour of isopropanol have been defined.

Isopropanol is rapidly absorbed from the gastrointestinal tract, with more than 80% absorbed within 30 min and 100% within 3 h. In contrast, absorption through the skin is low (Martinez *et al*., 1986; McGrath & Einterz, 1989) but may be sufficient to be involved in isopropanol toxicity. The volume of distribution of isopropanol is 0.6–0.7 L/kg, similar to total body water.

The metabolism of isopropanol is via oxidation by aldehyde dehydrogenase (ADH) to acetone. In common with other α-substituted (secondary) alcohols, isopropanol is a relatively poor substrate for ADH (WHO, 1990; Light *et al*., 1992). The primary metabolite, acetone, is eliminated in the expired air and in the urine and also undergoes further oxidation to acetate, formate and, ultimately, CO_2.

Isopropanol is excreted unchanged in the urine and the expired air, these routes together accounting for approximately 50% of the dose. In adults, the elimination half-life ranges from 2.9 to 16.2 h and this is shorter in alcoholics. Values observed in children poisoned with isopropanol fall within this range. The elimination of acetone formed from isopropanol is slower (Daniel *et al*., 1981), with levels remaining elevated up to 38 h after ingestion: it was not possible to calculate a half-life as the levels were essentially constant during the study period.

Acetonaemia is a clinical feature seen in diabetes and starvation and is thus not diagnostic of isopropanol exposure. However, Kawai *et al*. (1990) have suggested that urinary acetone levels provide a valuable index of workplace exposure to isopropanol. They found a good correlation (r = 0.84) between isopropanol exposure assessed by

diffusive samplers worn by workers during an 8-h shift and the acetone concentration in spot urine samples collected 6 h into the shift.

4.1.2 *Experimental systems*

Martinez *et al*. (1986) compared the absorption and metabolism of isopropanol in rabbits after oral, dermal and inhalation exposure. The highest blood levels were seen after oral dosing, lower after inhalation and lowest after dermal application. Blood levels after doses of 4 mL/kg were approximately twice those seen after 2 mL/kg, but concentrations of acetone were the same after both doses, regardless of the route of administration.

Jerrard *et al*. (1992) gave anaesthetized dogs 60 mL of 70% aqueous isopropanol (approximately 2 mL/kg) and determined blood levels of isopropanol and acetone for up to 6 h. The peak isopropanol level occurred at 3 h, while acetone concentrations increased throughout the 6-h experiment.

4.2 Toxic effects

4.2.1 *Humans*

Isopropanol has sensitizing properties but is not a dermal irritant. Volunteers inhaling this compound for several minutes developed irritation to the eyes and rhinopharynx. Oral intake of low doses (2.6–6.4 mg/kg bw) had no effect on blood cells, serum or urine and produced no symptoms (IARC, 1977).

Acute inhalation exposure to isopropanol can produce central nervous system depression that may be prolonged by acetone, a metabolite of isopropanol; lethalities have occurred in very young and newborn children (Mydler *et al.*, 1993; Vicas & Beck, 1993). Ingestion of isopropanol has been implicated in the deaths of a number of adults, particularly among alcoholics. Pulmonary congestion was the most frequent post-mortem finding and is typical, although not diagnostic or specific, of deaths involving drug-induced central nervous system depression (Alexander *et al.*, 1982).

4.2.2 *Experimental systems*

Oral administration of isopropanol increased the hepatotoxicity of chlorinated hydrocarbons in mice and led to accumulation of liver triglycerides in rats (IARC, 1977).

Rats continuously inhaling 8 ppm [20 mg/m^3] isopropanol for 86 days showed increased bromosulfophthalein retention, liver parenchymal dystrophy, enlarged spleen and degenerative changes in the brain (IARC, 1977). Groups of rats inhaling isopropanol at a concentration of 8000 ppm [20 000 mg/m^3] for 20 weeks showed no change in erythrocyte numbers. There was an increase in serum cholesterol levels throughout the dosing period, which returned to normal values within four weeks in a 12-week recovery period. Serum alanine aminotransferase and aspartate aminotransferase activities were significantly increased during the first 12 weeks of the dosing period, but had returned to normal values by the end of the dosing period. No effect on these parameters was observed at 4000 ppm [10 000 mg/m^3] (Nakaseko *et al.*, 1991).

Inhalation of 400 ppm [1000 mg/m^3] isopropanol by guinea-pigs for 24 h reduced the ciliary activity in the nasal mucosa, but recovery was complete within two weeks. Higher concentrations produced damage that required longer to repair (Ohashi *et al.*, 1987, 1988).

4.3 Reproductive and developmental effects

Isopropanol administered daily in the drinking-water of rats to achieve doses of 1500, 1400 and 1300 mg/kg bw in the parents and two successive generations, respectively, had no effect upon growth, reproductive function, intrauterine or postnatal development (IARC, 1977).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

In a single in-vivo study, isopropanol did not induce micronuclei in bone marrow of mice. In single studies conducted in mammalian cells *in vitro*, it did not induce sister chromatid exchanges or gene mutations.

Isopropanol did not induce aneuploidy in *Neurospora crassa* in a single study. It was not mutagenic to bacteria.

5. Summary of Data Reported and Evaluation[1]

5.1 Exposure data

Exposure to isopropanol may occur in its production, in the production of acetone and during its use as a solvent.

5.2 Human carcinogenicity data

An increased incidence of cancer of the paranasal sinuses and laryngeal cancer was observed in workers at factories where isopropanol was manufactured by the strong-acid process. One case–control study investigated the risk associated with occupational exposure to isopropanol, but for none of the investigated cancer sites was a significant increase in risk observed.

5.3 Animal carcinogenicity data

Isopropanol was tested for carcinogenicity in mice and rats by inhalation exposure. Although no increase in tumours was observed in mice, the study had some limitations in design and adequacy. A slight increase in interstitial cell adenomas of the testis was observed in male rats.

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[1] Summary (but not the evaluation) prepared by the Secretariat after the meeting.

Table 1. Genetic and related effects of isopropanol

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500	Shimizu *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	Zeiger *et al.* (1992)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500	Shimizu *et al.* (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	Zeiger *et al.* (1992)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500	Shimizu *et al.* (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Zeiger *et al.* (1992)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2500	Shimizu *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	Shimizu *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Zeiger *et al.* (1992)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	5000	Zeiger *et al.* (1992)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	–	2500	Shimizu *et al.* (1985)
NCN, *Neurospora crassa*, meiotic non-disjunction, aneuploidy	–	–	NG	Brockman *et al.* (1984)
GCO, Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	–	–	5000	Kapp *et al.* (1993)
SIC, Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	–	–	6000	von der Hude *et al.* (1987)
MVM, Micronucleus test, ICR mice bone-marrow cells *in vivo*	–		2500 ip × 1	Kapp *et al.* (1993)

[a] –, negative

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; ip, intraperitoneal

5.4 Other relevant data

Isopropanol is rapidly absorbed from the human gastrointestinal tract, whereas absorption through the skin is slow. It is metabolized by aldehyde dehydrogenase to acetone, but following human exposure, a large proportion is excreted unchanged in expired air and urine. It is a human sensitizer and is irritant to the eyes and rhinopharynx. Isopropanol is a central nervous system depressant and prolonged inhalation exposure of rats can produce degenerative changes in the brain. There is no evidence for genetic toxicity.

5.5 Evaluation

There is *inadequate evidence* for the carcinogenicity of isopropanol in humans.

There is *inadequate evidence* for the carcinogenicity of isopropanol in experimental animals.

Overall evaluation

Isopropanol is *not classifiable as to its carcinogenicity to humans (Group 3).*

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MALONALDEHYDE (MALONDIALDEHYDE)

Data were last reviewed in IARC (1985) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 542-78-9

Chem. Abstr. Name: Propanedial

IUPAC Systematic Name: Malonaldehyde

Synonym: Malondialdehyde

1.1.2 *Structural and molecular formulae and relative molecular mass*

$C_3H_4O_2$ Relative molecular mass: 72.07

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Solid (needles) (United States National Library of Medicine, 1997)

(*b*) *Melting-point*: 72–74°C (IARC, 1985)

(*c*) *Stability*: Highly pure malonaldehyde is quite stable under neutral conditions but not under acidic conditions such as those used to prepare it by hydrolysis of its bis(dialkyl)acetal. Since malonaldehyde has $pK_a = 4.46$, it exists under physiological conditions as its conjugate base ($^-$O–CH=CH–CHO), which is relatively stable to self-condensation (IARC, 1985).

(*d*) *Conversion factor*: $mg/m^3 = 2.95 \times ppm$

1.2 Production and use

Malonaldehyde is produced and used in small quantities, principally for research purposes (United States National Library of Medicine, 1997).

1.3 Occurrence

1.3.1 *Occupational exposure*

Exposure to malonaldehyde may occur in research laboratories.

1.3.2 *Environmental occurrence*

Malonaldehyde has been detected in the leaves of pea and cotton plants. It is found in many foodstuffs and can be present at high levels in rancid foods. It has been detected in fish meat, fish oil, rancid salmon oil, rancid nuts, rancid flour, orange juice essence, vegetable oils, fats, fresh frozen green beans, milk, milk fat, rye bread and in raw, cured and cooked meats (United States National Library of Medicine, 1997).

1.3.3 *Human tissues and secretions*

Malonaldehyde is found in human and animal tissue as an end-product of lipid peroxidation. It is also a side-product of prostaglandin and thromboxane biosynthesis. Malonaldehyde is present in blood platelets and in serum (IARC, 1985).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not proposed any occupational exposure limit for malonaldehyde in workplace air.

No international guideline for malonaldehyde in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Malonaldehyde and its bis(dimethylacetal) and sodium salt were tested for carcinogenicity in mice by skin application. Its bis(dimethylacetal) and sodium salt were tested in mice by oral administration in drinking-water. The two studies by oral administration were inadequate for evaluation. After topical application, no increase in the incidence of skin tumours was observed in one study. In one two-stage mouse-skin assay, a high dose of malonaldehyde (possibly containing impurities) showed initiating activity. In two other two-stage assays using lower doses, no initiating or promoting activity was observed (IARC, 1985).

3.1 Oral administration

3.1.1 *Mouse*

Three groups of 50 male and 50 female B6C3F_1 mice, eight weeks of age, were administered 0 (vehicle control), 60 or 120 mg/kg bw malonaldehyde sodium salt (purity, 63–79% malonaldehyde sodium salt; 22–38% water; and 1% impurities as chloride and acetone) in distilled water by oral gavage on five days per week for up to 105 weeks. Survival rates at termination for males were 23/50 control, 19/50 low-dose and 14/50

high-dose ($p < 0.02$, Cox's method) and for females were 41/50 control, 38/50 low-dose and 30/50 high-dose. Body weight was slightly (less than 10%) lower than that of the vehicle controls in high-dose males during the second half of the study. Body weight was slightly (approximately 10%) higher than that of the vehicle controls in high-dose females during most of the study. No tumour type was increased in incidence in malonaldehyde-exposed mice compared with the vehicle controls (United States National Toxicology Program, 1988). [The Working Group noted the high mortality in high-dose males.]

3.1.2 *Rat*

Three groups of 50 male and 50 female Fischer 344/N rats, seven weeks of age, were administered 0 (vehicle controls), 50 or 100 mg/kg bw malonaldehyde sodium salt (purity, 63–79% malonaldehyde sodium salt; 22–38% water; and 1% impurities as chloride and acetone) in distilled water by oral gavage on five days per week for up to 105 weeks, at which time the surviving animals were killed. Survival rates at termination for males were 37/50 control, 33/50 low-dose and 15/50 high-dose and for females were 37/50 control, 37/50 low-dose and 14/50 high-dose. The survival of the high-dose groups was significantly lower than that of the vehicle controls ($p < 0.001$). Mean body weights of high-dose male rats were 10–20% lower than those of the vehicle controls from week 33 to week 72 and 20–26% lower from week 72 to the end of the study. Mean body weights of low-dose male rats were 3–7% lower than those of vehicle controls from week 67 to the end of the study. Mean body weights of high-dose females were 10–20% lower than those of the vehicle controls from week 54 to week 72 and 21–36% lower from week 72 to the end of the study. The incidences of thyroid follicular-cell adenomas were 3/50 control males, 3/49 low-dose males and 9/50 high-dose males ($p < 0.05$) and 2/50 control females, 0/50 low-dose females and 5/50 high-dose females ($p < 0.05$); the incidences of follicular-cell carcinomas were 1/50 control males, 5/49 low-dose males and 5/50 high-dose males ($p < 0.05$) and 0/50 control females, 1/50 low-dose females and 2/50 high-dose females. Overall rates of thyroid follicular-cell tumours were 4/50 control males, 8/49 low-dose males and 13/50 high-dose males ($p = 0.015$) and 2/50 control females, 1/50 low-dose females and 7/50 high-dose females ($p = 0.03$). The incidences of pancreatic islet-cell adenomas in males were 0/49, 9/50 ($p < 0.002$) and 1/49 in the control, low- and high-dose groups, respectively (United States National Toxicology Program, 1988). [The Working Group noted the high mortality and the strong reduction in body weights in the high-dose males and females, indicating that the maximum tolerated dose was exceeded and also that the increased incidences of pancreatic islet-cell adenomas in males were not dose-related.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Two aldehyde dehydrogenases in the rat-liver cytosol fraction account for virtually all of the metabolizing activity for malonaldehyde (IARC, 1985).

Twelve hours after oral intubation of [1,3-^{14}C]malonaldehyde to rats, 60–70%, 5–15% and 9–17% of radioactivity was recovered in expired CO_2, faeces and urine, respectively (Siu & Draper, 1982).

After oral administration of malonaldehyde (158 mg/kg bw) to rats, increased quantities of formaldehyde, acetaldehyde, acetone and malonaldehyde itself were found in the urine. Additionally, methyl ethyl ketone, not found in control rats, was present in the urine of the animals that had received malonaldehyde (Akubue *et al.*, 1994).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Malonaldehyde, given in the drinking-water, induced morphological changes in the liver and mild dysplasia at all doses tested (2–500 mg/kg bw per day) in female ICR mice; pancreatic damage was observed at doses of 500 mg/kg bw per day (IARC, 1985). Histological changes were observed in the liver only of female ICR Swiss mice given 10–1000 μg/kg bw malonaldehyde in the drinking-water for one year, but no dose–response relationship was observed (Bird *et al.*, 1982a).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

Malonaldehyde reacts with DNA to form an adduct with 2′-deoxyguanosine which has been characterized as 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrimido[1,2-α]purin-10(3H)-one (M_1G-dR) (Marnett, 1994). This adduct is present at quantifiable levels in DNA from many human tissues.

Chaudhary *et al.* (1994) found M_1G-dR in DNA from human liver samples (four males, two females) at levels ranging from 5 to 11 adducts/10^7 bases, using gas chromatography/electron capture negative chemical ionization mass spectrometry.

Leuratti *et al.* (1997) found M_1G-dR in DNA from human gastric biopsy samples at levels ranging from 1 to 9 adducts/10^8 normal nucleotides, using a high-performance liquid chromatography/^{32}P-postlabelling method (results presented as an abstract).

Fang *et al.* (1996) found that levels of M_1G-dR were influenced by dietary fats, with men and women consuming unsaturated fats having higher levels of the adduct in DNA from peripheral white blood cells than those who consumed saturated fats.

4.4.2 *Experimental systems* (see Table 1 for references)

Malonaldehyde induced mutation in bacteria that were either DNA repair competent or were sensitive to oxidative DNA damage. It had been suggested previously that all or part of the activity might be attributable to impurities, which occur as a result of malonaldehyde instability. However, specially purified or specially synthesized malonaldehyde continued to show mutagenic activity in *S. typhimurium his* D3052 (Basu & Marnett, 1983).

In one study in *Drosophila melanogaster*, malonaldehyde induced somatic mutations but not sex-linked recessive lethal mutations.

Malonaldehyde formed adducts with purified rat liver DNA, dAMP and dGMP *in vitro*; these adducts were observed with the ^{32}P-postlabelling technique (Wang & Liehr, 1995).

Malonaldehyde induced dose-dependent increases in sister chromatid exchanges but did not produce chromosomal aberrations in Chinese hamster ovary cells, but did induce chromosomal aberrations and micronuclei in rat primary skin fibroblasts.

At concentrations of malonaldehyde which produce reversion to histidine prototrophy in *Salmonella typhimurium* (TA100), characteristic adducts were found in bacterial DNA. The adducts were formed in a dose-dependent manner.

Injection of [^{14}C]malonaldehyde into male C57BL/6 mice resulted in covalent binding to liver DNA and haemoglobin.

Agarwal and Draper (1992) found background levels of malonaldehyde–guanine adduct in rat liver DNA, using high-performance liquid chromatography–fluorescence. Similar results were found by Vaca *et al*. (1992) and Wang and Liehr (1995) using ^{32}P-postlabelling. Chaudhary *et al*. (1994) used gas chromatography/electron capture negative chemical ionization mass spectrometry to measure background levels in rat liver. The levels were significantly elevated following administration of carbon tetrachloride (0.1 mL/kg bw), which induced lipid peroxidation (Chaudhary *et al*., 1994; Wang & Liehr, 1995).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Malonaldehyde is found in many foodstuffs and can be present at high levels in rancid foods. It is present as a lipid metabolite in human and animal tissues. It is probably used only as a research chemical.

Table 1. Genetic and related effects of malonaldehyde

Test system	Results[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ERD, *Escherichia coli rec* strains, differential toxicity	+	NT	72	Yonei & Furui (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	NT	NG	Marnett & Tuttle (1980)
SA2, *Salmonella typhimurium* TA102, reverse mutation	+	NT	1000	Levin *et al.* (1982)
SA2, *Salmonella typhimurium* TA102, reverse mutation	+	NT	900	Marnett *et al.* (1985)
SA4, *Salmonella typhimurium* TA104, reverse mutation	+	NT	2520	Marnett *et al.* (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	NT	NG[d]	Mukai & Goldstein (1976)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	NT	NG	Marnett & Tuttle (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	NT	NG[d]	Mukai & Goldstein (1976)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	NG[d]	Mukai & Goldstein (1976)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	NG	Marnett & Tuttle (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	NG	Marnett & Tuttle (1980)
SAS, *Salmonella typhimurium his* G46, reverse mutation	–	NT	NG[d]	Mukai & Goldstein (1976)
SAS, *Salmonella typhimurium his* C3076, reverse mutation	+	NT	NG[d]	Mukai & Goldstein (1976)
SAS, *Salmonella typhimurium his* D3052, reverse mutation	+	NT	NG[d]	Mukai & Goldstein (1976)
SAS, *Salmonella typhimurium* TA1975, reverse mutation	+	NT	500	Shamberger *et al.* (1979)
SAS, *Salmonella typhimurium* TA1977, reverse mutation	+	NT	500	Shamberger *et al.* (1979)
SAS, *Salmonella typhimurium* TA1978, reverse mutation	+	NT	500	Shamberger *et al.* (1979)

Table 1 (contd)

Test system	Results[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SAS, *Salmonella typhimurium his* C207, reverse mutation	+	NT	500	Shamberger *et al.* (1979)
SAS, *Salmonella typhimurium his* C3076, reverse mutation	+	NT	500	Shamberger *et al.* (1979)
SAS, *Salmonella typhimurium his* D3052, reverse mutation	+	NT	500	Shamberger *et al.* (1979)
SAS, *Salmonella typhimurium his* G46, reverse mutation	–	NT	4000	Shamberger *et al.* (1979)
SAS, *Salmonella typhimurium his* D3052, reverse mutation	+	NT	94	Marnett & Tuttle (1980)
SAS, *Salmonella typhimurium his* D3052, reverse mutation	+[c]	NT	72	Basu & Marnett (1983)
ECF, *Escherichia coli* H/r30 (*uvr*$^+$*rec*$^+$), forward mutation, streptomycin resistance	+	NT	144	Yonei & Furui (1981)
ECR, *Escherichia coli* H/r30 (*uvr*$^+$*rec*$^+$), forward mutation, arginine prototrophy	+	NT	144	Yonei & Furui (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		6125 feed	Szabad *et al.* (1983)
DMM, *Drosophila melanogaster*, somatic mutations	+		6125 feed	Szabad *et al.* (1983)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	2.9	Yau (1979)
G51, Gene mutation, mouse lymphoma L5178Y cells, methotrexate resistance *in vitro*	+	NT	3.6	Yau (1979)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	15	Anderson *et al.* (1990)
MIA, Micronucleus test, Sprague-Dawley rat primary skin fibroblasts *in vitro*	+	NT	7.2	Bird *et al.* (1982)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	3270	Anderson *et al.* (1990)
CIR, Chromosomal aberrations, Sprague-Dawley rat primary skin fibroblasts *in vitro*	+	NT	7.2	Bird *et al.* (1982)
BID, Binding (covalent) to calf thymus DNA *in vitro*	+	NT	7200	Vaca *et al.* (1992)
BID, Binding (covalent) to DNA from *Salmonella typhimurium* TA100 *in vitro*	+	NT	721	Sevilla *et al.* (1997)

Table 1 (contd)

Test system	Results[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BVD, Binding (covalent) to liver DNA, male C57BL/6 mice *in vivo*	+		22.5 ip × 1	Kautiainen *et al.* (1993)
BVP, Binding (covalent) to haemoglobin, male C57BL/6 mice *in vivo*	+		22.5 ip × 1	Kautiainen *et al.* (1993)
BVD, Binding (covalent) to DNA, C57/BL6 mouse liver *in vivo*	+		1.3 ip × 1	Vaca *et al*. (1992)

[a] +, positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; ip, intraperitoneal injection
[c] Highly purified malonaldehyde
[d] Confusion over actual dose applied

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Malonaldehyde sodium salt was tested for carcinogenicity in one experiment in mice and in one experiment in rats by oral administration. No increase in tumour incidence was found in mice. In rats, the incidence of follicular-cell tumours of the thyroid was increased in both sexes at the high dose and the incidence of pancreatic islet-cell adenomas was increased in low-dose males.

Malonaldehyde, its bis(dimethylacetal) and its sodium salts were tested for carcinogenicity in mice by skin application; no carcinogenic activity was observed.

5.4 Other relevant data

Background exposures to malonaldehyde occur in experimental animals and humans, as determined by the presence of specific DNA adducts in blood and other tissues. It is mutagenic to bacteria.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of malonaldehyde were available.

There is *limited evidence* in experimental animals for the carcinogenicity of malonaldehyde.

Overall evaluation

Malonaldehyde is *not classifiable as to its carcinogenicity to humans (Group 3)*.

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4,4′-METHYLENEDIPHENYL DIISOCYANATE AND POLYMERIC 4,4′-METHYLENEDIPHENYL DIISOCYANATE

Data were last reviewed in IARC (1979) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

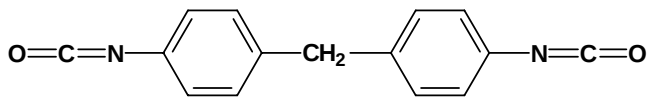
Chem. Abstr. Serv. Reg. No.: (a) 101-68-8 (monomer) and (b) 26447-40-5

Chem. Abstr. Name: 1,1′-Methylenebis(4-isocyanatobenzene)

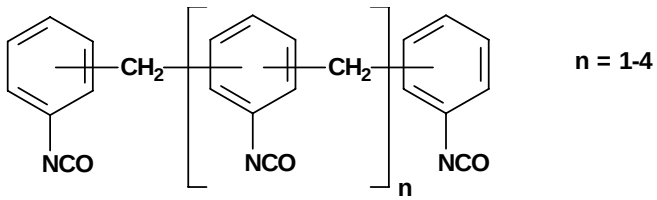
IUPAC Systematic Names: For 101-68-8: isocyanic acid, methylenedi-*para*-phenylene ester

Synonyms: Bis(1,4-isocyanatophenyl)methane; diphenylmethane diisocyanate; MDI; methylenedi-*para*-phenylene isocyanate and for 26447-40-5: crude MDI; polymeric MDI; PMDI; generic MDI; non-isomeric-specific MDI

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{15}H_{10}N_2O_2$ Relative molecular mass: 250.27



$C_{15}H_{10}N_2O_2.[C_8H_5NO]_n$ (polymeric)

1.1.3 *Chemical and physical properties of the pure substance*

(from United States National Library of Medicine (1997a), unless otherwise stated)

(*a*) *Description*: White to light yellow odourless flakes

(*b*) *Boiling-point*: 196°C at 665 Pa

(*c*) *Melting-point*: 38°C

(*d*) *Solubility*: Soluble in acetone, benzene, kerosene and nitrobenzene
(*e*) *Vapour pressure*: 0.13 Pa at 40°C

1.1.4 *'Polymeric MDI'*

The principal material of commerce is not pure 4,4′-methylenediphenyl diisocyanate but is a mixture containing 4,4′-methylenediphenyl diisocyanate, other methylenediphenyl diisocyanate isomers, and low oligomers of the general structure shown above.

This dark amber mixture is commonly called polymeric, or generic, or non-isomer-specific methylenediphenyl diisocyanate. Its composition is variable but typically is in the range of 40–50% 4,4′-methylenediphenyl diisocyanate, 2.5–4.0% 2,4′-methylenediphenyl diisocyanate, 0.1–0.2% 2,2′-methylenediphenyl diisocyanate, and the remainder (50–60%) higher homologues (Woods, 1990; European Union, 1999).

1.2 Production and use

The world production of methylenediphenyl diisocyanate all types included was 1200 thousand tonnes in 1991. In the European Union, approximately 790 thousand tonnes were manufactured in 1996, compared with 540 thousand tonnes in 1991 and 267 thousand tonnes in 1980; 215 thousand tonnes were processed in 1980 (European Union, 1999).

It is mainly used in the industrial production of rigid polyurethane foams. Many other uses are in the fields of coatings, adhesives, sealants and elastomers such as paints, adhesives, weather-resistant sealing materials and footwear. There is use also in the production of particle board (bonding of wood) and mould cores for the foundry industry (European Union, 1999).

1.3 Occurrence

1.3.1 *Occupational exposure*

Some data on levels of occupational exposure to 4,4′-methylenediphenyl diisocyanate have been presented in a previous *IARC Monograph* (IARC, 1979).

1.3.2 *Environmental occurrence*

4,4′-Methylenediphenyl diisocyanate can be released to the environment in waste stream emissions from sites of industrial manufacture and use. Toxic Release Inventory reports to the United States Environmental Protection Agency before at least the mid-1990s were subject to serious overestimation of the releases to the environment, because of errors in the way that the figures were calculated by industry. Within the European Union, total emissions from production sites in 1996 were about 43 kg and emissions from processing plants in the same year were about 7100 kg (European Union, 1999).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 0.051 mg/m^3 as the threshold limit value for occupational exposure to

4,4′-methylenediphenyl diisocyanate in the workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for 4,4′-methylenediphenyl diisocyanate in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

See monograph on toluene diisocyanate.

3. Studies of Cancer in Experimental Animals

No data were available to the Working Group with regard to preparations of monomeric 4,4′-methylenediphenyl diisocyanate alone. The following is a study of a preparation containing both monomeric and polymeric 4,4′-methylenediphenyl diisocyanate.

3.1 Inhalation

3.1.1 *Rat*

Groups of 60 male and 60 female Wistar rats, six weeks of age, were exposed to target concentrations of 0 (controls), 0.2, 1.0 or 6.0 mg/m^3 (analytical value, 0.19, 0.98 or 6.03 mg/m^3) respirable (particle size, 93.5% < 4.2 μm) polymeric 4,4′-methylenediphenyl diisocyanate aerosol (31.0–31.7% (w/w) isocyanate content, 0.06–0.12% hydrolysable chlorine, 0.20–0.37% total chlorine, 0.0001–0.0069% chlorobenzene, 0.003–0.005% phenyl isocyanate, 44.8–50.2% monomeric 4,4′-methylenediphenyl diisocyanate, 0.01% sediment content) for 6 h per day on five days per week for two years. The exposure concentrations were selected based on results of a 13-week study. Complete histological examination was performed and almost all organs and all grossly observed lesions were examined histologically. Survival at 104 weeks of study was 38/60, 38/60, 42/60 and 36/60 control, low-dose, mid-dose and high-dose males and 41/60, 42/60, 48/60 and 50/60 control, low-dose, mid-dose and high-dose females. In the high-dose group, pulmonary adenomas were found in 6/60 males ($p < 0.05$ by two-sided Fisher's exact test) and 2/59 females, and pulmonary adenocarcinoma was found in 1/60 males. No lung tumours were found in other groups. Accumulation of alveolar macrophages containing polymeric 4,4′-methylenediphenyl diisocyanate-associated refractile yellowish material, localized fibrosis, alveolar duct epithelialization and increased incidences of calcareous deposits and localized alveolar bronchiolization were observed in the lungs of the high-dose group (Reuzel *et al*., 1994). [The Working Group noted that the effects observed could not be attributed confidently either to a non-specific small inhaled particle effect or to the chemical composition of the particles.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

The air levels of 4,4′-methylenediphenyl diisocyanate in a factory manufacturing polyurethane products were measured using personal, work-room and work-station monitors. In most cases, the levels were below detection limits. Using gas chromatography/mass spectrometry methods, urinary base-extractable metabolites were above control levels in 15 of the 20 workers and ranged from 0.035 to 0.83 pmol/mL 4,4′-methylenedianiline. The level of the acetylated metabolite, *N*-acetyl-4,4′-methylenedianiline, ranged from 0.13 to 7.61 pmol/mL. The amount of 4,4′-methylenedianiline released after acid hydrolysis was on average 6.5 times higher than the amount of free 4,4′-methylenedianiline and *N*-acetyl-4,4′-methylenedianiline present in urine. 4,4′-Methylenedianiline was present as a haemoglobin adduct in all of the 20 subjects at levels ranging from 70 to 710 fmol/g haemoglobin. In one individual, the haemoglobin adduct of *N*-acetyl-4,4′-methylenedianiline was detected. Plasma 4,4′-methylenedianiline levels ranged from 0.25 to 5.4 pmol/mL; up to 120 fmol/mg were covalently bound to albumin (Sepai *et al*., 1995a).

4.1.2 *Experimental data*

Following topical administration of [^{14}C]4,4′-methylenediphenyl diisocyanate in acetone to female Wistar rats, 20% of the administered dose was eliminated in the faeces within 24 h, while less than 1% appeared in the urine (Vock & Lutz, 1997).

After inhalation exposure of female Wistar rats to 4,4′-methylene diisocyanate aerosols (0.26, 0.70 and 2.06 mg/m^3 chronically for three and 12 months), 4,4′-methylenedianiline and *N*-acetyl-4,4′-methylenedianiline were the major urinary metabolites. Haemoglobin adducts of these metabolites were also detected. The dose–response relationships for haemoglobin adducts and urinary metabolites were non-linear over this dose range. The amounts of 4,4′-methylenedianiline and, to a lesser extent, *N*-acetyl-4,4′-methylenedianiline found in urine correlated well with the corresponding amount determined as haemoglobin adducts for all dose groups. Similar results were obtained with rats exposed for three and 12 months, indicating that a steady state had been reached by three months. Haemoglobin adducts from rats after a one-week recovery period decreased by approximately 40% for all dosed groups, suggesting that erythrocytes containing modified haemoglobin have a shorter lifespan (Sepai *et al.*, 1995b).

4.2 Toxic effects

4.2.1 *Humans*

4,4′-Methylenediphenyl diisocyanate is irritating to the skin, eyes and respiratory tract and induces asthma in humans (IARC, 1979).

Isocyanate-induced asthma and hypersensitivity pneumonitis in humans have been reviewed (Baur, 1995; Bernstein, 1996). A case of fatal asthma of a 4,4′-methylenediphenyl diisocyanate-sensitized subject has been described (Carino *et al*., 1997). Exposure to 4,4′-methylenediphenyl diisocyanate is a frequent cause of occupational asthma (Liss *et al*., 1988; Vogelmeier *et al*., 1991; Bernstein *et al*., 1993) but may also induce hypersensitivity pneumonitis (Malo & Zeiss, 1982; Vandenplas *et al*., 1993) and inflammatory upper respiratory tract diseases (Liss *et al*., 1988; Littorin *et al*., 1994). Most patients with 4,4′-methylenediphenyl diisocyanate-induced asthma have elevated levels of IgG-class antibodies towards 4,4′-methylenediphenyl diisocyanate–albumin conjugates in the plasma, while IgE-class antibodies are rare (Liss *et al*., 1988).

4.2.2 *Experimental systems*

Intradermal injection and topical cutaneous administration of 4,4′-methylenediphenyl diisocyanate induced IgG1 antibodies towards 4,4′-methylenediphenyl diisocyanate and an acute pulmonary response towards inhalation challenge to a non-irritating concentration of 4,4′-methylenediphenyl diisocyanate in guinea-pigs, while no such reaction was observed after inhalation treatment (approximately 21.5 mg/m^3 for 3 h per day on five consecutive days) (Rattray *et al*., 1994). However, when guinea-pigs were exposed to a high concentration of 4,4′-methylenediphenyl diisocyanate (135 or 360 mg/m^3) once for 15 min, an immediate-onset respiratory sensitivity response was observed after challenge to a low (3.4 mg/m^3) concentration of 4,4′-methylenediphenyl diisocyanate; after intradermal sensitization, such a reaction was observed only upon challenge with an irritating concentration (60 mg/m^3) of 4,4′-methylenediphenyl diisocyanate (Pauluhn & Mohr, 1994). C57BL/6 mice injected intradermally with 4,4′-methylenediphenyl diisocyanate responded to local 4,4′-methylenediphenyl diisocyanate administration by ear swelling; this reaction could be transferred by transfusion of syngeneic mouse lymph node-derived T cells (Tanaka *et al*., 1987). Total IgE concentration in blood was elevated in a dose-dependent manner in BALB/c mice after dermal instillation of 4,4′-methylenediphenyl diisocyanate; the elevation became significant when the dose reached 1.35 mg (Potter & Wederbrand, 1995).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

When female Wistar rats were exposed by inhalation to 4,4′-methylenediphenyl diisocyanate (nominal concentrations of 1, 3 or 9 mg/m^3, 6 h per day) on days 6 through 15 of gestation, a slight increase of asymmetric sternebrae appeared at the highest dose but no adverse effect was observed on maternal weight gain, number of corpora lutea, implantation sites, pre- and postimplantation loss, fetal or placental weight, gross and visceral anomalies or degree of ossification (Buschmann *et al*., 1996).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

In one study, 4,4′-methylenediphenyl diisocyanate induced mutations in *Salmonella typhimurium* strain TA100 in the presence of exogenous metabolic activation but not in its absence. It induced a weak mutagenic response in *S. typhimurium* strain TA98 with exogenous metabolic activation but was not mutagenic in strains TA1535, TA1537, TA1538 or in *Escherichia coli* WP2 *uvrA*.

A second study reported that technical-grade 4,4′-methylenediphenyl diisocyanate (containing 25% 4,4′-methylenediphenyl triisocyanate and 30% unspecified higher molecular weight compounds) increased the frequency of sister chromatid exchanges and chromosomal aberrations in human lymphocytes in the presence or absence of exogenous metabolic activation.

Results from an inhalation study showed that a low level (5–10 adducts/10^9 nucleotides) of arylamine-derived DNA adducts was formed in the olfactory epithelium of female Wistar rats exposed to an average atmospheric concentration of 0.7–2.0 mg/m^3 4,4′-methylenediphenyl diisocyanate for 17 h per day on five days per week for one year. Adducts were not detected in DNA from lung, liver, bladder, kidney, respiratory epithelium or peripheral blood lymphocytes of exposed animals (Vock *et al*., 1996).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

4,4′-Methylenediphenyl diisocyanate is used for the production of polyurethane coatings and elastomers.

5.2 Human carcinogenicity data

The risk of cancer associated with occupational exposure to isocyanates has been examined in three industrial cohort studies and in a population-based case–control study of several types of cancer. No strong association or consistent pattern has emerged.

5.3 Animal carcinogenicity data

Polymeric 4,4′-methylenediphenyl diisocyanate containing 44.8–50.2% monomeric 4,4′-methylenediphenyl diisocyanate was tested for carcinogenicity by inhalation in rats. An increased incidence of lung tumours was observed.

5.4 Other relevant data

The major urinary metabolites of 4,4′-methylenediphenyl diisocyanate are 4,4′-methylenedianiline and *N*-acetyl-4,4′-methylenedianiline, both of which also form haemoglobin

Table 1. Genetic and related effects of 4,4′-methylenediphenyl diisocyanate

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	370	Shimizu *et al*. (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1850	Shimizu *et al*. (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1850	Shimizu *et al*. (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1850	Shimizu *et al*. (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	(+)	185	Shimizu *et al*. (1985)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	–	–	1850	Shimizu *et al*. (1985)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*[c]	(+)	(+)	2600	Mäki-Paakkanen & Norppa (1987)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*[c]	+	(+)	650	Mäki-Paakkanen & Norppa (1987)

[a] +, positive; (+), weakly positive;–, negative
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL
[c] Technical-grade product was tested

adducts in exposed workers and rats. 4,4′-Methylenediphenyl diisocyanate is an irritant and a sensitizer; exposure by inhalation produces asthma among workers.

4,4′-Methylenediphenyl diisocyanate forms low-level DNA adducts *in vivo* and induces mutations in bacteria and chromosomal aberrations and sister chromatid exchanges in human lymphocyte cultures.

5.5 Evaluation

There is *inadequate evidence* for the carcinogenicity of 4,4′-methylenediphenyl diisocyanate or polymeric 4,4′-methylenediphenyl diisocyanate in humans.

There is *limited evidence* in experimental animals for the carcinogenicity of a mixture containing monomeric and polymeric 4,4′-methylenediphenyl diisocyanate.

Overall evaluation

4,4′-Methylenediphenyl diisocyanate (industrial preparation) is *not classifiable as to its carcinogenicity to humans (Group 3).*

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METHYL METHANESULFONATE

Data were last reviewed in IARC (1974) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

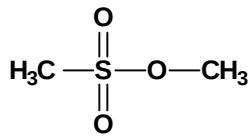
1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 66-27-3

Chem. Abstr. Name: Methanesulfonic acid, methyl ester

Synonym: MMS

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2H_6O_3S$ Relative molecular mass: 110.13

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid (Budavari, 1996)

(*b*) *Boiling-point*: 202.5°C (Lide, 1997)

(*c*) *Melting-point*: 20°C (Lide, 1997)

(*d*) *Solubility*: Soluble in water (1 part in 5 at 25°C), dimethylformamide and propylene glycol; slightly soluble in non-polar solvents (Budavari, 1996)

(*e*) *Conversion factor*: $mg/m^3 = 4.5 \times ppm$

1.2 Production and use

No indication was found that methyl methanesulfonate is produced commercially, although it has been produced for research purposes. Information available in 1995 indicated that it was produced in one country (India) (Chemical Information Services, 1995).

It is believed to be used currently only for research purposes.

1.3 Occurrence

No data were available to the Working Group.

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not proposed any occupational exposure limit for methyl methanesulfonate in work-place air.

No international guideline for methyl methanesulfonate in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Methyl methanesulfonate was tested for carcinogenicity in rats by subcutaneous and intraperitoneal injection, producing local tumours and tumours of the nervous system. Following oral administration to mice, it increased the incidence of lung tumours and of lymphomas. In rats, it produced neurogenic tumours after administration of a single dose as well as following prenatal exposure (IARC, 1974).

3.1 Inhalation exposure

Rat: Male Sprague-Dawley rats, 9–10 weeks of age, were exposed by whole-body inhalation to 50 ppm [225 mg/m^3] methyl methanesulfonate (purity, > 95%) for 6 h per day on five days per week for six weeks and were observed for life. Nasal tumours were found in 47/80 animals versus 0/98 air controls. The median life spans for the control and exposed groups were 613 days and 495 days, respectively. The median time of nasal tumour appearance was 513 days (range, 256–775) (Snyder *et al*., 1986; Sellakumar *et al*., 1987).

3.2 Oral or intraperitoneal administration

Mouse: Groups of 16 male and 16 female A/J strain mice, six to eight weeks of age, were dosed either orally or intraperitoneally with methyl methanesulfonate in tricaprylin three times per week for eight weeks and were then observed for an additional 16 weeks. The total cumulative doses were: gavage, 0 and 300 mg/kg bw; intraperitoneal injection, 0, 60, 150 and 300 mg/kg bw. Survival was similar in all groups. At the end of the experiment, in all groups, the numbers of animals with superficial lung adenomas and numbers of lung adenomas per animal were within the range of those observed in a variety of control groups (Stoner *et al*., 1986).

3.3 Multistage models

3.3.1 *Mouse*

Groups of 20 female NMRI mice, seven weeks of age, received a single skin application of either (*a*) 100 nmol 7,12-dimethylbenz[*a*]anthracene (DMBA), (*b*) 100 µmol methyl methanesulfonate or (*c*) 400 µmol methyl methanesulfonate (highest tolerated dose). One week later, all were treated with 10 nmol 12-*O*-tetradecanoylphorbol 13-acetate twice weekly for 24 weeks. While 90% of the DMBA group had skin tumours after 15 weeks, no methyl methanesulfonate-initiated mice had skin tumours after 24 weeks (Fürstenberger *et al*., 1989).

Groups of 20 female NMRI mice were treated with DMBA as above and subsequently treated with (*a*) acetone (the vehicle used for all substances in the experiment) 6 h before 10 nmol 12-*O*-retinoylphorbol 13-acetate, (*b*) 100 µmol methyl methanesulfonate 6 h before acetone, (*c*) 100 µmol methyl methanesulfonate 6 h before 10 nmol 12-*O*-retinoylphorbol 13-acetate, (*d*) 10 µmol methyl methanesulfonate 6 h before 10 nmol 12-*O*-retinoylphorbol 13-acetate or (*e*) 10 nmol 12-*O*-tetradecanoylphorbol 13-acetate 6 h before acetone. Two weeks after DMBA treatment, all groups received 10 nmol 12-*O*-retinoylphorbol 13-acetate once a week for 23 weeks. At the end of the experiment, > 90% of the mice were alive. The numbers of papillomas per survivor at 24 weeks [figures read from a graph] were (*a*) 0.4, (*b*) 1.6, (*c*) 1.7, (*d*) 2.9 and (*e*) 3.0 (Fürstenberger *et al*., 1989).

Following a single intraperitoneal injection of 120 mg/kg bw to an unspecified number of four-week-old AKR mice, all of the methyl methanesulfonate-treated mice had developed thymomas after 50 weeks versus 50% tumour incidence in the controls (Warren *et al*., 1990). [The Working Group noted the inadequate reporting.]

3.3.2 *Rat*

Groups of female Fischer 344 rats, six to eight weeks old, were either left untreated (group A) or received a single intravesicular instillation of 0.3 mg *N*-methyl-*N*-nitrosourea (group B), six intravesicular instillations of 2.5 mg methyl methanesulfonate at 14-day intervals (group C) or sequential treaments with 0.3 mg *N*-methyl-*N*-nitrosourea followed by six intravesicular instillations of 2.5 mg methyl methanesulfonate at 14-day intervals (group D). The numbers of rats with bladder tumours were (A) 0/25, (B) 7/29 (24%), (C) 2/27 (7%) and (D) 19/33 (58%) (Tudor *et al*., 1984).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Methyl methanesulfonate is rapidly distributed throughout the body of mice and rats, including the central nervous system, and rapidly crosses the placenta. After intravenous injection of 100 mg/kg bw methyl methanesulfonate to rats, none was detected in serum after 2 h (IARC, 1974).

In rats injected with [*methyl*-^{14}C]methyl methanesulfonate, about 30% of the label was exhaled as CO_2 within 30 h and 20% was found in the urine. The corresponding values for mice given an intraperitoneal dose were 27% and 34%, respectively (IARC, 1974).

Urinary metabolites recovered within the first 16 h and representing 80% of the excretion products resulted from an initial methylation of cysteine residues by methyl methanesulfonate. These were methylmercapturic acid sulfoxide, 2-hydroxy-3-methylsulfinylpropionic acid, methylsulfinylacetic acid, methylmercapturic acid and *N*-(methylthioacetyl)glycine. Glutathione conjugation has been shown to occur in rat liver (IARC, 1974).

4.2 Toxic effects

4.2.1 *Humans*

Therapeutic application to cancer patients of total doses ranging from 2.8 to 800 mg/kg bw over a period of up to 350 days led to significant gastrointestinal and hepatic toxic effects (IARC, 1974).

4.2.2 *Experimental systems*

Methyl methanesulfonate (250 μM) induced neurite formation in 71% of mouse neuroblastoma N-18 cells, when cell growth was inhibited by 83% (Yoda *et al*., 1982).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Groups of adult female (C3H/R1 × 101/R1)F_1 mice received a single intraperitoneal injection of 75 mg/kg bw methyl methanesulfonate within four days before mating or at 1, 6, 9 or 25 h after mating with untreated males. Control groups were treated with vehicle only (1 mL water) four days before mating or 6 or 25 h after mating. Control and treated females were killed and their uterine contents examined 17–18 days after mating. Resorptions were significantly increased ($p < 0.01$) following treatment at 1, 6, 9 or 25 h after mating (21.2%, 25.2%, 28.2% and 22.7%, respectively) in comparison with before mating and 6 h and 25 h after mating control group frequencies of 4.8%, 3.6% and 4.6%, respectively. Treatment before mating had no effect. Mid-gestational deaths were unaffected at any time, while late deaths were significantly increased only at 1 h (3.4%) in comparison with the 6 h control frequency of 0.7%. The incidences of live fetuses with malformations were (numbers of fetuses examined in parentheses): before-mating

control, 1.0% (298); treated, 0.0% (292); 1 h treated, 4.4% (411); 6 h after mating control, 0.8% (392); 6 h treated, 3.8% (448); 9 h treated, 3.6% (249); 25 h after mating control, 0.6% (350); 25 h treated, 2.6% (546). By comparison with other alkylating agents with similar DNA-binding properties but different effects upon exposed zygotes, there appeared to be no site-specific alkylation product identifiable as the critical target (Generoso *et al*., 1991).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Methyl methanesulfonate is a direct-acting alkylating agent that was active in all of the standard short-term tests for genetic and related effects *in vivo* and *in vitro*. It induced SOS response in the *umu* test using *Salmonella typhimurium* strain TA1535/pSK1002 and it induced mutations in strain TA100. In *Drosophila melanogaster*, somatic and sex-linked recessive lethal mutations were induced following exposure of adults or larvae to methyl methanesulfonate in their feed.

DNA damage was induced in rabbit alveolar macrophages *in vitro* and Clara cell cultures incubated with methyl methanesulfonate. DNA single-strand breaks and unscheduled DNA synthesis were induced in rat primary hepatocytes. Unscheduled DNA synthesis was also induced in rat tracheal epithelium, in Syrian hamster and mouse primary hepatocytes and in mouse epidermal keratinocytes *in vitro*. Methyl methanesulfonate induced gene mutations at the *hprt* locus in Chinese hamster ovary cells and lung V79 fibroblasts. One study reported gene mutation in V79 cells transfected with a retroviral vector carrying the *tag* gene of *Escherichia coli.* This gene encodes for 3-methyladenine DNA glycosylase I, which excises 3-alkyl-adenine. The results showed that the majority of the mutations induced by methyl methanesulfonate were GC→AT transitions. Gene mutations were induced at the *tk* locus in mouse lymphoma L5178Y cells, and ouabain-resistant mutants were induced in mouse C3H 10T½ and L5178Y cells *in vitro*. Methyl methanesulfonate increased the frequency of sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary and mouse lymphoma cells. It also induced micronuclei in mouse lymphoma cells in a single study. It induced morphological cell transformation in virally enhanced Syrian hamster ovary cell cultures but not in the same cell line without viral enhancement.

Methyl methanesulfonate induced DNA single-strand breaks and alkali-labile sites in human lymphocytes *in vitro*. It induced unscheduled DNA synthesis in human epidermal keratinocytes and in oral epithelial and fibroblast cell cultures. Methyl methanesulfonate induced gene mutations in human lymphoblasts at the *hprt* locus and sister chromatid exchanges and micronuclei in HepG2 human liver cells *in vitro*.

Methyl methanesulfonate induced DNA strand breaks in mouse kidney and spermatozoa and DNA fragmentation in rat brain cells following in-vivo treatment.

Table 1. Genetic and related effects of methyl methanesulfonate

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage, *umu* induction/SOS response/strand-breaks or cross-links	+	NT	27	Nakamura *et al*. (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	280	McCann *et al*. (1975)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	500	Bruce & Heddle (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	5	De Flora (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	15	Eder *et al*. (1989)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	100	Koch *et al*. (1994)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	NT	280	McCann *et al*. (1975)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	5	De Flora (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	330	Eder *et al*. (1989)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	150	De Flora (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	280	McCann *et al*. (1975)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	150	De Flora (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	280	McCann *et al*. (1975)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	150	De Flora (1981)
DMM, *Drosophila melanogaster*, somatic mutation	+		10 ppm feed	Mitchell *et al*. (1981)
DMM, *Drosophila melanogaster*, somatic mutation	+		275 µg/mL sol	Vogel & Zijlstra (1987)
DMM, *Drosophila melanogaster*, somatic mutation	+		275 µg/mL sol	Vogel (1989)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	?[c]		550ppm feed	Vogel & Zijlstra (1987)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		22 µg/mL sol	Vogel & Nivard (1997)
DIA, DNA single-strand breaks, rat hepatocytes *in vitro*	+	NT	33	Sina *et al*. (1983)
DIA, DNA damage, rabbit (macrophage, Clara and type II) lung cells *in vitro*	+	NT	5	Becher *et al*. (1993)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	NT	11	Kornbrust & Barfknecht (1984)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
UIA, Unscheduled DNA synthesis, male B6C3F$_1$ mouse hepatocytes *in vitro*	+	NT	80	McQueen *et al*. (1983)
UIA, Unscheduled DNA synthesis, male Syrian hamster hepatocytes *in vitro*	+	NT	80	McQueen *et al*. (1983)
UIA, Unscheduled DNA synthesis, Syrian hamster hepatocytes *in vitro*	+	NT	11	Kornbrust & Barfknecht (1984)
UIA, Unscheduled DNA synthesis, rat tracheal epithelium cells *in vitro*	+	NT	11	Doolittle & Butterworth (1984)
UIA, Unscheduled DNA synthesis, mouse epidermal keratinocytes *in vitro*	+	NT	0.1	Sawyer *et al*. (1988)
GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus *in vitro*	+	NT	5	Couch *et al*. (1978)
GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus *in vitro*	+	NT	11	Lee *et al*. (1986)
GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus *in vitro*	+	NT	6	Moore *et al*. (1989)
GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus *in vitro*	+	NT	5	Oberly *et al*. (1990)
GCO, Gene mutation, Chinese hamster ovary cells, *hprt* locus *in vitro*	+	NT	5	Moore *et al*. (1991)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	(+)	NT	50	Nishi *et al*. (1984)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	+	NT	175	Slamenova *et al*. (1990)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	7.5	Clive *et al*. (1979)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	5	Oberly *et al*. (1984)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	4	Moore *et al*. (1989)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	13.2	Cole *et al*. (1990)
G51, Gene mutation, mouse lymphoma L5178Y cells, *hprt* locus *in vitro*	NT	+	7.5	Clive *et al*. (1979)
G51, Gene mutation, mouse lymphoma L5178Y cells, ouabain resistance *in vitro*	+	NT	13.2	Cole *et al*. (1990)
GIA, Gene mutation, mouse lymphoma LC98.16 cells, *tk* locus *in vitro*	+	NT	7.5	Blazak *et al*. (1986)
GIA, Gene mutation, C3H10T½ mouse cells, ouabain resistance *in vitro*	(+)	NT	120	Smith *et al*. (1988)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
GIA, Gene mutation, Chinese hamster AS52/hprt cells, *hprt* locus *in vitro*	+	NT	20	Oberly *et al*. (1993)
GIA, Gene mutation, Chinese hamster fibroblasts, *hprt* locus *in vitro*	+[d]	NT	180	Klungland *et al*. (1995)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	NT	7.4	Natarajan *et al*. (1983)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	NT	5	Nishi *et al*. (1984)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	NT	5.5	Lee *et al*. (1986)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	NT	17.6	Darroudi *et al*. (1989)
SIM, Sister chromatid exchange, mouse fetal liver erythroblasts *in vitro*	+	NT	5	Cole *et al*. (1983)
SIT, Sister chromatid exchange, transformed cells (CHO 43-3B) *in vitro*	+	NT	17.6	Darroudi *et al*. (1989)
MIA, Micronucleus test, mouse lymphoma L5178Y cells *in vitro*	+	NT	13.2	Cole *et al*. (1990)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	NT	22	Natarajan *et al*. (1983)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	NT	11	Lee *et al*. (1986)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	NT	44	Darroudi *et al*. (1989)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	NT	33	Lin *et al*. (1989)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	NT	6	Moore *et al*. (1989)
CIM, Chromosomal aberrations, mouse lymphoma L5178Y cells *in vitro*	+	NT	7.5	Blazak *et al*. (1986)
CIM, Chromosomal aberrations, mouse lymphoma L5178Y cells *in vitro*	+	NT	4	Moore *et al*. (1989)
CIT, Chromosomal aberrations, transformed cells (CHO 43-3B) *in vitro*	+	NT	17.6	Darroudi *et al*. (1989)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	–	NT	330	Dusinska & Slamenova (1994)
T7S, Cell transformation, SA7/Syrian hamster embryo cells *in vitro*	+	NT	50	Casto *et al*. (1979)
DIH, DNA single-strand breaks/alkaline-labile sites, human lymphocytes *in vitro*	+	NT	5.5	Munzer *et al*. (1988)
UIH, Unscheduled DNA synthesis, human oral epithelium and fibroblasts *in vitro*	+	NT	11	Ide *et al*. (1982)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
UIH, Unscheduled DNA synthesis, human epidermal keratinocytes *in vitro*	+	NT	1.1	Lawrence & Benford (1993)
GIH, Gene mutation, GM6804 human lymphoblasts, *hprt* locus *in vitro*	+	NT	5	Aubrecht *et al*. (1995)
SIH, Sister chromatid exchange, human HepG2 liver cells *in vitro*	+	NT	44	Natarajan & Darroudi (1991)
MIH, Micronucleus test, human HepG2 liver cells *in vitro*	+	NT	88	Natarajan & Darroudi (1991)
HMM, Host-mediated assay, *Escherichia coli* K-12 in NMRI mice *in vivo*	+		83 po × 1	Hellmer & Bolcsfoldi (1992)
DVA, DNA single-strand breaks, NMRI mouse kidney *in vivo*	+		33 ip × 1	Solveig Walles & Erixon (1984)
DVA, DNA strand breaks, mouse spermatozoa *in vivo*	+		10 ip × 1	Sega *et al*. (1986)
DVA, DNA fragmentation, Sprague-Dawley rat brain *in vivo*	+		27.5 iv × 1	Robbiano & Brambilla (1987)
UVM, Unscheduled DNA synthesis, ICR mouse skin epithelial cells *in vivo*	+		6 sc × 1	Ishikawa *et al*. (1982)
UVR, Unscheduled DNA synthesis, Fischer 344 rat kidney cells *in vivo*	+[e]		100 ip × 1	Tyson & Mirsalis (1985)
UVR, Unscheduled DNA synthesis, Fischer 344 rat spermatocytes *in vivo*	+		10 ip × 1	Bentley & Working (1988)
GVA, Gene mutation, Fischer 344 rat fibroblasts, *hprt* locus *in vivo*	–		100 ip × 1	Khan & Heddle (1991)
GVA, Gene mutation (*lac*I), male Big Blue™ mouse liver cells, *in vivo*	–		20 ip × 21	Mirsalis *et al*. (1993)
GVA, Gene mutation (*lac*I or *Dbl*-1), male transgenic C57BL/6 mouse intestinal epithelium *in vivo*	+[f]		100 ip/wk × 10	Tao *et al*. (1993)
GVA, Gene mutation (*lacz*), Muta™ Mouse germ cells *in vivo*	–		40 ip × 1	Brooks & Dean (1997)
GVA, Gene mutation (*lac*I), Big Blue™ mouse germ cells *in vivo*	–		40 ip × 1	Gorelick *et al*. (1997)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
GVA, Gene mutation (*lacz*), Muta™ Mouse germ cells *in vivo*	–		80 ip × 1	Itoh *et al*. (1997)
GVA, Gene mutation (*lacz*), male Muta™ Mouse, germ and bone-marrow cells *in vivo*	–		40 ip × 1	Renault *et al*. (1997)
GVA, Gene mutation (*lacz*), male Muta™ Mouse germ cells *in vivo*	–		40 ip × 1	Suzuki *et al*. (1997)
GVA, Gene mutation (*lacz*), male Muta™ Mouse germ cells *in vivo*	–		100 ip × 1	Tinwell *et al*. (1997)
SLP, Mouse specific locus, postspermatogonia *in vivo*	(+)		7 ip × 1	Ehling (1978)
SLO, Mouse specific locus, other stages *in vivo*	–		5.25 ip × 1	Ehling (1978)
SVA, Sister chromatid exchange, fetal Porton albino mouse liver erythroblasts *in vivo*	+		30 ip × 1	Cole *et al*. (1983)
MVM, Micronucleus test, mouse erythroblasts *in vivo*	+		30 ip × 1	Jenssen & Ramel (1976)
MVM, Micronucleus test, (C57BL/6 × C3H/He)F_1 mice *in vivo*	+		500 ip × 5	Bruce & Heddle (1979)
MVM, Micronucleus test, fetal mouse liver erythroblasts *in vivo*	+		30 ip × 1	Cole *et al*. (1982)
MVM, Micronucleus test, fetal Porton albino mouse liver erythroblasts *in vivo*	+		30 ip × 1	Cole *et al*. (1983)
MVM, Micronucleus test, MS/Ae mouse erythrocytes *in vivo*	+		25 ip × 2	Aeschbacher (1986)
MVM, Micronucleus test, ddY mouse erythrocytes *in vivo*	+		46 inh 21 min × 1	Odagiri *et al*. (1986)
MVM, Micronucleus test, MS/Ae and CD-1 mouse erythrocytes *in vivo*	+		40 ip × 1	Tsuyoshi *et al*. (1989)
MVM, Micronucleus test, MS/Ae and CD-1 mouse erythrocytes *in vivo*	+		40 po × 1	Tsuyoshi *et al*. (1989)
MVM, Micronucleus test, female C57BL/6, DBA2 and BALB/c mouse erythrocytes *in vivo*	+		25 ip × 1	Sato *et al*. (1990)
MVM, Micronucleus test, NMRI mice (during skin carcinogenesis) *in vivo*	+		450 skin × 1	Haesen *et al*. (1993)
MVM, Micronucleus test, Big Blue™ mouse peripheral blood *in vivo*	+		40 ip × 1	Gorelick *et al*. (1997)
MVM, Micronucleus test, Muta™ Mouse reticulocytes *in vivo*	+		40 ip × 1	Suzuki *et al*. (1997)
MVR, Micronucleus test, Wistar rat hepatocytes *in vivo*	–		80 ip × 1	Tates *et al*. (1986)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVR, Micronucleus test, Wistar rat hepatocytes *in vivo*	+[g]		10 ip × 1	Tates & den Engelse (1989)
MVR, Micronucleus test, Fischer rat lung cells *in vivo*	+		50 ip × 1	Khan & Heddle (1991)
CBA, Chromosomal aberrations, female C57BL mouse bone marrow *in vivo*	+		120 ip × 1	Frei & Venitt (1975)
CVA, Chromosomal aberrations, female NMRI mouse epidermal cells *in vivo*	+		440 skin × 1	Furstenberger *et al*. (1989)
CCC, Chromosomal aberrations, mouse spermatocytes *in vivo*	+		30 ip × 1	Moutschen (1969)
CGC, Chromosomal aberrations, mouse spermatogonia treated *in vivo*, spermatocytes observed	–		50 ip × 1	Leonard & Linden (1972)
COE, Chromosomal aberrations, CD1/CR mouse oocytes or embryos *in vivo*	+		50 iv × 1	Brewen *et al*. (1975)
COE, Chromosomal aberrations, NMRI mouse oocytes or embryos *in vivo*	+		25 ip × 1	Braun *et al*. (1986)
DLM, Dominant lethal test, male mice	+		50 ip × 1	Partington & Bateman (1964)
DLM, Dominant lethal test, male mice	+		50 ip × 1	Ehling *et al*. (1968)
DLM, Dominant lethal test, mice	+		30 ip × 1	Moutschen (1969)
DLM, Dominant lethal test, mice	+		50 ip × 1	Beliles *et al*. (1973)
DLM, Dominant lethal test, male albino mice	+		12.5 ip × 1	Arnold *et al*. (1976)
DLM, Dominant lethal test, male CD1 mice	+		20 ip × 1	Dean & Johnstone (1977)
DLM, Dominant lethal test, male (101 × C3H)F_1 mice	+		10 ip × 1	Ehling (1977)
DLM, Dominant lethal test, male NMRI mice	+		40 ip × 1	Lang & Adler (1977)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MHT, Mouse heritable translocation test	+		40 ip × 1	Lang & Adler (1977)
MHT, Mouse heritable translocation test	+		20 ip × 1	Adler (1980)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; sol, solution; po, oral; ip, intraperitoneal; sc, subcutaneous; iv, intravenous; wk, week; inh, inhalation

[c] Significant increase only with treatments of female larvae at the highest dose

[d] Mutation frequency lower in cells transfected with *E. coli tag*-expressing vector (*tag* gene encodes 3-methyladenine DNA glycosylase I activity)

[e] Negative for gavage treatment

[f] *Dbl-1* more sensitive than *lac*I; negative for both loci following acute exposure

[g] Rats received partial hepatectomy 17 h before treatment; bone marrow analysed 24 h after treatment. A weak positive response was also seen in hepatocytes two to three days after treatment.

Unscheduled DNA synthesis was induced in mouse skin epithelium after a single subcutaneous injection of methyl methanesulfonate and in rat kidney cells and spermatocytes after a single intraperitoneal injection of this compound.

Methyl methanesulfonate did not induce mutations at the *hprt* locus in Fischer 344 rat fibroblasts *in vivo*. In a single study, it induced *lac*I and *Dbl*-1 mutations in intestinal epithelium of transgenic mice given 10 weekly injections but did not induce *lac*Z or *lac*I mutations in germ cells of transgenic mice from acute exposure studies. It increased the frequencies of micronuclei in mouse peripheral blood, skin keratinocytes and fetal liver erythrocytes and in rat hepatocytes and lung fibroblasts *in vivo*. It also induced sister chromatid exchanges in fetal mouse liver and chromosomal aberrations in mouse bone marrow and skin epidermal cells after a single intraperitoneal injection. Methyl methanesulfonate was not mutagenic to mouse germ cells: it induced specific locus mutations in only postspermatogonial stages, heritable translocations and chromosomal aberrations in spermatocytes and embryonic cells, and mouse dominant lethal mutations.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Methyl methanesulfonate is a laboratory chemical that has been produced for research purposes. No information was available to the Working Group on potential human exposures.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Methyl methanesulfonate was tested in rats by inhalation exposure and by subcutaneous and intraperitoneal administration, producing nasal tumours, tumours of the nervous system and tumours at the injection site. In rats, it was carcinogenic after administration of a single dose as well as following prenatal exposure. Following instillation into the bladder of rats, it potentiated the effect of *N*-methyl-*N*-nitrosourea. In one study, following oral administration in mice, it increased the incidence of lung tumours and of lymphomas. A subsequent experiment with oral and intraperitoneal administration to mice failed to increase the incidence of lung adenomas in A/J mice. In a multistage mouse skin model, it was not an initiator but was found to be a stage I tumour promoter. It accelerated the occurrence of thymic lymphomas in AKR mice.

5.4 Other relevant data

Methyl methanesulfonate caused an increased frequency of resorptions and congenital malformations after treatment of females 1–25 h after mating.

Methyl methanesulfonate induced mouse germ cell mutations and chromosomal aberrations, and DNA damage, micronuclei, sister chromatid exchanges and chromosomal aberrations in somatic cells of rodents *in vivo*. It increased the frequency of DNA damage, gene mutation, sister chromatid exchanges and micronuclei in human and rodent cell cultures, as well as chromosomal aberrations in rodent cells *in vitro*. Methyl methanesulfonate induced somatic and sex-linked mutations in *Drosophila.* It induced DNA damage in *Escherichia coli* and was mutagenic in bacteria.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of methyl methanesulfonate were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of methyl methanesulfonate.

Overall evaluation

Methyl methanesulfonate is *probably carcinogenic to humans (Group 2A)*.

In making the overall evaluation, the Working Group took into consideration that methyl methanesulfonate is a direct-acting methylating agent which is mutagenic in a wide range of in-vivo and in-vitro test systems.

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2-NITROPROPANE

Data were last reviewed in IARC (1982) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

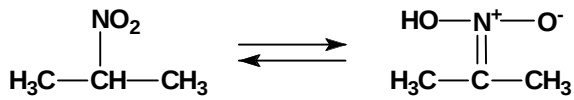
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 79-46-9
Chem. Abstr. Name: 2-Nitropropane
IUPAC Systematic Name: 2-Nitropropane
Synonyms: Dimethylnitromethane; isonitropropane

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_3H_7NO_2$ Relative molecular mass: 89.09

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid (Lewis, 1993)
(*b*) *Boiling-point*: 120.2°C (Lide, 1997)
(*c*) *Melting-point*: –91.3°C (Lide, 1997)
(*d*) *Solubility*: Slightly soluble in water (1.7 mL/100 mL); miscible with many organic solvents (Budavari, 1996)
(*e*) *Vapour pressure*: 1.7 kPa at 20°C; relative vapour density (air = 1), 3.06 (WHO, 1992; Lewis, 1993)
(*f*) *Flash point*: 38°C, open cup (WHO, 1992)
(*g*) *Explosive limits*: Lower flammability limit, 2.6% by volume in air (WHO, 1992)
(*h*) *Conversion factor*: $mg/m^3 = 3.65 \times ppm$

1.2 Production and use

In 1977, production of 2-nitropropane in the United States was estimated to be 13 600 tonnes. 2-Nitropropane is reportedly produced by two companies in the United States and one company in France (WHO, 1992).

A major use of 2-nitropropane is as an industrial solvent. It is used in vinyl inks, generally at low concentrations, for printing, flexography and photogravure, and in adhesives and electrostatic paints (IARC, 1982; Markofsky, 1991). 2-Nitropropane, often mixed with alcohols, dissolves a large number of resins such as epoxy, polyurethane, polyester, vinyl, urea–formaldehyde and phenolic. These solvent–resin mixtures are used for coatings, such as for beverage cans (Markofsky, 1981; WHO, 1992). Minor uses of 2-nitropropane are as a solvent for chemical reactions, a processing solvent to separate natural products, an intermediate for the manufacture of several propane derivatives, a component of explosives and propellants, and in fuels for internal combustion engines (WHO, 1992).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), as many as 10 000 workers in the United States were potentially exposed to 2-nitropropane (see General Remarks). Occupational exposures may occur in its production and use as a solvent.

1.3.2 *Environmental occurrence*

2-Nitropropane may enter the atmosphere during its manufacture and use as a solvent. A major contributor to these releases is evaporation of 2-nitropropane used as a solvent in printing ink and surface coatings (United States National Library of Medicine, 1997a; WHO, 1992). According to the United States Environmental Protection Agency Toxic Chemical Release Inventory, industrial releases of 2-nitropropane from manufacturing and processing facilities in the United States were approximately 211 000 kg in 1987 and 14 000 kg in 1995 to the atmosphere; 1860 kg in 1987 and 1360 kg in 1995 for water release; and 76 000 kg for 1987 and none for 1995 for underground release (United States National Library of Medicine, 1997b). There appear to be no reports of occurrence of 2-nitropropane in outdoor air or water away from areas of manufacture and use (WHO, 1992).

2-Nitropropane has been detected in cigarette smoke (IARC, 1986).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 36 mg/m^3 as the 8-h time-weighted average threshold limit value for occupational exposures to 2-nitropropane in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for 2-nitropropane in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

The only epidemiological study available was published as an abstract and was considered inadequate for evaluation (IARC, 1982).

3. Studies of Cancer in Experimental Animals

2-Nitropropane was tested for carcinogenicity in two experiments in rats by inhalation exposure. Hepatocellular carcinomas were produced in one experiment and an increased incidence of hepatocellular nodules in the other. An inhalation study in rabbits was considered to be inadequate for evaluation (IARC, 1982).

3.1 Oral administration

Rat: 2-Nitropropane (redistilled) was administered by gavage to weanling male Sprague-Dawley rats at a dose of 1 mmol/kg bw three times per week for 16 weeks. All surviving rats were killed at week 77. Benign liver tumours appeared in 4/22 treated animals versus 1/29 controls and malignant liver tumours occurred in 22/22 treated rats versus 0/29 controls ($p < 0.001$) (Fiala *et al*., 1987a).

3.2 Two-stage initiation/promotion models

Rat: Male and female Sprague-Dawley rats, four to six days of age, were exposed by whole-body inhalation to concentrations of 0, 25, 40, 50, 80 and 125 ppm [0, 91, 146, 182, 292 and 456 mg/m^3] 2-nitropropane (99% pure) for 6 h per day on five days per week for three weeks. One week later, a polychlorinated biphenyl (Clophen A50) was administered orally at a dose of 10 mg/kg bw twice per week for eight weeks. Thirteen weeks after the start of the experiment, the number of preneoplastic adenosine-5-triphosphatase-deficient foci in the liver was found to increase linearly with the exposure concentration, demonstrating the initiating activity of 2-nitropropane (Denk *et al*., 1990).

Male Wistar rats, three to four weeks of age, received six intraperitoneal injections of 25, 50 or 100 mg/kg bw 2-nitropropane (95% pure) every two days. Between the 42nd and 56th day of the experiment, 2-acetylaminofluorene (2-AAF) dissolved in corn oil was added to the diet at a concentration of 50 mg/kg diet (ppm). In the middle of the 2-AAF treatment period (on day 49), rats were subjected to partial hepatectomy. From day 56, phenobarbital sodium was added to the diet at a concentration of 500 ppm for two weeks. After 70 days of the experiment, rats were killed and the livers examined for γ-glutamyl-transpeptidase (γ-GT) and for glutathione *S*-transferase (GST) foci. The numbers of γ-GT-positive foci per cm^2 were 0.6, 3.7, 5.5 and 22.2 in the control, low-dose, mid-dose and high-dose groups, respectively. The numbers of GST-positive foci were 1.4, 10.8, 10.7 and 29.9 in the four groups, respectively (Astorg *et al*., 1994).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Nolan *et al.* (1982) administered 2-nitro[^{14}C]propane to rats by inhalation at levels of 73 or 562 mg/m^3 for 6 h. Over 40% of the administered dose was retained in the body and the bulk of the absorbed dose was rapidly metabolized and excreted. The elimination of total ^{14}C from the blood and by exhalation was biphasic and the two sets of parameters were broadly comparable at the two dose levels. The two elimination half-lives from blood were 71 min and 13.2 h at the high dose and 64 min and 16.4 h at the low dose, while for exhalation the corresponding values were 172 min and 36 h (high dose) and 354 min and 35 h (low dose). At the high dose, the elimination half-life of 2-nitropropane from blood was 48 min; the value could not be calculated at the low dose, since 2-nitropropane levels were 1/12th of those at the high dose and only two levels could be measured before the concentration fell below the detection limit. The blood levels of 2-nitropropane and ^{14}C at the end of the 6-h inhalation exposure at the high dose were 12 and nine times those after the low dose, values to be compared with the 7.5-fold difference between the doses. A 100% recovery of the absorbed dose was obtained in excretion balance studies conducted 48 h after the 6-h inhalation exposure. Some 10% of the dose was excreted in the urine, with a further 5% (high dose) or 10% (low dose) in the faeces. Fifty per cent of the dose was exhaled as $^{14}CO_2$ and 3% (low dose) or 22% (high dose) as 2-nitropropane. The balance of the dose (25%, low dose; 11%, high dose) was present in the carcass.

Suggestions about the metabolic pathway leading to $^{14}CO_2$ were provided by the work of Marker and Kulkarni (1985, 1986). They showed that the microsomal metabolism of 2-nitropropane in mice resulted in the release of nitrite in a cytochrome P450-dependent reaction. In contrast to earlier results in rats, enzymatic denitrification in mice did not require enzyme induction.

2-Nitropropane exists in equilibrium with its tautomer propane-2-nitronic acid, present in physiological media as the anion propane-2-nitronate. Dayal *et al.* (1991) have investigated the metabolism of both 2-nitropropane and propane-2-nitronate in mouse microsomes and found that the oxidative denitrification of propane-2-nitronate was 5–10 times more extensive than that of 2-nitropropane. Acetone formed by this reaction affords the source of $^{14}CO_2$ detected when 2-nitro[^{14}C]propane was administered *in vivo*. However, neither 2-nitropropane nor propane-2-nitronate was cytotoxic to mouse hepatocytes.

After administration of 2-nitropropane to rats, oxidative damage to DNA occurs, notably an increase in 8-hydroxy- and 8-aminodeoxyguanine (Fiala *et al.*, 1989). The

mechanism of this damage is hard to relate to the oxidation of 2-nitropropane (Bors *et al.*, 1993; Kohl *et al.*, 1995). Sodum *et al.* (1994) have advanced a novel pathway of metabolism for both 2-nitropropane and propane-2-nitronate in which an *N*-hydroxy form is *O*-sulfated, giving an amino radical and acetone. Damage to DNA and RNA was markedly inhibited by the sulfotransferase inhibitors pentachlorophenol and 2,6-dichloro-4-nitrophenol. However, the mechanisms of 2-nitropropane metabolism and associated DNA damage remain uncertain (Kohl *et al.*, 1995).

4.2 Toxic effects

4.2.1 *Humans*

An employee health examination including workers exposed to personal time-weighted average levels below 25 ppm [91 mg/m^3] 2-nitropropane revealed no adverse effects in the lung, liver, kidney, skin or haematopoietic and cardiovascular systems (Crawford *et al*., 1985).

Harrison *et al*. (1985, 1987) reported on two construction workers who were exposed to 2-nitropropane while applying epoxy resin coating. One man died 10 days after exposure from fulminant hepatitis, the other man had persistently elevated serum aminotransferase activity. Serum concentrations of 2-nitropropane on admission were 13 mg/L in the man who died and 8.5 mg/L in his co-worker.

4.2.2 *Experimental systems*

Dayal *et al*. (1989) treated BALB/c mice intraperitoneally with a single dose of 9 mmol/kg bw 2-nitropropane. In male mice, plasma activities of the hepatic enzymes sorbitol dehydrogenase, alanine aminotransferase and aspartate aminotransferase were significantly elevated, while doses of 6.7 mmol/kg bw were ineffective. In female mice, a dose of 6.7 mmol/kg bw was sufficient to cause hepatotoxicity. Histopathological evaluation revealed hepatic damage, particularly in the periportal region.

Griffin *et al*. (1981) exposed male and female Sprague-Dawley rats by inhalation to 25 ppm [91 mg/m^3] 2-nitropropane for 7 h per day on five days per week over a period of 22 months. Histopathological examination of all major organs did not show any lesions.

Cunningham and Matthews (1991) treated male Fischer 344 rats with 0.5, 1 or 2 mmol/kg bw 2-nitropropane daily for 10 days by gavage. At the higher dose levels, but not at 0.5 mmol/kg bw, increased hepatic DNA synthesis was found, together with moderate signs of cholestasis and hepatotoxicity.

Inhalation exposure of male Sprague-Dawley rats to 2-nitropropane at air concentrations of 100 ppm [365 mg/m^3] for 7 h per day on four consecutive days did not result in increased hepatic microsomal malonaldehyde content as a measure of lipid peroxidation, or increased levels of serum aspartate transferase or of glutamic oxaloacetic transaminase. Total hepatic glutathione was enhanced by 2-nitropropane treatment (Haas-Jobelius *et al*., 1992).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Sprague-Dawley rats were treated intraperitoneally with 170 mg/kg bw per day on days 1–15 of gestation. The treatment resulted in reduced pre- and postimplantation survival and reduced fetal body weight or length. No signs of maternal toxicity or teratogenicity were observed (Hardin *et al.*, 1981).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

2-Nitropropane was mutagenic to bacteria both in the presence and in the absence of an exogenous metabolic system.

In vitro, in the absence of an exogenous metabolic system, 2-nitropropane induced unscheduled DNA synthesis in rat and mouse liver cells. It induced gene mutations in Chinese hamster cells and rat hepatoma cells in the absence of an exogenous metabolic system. In the absence of an exogenous metabolic system, 2-nitropropane induced micronuclei in three rat hepatoma cell lines but not in Chinese hamster cells.

In one study, 2-nitropropane induced unscheduled DNA synthesis *in vitro* in hepatocytes from three of six people. In human peripheral lymphocytes, 2-nitropropane induced sister chromatid exchanges and chromosomal aberrations in the presence of an exogenous metabolic system.

The genotoxic effects of 2-nitropropane have been reviewed (WHO, 1992). 2-Nitropropane is genotoxic to a wide range of organisms *in vitro* and *in vivo*. It induced DNA modifications (8-hydroxydeoxyguanosine and 8-aminodeoxyguanosine) in rat liver *in vivo* but not in the kidney. In one study, levels of 8-hydroxydeoxyguanosine in rabbit liver DNA were lower than in DNA from rat liver in animals treated with 2-nitropropane *in vivo*. In another study, 2-nitropropane induced DNA strand breaks in liver, but not in lung, kidney, bone marrow and brain of rats treated *in vivo*. DNA strand breaks and 8-hydroxydeoxyguanosine were detected, in a single study, in bone marrow of rats *in vivo*. It provoked unscheduled DNA synthesis in liver of rats treated *in vivo*. In single studies conducted *in vivo*, 2-nitropropane failed to induce micronuclei in the bone marrow of mice or rats, but did induce them in rat liver.

The production of oxygen free radicals by one-electron oxidation of the nitronate form (De Rycker & Halliwell, 1978; Porter & Bright, 1983; Kido & Soda, 1984) could explain the relatively high mutagenicity in the oxidant-sensitive *Salmonella typhimurium* strain TA102 and the production of 8-hydroxydeoxyguanosine in rat liver *in vivo*. Another modified deoxynucleoside, 8-aminodeoxyguanosine, has been identified in rat

Table 1. Genetic and related effects of 2-nitropropane

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	1410	Hite & Skeggs (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	1270	Speck *et al.* (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	1280	Haworth *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	2207	Fiala *et al.* (1987b)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	233	Göggelmann *et al.* (1988)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	1320	Conaway *et al.* (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	90	Kohl *et al.* (1994)
SA2, *Salmonella typhimurium* TA102, reverse mutation	+	+	1070	Fiala *et al.* (1987b)
SA2, *Salmonella typhimurium* TA102, reverse mutation	+	+	2640	Conaway *et al.* (1991)
SA2, *Salmonella typhimurium* TA102, reverse mutation	+	NT	90	Kohl *et al.* (1994)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	?	3850	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	(+)	38150	Hite & Skeggs (1979)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	3850	Haworth *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	4200	Hite & Skeggs (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	385	Speck *et al.* (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	1280	Haworth *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2140	Fiala *et al.* (1987b)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	233	Göggelmann *et al.* (1988)
SAS, *Salmonella typhimurium* TA92, reverse mutation	+	+	11450	Hite & Skeggs (1979)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	NT	8.9	Davies *et al.* (1993)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	NT	22	Kohl *et al.* (1994)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	NT	0.89	Fiala *et al.* (1995)
UIA, Unscheduled DNA synthesis, mouse primary hepatocytes *in vitro*	+	NT	89	Davies *et al.* (1993)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	+	NT	45	Roscher *et al.* (1990)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	+	NT	267	Haas-Jobelius *et al.* (1991)
GIA, Gene mutation, rat hepatoma H4IIEC3/G$^-$ cells, *hprt* locus *in vitro*	+[c]	NT	89	Roscher *et al.* (1990)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	–	5000	Galloway *et al*. (1987)
MIA, Micronucleus test, H4IIEC3/G$^-$ rat hepatoma cell line *in vitro*	+[c]	NT	267	Roscher *et al.* (1990)
MIA, Micronucleus test, 2sFou rat hepatoma cell line *in vitro*	+[c]	NT	267	Roscher *et al.* (1990)
MIA, Micronucleus test, C_2Rev7 rat hepatoma cell line *in vitro*	+[c]	NT	267	Roscher *et al.* (1990)
MIA, Micronucleus test, Chinese hamster lung V79 cells *in vitro*	–	NT	891	Roscher *et al.* (1990)
MIA, Micronucleus test, Chinese hamster lung V79 cells *in vitro*	–	NT	445	Haas-Jobelius *et al.* (1991)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	–	5000	Galloway *et al*. (1987)
UIH, Unscheduled DNA synthesis, human primary hepatocytes *in vitro*	(+)	NT	89	Davies *et al.* (1993)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	+	668	Bauchinger *et al.* (1987)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	+	7120	Göggelmann *et al*. (1988)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	–	+	5345	Bauchinger *et al.* (1987)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	–	+	7120	Göggelmann *et al*. (1988)
DVA, 8-Hydroxydeoxyguanosine in Sprague-Dawley rat liver DNA *in vivo*	+		100 ip × 1	Fiala *et al.* (1989)
DVA, 8-Hydroxydeoxyguanosine in Sprague-Dawley rat liver DNA *in vivo*	+		100 ip × 1	Guo *et al.* (1990)
DVA, 8-Hydroxydeoxyguanosine in Sprague-Dawley rat kidney DNA *in vivo*	–		100 ip × 1	Guo *et al.* (1990)
DVA, DNA strand breaks, Sprague-Dawley rat liver *in vivo*	+		45 po × 1	Robbiano *et al.* (1991)
DVA, DNA strand breaks, Sprague-Dawley rat lung, kidney, bone marrow and brain *in vivo*	–		713 po × 1	Robbiano *et al.* (1991)
DVA, 8-Hydroxydeoxyguanosine in Wistar rat liver DNA *in vivo*	+		80 ip × 1	Adachi *et al.* (1993)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DVA, 8-Amino- and 8-hydroxydeoxyguanosine in Sprague-Dawley rat liver DNA *in vivo*	+		100 ip × 1	Fiala *et al.* (1993)
DVA, 8-Amino- and 8-hydroxydeoxyguanosine in New Zealand White rabbit liver DNA *in vivo*	(+)		100 ip × 1	Fiala *et al.* (1993)
DVA, 8-Hydroxydeoxyguanosine in Fischer 344 rat liver DNA *in vivo*	+		100 ip × 1	Hasegawa *et al.* (1995)
DVA, 8-Aminodeoxyguanosine in Fischer 344 rat liver DNA *in vivo*	+		100 ip × 1	Sodum *et al.* (1993)
DVA, 8-Amino- and 8-hydroxydeoxyguanosine in Fischer 344 rat liver DNA *in vivo*	+		100 ip × 1	Sodum *et al.* (1994)
DVA, 8-Hydroxydeoxyguanosine in Fischer 344 rat liver DNA *in vivo*	+		100 ip × 1	Takagi *et al.* (1995)
DVA, DNA strand breaks (comet assay), Wistar rat bone-marrow DNA *in vivo*	+		100 ip × 1	Deng *et al.* (1997)
DVA, 8-Hydroxydeoxyguanosine in Wistar rat bone-marrow DNA *in vivo*	+		100 ip × 1	Deng *et al.* (1997)
DVA, 8-Hydroxydeoxyguanosine in Fischer 344 rat liver DNA *in vivo*	+		100 ip × 1	Fiala *et al.* (1997)
UPR, Unscheduled DNA synthesis, Sprague-Dawley rat hepatocytes *in vivo*	+		50 po × 1	George *et al.* (1989)
MVM, Micronucleus test, CD-1 mouse bone-marrow cells *in vivo*	–		300 po × 2	Hite & Skeggs (1979)
MVM, Micronucleus test, (101/E1 × C3H/E1)F_1 mouse bone-marrow cells *in vivo*	–		300 po × 1	Kliesch & Adler (1987)
MVR, Micronucleus test, Sprague-Dawley rat hepatocytes *in vivo*	+		25 po × 1	George *et al.* (1989)
MVR, Micronucleus test, Sprague-Dawley rat bone-marrow cells *in vivo*	–		300 po × 1	George *et al.* (1989)
8-Hydroxyguanosine in Sprague-Dawley rat liver RNA *in vivo*	+		100 ip × 1	Fiala *et al.* (1989)
8-Hydroxyguanosine in Sprague-Dawley rat liver and kidney RNA *in vivo*	+		100 ip × 1	Guo *et al.* (1990)
8-Amino- and 8-hydroxyguanosine in Sprague-Dawley rat liver RNA *in vivo*	+		100 ip × 1	Fiala *et al.* (1993)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
8-Amino- and 8-hydroxyguanosine in New Zealand White rabbit liver RNA *in vivo*	(+)		100 ip × 1	Fiala *et al.* (1993)
8-Aminoguanosine in Fischer 344 rat liver RNA *in vivo*	+		100 ip × 1	Sodum *et al.* (1993)
8-Amino- and 8-hydroxyguanosine in Fischer 344 rat liver RNA *in vivo*	+		100 ip × 1	Sodum *et al.* (1994)
8-Hydroxyguanosine in Fischer 344 rat liver RNA *in vivo*	+		100 ip × 1	Fiala *et al.* (1997)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; ip, intraperitoneal; po, oral
[c] Cells pre-incubated for 24 h with 2 µM dexamethasone, an inducer of liver-specific cytochrome P450 isoforms; results were negative without this pretreatment.

liver DNA following treatment with 2-nitropropane *in vivo* (Sodum *et al*., 1993, 1994) and in rat hepatocytes treated *in vitro* (Davies *et al*., 1993).

4.4.3 *Mechanistic considerations*

Nitroalkanes are acidic compounds; the dissociation of a proton from a nitroalkane produces the nitroalkane anion, or nitronate, whose chemical and physical properties differ from those of the parent nitroalkane. The nitronate form of 2-nitropropane is more mutagenic in *S. typhimurium* TA100 and TA102 than is the neutral parent compound (Fiala *et al*., 1987b; Dayal *et al*., 1989; Kohl *et al*., 1994), suggesting that propane 2-nitronate may act as an intermediate in the mechanism by which 2-nitropropane exerts its genotoxic and carcinogenic effects. This hypothesis is supported by studies indicating that both bacterial mutagenicity and induction of unscheduled DNA synthesis in rat hepatocytes are decreased by conditions (low pH or deuteration of the secondary carbon atom) that limit formation of the nitronate tautomer, and that the tautomerization of 2-nitropropane can be influenced by hepatic enzymes (Kohl *et al*., 1994).

It has been proposed that 8-aminodeoxyguanosine is formed from the nitronate tautomer of 2-nitropropane either by base nitrosation followed by reduction, or via an enzyme-mediated conversion of the nitronate anion to hydroxylamine-*O*-sulfonate or acetate, which yields the highly reactive nitrenium ion NH_2^+ (Sodum *et al*., 1993). Sodum *et al*. (1994) have provided evidence for the activation of 2-nitropropane to an aminating species by rat liver aryl sulfotransferase *in vitro* and *in vivo*. Pretreatment of rats with the aryl sulfotransferase inhibitors pentachlorophenol or 2,6-dichloro-4-nitrophenol significantly decreased the levels of nucleic acid modifications produced in the liver by 2-nitropropane treatment. Partially purified rat liver aryl sulfotransferase activated 2-nitropropane and its nitronate at neutral pH to a reactive species that aminated guanosine at the C^8 position. This activation was dependent on the presence of the enzyme, its specific cofactor adenosine 3′-phosphate 5′-phosphosulfate, and mercaptoethanol. It was inhibited by 2,6-dichloro-4-nitrophenol.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

2-Nitropropane is produced in low volume and occupational exposures occur primarily in its production and use as a solvent in inks, adhesives, paints and coatings. Exposures of the general population may occur in ambient air and water near industrial sites manufacturing or using 2-nitropropane, in cigarette smoke, and possibly from its solvent uses.

5.2 Human carcinogenicity data

No adequate epidemiological data were available to the Working Group.

5.3 Animal carcinogenicity data

2-Nitropropane was tested for carcinogenicity in one experiment in rats by oral administration and two experiments in rats by inhalation exposure. It induced benign and malignant liver tumours following oral administration and hepatocellular carcinomas in one inhalation experiment and an increased incidence of hepatocellular nodules in the other. 2-Nitropropane showed initiating activity in rat liver in two experiments.

5.4 Other relevant data

Nitropropane shows mainly hepatotoxicity in rats.

It is mutagenic in a wide variety of in-vitro and in-vivo systems by a direct action. It leads to formation of 8-hydroxydeoxyguanosine in DNA *in vivo*.

5.5 Evaluation

There is *inadequate evidence* for the carcinogenicity of 2-nitropropane in humans.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2-nitropropane.

Overall evaluation

2-Nitropropane is *possibly carcinogenic to humans (Group 2B)*.

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1,3-PROPANE SULTONE

Data were last reviewed in IARC (1974) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

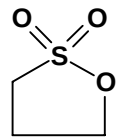
1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 1120-71-4

Chem. Abstr. Name: 1,2-Oxathiolane, 2,2-dioxide

Synonyms: 3-Hydroxy-1-propanesulfonic acid, γ-sultone; propane sultone

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_3H_6O_3S$ Relative molecular mass: 122.14

1.1.3 *Chemical and physical properties of the pure substance*

(from American Conference of Governmental Industrial Hygienists, 1992)

(*a*) *Description*: White crystalline solid or colourless liquid

(*b*) *Boiling-point:* 112°C

(*c*) *Melting-point:* 31°C

(*d*) *Solubility:* Moderately soluble in water (100 g/L) and most organic solvents; insoluble in aliphatic hydrocarbons

(*e*) *Conversion factor:* $mg/m^3 = 5.0 \times ppm$

1.2 Production and use

No information on the global production of 1,3-propane sultone was available to the Working Group.

1,3-Propane sultone has been used as a chemical intermediate to introduce the propyl-sulfonate ($–CH_2CH_2CH_2SO_3^–$) group into molecules and to confer water solubility and anionic character. It is also a chemical intermediate in the production of fungicides, insecticides, cation-exchange resins, dyes, vulcanization accelerators and a variety of other

chemicals (American Conference of Governmental Industrial Hygienists, 1992; Lewis, 1993).

1.3 Occurrence

No data were available to the Working Group.

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not proposed any occupational exposure limit for 1,3-propane sultone in workplace air but does list it as an animal carcinogen. Australia, Belgium, Finland, France, Germany, Sweden and Switzerland list 1,3-propane sultone as a probable human carcinogen (International Labour Office, 1991).

No international guideline for 1,3-propane sultone in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

1,3-Propane sultone was tested for carcinogenicity by oral, intravenous and prenatal exposure in rats, producing tumours at various sites; a local carcinogenic effect was induced in mice and rats when it was given subcutaneously. It was carcinogenic in rats after single-dose exposures (IARC, 1974).

3.1 Oral administration

Rat: 1,3-Propane sultone (purity 91%) was administered orally by gavage to groups of 26 male and 26 female weanling Sprague-Dawley rats at doses of 28 and 56 mg/kg bw per day twice per week for 60 weeks or 32 weeks. The animals were then observed without further dosing up to 60 weeks. Two groups of rats, one of 16 males and 16 females and one of 26 males and 26 females, were used as matched and pooled controls. Survival at 52 weeks among male and female rats, respectively, was 62% and 39%, in the 28 mg/kg bw group and 15% and 23%, respectively, in the 56 mg/kg bw group. Administration of the high dose was stopped at week 32 because numerous mammary tumours had developed in the females from week 18 and there was high mortality among the males. Significant increases in the incidence of certain tumours were found. The incidences in the matched control, low-dose and high-dose groups, respectively, were: male rats—malignant glioma (cerebrum), 1/16, 10/26 and 11/26; malignant glioma (cerebellum), 0/16, 6/26 and 11/26; and female rats—malignant glioma (cerebrum), 1/16,

12/26 and 12/26; malignant glioma (cerebellum), 0/16, 8/26 and 4/26; mammary adenocarcinoma, 0/16, 6/26 and 13/26 (Weisburger *et al*., 1981).

3.2 Subcutaneous administration

Rat: Eighty random-bred male albino rats (weighing 70–140 g) were divided into groups of five or 10 [no controls] and given 1–7 subcutaneous injections of 1,3-propane sultone at doses of 62, 125 or 166 mg/kg bw. Multiple doses were given at 15-day intervals. Neoplastic lesions varying from well differentiated to anaplastic adenocarcinomas were seen in the lungs of 17/73 rats 21–25 weeks after injection of 1,3-propane sultone (Gupta *et al*., 1981). [The Working Group noted the limited reporting of the data.]

3.3 Skin application

Mouse: Groups of 25 male and 25 female mice of each of three strains (CF1, C3H and CBah, a hairless strain), six weeks of age, were treated twice weekly by painting with approximately 0.05–0.1 mL benzene per mouse for four weeks and then toluene for one year or with 2.5% w/v 1,3-propane sultone (purity, 99.9%) in the same solvents and for the same time; control groups were left untreated. In the control groups, survival at the end of the experiment (58 weeks) was at least 60%. No CF1 or C3H mice survived exposure to 1,3-propane sultone for 58 weeks and only 12% of the CBah mice survived to this time. No skin tumours were seen in the untreated or solvent control groups, whereas, in the 1,3-propane sultone-treated groups of male and female mice, respectively, the numbers of tumour-bearing mice were: CF1, 15/21, 3/24; C3H, 20/22, 6/25; CBah, 20/23, 18/25. In addition, there was clearly a higher proportion of CF1 mice with lymphoreticular neoplasms: untreated control males, 1/24, females, 1/23; solvent control males, 0/22, females, 3/25; 1,3-propane sultone-treated males, 12/21, females, 17/24. No significant increase in these neoplasms was seen in either the C3H or the CBah strains of mice (Doak *et al*., 1976).

Groups of 48 male and 48 female CF1 mice were painted with either approximately 0.05–0.1 mL per mouse toluene or 1,3-propane sultone in toluene administered as a single application of 2.5% or 25% w/v or as 10 applications of a 2.5% w/v solution on alternate days. The experiment was terminated after 78 weeks. No skin tumour was found in the toluene controls of either sex, whereas in the 1,3-propane sultone-treated groups, the numbers of tumour-bearing mice were: single application of 2.5%, 0/48 males and 1/48 females; 10 applications of 2.5%, 3/48 males and 2/48 females; single application of 25%, 29/36 males and 26/46 females (Doak *et al*., 1976).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Toxic effects

No data were available to the Working Group.

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

1,3-Propane sultone causes DNA damage and mutation in bacteria and induces mitotic recombination in yeast. It induces mutations and chromosomal aberrations in plant cells. In cultured mammalian cells, it induces chromosomal aberrations, sister chromatid exchanges and, according to single studies, cell transformation in C3H 10T½ cells, but not in Syrian hamster embryo cells. DNA strand breaks are induced in brain cells from rats injected with 1,3-propane sultone.

1,3-Propane sultone reacts with guanosine and DNA at pH 6–7.5 *in vitro*, with *N*7-alkylguanosine accounting for > 90% of the total reaction products. Minor products that have been identified are *N*1-alkylguanosine (approx. 1.6%) and O^6-alkylguanosine (approx. 0.5%) (Hemminki, 1983).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

1,3-Propane sultone has been used as an intermediate in the production of a variety of chemical products.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

1,3-Propane sultone is carcinogenic in rats by all routes of administration (oral, dermal, intravenous, subcutaneous or prenatal), producing tumours at various sites

Table 1. Genetic and related effects of 1,3-propane sultone

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage, *umu* induction, SOS repair test, DNA strand breaks, cross-links or related damage	+	NT	16	Nakamura *et al*. (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	2.5	Simmon (1979a)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	6	Bartsch *et al*. (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	2.5	Simmon (1979a)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	6	Bartsch *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	NT	NG	Simmon (1979a)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	NG	Simmon (1979a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	NG	Simmon (1979a)
SAS, *Salmonella typhimurium* TA1536, reverse mutation	–	NT	NG	Simmon (1979a)
SCH, *Saccharomyces cerevisiae*, homozygosis by mitotic recombination or gene conversion	+	+	1000	Simmon (1979b)
HSM, *Hordeum* species (barley), mutation	+	NT	611	Kaul & Tandon (1981)
HSM, *Hordeum* species (barley), mutation	+	NT	975	Singh & Kaul (1985)
HSC, *Hordeum* species (barley), chromosomal aberrations	(+)	NT	611	Kaul & Tandon (1981)
SIC, Sister chromatid exchange, Chinese hamster lung fibroblasts *in vitro*	+	NT	1.2	Abe & Sasaki (1977)
CIC, Chromosomal aberrations, Chinese hamster lung fibroblasts *in vitro*	+	NT	12	Abe & Sasaki (1977)
CIC, Chromosomal aberrations, Chinese hamster lung Don cells *in vitro*	+	NT	63	Ishidate (1988)
TCM, Cell transformation, C3H 10T½ CL8 mouse cells *in vitro*	(+)	NT	50	Oshiro *et al*. (1981)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay *in vitro*	–	NT	10	Pienta *et al*. (1977)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	61	Kaul (1985)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	122	Kaul (1985)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
TIH, Cell transformation, human newborn foreskin epithelial cells	+	NT	7.5	Milo *et al*. (1981)
HMM, Host-mediated assay, *Salmonella typhimurium* TA1530 and TA1538 in Swiss-Webster mice	+		12 im × 1	Simmon *et al*. (1979)
DVA, DNA strand breaks, male Sprague-Dawley rat brain cells *in vivo* (alkaline elution assay)	+		31 ip × 1	Robbiano & Brambilla (1987)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; NG, not given; im, intramuscular; ip, intraperitoneal

including the brain and mammary gland. In mice, it was carcinogenic after skin application and subcutaneous injection producing local tumours.

5.4 Other relevant data

1,3-Propane sultone is mutagenic in bacteria. It is positive for many genetic activity end-points *in vitro* in rodent and human cells. 1,3-Propane sultone induces DNA strand breaks *in vivo* in rat brain cells.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of 1,3-propane sultone were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,3-propane sultone.

Overall evaluation

1,3-Propane sultone is *possibly carcinogenic to humans (Group 2B)*.

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β-PROPIOLACTONE

Data were last reviewed in IARC (1974) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

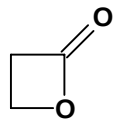
1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 57-57-8

Chem. Abstr. Name: 2-Oxetanone

Synonyms: Hydracrylic acid, β-lactone; 3-hydroxypropionic acid, lactone; 3-hydroxypropionic acid, β-lactone; propanolide; 3-propanolide; propiolactone; 3-propiolactone; β-propionolactone

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_3H_4O_2$ Relative molecular mass: 72.06

1.1.3 *Chemical and physical properties of the pure substance*

(from American Conference of Governmental Industrial Hygienists (1992), unless otherwise noted)

(*a*) *Description*: Colourless liquid with a slightly sweetish odour (Budavari, 1996)

(*b*) *Boiling-point:* 162°C (Lide, 1997)

(*c*) *Melting-point:* –33.4°C (Lide, 1997)

(*d*) *Solubility:* Soluble in water (37 mL/100 mL at 25°C) with hydrolysis; miscible with acetone, chloroform, diethyl ether, ethanol and other common organic solvents (American Conference of Governmental Industrial Hygienists, 1992; Budavari, 1996; Lide, 1997)

(*e*) *Vapour pressure:* 452 Pa at 25°C; relative vapour density (air = 1), 2.5

(*f*) *Flash point:* 74°C, closed cup

(*g*) *Reactivity*: Polymerizes during storage

(*h*) *Explosive limits:* Lower, 2.9% by volume in air

(*i*) *Conversion factor*: $mg/m^3 = 2.95 \times ppm$

1.2 Production and use

No information on the global production of β-propiolactone was available to the Working Group.

β-Propiolactone has been used as a vapour sterilant for plasma, vaccines, tissue grafts, surgical instruments and enzymes; as a vapour-phase disinfectant in enclosed spaces; and in organic synthesis. Its sporicidal action is used against vegetative bacteria, pathogenic fungi, and viruses. It has been used as an intermediate in the production of acrylic acid and esters (American Conference of Governmental Industrial Hygienists, 1992; United States National Library of Medicine, 1998).

1.3 Occurrence

No data were available to the Working Group.

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 1.5 mg/m^3 as the 8-h time-weighted average threshold limit value for occupational exposures to β-propiolactone in workplace air and lists it as an animal carcinogen. Similar values have been used as standards or guidelines in many countries. Australia, Belgium, Finland, France, Germany, Sweden and Switzerland list β-propiolactone as a probable human carcinogen (International Labour Office, 1991).

No international guideline for β-propiolactone in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

β-Propiolactone was tested for carcinogenicity in mice following skin application or subcutaneous or intraperitoneal injection, and in rats following subcutaneous injection, producing local tumours. It is carcinogenic to mice after single-dose exposure. Oral administration to rats gave some indication of carcinogenic activity. The results obtained in Syrian hamsters and guinea-pigs are equivocal (IARC, 1974).

3.1 Inhalation exposure

Rat: A group of 50 male Sprague-Dawley rats [age unspecified] was exposed by whole-body inhalation to 10 ppm [30 mg/m^3] β-propiolactone [purity unspecified] for 6 h

per day on five days per week for six weeks. A control group of 150 rats was exposed to filtered air using the same exposure protocol. After treatment, animals were observed for lifespan. The mortality-corrected incidence of nasal cancer 480 days after the start of exposure was 60%. At the end of the experiment (around 720 days), all β-propiolactone-exposed rats had developed nasal cancer [histology unspecified]. No nasal cancer was observed in control rats (Snyder *et al.*, 1986).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Toxic effects

No data were available to the Working Group.

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

β-Propiolactone is a direct-acting alkylating agent that reacts with polynucleotides and DNA, mainly at N7 of guanine and N1 of adenine, to form carboxyethyl derivatives. It also forms adducts with the N^3 of cytosine and thymine (Hemminki, 1981; Lawley, 1984). It is genotoxic to a wide range of organisms *in vitro* and *in vivo.*

β-Propiolactone was mutagenic to bacteria. In yeast, it induced mitotic gene conversion, aneuploidy and mutations. It produced heritable translocations and sex-linked recessive lethal mutations in *Drosophila melanogaster*. *In vitro*, it induced cell transformation and gene mutations in human cells, and cell transformation, gene mutations, chromosomal aberrations and sister chromatid exchanges in mammalian cells.

In single studies, when given *in vivo*, β-propiolactone induced gene mutations in the stomach and liver in the Muta™ Mouse, and DNA strand breaks in rat liver and mouse skin keratinocytes. In a single study, it induced chromosomal aberrations in rat bone-marrow cells *in vivo*. β-Propiolactone bound covalently to mouse skin DNA and RNA *in vivo.* It induced chromosomal aberrations or micronuclei in oocytes, spermatids, hepatocytes and splenocytes in mice *in vivo.*

Table 1. Genetic and related effects of β-propiolactone

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage, *umu* induction, SOS repair test, DNA strand breaks, cross-links	+	NT	8.3	Nakamura *et al.* (1987)
SAF, *Salmonella typhimurium*, forward mutation, 8-azaguanine	+	NT	3	Castellino *et al.* (1978)
SAF, *Salmonella typhimurium*, forward mutation, 8-azaguanine	+	NT	2.9	Penman *et al.* (1979)
SAF, *Salmonella typhimurium*, forward mutation, 8-azaguanine	NT	+	100	Skopek *et al.* (1981)
SAF, *Salmonella typhimurium*, forward mutation, 8-azaguanine	+	NT	0.7	Skopek & Thilly (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	+	50	Anderson & Styles (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	3	Castellino *et al.* (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	50	Simmon (1979a)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	18	Drinkwater *et al.* (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	37	Baker & Bonin (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	NG	Brooks & Dean (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	6.9	Garner *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation, fluctuation test	+	+	1	Hubbard *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	7.5	MacDonald (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	25	Martire *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	5	Nagao & Takahashi (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	5	Richold & Jones (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	50	Rowland & Severn (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	NG	Simmon & Shepherd (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	NG	Venitt & Crofton-Sleigh (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	36	Bartsch *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	91	Wattenberg *et al.* (1987)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA5, *Salmonella typhimurium* TA1535, reverse mutation	NT	–	NG	Anderson & Styles (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	3	Castellino *et al.* (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	50	Simmon (1979a)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	NG	Baker & Bonin (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	1.5	Brooks & Dean (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	NG	Garner *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	NT	+	633 μg/m^3 vap.	Pincus *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	50	Richold & Jones (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	10	Simmon & Shepherd (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	36	Bartsch *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	NT	NG	Simmon (1979a)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	+	NG	Baker & Bonin (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	+	+	NG	Brooks & Dean (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Garner *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	?	–	NG	Martire *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	+	+	NG	Nagao & Takahashi (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	50	Richold & Jones (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Simmon & Shepherd (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	NT	+	50	Anderson & Styles (1978)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	NG	Simmon (1979a)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	370	Baker & Bonin (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	+	+	NG	Brooks & Dean (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	50	Richold & Jones (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	NG	Simmon & Shepherd (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	NT	–	NG	Anderson & Styles (1978)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	NG	Simmon (1979a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	370	Baker & Bonin (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	–	NG	Brooks & Dean (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Garner *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation, fluctuation test	–	+	2	Gatehouse (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation, fluctuation test	+	+[c]	1	Hubbard *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	25	MacDonald (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Martire *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Nagao & Takahashi (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	5	Richold & Jones (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Simmon & Shepherd (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	185	Venitt & Crofton-Sleigh (1981)
SAS, *Salmonella typhimurium* TA1536, reverse mutation	–	NT	NG	Simmon (1979a)
SAS, *Salmonella typhimurium* TA92, reverse mutation	+	+	NG	Brooks & Dean (1981)
ECW, *Escherichia coli* WP2 *uvr*ApKM101, reverse mutation	+	+	9	Matsushima *et al.* (1981)
ECW, *Escherichia coli* WP2 *uvr*ApKM101, reverse mutation	+	+	NG	Venitt & Crofton-Sleigh (1981)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	+	+	9	Matsushima *et al.* (1981)
EC2, *Escherichia coli* WP2B/r, reverse mutation	+	+	22	Matsushima *et al.* (1981)
SCR, *Saccharomyces cerevisiae* XV185-14C, reverse mutation,	+	+	89	Mehta & von Borstel (1981)
SZF, *Schizosaccharomyces pombe*, forward mutation	+	+	0.1	Loprieno (1981)
SCN, *Saccharomyces cerevisiae* D6, mitotic aneuploidy induction	+	+	25	Parry & Sharp (1981)
SCH, *Saccharomyces cerevisiae* D3, homozygosis by mitotic gene conversion	+	+	100	Simmon (1979b)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SCH, *Saccharomyces cerevisiae* JD1, homozygosis by mitotic gene conversion	+	+	25	Sharp & Parry (1981)
SCH, *Saccharomyces cerevisiae* D7, homozygosis by mitotic gene conversion	+	+	14	Zimmermann & Scheel (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		720 ppm feed or inj	Kortselius (1979)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		2300 µg/mL inj	Vogel *et al.* (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		250 ppm feed	Woodruff *et al.* (1984)
DMH, *Drosophila melanogaster*, heritable translocation test	+		1800 ppm feed	Kortselius (1979)
DMH, *Drosophila melanogaster*, heritable translocation test	+		3000 ppm feed	Woodruff *et al.* (1984)
DIA, DNA damage (comet assay), male CBA mouse keratinocytes *in vitro*	+	NT	100	Yendle *et al.* (1997)
GCL, Gene mutation, Chinese hamster lung g 12 transgenic cells, *gpt* locus *in vitro*	+	NT	100	Klein & Rossman (1990)
GCO, Gene mutation, Chinese hamster ovary cells *in vitro,* five different loci	+	NT	10	Gupta & Singh (1982)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	+	NT	5	Nishi *et al.* (1984)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	+	NT	50	Klein & Rossman (1990)
SIC, Sister chromatid exchange, Chinese hamster lung Don cells *in vitro*	+	NT	0.072	Abe & Sasaki (1977)
SIC, Sister chromatid exchange, Chinese hamster lung Don cells *in vitro*	+	NT	11.5	Baker *et al.* (1983)
SIC, Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	+	NT	5	Nishi *et al.* (1984)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CIC, Chromosomal aberrations, Chinese hamster lung Don cells *in vitro*	+	NT	72	Abe & Sasaki (1977)
CIC, Chromosomal aberrations, Chinese hamster lung fibroblasts *in vitro*	+	NT	30	Ishidate *et al*. (1988)
TBM, Cell transformation, BALB/c 3T3 'A31 clone' mouse cells	+	NT	1	Atchison *et al.* (1982)
TBM, Cell transformation, BALB/c 3T3 'A31 clone' mouse cells	+	NT	2.5	Baturay & Kennedy (1986)
TCM, Cell transformation, mouse C3H 10T½ cells *in vitro*	+	NT	18	Oshiro *et al.* (1981)
HMM, Host-mediated assay, *Salmonella typhimurium* TA1535 in Swiss-Webster mice	+		405 po	Simmon *et al.* (1979)
GIH, Gene forward mutation, human fibroblasts (MIT-2 cells), *hprt* locus *in vitro*	+	NT	6.5	Penman *et al.* (1979)
GIH, Gene mutation, human fibroblasts, diphtheria toxin resistance (HF Dip^r) *in vitro*	+	NT	15	Gupta & Goldstein (1981)
TIH, Cell transformation, human foreskin epithelial cells	+	NT	7.5	Milo *et al.* (1981)
DVA, DNA strand breaks, Wistar rat liver *in vivo*	+		500 po × 1	Stewart (1981)
DVA, DNA strand breaks, male CBA mouse skin keratinocytes *in vivo*	+		800 µg/cm^2 skin × 1	Yendle *et al.* (1997)
GVA, Gene mutation, Muta™ Mouse stomach and liver *in vivo*	+		150 po × 1	Brault *et al.* (1996)
MVM, Micronucleus test, B6C3F$_1$ mouse bone marrow *in vivo*	?		~ 80 ip (80% LD_{50}) × 1	Salamone *et al.* (1981)
MVM, Micronucleus test, CD-1 mouse bone marrow *in vivo*	–		46 ip × 2	Tsuchimoto & Matter (1981)
MVM, Micronucleus test, CD-1 mouse hepatocytes *in vivo*	+		27 ip × 2	Cliet *et al.* (1989)
MVM, Micronucleus test, CD-1 mouse bone-marrow cells *in vivo*	–		162 ip × 1	Cliet *et al.* (1993)
MVM, Micronucleus test, CD-1 mouse spermatids *in vivo*	+		54 ip × 1	Cliet *et al.* (1993)
MVM, Micronucleus test, CD-1 mouse splenocytes *in vivo*	+		53.7 ip × 1	Benning *et al.* (1994)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CBA, Chromosomal aberrations, Sprague-Dawley rat bone-marrow cells *in vivo*	(+)		100 iv × 1	Rees *et al.* (1979)
CBA, Chromosomal aberrations, Long-Evans rat bone-marrow cells *in vivo*	(+)		100 iv × 1	Rees *et al.* (1979)
COE, Chromosomal aberrations, C57BL/6J × CBA/Ca F_1 mouse oocytes *in vivo*	+		2 ip × 1	Santalo *et al.* (1987)
COE, Chromosomal aberrations, C57BL/6J × CBA/Ca F_1 mouse embryos *in vivo*	+		2 ip × 1	Santalo *et al.* (1987)
BID, Formation of DNA adducts *in vitro*	+	NT	2559	Chen *et al.* (1981)
BID, Formation of DNA adducts *in vitro*	+	NT	3603	Hemminki (1981)
BID, Formation of DNA adducts *in vitro*	+	NT	18 735	Randerath *et al.* (1981)
BVD, Binding (covalent) to DNA, STS mouse skin *in vivo*	+		~ 1150 μg/cm^2 skin	Colburn & Boutwell (1968)
BVP, Binding (covalent) to RNA and proteins, STS mouse skin *in vivo*	+		~ 1150 μg/cm^2 skin	Colburn & Boutwell (1968)
Apurinic/apyrimidinic site production in SV40 DNA *in vitro*	+	NT	2.0	Drinkwater *et al.* (1980)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; NG, not given; inj, injection; po, oral; ip, intraperitoneal; iv, intravenous; vap, vapour

[c] Freshly isolated rat hepatocytes used for metabolic activation

4.4.3 *Mechanistic considerations*

DNA from skin carcinomas and fibrosarcomas induced in mice by β-propiolactone was tested for its ability to transform NIH3T3 cells by DNA transfection. One of two squamous-cell carcinomas and one of three fibrosarcomas gave positive results in the transfection assay. The transformed phenotype of the positive transfectants was confirmed by the observation of anchorage-independent growth, tumorigenicity in nude mice and secondary transfection. One of the two β-propiolactone-induced squamous-cell skin carcinomas was found to contain an activated H-*ras* oncogene with an A→T transversion at the second nucleotide of codon 61. The mutation was detected in the NIH3T3 transfectant and in the original tumour. The mutation was not seen in the liver of the same animal. The A→T transversion mutation is consistent with a direct miscoding effect of a specific β-propiolactone–DNA adduct (Garte *et al.* 1985; Hochwalt *et al.* 1988).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

The main use of β-propiolactone has been as an intermediate in the production of acrylic acid and its esters. It has also been used for the sterilization of vaccines and blood products.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

β-Propiolactone was tested for carcinogenicity in mice by skin application and subcutaneous or intraperitoneal injection and in rats by inhalation exposure and subcutaneous injection, producing local tumours. The results obtained in studies in hamsters and guinea-pigs were equivocal.

5.4 Other relevant data

β-Propiolactone is a direct-acting alkylating agent. It forms DNA adducts. It is mutagenic in a wide variety of in-vitro and in-vivo systems, both in somatic and germ cells.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of β-propiolactone were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of β-propiolactone.

Overall evaluation

β-Propiolactone is *possibly carcinogenic to humans (Group 2B)*.

6. References

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RESORCINOL

Data were last reviewed in IARC (1977) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

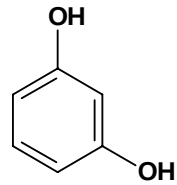
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 108-46-3
Chem. Abstr. Name: 1,3-Benzenediol
IUPAC Systematic Name: Resorcinol
Synonyms: *meta*-Benzenediol; resorcin

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_6H_6O_2$ Relative molecular mass: 110.11

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: White, needle-like crystals with a sweetish taste (Budavari, 1996)
(*b*) *Boiling-point*: 280°C (Budavari, 1996)
(*c*) *Melting-point*: 111°C (Lide, 1997)
(*d*) *Solubility*: Soluble in water, ethanol, diethyl ether and glycerol; slightly soluble in chloroform (Budavari, 1996)
(*e*) *Vapour pressure*: 665 Pa at 138°C; relative vapour density (air = 1), 3.79 (Verschueren, 1996)
(*f*) *Flash point*: 127°C, closed cup (Lewis, 1993)
(*g*) *Explosive limits*: Lower explosive limit (199°C), 1.4% by volume in air (Schmiedel & Decker, 1993)
(*h*) *Conversion factor*: $mg/m^3 = 4.5 \times ppm$

1.2 Production and use

Worldwide production of resorcinol in 1994 was estimated to be 30 000–35 000 tonnes. Countries producing resorcinol include Germany, Italy, Japan, the United Kingdom and the United States (Krumenacker *et al*., 1995).

Resorcinol is used primarily in the rubber industry for tyres and reinforced rubber products (conveyer belts, driving belts) and in high-quality wood adhesives, which are made from resorcinol, phenol and formaldehyde. It is also used in the preparation of dyes and pharmaceuticals, as a cross-linking agent for neoprene and a rubber tackifier, and in cosmetics (Lewis, 1993; Schmiedel & Decker, 1993; Krumenacker *et al.*, 1995).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), as many as 100 000 workers in the United States were potentially exposed to resorcinol (see General Remarks). Occupational exposures to resorcinol may occur in its production, in the manufacture of wood adhesives, rubber, wood products, dyes and pharmaceuticals, and in coal processing.

1.3.2 *Environmental occurrence*

Resorcinol may be released to the environment in waste effluents associated with coal gasification and conversion, coal-tar production and shale oil processing and from the combustion of wood and tobacco. It has been detected in low levels in groundwater samples (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 45 mg/m^3 as the 8-h time-weighted average threshold limit value for occupational exposures to resorcinol in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for resorcinol in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Resorcinol showed no carcinogenic effect in mice when tested by repeated skin application (IARC, 1977).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 60 male and 60 female B6C3F$_1$ mice, seven to eight weeks of age, were administered resorcinol (purity, > 99%) by oral gavage in deionized water at dose levels of 0, 112 or 225 mg/kg bw on five days per week for 104 weeks. Groups of 10 animals of each sex were killed after 15 months. Mean body weights of high-dose female mice were 10–15% lower than those of controls from week 85 to the end of the study, whereas those of the remaining exposed groups were similar to those of the controls. Survival of exposed mice was similar to that of controls. All mice were necropsied and tissues examined histologically. There was no treatment-related increase in the incidence of neoplasms or non-neoplastic lesions (United States National Toxicology Program, 1992).

3.1.2 *Rat*

Groups of 60 male and 60 female Fischer 344 rats, six to seven weeks of age, were administered resorcinol (purity, > 99%) by oral gavage in deionized water on five days per week for 104 weeks at dose levels of 0, 112 or 225 mg/kg bw for males and 0, 50, 100 or 150 mg/kg bw for females. Groups of 10 animals of each sex were killed after 15 months. Mean body weights of high-dose male rats were 10–15% lower than those of the controls from week 87 to the end of the study. Mean body weights of high-dose females were 11–14% lower than those of controls from week 95 to the end of the study. Mean body weights of other exposed rats were similar to those of controls. Survival of high-dose males and females was significantly lower than that of controls. Decreased survival in the high-dose groups was attributed to chemical-related toxicity. All rats were necropsied and tissues were examined histologically. There was no treatment-related increase in the incidence of neoplasms or non-neoplastic lesions (United States National Toxicology Program, 1992).

3.2 Administration with known carcinogens

3.2.1 *Rat*

Groups of 15 male Fischer 344 rats, six weeks of age, were given resorcinol [purity unspecified] at concentrations of 0 or 0.2% in the diet for 22 weeks either after ligation of one ureter to enhance bladder carcinogenesis or after exposure to 0.05% *N*-nitrosobutyl-*N*-(4-hydroxybutyl)amine in the drinking-water for two weeks followed by ureteric ligation to initiate bladder carcinogenesis. All animals were killed at 24 weeks. Resorcinol administration did not affect body weight. Resorcinol alone did not induce bladder lesions and did not increase the incidence of any type of tumour when given after the initiator (Miyata *et al*., 1985).

Groups of 16 male Fischer 344 rats, six weeks of age, were given resorcinol (purity, > 99%) at concentrations of 0 or 0.8% in the diet for 51 weeks. Other groups were given 0 or 0.8% resorcinol in the diet for 51 weeks beginning one week after oral gavage of 50 mg/kg bw *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine to initiate gastric carcinogenesis. Resorcinol reduced the body weights of rats given no initiator and of those given initiator.

Resorcinol alone induced a low incidence of mild hyperplasia in the forestomach, but no forestomach tumours. Resorcinol given after the initiator did not increase the incidence of forestomach papillomas or squamous-cell carcinomas induced by the initiator (Hirose *et al*., 1989).

Groups of 9–11 male Sprague-Dawley rats, weighing 200 g, were given 0 or 100 mg/kg resorcinol (purity, > 99%) in the diet for six weeks beginning one week after partial hepatectomy and intraperitoneal injection of 300 mg/kg bw *N*-nitrosodiethylamine to initiate liver carcinogenesis. Resorcinol treatment after initiation did not increase the multiplicity of enzyme-altered (γ-glutamyltranspeptidase) foci (Stenius *et al*., 1989).

Groups of 11–12 male Fischer 344 rats, five weeks of age, were given resorcinol (purity, > 99%) at concentrations of 0 or 0.8% in the diet for 49 weeks either alone or one week after intraperitoneal injection of *N*-nitrosomethyl-*n*-amylamine to initiate upper digestive tract carcinogenesis. Resorcinol given after the initiator reduced body weight gain compared with rats given initiator and increased kidney weights. In the group given resorcinol after initiation, the incidence of papillomas of the tongue was increased to 6/12 ($p < 0.05$) compared with 1/11 in the group given initiator and incidence of oesophageal carcinoma was increased to 7/12 ($p < 0.01$) compared with 0/11 in controls. The incidence of lung alveolar cell hyperplasia was reduced (Yamaguchi *et al*., 1989). [The Working Group noted that no data were reported on the group given resorcinol only.]

Groups of 10 or 20 male Fischer 344 rats, six weeks of age, were given resorcinol (purity, > 99.5%) at concentrations of 0 or 0.8% in the diet for 36 weeks either alone or after exposure to 0.05% *N*-nitrosobutyl-*N*-(4-hydroxybutyl)amine in the drinking-water for four weeks to initiate bladder carcinogenesis. Resorcinol did not affect body or bladder weight either when given alone or after initiator. Resorcinol exposure alone did not induce bladder lesions. Feeding of resorcinol after initiation did not increase the incidence or multiplicity of bladder neoplasms resulting from initiation (Kurata *et al*., 1990).

Groups of 15 or 20 six-week-old male Wistar/Crj rats were given resorcinol [purity unspecified] at concentrations of 0 or 0.8% in the diet for 36 weeks alone or starting one week after exposure to 0.1% *N*-nitrosoethyl-*N*-hydroxyethylamine in the drinking-water for three weeks to initiate liver and kidney carcinogenesis. The final body weights of rats given resorcinol were lower than those of rats given basal diet alone or with initiator. Resorcinol also increased the relative liver and kidney weights. Resorcinol treatment after initiation did not affect the incidence of liver or kidney tumours induced by the initiator, but reduced the multiplicity of liver tumours (Okazaki *et al*., 1993).

3.2.2 *Hamster*

Groups of 10 or 20 female Syrian golden hamsters were given resorcinol (purity, > 99.5%) at concentrations of 0 or 1.5% in the diet for 16 weeks alone or after two subcutaneous injections of 70 mg/kg bw *N*-nitrosobis(2-oxopropyl)amine to initiate pancreatic carcinogenesis. Resorcinol given either alone or after the initiator did not affect body weight gain or pancreas weight; when given after the initiator, it did not affect liver weight, but when resorcinol was given alone, liver weight was reduced. Resorcinol alone

did not induce pancreatic neoplasms. When given after the initiator, it did not affect the incidence of pancreatic adenocarcinomas, but reduced the incidence of atypical ductal hyperplasia. Resorcinol alone did not induce liver lesions. When given after the initiator, it did not affect the incidence of liver carcinomas or cholangiocarcinomas or gall-bladder carcinomas induced by the initiator. In the forestomach and glandular stomach, resorcinol alone or after initiator increased the frequency of epithelial hyperplasia [data not given] (Maruyama *et al*., 1991).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, excretion and metabolism

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

The pharmacokinetics of [^{14}C]resorcinol were studied after subcutaneous injection (single dose of 10–100 mg/kg bw, or repeated dosing at 100 mg/kg bw per day) in Sprague-Dawley rats (Merker *et al*., 1982). Since most measurements were of radioactivity, no distinction was made between resorcinol and its metabolites. The radioactivity was very rapidly cleared, mainly in urine as conjugates. Repeated administration of resorcinol did not change its elimination characteristics as judged by radioactivity.

4.2 Toxic effects

The toxicity of resorcinol has been reviewed (GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance (BUA), 1993).

4.2.1 *Humans*

Concentrated solutions of resorcinol are irritating to the skin and ingestion of large doses may induce hypothermia, hypotension, decreased respiration, tremors, icterus and haemoglobinuria in children (IARC, 1977).

Several cases of serious and even fatal intoxication after dermal application of resorcinol have been described. Methaemoglobinaemia, haemolysis and convulsions have often been noted (Cunningham, 1956; Lundell & Nordman, 1973; Bontemps *et al*., 1995). Resorcinol is a rare cause of allergic contact dermatitis (Vilaplana *et al*., 1991; Pecegueiro, 1992; Serrano *et al*., 1992; Massone *et al*., 1993) and may also induce generalized eczema, urticaria and angioneurotic oedema (Barbaud *et al*., 1996).

4.2.2 *Experimental systems*

In a 17-day gavage study (27.5–450 mg/kg bw per day) in Fischer 344/N rats, hyper-excitability was observed in males at doses of ≥ 225 mg/kg bw and in females at levels

of ≥ 55 mg/kg bw, and tachypnoea at levels of 450 and 110 mg/kg bw, respectively; no gross or microscopic lesions attributable to resorcinol were observed. In a 13-week study at dose levels of 32–520 mg/kg bw per day, no gross or microscopic lesions or haematological or clinical chemistry changes were observed in rats. However, all rats given the highest dose died, and there was mortality also at the dose of 260 mg/kg bw per day (United States National Toxicology Program, 1992).

In the two-year carcinogenicity study (see Section 3.1.2) with dose levels of 112 and 225 mg/kg bw per day, no gross or microscopic lesions were observed in rats, although survival of male and female rats was significantly lower than that of the controls in the high-dose groups (225 and 150 mg/kg bw per day, respectively) (United States National Toxicology Program, 1992).

In mice, no treatment-related gross or microscopic lesions, or haematological or clinical chemistry changes were observed in 17-day (≤ 600 mg/kg bw per day), 13-week (≤ 420 mg/kg bw per day) or two-year (112 or 225 mg/kg bw per day) studies. Survival was decreased at the highest dose in 17-day and 13-week studies, but not in the long-term study (United States National Toxicology Program, 1992).

Administration of resorcinol (0.25% in the diet) for 20 weeks did not induce hyperplasia or papillomatous lesions in the forestomach in Syrian golden hamsters. Labelling index, after an intraperitoneal dose of [*methyl*-^{3}H]thymidine, was not elevated in the pyloric region, forestomach or urinary bladder (Hirose *et al*., 1986).

In male Fischer rats, oral administration of resorcinol for eight weeks (0.8% in the diet) did not induce hyperplasia or DNA synthesis, as measured by a bromodeoxyuridine-labelling index, in the forestomach epithelium. No cell proliferation, increased DNA synthesis or increase in pepsinogen-altered neoplastic foci was observed in the pyloric mucosa (Shibata *et al*., 1990).

Resorcinol (≤ 1.0 μg/mL) had no effect on the elimination by apoptosis of G418-resistant, transformed Swiss 3T3 MxCl1 cells by co-cultured TGF-β-treated C3H 10T½c8 cells (Schaefer *et al*., 1995).

Resorcinol (200 mg/kg bw as a single oral dose) administered to male Sprague-Dawley rats did not affect the urinary excretion of malonaldehyde, did not increase hepatic ornithine decarboxylase activity and at 0.3 mmol/L did not induce depletion of glutathione content in isolated hepatocytes (Stenius *et al*., 1989).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Resorcinol (125, 250 or 500 mg/kg bw per day) administered to rats by gavage on days 6 through 15 of the gestation induced no embryotoxicity or fetal external, visceral or skeletal anomalies in the fetuses; no toxicity to the dams was observed (DiNardo *et al*., 1985). No embryotoxicity or teratogenic effects were observed in rats (40, 80 or 250

mg/kg bw per day, days 6 through 15 of gestation) or in rabbits (25, 50 or 100 mg/kg bw per day, days 6 through 18 of gestation) in a study reported as an abstract (Spengler *et al*., 1986).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Resorcinol did not induce gene mutations in *Salmonella typhimurium* or in *Escherichia coli* strains in either the presence or absence of an exogenous metabolic activation system.

In-vitro exposure to resorcinol did not induce sister chromatid exchanges either in Chinese hamster ovary CHO cells or in human lymphocytes. Chromosomal aberrations were induced *in vitro* in human lymphocytes and amniotic cells but not in CHO cells or human fibroblasts.

In rats *in vivo*, neither sister chromatid exchanges nor micronuclei were induced in bone marrow.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to resorcinol may occur in its production, in the manufacture of adhesives, rubber, wood products, dyes and pharmaceuticals. It has been detected at low levels in groundwater and occurs in wood smoke and tobacco smoke.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Resorcinol was tested for carcinogenicity in one experiment in mice and in one experiment in rats by oral administration. It was also tested in mice by skin application. No carcinogenic effect was observed in these experiments.

In several experiments in rats and hamsters, resorcinol was tested for promoting activity after initiation by known carcinogens. It did not enhance the incidence of tumours of the bladder, forestomach, liver or kidney.

In one study, resorcinol increased the incidence of tongue and oesophageal tumours after initiation with *N*-nitrosomethyl-*n*-amylamine.

Table 1. Genetic and related effects of resorcinol

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500	JETOC (1997)
SA2, *Salmonella typhimurium* TA102, reverse mutation	–	–	2500	JETOC (1997)
SA4, *Salmonella typhimurium* TA104, reverse mutation	–	–	2500	JETOC (1997)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500	JETOC (1997)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500	JETOC (1997)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	JETOC (1997)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	–	–	2500	JETOC (1997)
ECW, *Escherichia coli* WP2 *uvrA*/pKM 101, reverse mutation	–	–	2500	JETOC (1997)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–[c]		11 000 ppm feed	Foureman *et al*. (1994)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	250	McGregor *et al*. (1988)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	2035	Darroudi & Natarajan (1983)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	2035	Darroudi & Natarajan (1983)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	NT	125	Darroudi & Natarajan (1983)
CHF, Chromosomal aberrations, human fibroblasts *in vitro*	–	NT	64	Darroudi & Natarajan (1983)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	25	Darroudi & Natarajan (1983)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	80	Schulz *et al*. (1982)
CHL, Chromosomal aberrations, human trisomy 21-lymphocytes *in vitro*	+	NT	80	Schulz *et al*. (1982)
CIH, Chromosomal aberrations, amniotic cells *in vitro*	+	NT	40	Schulz *et al*. (1982)

Table 1 (contd)

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SVA, Sister chromatid exchange, Sprague-Dawley rat bone-marrow cells *in vivo*	–		100 ip × 1	Bracher *et al*. (1981)
SVA, Sister chromatid exchange, Sprague-Dawley rat bone-marrow cells *in vivo*	–		100 po × 1	Bracher *et al*. (1981)
SVA, Sister chromatid exchange, Sprague-Dawley rat bone-marrow cells *in vivo*	–		200 topical × 1	Bracher *et al*. (1981)
MVM, Micronucleus test, mouse bone-marrow cells *in vivo*	–		300 ip × 1	Darroudi & Natarajan (1983)

[a] +, positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; po, oral; ip, intraperitoneal
[c] Inconclusive results with injection of 12 000 ppm resorcinol

5.4 Other relevant data

Resorcinol is water-soluble and readily conjugated and eliminated. The chemical has no known potential for formation of electrophilic reactive intermediates comparable to those derived from the other dihydroxybenzenes. Resorcinol was tested in various genetic toxicology assays, including in-vitro bacterial and mammalian assays and in-vivo mammalian assays. It gave negative results in all studies, with the exception of a positive response in the two in-vitro studies that assessed chromosomal aberrations in human lymphocytes from whole blood cultures; however, resorcinol did not induce chromosomal aberrations in human fibroblasts.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of resorcinol were available.

There is *inadequate evidence* in experimental animals for the carcinogenicity of resorcinol.

Overall evaluation

Resorcinol is *not classifiable as to its carcinogenicity to humans (Group 3)*.

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1,1,1,2-TETRACHLOROETHANE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

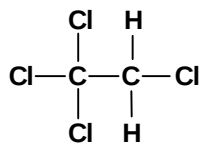
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 630-20-6
Chem. Abstr. Name: 1,1,1,2-Tetrachloroethane
IUPAC Systematic Name: 1,1,1,2-Tetrachloroethane
Synonym: (Chloromethyl)trichloromethane

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2H_2Cl_4$ Relative molecular mass: 167.85

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid (United States National Library of Medicine, 1997)
(*b*) *Boiling-point*: 130.5°C (Lide, 1995)
(*c*) *Melting-point*: –70.2°C (Lide, 1995)
(*d*) *Solubility*: Slightly miscible with water (1.1 g/L at 25°C); miscible in acetone, benzene, chloroform, diethyl ether and ethanol (Lide, 1995; United States National Library of Medicine, 1997)
(*e*) *Vapour pressure*: 1.9 kPa at 25°C (United States National Library of Medicine, 1997)
(*f*) *Conversion factor*: $mg/m^3 = 6.87 \times ppm$

1.2 Production and use

1,1,1,2-Tetrachloroethane has been used as a solvent and in the manufacture of insecticides, herbicides, soil fumigants, bleaches, other chlorocarbon solvents and paints and

varnishes (United States National Library of Medicine, 1997). It is present as an unisolated intermediate in some processes for the manufacture of trichloroethylene and tetrachloroethylene from 1,2-dichloroethane (IARC, 1986).

1.3 Occurrence

1.3.1 *Occupational exposure*

No data were available to the Working Group.

1.3.2 *Environmental occurrence*

Although 1,1,1,2-tetrachloroethane apparently is not produced or used commercially in large quantities, it may be formed incidentally during the manufacture of other chlorinated ethanes and released into the environment as air emissions or in wastewater. It has been detected at low levels in urban air, ambient air, drinking-water, ambient water, groundwater, wastewater and soil samples (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not recommended a threshold limit value for occupational exposures to 1,1,1,2-tetrachloroethane in workplace air.

No international guideline for 1,1,1,2-tetrachloroethane in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

1,1,1,2-Tetrachloroethane was tested for carcinogenicity by oral gavage in one study in mice and one study in rats. An increased incidence of hepatocellular adenomas was observed in mice of each sex and of hepatocellular carcinomas in females. The experiment in male rats gave negative results and that in female rats was inconclusive (IARC, 1986).

3.1 Oral administration

Rat: In a rat liver foci assay for tumour-initiating activity, groups of 10 male Osborne-Mendel rats were subjected to two-thirds partial hepatectomies and, 24 h later, were given 1,1,1,2-tetrachloroethane by gavage at the maximum tolerated dose (MTD) in corn oil. Six days after partial hepatectomy, the rats received 0.05% phenobarbital in

the diet for seven weeks, then control diets for seven further days, after which they were killed and their livers examined. The numbers of enzyme-altered foci in the liver were 0.77 ± 0.34 and 0.26 ± 0.19 foci/cm^2 (mean ± standard error) in the test and control (corn oil) groups, respectively. It was concluded that 1,1,1,2-tetrachloroethane did not show initiating activity in this system (Milman *et al*., 1988).

In a promotion study, groups of 10 rats were given an intraperitoneal injection of 30 mg/kg bw *N*-nitrosodiethylamine (NDEA) 24 h after a two-thirds partial hepatectomy. Six days later, the rats received 1,1,1,2-tetrachloroethane in corn oil at the MTD by gavage on five days per week for seven weeks. The rats were held for an additional seven days and then killed and the livers were examined. The numbers of enzyme-altered foci were 1.68 ± 0.44 foci/cm^2 in the treated group and 1.77 ± 0.49 foci/cm^2 in the control (corn oil) group. No promoting activity was observed (Milman *et al*., 1988).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

The metabolism of 1,1,1,2-tetrachloroethane and related chloroethanes has been reviewed (IARC, 1986).

In mice given a subcutaneous dose of 1.2–2.0 g/kg bw 1,1,1,2-tetrachloroethane, 21–62% was eliminated unchanged in exhaled air within 72 h. The major urinary metabolites in mice, rats, rabbits and guinea-pigs were trichloroethanol and its glucuronide conjugate; trichloroacetic acid was also excreted (IARC, 1986).

In the presence of oxygen, NADPH and rat liver microsomes, 1,1,1,2-tetrachloroethane undergoes little dechlorination. In contrast, NADPH-dependent reductive metabolism of 1,1,1,2-tetrachloroethane by hepatic microsomal fractions from rats yields 1,1-dichloroethylene as the major metabolite and 1,1,2-trichloroethane as a minor metabolite (IARC, 1986).

4.2 Toxic effects

The toxicity of 1,1,1,2-tetrachloroethane has been reviewed (Luotamo & Riihimäki, 1996).

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Short- and long-term administration of 1,1,1,2-tetrachloroethane induced hepatic damage; in long-term studies, central nervous system effects and renal mineralization were also observed (IARC, 1986).

When 1,1,1,2-tetrachloroethane was administered to male Fischer 344/N rats by gavage at 0.62 or 0.124 mmol/kg once daily for 21 days, hyaline nephropathy, consisting of hyaline droplet accumulation, and an increased incidence of tubule regeneration were observed. Granular casts and an increased proliferating cell nuclear antigen labelling index were observed at the higher dose level (United States National Toxicology Program, 1996).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

1,1,1,2-Tetrachloroethane induced reverse but not forward mutation in *Salmonella typhimurium*: one of two studies reported that 1,1,1,2-tetrachloroethane induced mutations in strain TA100 and TA98 in the presence or absence of exogenous metabolic activation. A weak mutagenic response was reported for strain TA104 and results for strain TA97 were positive in the presence of exogenous metabolic activation. 1,1,1,2-Tetrachloroethane induced recombination but not mutation or aneuploidy in *Saccharomyces cerevisiae* and induced genetic crossing-over and aneuploidy in *Aspergillus nidulans* in the absence of metabolic activation. Sex-linked recessive lethal mutations were not induced in *Drosophila melanogaster*.

1,1,1,2-Tetrachloroethane induced gene mutations in the mouse lymphoma $tk^{+/-}$ assay only in the presence of an exogenous metabolic activation system. It did not increase the frequency of chromosomal aberrations in Chinese hamster lung fibroblasts or ovary cells but did induce sister chromatid exchanges in Chinese hamster ovary cells and aneuploidy in Chinese hamster lung fibroblasts in the absence of exogenous activation. 1,1,1,2-Tetrachloroethane did not induce cell transformation in BALB/c-3T3 cells.

One study reported that 1,1,1,2-tetrachloroethane covalently bound to DNA in rat and mouse lung, liver, kidney and stomach following a single treatment by intraperitoneal injection.

Table 1. Genetic and related effects of 1,1,1,2-tetrachloroethane

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SAF, *Salmonella typhimurium*, forward mutation, arabinose resistance	–	–	150	Roldán-Arjona *et al*. (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	166	Haworth *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	5	Strobel & Grummt (1987)
SA4, *Salmonella typhimurium* TA104, reverse mutation	(+)	(+)	25	Strobel & Grummt (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	166	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	166	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	166	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	125	Strobel & Grummt (1987)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	+	5	Strobel & Grummt (1987)
SCG, *Saccharomyces cerevisiae* strain D7, gene conversion, *trp* locus	+	–	168	Bronzetti *et al*. (1989)
ANG, *Aspergillus nidulans* strain P1, genetic crossing-over	+	NT	400	Crebelli *et al*. (1988)
SCR, *Saccharomyces cerevisiae*, reverse mutation, *ilv* locus	–	–	1679	Bronzetti *et al*. (1989)
SCN, *Saccharomyces cerevisiae* strain D61.M, aneuploidy	–	NT	1340	Whittaker *et al*. (1990)
ANN, *Aspergillus nidulans* strain P1, aneuploidy	+	NT	200	Crebelli *et al*. (1988)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		1500 µg/mL inj	Foureman *et al*. (1994)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	+	200	McGregor *et al*. (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	?	200	Sofuni *et al*. (1996)
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	–	248	Galloway *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	–	506	Galloway *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster lung fibroblasts *in vitro*	–	–	200	Matsuoka *et al*. (1996)
AIA, Aneuploidy, Chinese hamster lung fibroblasts *in vitro*	+	+	100	Matsuoka *et al*. (1996)
TBM, Cell transformation, BALB/c-3T3 mouse cells	–	NT	250	Tu *et al*. (1985)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
BID, Binding (covalent) to calf thymus DNA *in vitro*	NT	+	9.6	Colacci *et al*. (1989)
BVD, Binding (covalent) to DNA, BALB/c mouse lung, liver, kidney and stomach *in vivo*	+		1.46 ip × 1	Colacci *et al*. (1989)
BVD, Binding (covalent) to DNA, Wistar rat lung, liver, kidney and stomach *in vivo*	+		1.46 ip × 1	Colacci *et al*. (1989)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested; ?, inconclusive
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw /day; inj, injection; ip, intraperitoneal

5. Summary of Data Reported and Evaluation

5.1 Exposure data

1,1,1,2-Tetrachloroethane is an intermediate in one process for the manufacture of trichloroethylene and tetrachloroethylene and has been reported to occur as an impurity in these widely used products. It has been detected at low levels in ambient air and in drinking-water.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

1,1,1,2-Tetrachloroethane was tested for carcinogenicity by oral administration by gavage in one study in mice and one study in rats. An increased incidence of hepatocellular adenomas was observed in mice of each sex and of hepatocellular carcinomas in females. The experiment in male rats gave negative results and that in female rats was inconclusive. In one small experiment in rats, no initiating or promoting activity of 1,1,1,2-tetrachloroethane was demonstrated.

5.4 Other relevant data

In a single study, 1,1,1,2-tetrachloroethane bound covalently to DNA in rats and mice *in vivo*. It induced gene mutations, sister chromatid exchanges and aneuploidy, but not chromosomal aberrations, in rodent cell cultures. It did not induce sex-linked recessive mutation in *Drosophila* or mutations or aneuploidy in yeast. 1,1,1,2-Tetrachloroethane induced gene conversion in yeast, genetic crossing-over and aneuploidy in fungus and gene mutations in bacteria.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of 1,1,1,2-tetrachloroethane were available.

There is *limited evidence* in experimental animals for the carcinogenicity of 1,1,1,2-tetrachloroethane.

Overall evaluation

1,1,1,2-Tetrachloroethane is *not classifiable as to its carcinogenicity to humans (Group 3)*.

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TETRAFLUOROETHYLENE

Data were last reviewed in IARC (1979) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

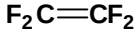
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 116-14-3
Chem. Abstr. Name: Tetrafluoroethene
IUPAC Systematic Name: Tetrafluoroethylene
Synonyms: Perfluoroethene; perfluoroethylene; 1,1,2,2-tetrafluoroethylene

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_2F_4 Relative molecular mass: 100.02

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless gas (Lewis, 1993)
(*b*) *Boiling-point*: –75.9°C (Lide, 1997)
(*c*) *Melting-point*: –142.5°C (Lide, 1997)
(*d*) *Solubility*: Insoluble in water (Lide, 1997)
(*e*) *Reactivity*: May polymerize in the absence of an inhibitor, especially when heated or in the presence of oxygen (United States National Library of Medicine, 1998)
(*f*) *Explosive limits*: Upper, 60%; lower, 11% by volume in air (United States National Library of Medicine, 1998)
(*g*) *Conversion factor*: $mg/m^3 = 4.1 \times ppm$

1.2 Production and use

Worldwide production of tetrafluoroethylene in 1977 was estimated to have been 15–20 thousand tonnes. No information on current production quantities was available to the Working Group. Information available in 1995 indicated that it was produced in Germany and the United States (Chemical Information Services, 1995).

Tetrafluoroethylene has been used primarily as a monomer for polytetrafluoroethylene and other fluorinated resins, and as a chemical intermediate for hexafluoropropylene synthesis (United States National Library of Medicine, 1998).

1.3 Occurrence

No data were available to the Working Group.

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not proposed any occupational exposure limit for tetrafluoroethylene in workplace air. Russia has a short-term exposure limit of 30 mg/m^3 for exposure in workplace air (International Labour Office, 1991).

No international guideline for tetrafluoroethylene in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3.1 Inhalation exposure

3.1.1 *Mouse*

Groups of 58 male and 58 female B6C3F$_1$ mice, seven weeks of age, were administered tetrafluoroethylene (purity, > 98%) by whole-body inhalation at concentrations of 0, 312, 625 or 1250 ppm [0, 1280, 2560 or 5125 mg/m^3] for 6 h per day on five days per week for 95–96 weeks. Ten male and 10 female mice from each exposure group were evaluated at 15 months. Survival in all of the dose groups of mice was significantly reduced, so that, at 90 weeks, the survivors in the control, low-, mid- and high-dose groups, respectively, were: males—41/48, 17/48, 9/48, 7/48; females—38/48, 20/48, 18/48, 10/48. All mice, except one in each of the middle and high-dose groups of females, were necropsied and tissues were examined histologically. At the 15-month evaluation, haemangiosarcomas in the liver were found in three males of the high-dose group and one female of the low-dose group. At termination, the incidences of haemangiosarcomas in the liver in the control, low-, mid- and high-dose groups, respectively, were: males—0/48, 21/48, 27/48, 37/48 ($p \leq 0.01$ for all dose groups, Fisher's exact test); and females—0/48, 27/48, 27/47, 34/47 ($p \leq 0.01$ for all dose groups). The incidences of hepatocellular adenomas were: males—17/48, 17/48, 12/48, 20/48; and females—15/48, 17/48, 20/47 ($p \leq 0.05$), 15/47. The incidences of hepatocellular carcinomas were, for males, 11/48, 20/48, 33/48, 26/48 ($p \leq 0.01$ for all dose

groups) and, for females, 4/48, 28/48, 22/47, 20/47 ($p \leq 0.01$ for all dose groups). The incidences of histiocytic sarcomas (all organs combined) also were increased as follows: males—0/48, 12/48, 7/48, 7/48 ($p < 0.001$ for all dose groups, life table test); and females—1/48, 21/48, 19/47, 18/47 ($p < 0.001$ for all dose groups, life table test) (United States National Toxicology Program, 1997).

3.1.2 *Rat*

Groups of 60 male Fischer 344 rats, seven weeks of age, were administered tetrafluoroethylene (purity, > 98%) by whole-body inhalation at concentrations of 0, 156, 312 or 625 ppm [0, 640, 1280 or 2560 mg/m^3] and groups of 60 female Fischer 344 rats were administered concentrations of 0, 312, 625 or 1250 ppm [0, 1280, 2560 or 5125 mg/m^3] for 6 h per day on five days per week for 104 weeks. Ten male and 10 female rats from each exposure group were evaluated at 15 months. Survival in the high-dose male rats was significantly reduced, so that, at two years, the survivors in the control, low-, mid- and high-dose groups, respectively, were: males—17/50, 12/50, 17/50 and 1/50; females—28/50, 16/50, 15/50 and 18/50. Mean body weights in high-dose males were reduced from week 81 until the end of the study, while there was a marginal reduction in body weight in females of the high-dose group at the end of the study. All rats were necropsied and tissues examined histologically. The incidences of renal tubule neoplasms in the control, low-, mid- and high-dose groups, respectively, were: males—adenomas, 2/50, 4/50, 9/50 ($p \leq 0.05$, Fisher's exact test), 13/50 ($p \leq 0.01$); carcinomas, 1/50, 1/50, 2/50, 0/50; and, females—adenomas, 0/50, 3/50, 3/50, 8/50 ($p \leq 0.01$); carcinomas, 0/50, 0/50, 0/50, 3/50. Liver neoplasms were also increased in both males and females. The incidences were: males—hepatocellular carcinomas only, 1/50, 1/50, 10/50 ($p \leq 0.01$), 3/50; hepatocellular adenomas and carcinomas combined, 4/50, 7/50, 15/50, 8/50; and females—hepatocellular carcinomas only, 0/50, 4/50 ($p \leq 0.05$), 9/50 ($p \leq 0.01$), 2/50; hepatocellular adenomas and carcinomas combined, 0/50, 7/50, 12/50, 8/50 (United States National Toxicology Program, 1997).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

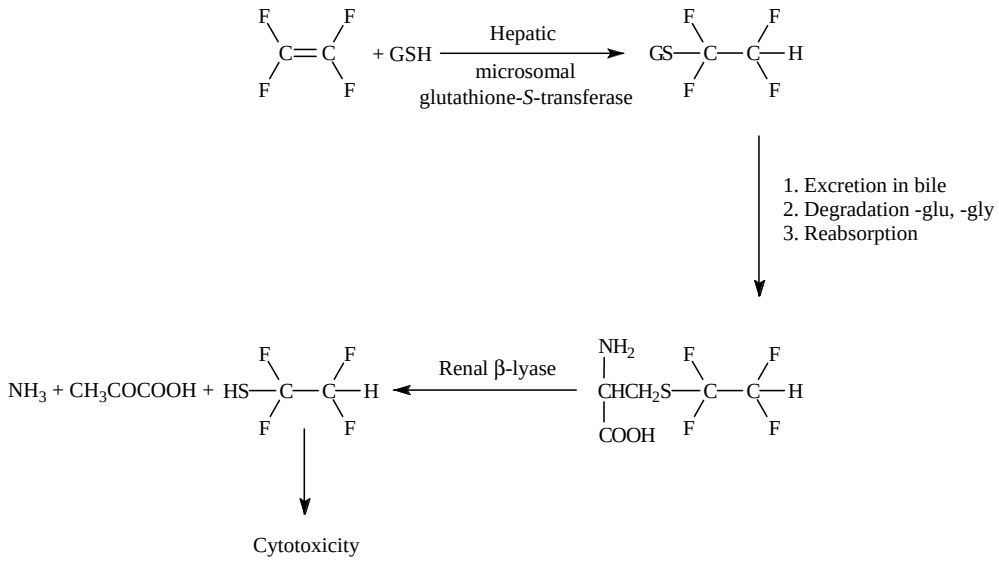
No data were available to the Working Group.

4.1.2 *Experimental systems*

Male rats exposed to 14 000 mg/m^3 tetrafluoroethylene in air for 30 min excreted small amounts of fluoride ion in the urine over a 14-day period, indicating that metabolism can occur (IARC, 1979).

The metabolism of tetrafluoroethylene has been studied in rat liver fractions; both microsomal and cytosolic glutathione *S*-transferases catalyse the formation of *S*-(1,1,2,2-tetrafluoroethyl)glutathione. The rate with microsomes was four times greater than with cytosol. Fluoride ion release was equivalent to approximately 20% of the glutathione used. The quantities of glutathione used and of fluoride ion released in incubations were identical whether cytochrome P450 was active or had been inactivated with carbon monoxide. Thus, cytochrome P450 oxidation, a pathway common to many haloalkenes, does not appear to be involved in the metabolism of tetrafluoroethylene. Evidence for the glutathione conjugation pathway *in vivo* comes from the identification of the cysteinyl-glycine and cysteine conjugates of tetrafluoroethylene in rat bile, following oral administration of L-[^{35}S]cysteine and then inhalation exposure to 6000 ppm [24 600 mg/m^3] tetrafluoroethylene for 6 h. *S*-(1,1,2,2-Tetrafluoroethyl)-L-cysteine is metabolized *in vitro* in rat renal cortex slices to give pyruvate and ammonia, a reaction that is catalysed by β-lyase and which also generates a reactive thiol (Figure 1) that is probably important in renal cytotoxicity (Odum & Green, 1984; Green & Odum, 1985).

Figure 1. Proposed mechanism for the metabolic activation of tetrafluoroethylene



From Odum and Green (1984)

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

No gross lesions were noted in any of the organs of male rats exposed to 14 000 mg/m^3 tetrafluoroethylene in air for 30 min (IARC, 1979).

In male Alderley Park rats exposed to a tetrafluoroethylene atmosphere of 6000 ppm [24 600 mg/m^3] for 6 h, there was marked renal necrosis involving the pars recta of the proximal tubules (Odum & Green, 1984). In addition, a review of largely unpublished data on the toxicity of tetrafluoroethylene (Kennedy, 1990) indicates that, in rats exposed to 2500 ppm [10 250 mg/m^3] for 6 h per day on five days per week for two weeks, or to 2000 ppm [8200 mg/m^3] for 6 h per day on five days per week for 18 weeks, there was decreased body weight gain and renal proximal tubule damage, which was more severe after the longer-duration exposure. Renal toxicity was also observed in Fischer 344/N rats in the 16-day and 13-week studies preliminary to the carcinogenicity test described above (Section 3.1.2) at 625 ppm [2560 mg/m^3] and higher concentrations; the damage was located predominantly at the corticomedullary junction. In the 13-week study, liver weights were increased in both male and female rats exposed to 5000 ppm [20 500 mg/m^3]. In the two-year carcinogenicity study, increases in renal degeneration were observed in male rats at 156 ppm [640 mg/m^3] and in female rats at 625 ppm and increases in renal hyperplasia were observed in both male and female rats at 625 ppm (United States National Toxicology Program, 1997).

According to the review by Kennedy (1990), Syrian hamsters exposed to 2500 ppm [10 250 mg/m^3] for 6 h per day on five days per week for two weeks or to 2000 ppm [8200 mg/m^3] for 6 h per day on five days per week for 18 weeks showed no sign of renal toxicity, but there was testicular atrophy. No sign of toxicity was observed in dogs exposed to 1000 ppm [4100 mg/m^3] for 6 h per day on five days per week for six weeks (reviewed by Kennedy, 1990).

In the 16-day toxicity study with B6C3F$_1$ mice preliminary to the carcinogenicity study described above (Section 3.1.1), there were increases in liver weight of female mice exposed to 2500 ppm [10 250 mg/m^3] or more and in kidney weight of females exposed to 5000 ppm [20 500 mg/m^3]. Renal tubule karyomegaly was observed, mainly in the inner cortex, of males and females exposed to 1250 ppm [5125 mg/m^3] or more. Karyomegaly was observed in the same region in the subsequent 13-week study at the same concentrations. In the two-year carcinogenicity test, renal tubule karyomegaly was increased at 625 ppm in male mice and at 1250 ppm in female mice. In the same study, liver angiectasis was increased at or above 312 ppm in both male and female mice, while there was increased liver and spleen haematopoietic cell proliferation in female mice at these dose levels (United States National Toxicology Program, 1997).

Identical clinical evidence of nephrotoxicity comes from inhalation of tetrafluoroethylene at atmospheric concentrations greater than 2000 ppm [8200 m/gm^3] and from oral administration of tetrafluoroethylcysteine to male rats, suggesting that the latter is an important metabolite in the cytotoxic response. The cytotoxicity of the cysteine conjugate has also been demonstrated *in vitro* in rat kidney slices by reduced uptake of both

organic anion, *para*-aminohippuric acid and the cation, tetraethylammonium bromide (Odum & Green, 1984; Green & Odum, 1985).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

No increases were observed in the frequency of micronucleated erythrocytes from the peripheral blood of mice exposed by inhalation for 13 weeks. This appears to be the only study with tetrafluoroethylene itself. However, *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine was not mutagenic to *Salmonella typhimurium* in either the presence or absence of an exogenous metabolic system based upon rat kidney S9.

Modifications to oncogenes and tumour-suppressor genes

The mutational spectrum of H-*ras* codon 61 was examined in hepatocellular neoplasms of male and female B6C3F$_1$ mice exposed to tetrafluoroethylene in the carcinogenicity study described in Section 3. A low frequency (9/59, 15%) of H-*ras* mutations was detected among the tumours from the treated groups, compared with a high frequency in the concurrent controls (10/17, 59%) and in historical controls (183/333, 56%). The proportion of H-*ras* mutations in the tumours from the treated groups was 1:1:1 for codon 61 AAA, CGA and CTA mutations, respectively, compared with 3:6:1 for the concurrent control tumours and 5:2:1 in the historical control tumours. There were no important differences in the mutation frequencies and spectra between hepatocellular adenomas and carcinomas. No K-*ras* mutations were found. The decreased frequency of H-*ras* mutations in hepatic tumours of the exposed group in this study was similar to that found in B6C3F$_1$ mice exposed to tetrachloroethylene (24%) (Anna *et al*., 1994). The data suggest that the tumours arise by a *ras*-independent pathway (United States National Toxicology Program, 1997).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Tetrafluoroethylene is used in the manufacture of polytetrafluoroethylene and other polymers. No information on potential human exposure is available.

5.2 Human carcinogenicity data

No data were available to the Working Group.

Table 1a. Genetic and related effects of tetrafluoroethylene

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVM, Micronucleus test, $B6C3F_1$ mouse peripheral erythrocytes *in vivo*	–		5000 ppm, 6 h/d, 5 d/wk, 13 wk	US National Toxicology Program (1997)

Table 1b. Genetic and related effects of *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	250	Green & Odum (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	250	Green & Odum (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	250	Green & Odum (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	250	Green & Odum (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250	Green & Odum (1985)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	250	Green & Odum (1985)

[a] –, negative
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day

5.3 Animal carcinogenicity data

Tetrafluoroethylene was tested for carcinogenicity in one study in mice and one study in rats by inhalation. In both sexes of mice, it increased the incidence of hepatocellular carcinomas, histiocytic sarcomas and haemangiosarcomas in the liver. In rats of both sexes, it increased the incidence of hepatocellular carcinomas and kidney tubule cell adenomas.

5.4 Other relevant data

Tetrafluoroethylene is metabolized by hepatic glutathione *S*-transferase and the resulting cysteine conjugate is further metabolized by renal β-lyase. This pathway results in the formation of a reactive thiol that causes kidney toxicity in rats.

Tetrafluoroethylene did not induce micronuclei in mouse erythrocytes and the metabolite tetrafluoroethylcysteine was not mutagenic in *Salmonella typhimurium*.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of tetrafluoroethylene were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of tetrafluoroethylene.

Overall evaluation

Tetrafluoroethylene is *possibly carcinogenic to humans (Group 2B).*

6. References

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United States National Library of Medicine (1998*) Hazardous Substances Data Bank (HSDB)*, Bethesda, MD [Record No. 844]

WHO (1993) *Guidelines for Drinking Water Quality*, 2nd Ed., Vol. 1, *Recommendations*, Geneva

1,1,2-TRICHLOROETHANE

Data were last evaluated in IARC (1991).

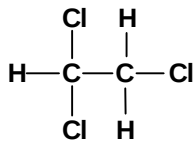
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 79-00-5
Chem. Abstr. Name: 1,1,2-Trichloroethane
IUPAC Systematic Name: 1,1,2-Trichloroethane
Synonym: Vinyl trichloride

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2H_3Cl_3$ Relative molecular mass: 133.41

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid with pleasant odour (Budavari, 1996)
(*b*) *Boiling-point*: 113.8°C (Lide, 1995)
(*c*) *Melting-point*: –36.6°C (Lide, 1995)
(*d*) *Solubility*: Insoluble in water; soluble in chloroform, diethyl ether, ethanol and many other organic liquids (Lide, 1995; Budavari, 1996)
(*e*) *Vapour pressure*: 2.5 kPa at 20°C; relative vapour density (air = 1), 4.6 (Verschueren, 1996)
(*f*) *Conversion factor*: $mg/m^3 = 5.46 \times ppm$

1.2 Production and use

Annual production of 1,1,2-trichloroethane in the United States in the early 1980s was estimated to be 186 000 tonnes (American Conference of Governmental Industrial Hygienists, 1992).

1,1,2-Trichloroethane is used primarily as an intermediate in the production of vinylidene chloride; other minor uses include as a solvent for fats, oils, waxes, resins and

other products, and as a process solvent in pharmaceutical manufacture (American Conference of Governmental Industrial Hygienists, 1992; Lewis, 1993; Snedecor, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

No national estimates of exposure were available to the Working Group.

1.3.2 *Environmental occurrence*

1,1,2-Trichloroethane may enter the atmosphere from its use in the manufacture of vinylidene chloride and its use as a solvent. It may also be discharged in wastewater associated with these uses and in leachates and volatile emissions from landfills. It has been detected at low levels in groundwater, drinking-water, wastewater, ambient water and ambient air (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 55 mg/m^3 as the threshold limit value for occupational exposures to 1,1,2-trichloroethane in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for 1,1,2-trichloroethane in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

1,1,2-Trichloroethane was tested for carcinogenicity in a two-year study in male and female B6C3F$_1$ mice and Osborne-Mendel rats by oral administration and in Sprague-Dawley rats by subcutaneous injection. In the study by oral administration, 1,1,2-trichloroethane produced hepatocellular neoplasms and adrenal phaeochromocytomas in mice of each sex but did not significantly increase the proportion of rats with neoplasms at any site relative to untreated controls. In the study in rats by subcutaneous injection, 1,1,2-trichloroethane did not increase the incidence of neoplasms.

In a screening assay for γ-glutamyltranspeptidase-positive foci in the liver of male Osborne-Mendel rats, 1,1,2-trichloroethane did not increase the number of foci in the liver after the initiation protocol (single injection), but the number was increased after the promotion protocol (repeated injections), after partial hepatectomy or after partial hepatectomy followed by initiation with *N*-nitrosodiethylamine (IARC, 1991).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

When [^{38}Cl]1,1,2-trichloroethane was administered by inhalation at a dose of about 5 mg per subject, about 3% of the compound was eliminated in the breath within 1 h, and urinary excretion of ^{38}Cl amounted less than 0.01% of the dose/min (IARC, 1991).

4.1.2 *Experimental systems*

1,1,2-Trichloroethane is rapidly absorbed after inhalation, oral administration and application to the skin in rodents. 1,1,2-Trichloroethane is extensively metabolized in mice given 100–200 mg/kg bw by intraperitoneal injection, 73–87 % of the dose being eliminated in the urine and 16–22% in expired air. Several urinary metabolites have been identified: chloroacetic acid, *S*-carboxymethyl-L-cysteine, thiodiacetic acid, 2,2-dichloroethanol and oxalic acid (IARC, 1991).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Upon administration of lethal doses of 1,1,2,-trichloroethane, signs of toxicity included sedation, gastric irritation, lung haemorrhage and liver and kidney damage, but at continued dosing at dose levels compatible with survival, no significant organ toxicity was observed (IARC, 1991). This was substantiated in a carcinogenicity study (United States National Cancer Institute, 1978), in which no gross or microscopic non-neoplastic changes were associated with administration of 1,1,2-trichloroethane (time-weighted average dose, 46 or 92 mg/kg bw per day by gavage for 78 weeks) to Osborne-Mendel rats of each sex and 195 or 390 mg/kg bw per day to B6C3F$_1$ mice of each sex.

A small but significant elevation of serum sorbitol dehydrogenase activity was observed 18 h after an intraperitoneal dose of 51 mg/kg bw 1,1,2-trichloroethane (1/8 of the LD_{50}), but not at a dose level of 25 mg/kg bw (Lundberg *et al*., 1986). After a single intragastric dose of 667 mg/kg bw 1,1,2 trichloroethane, serum glutamic pyruvic transaminase, sorbitol dehydrogenase and glutamate dehydrogenase activities showed elevations which peaked at 24 h in female Wistar rats. Cloudy swelling, vacuolar degeneration and scattered necrosis were observed in the liver. Electron spin resonance spectra from liver specimens were consistent with increased concentrations of free radicals (Liangfu & Tianju, 1992). In Sprague-Dawley rats, a single intraperitoneal dose of 200 mg/kg bw 1,1,2-trichloroethane induced an elevation of serum glutamic pyruvic transaminase activity and histological liver damage, while a dose of 167 mg/kg bw produced practically no effect. The hepatotoxicity of 1,1,2-trichloroethane was markedly potentiated by pre-

treatment with acetone. Hepatic glutathione content showed a slight decrease 2 h after administration of 1,1,2-trichloroethane; a very marked decrease was observed in acetone-pretreated animals (MacDonald *et al*., 1982).

When CD-1 mice were given 1,1,2-trichloroethane (4.4, 46 or 305 mg/kg bw for males and 3.9, 44 or 384 mg/kg bw for females) in the drinking-water for 90 days, very minor effects on weight gain or haematological or clinical chemical parameters were seen. A dose-dependent decrease in hepatic cytochrome P450 content and in aniline hydroxylase activity was observed in females (White *et al*., 1985). Cell-mediated immunity (delayed type hypersensitivity, popliteal lymph node proliferation, spleen lymphocyte response to concanavalin A) was not affected in these mice, while haemagglutination titres to sheep red blood cells were depressed in a dose-dependent manner in both sexes and the responsiveness of splenic lymphocytes to the B-cell mitogen lipopolysaccharide was decreased in females. In males, peritoneal exudate cells exhibited a decreased phagocytic capacity at the highest dose (Sanders *et al*., 1985).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

The genetic and related effects of 1,1,2-trichloroethane have been reviewed (Infante & Tsongas, 1982).

1,1,2-Trichloroethane gave conflicting results in the *Salmonella typhimurium* reverse mutation assay. Positive results were reported in only one study for strains TA100, TA104, and TA97. 1,1,2-Trichloroethane caused chromosome malsegregation in *Aspergillus nidulans* and morphological transformation of BALB/c-3T3 cells. It induced DNA damage in human lymphocyte cultures in the presence or absence of exogenous metabolic activation and micronuclei in the absence of metabolic activation. It bound to DNA, RNA and protein of lung, liver, kidney and stomach following treatment of rats and mice *in vivo*. Strong S-phase induction was observed in livers of treated mice, but unscheduled DNA synthesis was not seen in mouse or rat hepatocytes *in vivo*.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

1,2-Trichloroethane is used in the manufacture of vinylidene chloride. It has been detected in ground-, drinking-, waste- and ambient water and ambient air.

Table 1. Genetic and related effects of 1,1,2-trichloroethane

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SAF, *Salmonella typhimurium*, forward mutation (Ar2 test)	–		500	Roldán-Arjona *et al*. (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–[c]	–	4000	Barber *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	–	5	Strobel & Grummt (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–[d]	–	NG	Mersch-Sunderman (1989)
SA2, *Salmonella typhimurium* TA102, reverse mutation	–[c]	–	NG	Mersch-Sunderman (1989)
SA4, *Salmonella typhimurium* TA104, reverse mutation	–	+	5	Strobel & Grummt (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	3000	Rannug *et al*. (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–[c]	–	4000	Barber *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–[c]	–	4000	Barber *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Strobel & Grummt (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–[d]	–	NG	Mersch-Sunderman (1989)
SAS, *Salmonella typhimurium* TA97, reverse mutation	+	+	5	Strobel & Grummt (1987)
SAS *Salmonella typhimurium* TA97, reverse mutation	–[d]	–	NG	Mersch-Sunderman (1989)
ANG, *Aspergillus nidulans* strain P1, genetic crossing-over	–	NT	1000	Crebelli *et al.* (1988)
ANN, *Aspergillus nidulans* strain P1, aneuploidy	+	NT	1000	Crebelli *et al.* (1988)
TBM, Cell transformation, BALB/c-3T3 cells	(+)[c]	NT	25	Tu *et al*. (1985)
DIH, DNA damage, human lymphocytes *in vitro*	+	+	333	Tafazoli & Kirsch-Volders (1996)
MIH, Micronucleus test, human lymphocytes *in vitro*	(+)	–	133	Tafazoli & Kirsch-Volders (1996)
UPR, Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	–		1000 po × 1	Mirsalis *et al*. (1989)
UVM, Unscheduled DNA synthesis, B6C3F$_1$mouse hepatocytes *in vivo*	–		1000 po × 1	Mirsalis *et al*. (1989)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
BVD, DNA binding (covalent), BALB/c mouse liver, kidney, lung and stomach *in vivo*	+		0.8 ip × 1	Mazzullo *et al*. (1986)
BVD, DNA binding (covalent),Wistar rat liver, kidney, lung and stomach *in vivo*	+		0.8 ip × 1	Mazzullo *et al*. (1986)
BVP, Binding to RNA/protein, BALB/c mouse liver, kidney, lung and stomach *in vivo*	+		0.8 ip × 1	Mazzullo *et al*. (1986)
BVP, Binding to RNA/protein, Wistar rat liver, kidney, lung and stomach *in vivo*	+		0.8 ip × 1	Mazzullo *et al*. (1986)
S-phase synthesis induction, mouse hepatocytes *in vivo*	+		500 po × 1	Mirsalis *et al*. (1989)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest effective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw /day; NG, not given; po, oral; ip, intraperitoneal

[c] Closed container

[d] Negative in closed container, standard test or spot

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

1,1,2-Trichloroethane was tested for carcinogenicity in a two-year study in male and female B6C3F_1 mice and Osborne-Mendel rats by oral administration and in Sprague-Dawley rats by subcutaneous injection. In the study by oral administration, 1,1,2-trichloroethane produced hepatocellular neoplasms and adrenal phaeochromocytomas in mice of each sex but did not significantly increase the proportion of rats with neoplasms at any site relative to untreated controls. In the study in rats by subcutaneous injection, 1,1,2-trichloroethane did not increase the incidence of neoplasms.

5.4 Other relevant data

1,1,2-Trichloroethane bound to DNA, RNA and protein and caused strong S-phase induction but not unscheduled DNA synthesis in rodents *in vivo*. It induced DNA damage and micronuclei in human lymphocytes and cell transformation in BALB/c-3T3 cells *in vitro*. 1,1,2-Trichloroethane caused chromosomal malsegregation in fungi and showed some evidence of mutagenicity in bacteria.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of 1,1,2-trichloroethane were available.

There is *limited evidence* in experimental animals for the carcinogenicity of 1,1,2-trichloroethane.

Overall evaluation

1,1,2-Trichloroethane is *not classifiable as to its carcinogenicity to humans (Group 3).*

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VINYLIDENE CHLORIDE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

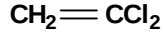
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 75-35-4
Chem. Abstr. Name: 1,1-Dichloroethene
IUPAC Systematic Name: 1,1-Dichloroethylene
Synonym: Asym-dichloroethylene

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2H_2Cl_2$ Relative molecular mass: 96.94

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid with sweet, chloroform-like odour (Budavari, 1996)
(*b*) *Boiling-point*: 31.6°C (Lide, 1995)
(*c*) *Melting-point*: –122.5°C (Lide, 1995)
(*d*) *Solubility*: Insoluble in water; soluble in acetone, ethanol and many organic solvents; very soluble in diethyl ether (Lide, 1995; Budavari, 1996; Verschueren, 1996)
(*e*) *Vapour pressure*: 67 kPa at 20°C; relative vapour density (air = 1), 3.25 (Verschueren, 1996)
(*f*) *Flash point*: –19°C, closed cup; –15°C, open cup (American Conference of Governmental Industrial Hygienists, 1992)
(*g*) *Explosive limits*: Upper, 16%; lower, 5.6% by volume in air (American Conference of Governmental Industrial Hygienists, 1993)
(*h*) *Conversion factor*: $mg/m^3 = 3.96 \times ppm$

1.2 Production and use

In 1967, world production of vinylidene chloride was estimated to be 220–330 thousand tonnes; by the early 1980s, it was approximately 306 thousand tonnes. A more recent estimate of worldwide production is 290 thousand tonnes (WHO, 1990).

Vinylidene chloride is used principally in copolymers with vinyl chloride, acrylonitrile and other monomers for packaging materials, adhesives and synthetic fibres (Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

The National Occupational Exposure Survey (NOES, 1997) estimated that 2675 workers in the United States were potentially exposed to vinylidene chloride between 1981 and 1983.

National estimates on exposure were not available from other countries.

1.3.2 *Environmental occurrence*

Vinylidene chloride can enter the atmosphere as emissions from its production and use in the manufacture of plastics. It has been detected in wastewater from plastics manufacturing and metal finishing (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 20 mg/m^3 as the threshold limit value for occupational exposures to vinylidene chloride in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

The World Health Organization has established an international drinking-water guideline for vinylidene chloride of 30 µg/L (WHO, 1993).

2. Studies of Cancer in Humans

In one epidemiological study of 138 workers exposed to vinylidene chloride in the United States, no excess of cancer was found, but follow-up was incomplete, and nearly 40% of the workers had less than 15 years' latency since first exposure (IARC, 1986). In a study in the Federal Republic of Germany of 629 workers exposed to vinylidene chloride, seven deaths from cancer (five bronchial carcinomas) were reported; this number was not in excess of the expected value. Two cases of bronchial carcinoma were found in workers, both of whom were 37 years old, whereas 0.07 were expected for persons aged 35–39 years (Thiess *et al*., 1979). The limitations of these two studies preclude assessment of the carcinogenicity of the agent to humans. No specific association was found between exposure to vinylidene chloride and an excess of lung cancer observed in a synthetic chemicals plant in the United States.

3. Studies of Cancer in Experimental Animals

Vinylidene chloride was tested for carcinogenicity in mice and rats by oral administration and by inhalation, in mice by subcutaneous administration and by topical application, and in hamsters by inhalation. Studies in mice and rats by oral administration gave negative results. In inhalation studies, no treatment-related neoplasm was observed in rats or hamsters. In mice, a treatment-related increase in the incidence of kidney adenocarcinomas was observed in male mice, as were increases in the incidence of mammary carcinomas in females and of pulmonary adenomas in male and female mice. In skin-painting studies in female mice, vinylidene chloride showed activity as an initiator, but, in a study of repeated skin application, no skin tumour occurred. No tumour at the injection site was seen in mice given repeated subcutaneous administrations (IARC, 1986).

3.1 Inhalation exposure

Rat: Groups of 54 and 60 female Sprague-Dawley breeder rats, 13 weeks of age, were exposed by whole-body inhalation to 0 or 100 ppm [0 or 400 mg/m^3] vinylidene chloride (purity, > 99.9%) for 4 h per day on five days per week for seven weeks and then for 7 h per day for 97 weeks. Also groups of 62 male and 61 female offspring were exposed transplacentally beginning at day 12 of gestation and postnatally to the same concentrations for a total of 104 weeks, with 158 male and 149 female rats serving as unexposed controls. In breeders, vinylidene chloride-exposed females exhibited a 7.4% incidence of malignant mammary tumours compared with 3.3% in controls [no statistical analysis provided]. The incidence of phaeochromocytomas was 7.4% compared with 18.3% in controls and the incidence of leukaemias was also lower in exposed rats. In offspring exposed to vinylidene chloride, malignant mammary tumours were observed in 4.9% of females [no statistical analysis provided] compared with 1.9% in controls. An increased incidence of leukaemia was found in offspring exposed for 104 weeks (16.1% and 6.5% in treated male and female rats, compared with 7.6% and 0.7% in male and female controls, respectively. These percentages roughly correspond to 10/62, 4/61, 12/158 and 1/149 rats with leukaemia/no. of rats at the start) (Cotti *et al.*, 1988). [The Working Group noted that details on survival and the absolute tumour data were not reported. Also, the time of appearance of tumours was not reported and no appropriate statistical analysis, taking into account the survival, was performed.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

Large reviews (ECETOC, 1985; WHO, 1990) have analysed and summarized the data including studies assessed by the previous IARC Working Group (IARC, 1986).

However, all of the additional studies reviewed are from the same period (1977–82) and were conducted by the same groups as the studies evaluated by the IARC Working Group and they do not affect the previous evaluation. WHO (1990) reviewed a number of additional studies which have also been assessed here.

4.1.1 *Humans*

Vinylidene chloride has been shown to be activated by human liver S9 supernatant in the Ames assay, suggesting the presence of a cytochrome P450 enzyme. Also, the formation of dichloroacetaldehyde was demonstrated in two human liver microsomal preparations; the rate of formation was approximately the same as in rat liver microsomes (WHO, 1990).

4.1.2 *Experimental systems*

In rats, metabolism, which probably proceeds via a cytochrome P450-generated epoxide intermediate and subsequent either direct or indirect glutathione conjugation, is saturated between doses of 1–50 mg/kg bw orally or 10–200 ppm [40–800 mg/m^3] by inhalation, resulting in a dose-dependent increased elimination via the lungs as unchanged vinylidene chloride. Mice metabolize vinylidene chloride to a greater extent than rats. Also the alkylation of proteins by vinylidene chloride metabolites is greater in mice than in rats. Metabolic activation via epoxidation was suggested (IARC, 1986).

In rats and mice, vinylidene chloride is very quickly absorbed after oral administration; in mice, elimination is tri-exponential (Putcha *et al*., 1986).

Glutathione levels and glutathione *S*-transferase activity are important determinants of metabolic inactivation of primary vinylidene chloride metabolites (Okine *et al*., 1985; Cossec *et al*., 1996). Covalent binding of vinylidene chloride metabolites is highest in kidney, liver and lung in mice and can be modified by glutathione depletion (Okine *et al*., 1985; Okine & Gram, 1986).

CYP2E1 is the principal catalyst of vinylidene chloride metabolism and metabolic activation. This conclusion is based on the following findings. Inducers (ethanol, acetone) and inhibitors (diethyldithiocarbamate) increase and decrease, respectively, metabolic activation of vinylidene chloride in mouse liver microsomes and in isolated mouse hepatocytes (Kainz *et al*., 1993; Lee & Forkert, 1994; Dowsley *et al*., 1995). Vitamin A treatment increases CYP2E1 activity in rat liver microsomes and also potentiates vinylidene chloride toxicity in liver slices from treated animals (Wijeweera *et al*., 1996). In mouse liver microsomes *in vitro*, vinylidene chloride decreases CYP2E1 levels (Lee & Forkert, 1994).

Variations in the tissue expression of CYP2E1 appear to underlie the tissue selectivity of vinylidene chloride toxicity. A sex difference in CYP2E1-mediated metabolism of vinylidene chloride correlates with the incidence of renal tumours (Speerschneider & Dekant, 1995). Metabolism in kidney microsomes from male mice was at least six times that in females, while tumours were seen only in males (see Section 3). CYP2E1 could not be detected in rat kidney and no renal tumours were seen in this species. In addition,

there is indirect evidence for a comparable basis for the lung toxicity of vinylidene chloride in mice. The rank order of CYP2E1-supported metabolic activation of vinylidene chloride in mouse lung microsomes (Lee & Forkert, 1995) correlates with the severity of its toxicity to the bronchiolar Clara cells, in the rank order adult female > weanling male = weanling female > adult male (Forkert *et al*., 1996a).

Metabolism of vinylidene chloride, and presumably metabolic activation, is about six times higher in liver microsomes from mice than in those from rats (Dowsley *et al*., 1995).

4.1.3 *Comparison of human and rodent data*

CYP2E1, an enzyme catalysing vinylidene chloride activation, is expressed in various human tissues, including liver, although functional evidence is lacking for many tissues. The level expressed in human liver is similar to that in rodents. CYP2E1 seems to be differentially expressed in kidney microsomes from humans (no expression), rats (no expression) and mice (high expression in males).

4.2 Toxic effects

4.2.1 *Humans*

Vinylidene chloride is a central nervous system depressant. Repeated exposure to low concentrations of vinylidene chloride may cause liver and renal dysfunction (Torkelson & Rowe, 1981). Skin contact with vinylidene chloride causes irritation, which may be due partly to the presence of an inhibitor, hydroquinone monomethyl ether (Chivers, 1972). In one study, spirometry, blood clinical chemistry for liver and renal toxicity, haematological parameters and blood pressure measurements did not differ between vinylidene chloride-exposed workers and controls. Measured past time-weighted average vinylidene chloride concentrations ranged from < 5 to 70 ppm [< 20–280 mg/m^3] (Ott *et al*., 1976).

4.2.2 *Experimental systems*

In a two-year study of male and female Sprague-Dawley rats receiving 60–230 mg/L vinylidene chloride in the drinking-water, no toxicological effect was noted except for a non-dose-related decrease in survival of male rats at 18 and 24 months (Norris, 1977).

Male C57BL/6 mice treated orally with an acute dose of 200 mg/kg bw vinylidene chloride exhibited necrosis and exfoliation of Clara cells in the lung within 24 h (Forkert *et al*., 1985). The severity of the response was increased at two days but was no longer evident seven days after exposure. Intraperitoneal administration of 125 mg/kg vinylidene chloride to male CD-1 mice also resulted in necrosis and covalent binding of vinylidene chloride-derived radioactivity in lung Clara cells (Forkert *et al*., 1986a, 1990). Increased Clara cell toxicity was noted following pretreatment with piperonyl butoxide, while SKF 525-A administration protected against vinylidene chloride-induced pulmonary damage (Forkert *et al*., 1986b).

Male CD-1 mice treated with 75, 125, 175 and 225 mg/kg bw vinylidene chloride by intraperitoneal injection exhibited glutathione (GSH) depletion in lung Clara cells at 75 and 125 mg/kg, with complete loss of GSH at the two highest doses (Moussa &

Forkert, 1992). Administration of 125 mg/kg bw vinylidene chloride to male CD-1 mice by intraperitoneal injection caused a decrease in GSH levels in lung within 6 h after exposure (Forkert & Moussa, 1993). However, GSH concentrations returned to normal levels by 24 h.

Pulmonary toxicity of vinylidene chloride was related to levels of CYP2E1 activity in male and female adult and weanling CD-1 mice following exposure to 50, 75 and 100 mg/kg bw vinylidene chloride (Forkert *et al.*, 1996a). For a given dose of vinylidene chloride, cytotoxicity was greatest in the lungs of male mice, followed in severity by male and female weanling mice and then female mice. Levels of CYP2E1 also followed this pattern, with adult male mice having the lowest concentrations of CYP2E1 in lung tissue and female adult mice having the greatest amount of the enzyme. Inhibition of CYP2E1 in male CD-1 mice by diallyl sulfone pretreatment resulted in an absence of pulmonary cytotoxicity following intraperitoneal injection of 75 mg/kg bw vinylidene chloride (Forkert *et al.*, 1996b).

Male Sprague-Dawley rats exposed for 4 h to 0–400 ppm [0–1600 mg/m^3] vinylidene chloride exhibited increased kidney weight to body weight ratios, serum nitrogen and creatinine levels 24 h after exposure to concentrations of 250 ppm [1000 mg/m^3] and above (Jackson & Conolly, 1985). Phenobarbital or polychlorinated biphenyl pretreatment antagonized vinylidene chloride-induced nephrotoxicity. Intraperitoneal injection of 25 and 50 mg/kg bw vinylidene chloride to female and male C57BL/6 mice caused mild tubular dilation in the S1 and S2 segments of the kidney proximal tubules 24 h after exposure (Brittebo *et al.*, 1993). Buthionine sulfoximine (a GSH depleter and inhibitor of γ-glutamylcysteine synthetase) or probenecid (an anionic transport inhibitor) pretreatment markedly increased the covalent binding of vinylidene chloride to renal tissue. Pretreatment with carboxymethoxyl amine, metyrapone or piperonyl butoxide had no effect on vinylidene chloride-induced kidney binding in this study. In contrast, probenecid and acivin (a γ-glutamyltranspeptidase inhibitor) had no effect on kidney toxicity (assessed by histopathology) caused by a single oral dose of 200 mg/kg bw vinylidene chloride administered to male Swiss OF1 mice (Ban *et al.*, 1995). Proximal tubular damage caused by 200 mg/kg vinylidene chloride was decreased in this study following pretreatment with the cysteine conjugate *S*-oxidase inhibitor methimazole or aminooxyacetic acid (an inhibitor of β-lyase).

Similar trends were noted in fasted male Sprague-Dawley rats following a 4-h inhalation exposure to 180–200 ppm [720–800 mg/m^3] vinylidene chloride (Cavelier *et al.*, 1996). Analysis of urine and serum for biochemical markers of toxicity 24 h after exposure revealed increased toxicity following vinylidene chloride exposure. Levels of marker enzymes were similar to control values following pretreatment with aminooxyacetic acid. No effect was noted on vinylidene chloride toxicity following acivicin treatment and only slight increases in vinylidene chloride toxicity were observed following methimazole pretreatment.

A single oral gavage dose of 2 mmol/kg (194 mg/kg bw) vinylidene chloride to fasted male Sprague-Dawley rats produced liver damage, as indicated by increases in serum

markers of toxicity (Cossec *et al*., 1996). Urinary activities of renal toxicity markers were also increased 24 h after administration of this dose of vinylidene chloride. No toxicity was noted in this study at a dose of 0.5 mmol/kg (48 mg/kg bw) vinylidene chloride.

Male and female Swiss-Webster mice were exposed to 60 ppm [240 mg/m^3] vinylidene chloride for 4 h (Speerschneider & Dekant, 1995). Urine was collected over a 48-h period and animals were then killed. Male mice were more sensitive to vinylidene chloride-induced nephrotoxicity, as assessed by changes in urinary volume, creatinine, glucose and γ-glutamyltranspeptidase levels. Increased necrosis was observed in exposed male mice and female mice pretreated with testosterone. Female mice had no observable kidney lesions or alteration in urinary parameters, suggesting the role of CYP2E1 in vinylidene chloride-induced nephrotoxicity.

Male Wistar rats exposed by inhalation to 2000 ppm [8000 mg/m^3] vinylidene chloride for 6 h exhibited only slight and insignificant elevation of serum hepatotoxic enzyme activities 24 h after exposure (Siegers *et al*., 1985a). Exposure to equal concentrations of vinylidene chloride under low oxygen conditions resulted in no vinylidene chloride-induced effects. Inhalation exposure of male Wistar rats to 1000 ppm [4000 mg/m^3] vinylidene chloride for 3 h caused only small increases in serum markers of liver toxicity 24 h after exposure. Toxicity was enhanced following pretreatment with phorone, a GSH-depleting agent (Siegers *et al*., 1985b). Oral administration of 1.5 mL/kg (1820 mg/kg bw) vinylidene chloride to male Sprague-Dawley rats caused increased activity of liver toxicity markers 8 and 24 h after gavage (Long *et al*., 1989). Decreases in calcium pump activity in liver endoplasmic reticulum were also noted 0.5–8 h after exposure.

Administration of 0–225 mg/kg vinylidene chloride to male CD-1 mice resulted in GSH depletion in both lung and liver, increased covalent binding to liver tissue and necrosis 1 h after exposure (Forkert & Moussa, 1993). In male Sprague-Dawley rats, GSH depletion was also noted following intraperitoneal injection of 50 mg/kg vinylidene chloride and was more pronounced in fasted rats than in fed animals (Kanz *et al*., 1988). Exposure of BALB/c mouse hepatocytes to vinylidene chloride *in vitro* indicated that GSH conjugation is a critical detoxification step (Kainz *et al*., 1993) and CYP2E1 is involved in vinylidene chloride metabolic activation. Similar results were noted *in vivo* when male Sprague-Dawley rats pretreated with high levels of vitamin A (an inducer of CYP2E1) exhibited a dose-dependent increase in the liver toxicity marker alanine aminotransferase 24 h after intraperitoneal treatment with 0–200 mg/kg bw vinylidene chloride (Wijeweera *et al*., 1996). In the same study, inactivation of Kupffer cells with gadolinium chloride decreased the toxicity of vinylidene chloride in the liver of vitamin A-pretreated animals. Vinylidene chloride-induced liver toxicity (assessed by histopathology) in male Wistar rats was noted to be greater following total inhalation exposure over a four-week period to 33 533 ppm.h at a nominal concentration of 50 ppm [200 mg/m^3] vinylidene chloride in a constant profile group compared with a fluctuating exposure scenario (Plummer *et al*., 1990).

Hyperthyroidism was noted to increase the hepatotoxicity, covalent binding and biliary clearance of vinylidene chloride in male Sprague-Dawley rats following oral administration of 50 mg/kg (Kanz *et al*., 1988, 1994). Increased serum levels of markers of liver toxicity and decreases in hepatic glutathione *S*-transferase and alcohol dehydrogenase levels were noted in hypothyroid rats (Kanz *et al*., 1991). Vinylidene chloride administered orally at doses of 50 or 200 mg/kg bw caused alterations in biliary excretion of inulin and other marker solutes (indocyanine green and phenolphthalein glucuronide) (Moslen *et al*., 1985; Moslen & Kanz, 1993). These vinylidene chloride doses were also reported to cause damage to the bile canaliculi, with fasted rats exhibiting greater damage than fed rats (Moslen *et al*., 1985, 1989; Moslen & Kanz, 1993).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

In a dominant lethal assay, male CD rats were exposed to 55 ppm [220 mg/m^3] vinylidene chloride for 6 h per day on five days per week (Short *et al*., 1977). On week 11 of exposure, males were mated with untreated females. There was no evidence of pre- or post-implantation loss in the pregnant females. Male CD-1 mice exposed to 10, 30 or 50 ppm [40, 120 or 200 mg/m^3] vinylidene chloride for 6 h per day for five days and subsequently mated with untreated females exhibited no pre- or post-implantation loss (Anderson *et al*., 1977), indicating an absence of adverse effects on male reproduction.

Sprague-Dawley rats were exposed by inhalation to 80, 316 or 630 mg/m^3 vinylidene for 7 h per day on days 6–15 of pregnancy. New Zealand rabbits were exposed to 316 or 630 mg/m^3 on days 6–18 of pregnancy (Murray *et al*., 1979). Toxicity was noted in the dams at 316 mg/m^3 in rats and 630 mg/m^3 in rabbits. Resorptions in dams and skeletal variations in pups were increased in rabbits at 630 mg/m^3. Skeletal variations were also noted in rats exposed to 316 mg/m^3 and 630 mg/m^3.

No adverse effects were noted in Sprague-Dawley rats exposed to 200 mg/L vinylidene chloride in the drinking-water on days 6–15 of pregnancy (Murray *et al*., 1979). In a three-generation reproductive toxicity study in which Sprague-Dawley rats were exposed to 50, 100 or 200 mg/L vinylidene chloride in the drinking-water, no adverse effects were noted in the reproductive capacity of either sex (Nitschke *et al*., 1983).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Vinylidene chloride induced mutations in *Salmonella typhimurium* and *Escherichia coli* in the absence of an exogenous metabolic system. In *Saccharomyces cerevisiae*, vinylidene chloride induced reverse mutation and mitotic gene conversion *in vitro* and in a host-mediated assay in mice. In a single study in *Saccharomyces*, it induced aneuploidy in the presence and absence of metabolic activation. *In vitro*, gene mutations were increased in mouse lymphoma cells but not in Chinese hamster lung cells with or without an exogenous metabolic system. In a single study, vinylidene chloride induced sister chromatid exchanges in Chinese hamster lung cells in the presence of an exogenous metabolic system but not in its absence. In single studies *in vivo*, it did not induce micronuclei or chromosomal aberrations in bone marrow or in fetal erythrocytes of mice, nor dominant lethal mutations in mice or rats.

5. Summary of Data Reported and Evaluation[1]

5.1 Exposure data

Exposure to vinylidene chloride may occur during its production and in the production of copolymers. It has been detected in wastewater.

5.2 Human carcinogenicity data

Two cohort studies were performed in workers exposed to vinylidene chloride. Both studies have major limitations and do not allow evaluation of the carcinogenicity of the compound.

No specific association was found between exposure to vinylidene chloride and an excess of lung cancer observed in a synthetic chemical plant in the United States.

5.3 Animal carcinogenicity data

Vinylidene chloride was tested for carcinogenicity in mice and rats by oral administration and inhalation exposure, in mice by subcutaneous administration and topical application and in hamsters by inhalation. Studies in mice and rats by oral administration gave negative results. In inhalation studies, no treatment-related neoplasm was observed in rats or hamsters. In mice, treatment-related increases in the incidence of kidney adenocarcinomas were observed in male mice, as were increases in mammary carcinomas in females and pulmonary adenomas in male and female mice. In skin-painting studies in female mice, vinylidene chloride showed activity as an initiator, but in a study of repeated skin application, no skin tumour occurred. No tumour at the injection site was seen in mice given repeated subcutaneous administration.

[1] Summary (but not the evaluation) prepared by the Secretariat after the meeting.

Table 1. Genetic and related effects of vinylidene chloride

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SAF, *Salmonella typhimurium* BA13/BAL13, forward mutation	–	+	500	Roldán-Arjona *et al*. (1991)
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	+	2% in air	Malaveille *et al*. (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	+	5% in air	Jones & Hathway (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	5% in air	Simmon & Tardiff (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	5% in air	Waskell (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	+	2% in air	Bartsch *et al*. (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	375 ppm in air	Oesch *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	+	125	Strobel & Grummt (1987)
SA4, *Salmonella typhimurium* TA104, reverse mutation	–	–	500	Strobel & Grummt (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	3% in air	Baden *et al*. (1977)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	NT	+	5% in air	Jones & Hathway (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	375 ppm in air	Oesch *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	(+)	375 ppm in air	Oesch *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	375 ppm in air	Oesch *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	(+)	125	Strobel & Grummt (1987)
SAS, *Salmonella typhimurium* TA92, reverse mutation	–	+	375 ppm in air	Oesch *et al*. (1983)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	+	5	Strobel & Grummt (1987)
ECK, *Escherichia coli* K12, forward or reverse mutation	–	(+)	242	Greim *et al*. (1975)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	+	375 ppm in air	Oesch *et al*. (1983)
SCG, *Saccharomyces cerevisiae* D7, gene conversion	–	+	2910	Bronzetti *et al*. (1983)
SCG, *Saccharomyces cerevisiae* D7, mitotic gene conversion	+[c]	–	7300	Koch *et al*. (1988)
SCR, *Saccharomyces cerevisiae* D7, reverse mutation	–	+	2910	Bronzetti *et al*. (1981)
SCR, *Saccharomyces cerevisiae* D7, reverse mutation	+[c]	+	4876	Koch *et al*. (1988)
SCN, *Saccharomyces cerevisiae* D61.M, aneuploidy	+	+	2435	Koch *et al*. (1988)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
G9H, Gene mutation, Chinese hamster lung V79 cells, *hrpt* locus *in vitro*	–	–	10% in air	Drevon & Kuroki (1979)
G9O, Gene mutation, Chinese hamster lung V79 cells, ouabain resistance *in vitro*	–	–	10% in air	Drevon & Kuroki (1979)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	?	+	0.16% in air	McGregor *et al*. (1991)
SIC, Sister chromatid exchange, Chinese hamster lung cells *in vitro*	–	+	75	Sawada *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster DON-6 cells *in vitro*	–	NT	2910	Sasaki *et al*. (1980)
CIC, Chromosomal aberrations, Chinese hamster fibroblast CHL cells *in vitro*	–	NT	2000	Ishidate *et al*. (1983)
CIC, Chromosomal aberrations, Chinese hamster lung cells *in vitro*	–	+	250	Sawada *et al*. (1987)
HMM, Host-mediated assay, *Saccharomyces cerevisiae* D7 in CD mouse hosts	+	NT	100 po × 23	Bronzetti *et al*. (1981)
HMM, Host-mediated assay, *Saccharomyces cerevisiae* D7 in CD mouse hosts	+	NT	400 po × 1	Bronzetti *et al*. (1981)
MVM, Micronucleus test, mouse bone marrow *in vivo*	–		200 po × 1	Sawada *et al*. (1987)
MVM, Micronucleus test, mouse fetal erythrocytes *in vivo*	–		100 ip × 1	Sawada *et al*. (1987)
CBA, Chromosomal aberrations, Sprague-Dawley rat bone marrow *in vivo*	–		75 ppm inh 6 h/d 3 d/wk 2 y	Rampy *et al*. (1977)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DLM, Dominant lethal test, male CD-1 mice	–		50 ppm inh 6 h/d 5 d	Anderson *et al*. (1977)
DLR, Dominant lethal test, CD rats	–		55 ppm inh 6 h/d 5 d/wk 11 wk	Short *et al*. (1977)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; po, oral; ip, intraperitoneal; inh, inhalation
[c] Positive in cells growing in logarithmic phase

5.4 Other relevant data

Vinylidene chloride is oxidized principally by CYP2E1, the activity of this cytochrome P450 being higher in those tissues (particularly mouse Clara cells and male mouse kidney) that are targets for toxicity of vinylidene chloride. Glutathione levels and conjugation are important in its inactivation and protect against covalent binding. It causes gene mutations in microorganisms, but its genetic activity has not been extensively studied in mammalian cells.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of vinylidene chloride.

There is *limited evidence* in experimental animals for the carcinogenicity of vinylidene chloride.

Overall evaluation

Vinylidene chloride is *not classifiable as to its carcinogenicity to humans (Group 3).*

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N-VINYL-2-PYRROLIDONE AND POLYVINYL PYRROLIDONE

Data were last reviewed in IARC (1979) and the compounds were classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

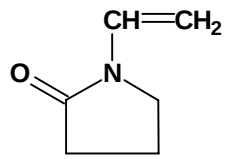
1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 88-12-0

Chem. Abstr. Name: 1-Ethenyl-2-pyrrolidinone

Synonyms: Vinylbutyrolactam; vinylpyrrolidinone; 1-vinylpyrrolidinone; *N*-vinyl-pyrrolidinone; 1-vinyl-2-pyrrolidinone; *N*-vinyl-2-pyrrolidinone; vinylpyrrolidone; *N*-vinylpyrrolidone; 1-vinyl-2-pyrrolidone

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_6H_9NO Relative molecular mass: 111.1

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid (Lewis, 1993)

(*b*) *Boiling-point*: 193°C (at 53 kPa) (Lide, 1997)

(*c*) *Melting-point*: 13.5°C (Lide, 1997)

(*d*) *Solubility*: Miscible with water and most organic solvents; partially miscible with aliphatic hydrocarbons (Harreus, 1993)

(*e*) *Vapour pressure*: 12 Pa at 20°C (Harreus, 1993)

(*f*) *Stability*: Flash-point, 98.4°C; polymerizes readily in the presence of oxygen (Lewis, 1993)

(*g*) *Conversion factor*: $mg/m^3 = 4.54 \times ppm$

1.2 Production and use

Information available in 1995 indicated that *N*-vinyl-2-pyrrolidone was produced in four countries (China, Germany, United Kingdom, United States) (Chemical Information Services, 1995).

It is used in the manufacture of polyvinylpyrrolidone (PVP), in the manufacture of copolymers with, for example, acrylic acid, acrylates, vinyl acetate and acrylonitrile and in the synthesis of phenolic resins. About 10–15% of the monomer is used in the pharmaceutical industry for the production of PVP–iodine complex used as a disinfectant. It is also used as a reactive solvent of ultraviolet-curable resins for the production of printing inks and paints as paper and textile auxiliaries, and as an additive in the cosmetics industry (Harreus, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 6000 workers in the United States were potentially exposed to *N*-vinyl-2-pyrrolidone (see General Remarks).

1.3.2 *Environmental occurrence*

No data were available to the Working Group.

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not proposed any occupational exposure limit for *N*-vinyl-2-pyrrolidone in workplace air. Russia has a short-term exposure limit of 1 mg/m^3 for exposure in workplace air, with a skin notation (International Labour Office, 1991).

No international guideline for *N*-vinyl-2-pyrrolidone in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Polyvinyl pyrrolidone was tested in mice, rats and rabbits by several routes of administration, using materials of various molecular weights. Repeated subcutaneous injections of an aqueous solution of polyvinyl pyrrolidone to rats caused local sarcomas. Single or several subcutaneous or intraperitoneal implantations of polyvinyl pyrrolidone powder resulted in a low incidence of local tumours. After several intravenous injections

or after intraperitoneal implantation of polyvinyl pyrrolidone, tumours occurred in rats at distant sites, including the reticuloendothelial system; the results of these experiments do not permit an evaluation of a possible association of these distant tumours with the treatment (IARC, 1979).

3.1 Inhalation exposure

Rat: Groups of 60 male and 60 female Sprague-Dawley rats, 37–39 days of age, were administered *N*-vinyl-2-pyrrolidone (purity, 99.9%) by whole-body exposure at concentrations of 5, 10 or 20 ppm [22, 45 or 90 mg/m^3] vapour for 6 h per day on five days per week for 24 months. A group of 70 males and 70 females exposed to air alone served as controls. Macroscopic examination was carried out on all animals, and histopathological examination was performed on almost all organs and all gross lesions. Exposed animals displayed a significant reduction in body weight gain. No difference in survival was noted in any group. As shown in Table 1, adenomas and adenocarcinomas of the nasal cavity and hepatocellular carcinomas occurred with significantly positive dose-related trends in treated groups of each sex. In addition, squamous carcinomas of the larynx were found in rats of the high-dose group. Increased incidences of inflammation, atrophy of the olfactory epithelium, hyperplasia and metaplasia in the nasal cavity, focal hyperplasia, foci of cellular alteration and spongiosis hepatis in the liver, and epithelial hyperplasia in the larynx were also seen in the exposed groups (Klimisch *et al*., 1997a).

Groups of 30 male and 30 female Sprague-Dawley rats, 37–39 days of age, were administered *N*-vinyl-2-pyrrolidone (purity, 99.9%) by whole-body inhalation at concen-

Table 1. Incidence of neoplasms in *N*-vinyl-2-pyrrolidone-treated Sprague-Dawley rats

Sex	Male				Female			
Exposure concentration (ppm)	0	5	10	20	0	5	10	20
Number	70	60	60	60	70	60	60	60
Nasal cavity								
Adenoma	0	9	9	11*	0	2	8	14**
Adenocarcinoma	0	0	4	6**	0	0	0	4*
Liver								
Hepatocellular carcinoma	1	6	5	17**	1	3	6	26**
Larynx								
Squamous carcinoma	0	0	0	4	0	0	0	4

From Klimisch *et al*. (1997a)

* Peto's analysis for trend, $p < 0.001$

** Peto's analysis for trend, $p < 0.0001$

trations of 0, 5, 10 or 20 ppm [0, 22, 45 or 90 mg/m^3] vapour for 6 h per day on five days per week for 12 months; 10 males and 10 females of each group were killed after three months of treatment. Macroscopic examination was carried out on all animals, and histopathological examination was performed on the liver, nasal cavity and pancreas. Adenomas of the nasal cavity were seen in one male in the low-dose group and one male and one female in the high-dose group at 12 months of treatment. Foci of cellular alteration (clear cell areas) of the liver were observed in low-dose females at 12 months, and in mid- and high-dose males and females at three and 12 months [incidence and statistical significance unspecified]. Also increased liver weight, spongiosis hepatis and nasal lesions (inflammation, atrophy of the olfactory epithelium, hyperplasia of basal cells of the respiratory and olfactory epithelium, and hyperplasia of the submucosal glands) were observed in the treated groups (Klimisch *et al*., 1997b).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Polyvinyl pyrrolidone of molecular weight of less than 25 000 can be excreted via the kidneys. Following intravenous administration to terminal cancer patients of polyvinyl pyrrolidone (average molecular weight 40 000), approximately one-third was eliminated in urine in 6 h and another one-third in the following 18 h. At autopsy, accumulation was observed in the kidneys, lung, liver, spleen and lymph nodes (IARC, 1979).

4.1.2 *Experimental systems*

The disposition of *N*-[^{14}C]vinyl-2-pyrrolidone has been studied in male Sprague-Dawley rats following a single intravenous injection. The plasma half-life was 1.9 h. Up to 6 h after dosing, the highest tissue concentrations of radioactivity were found in the liver and small intestines. By that time, about 19% of the dose had been excreted in bile, yet, by 12 h, only about 0.4% had been excreted in faeces while about 75% had been excreted in urine. Thus, there appeared to be substantial enterohepatic recirculation of biliary metabolites. Very small quantities of the administered material were excreted unchanged. In a single rat, 12% of the urinary radioactivity was present as acetic acid. Other metabolites were not identified (McClanahan *et al*., 1984).

There was no significant metabolism of polyvinyl pyrrolidone injected intravenously into rats, rabbits or dogs. Retention is proportional to molecular weight (IARC, 1979).

4.2 Toxic effects

4.2.1 *Humans*

In liver biopsies from people who had received polyvinyl pyrrolidone intravenously, basophilic globular deposits were observed in Kupffer cells, occasionally accompanied by

mild inflammation. Thesaurismosis (a foam-cell storage phenomenon characterized by swollen cells with reticulated nuclei loaded with vacuoles or deposits of polyvinyl pyrrolidone) has been observed after inhalation of hair sprays containing polyvinyl pyrrolidone and may be accompanied by pulmonary fibrosis and pneumonia (IARC, 1979).

4.2.2 *Experimental systems*

A thesaurismotic reaction has been found in many organs, but particularly the spleen, in mice, rats, rabbits and dogs treated with polyvinyl pyrrolidone (IARC, 1979).

The toxicology of *N*-vinyl-2-pyrrolidone in rodents described in unpublished reports has been summarized (Klimisch *et al*., 1997a,b). Most of these studies involved inhalation exposure to concentrations of up to 120 ppm [545 mg/m^3] (a lethal concentration) for periods ranging from one week to one year. Oral administration studies (gavage and drinking-water) have also been conducted in rats at dose levels of up to 100 mg/kg bw per day. *N*-Vinyl-2-pyrrolidone is an irritant to skin and mucous membranes, causes hepatotoxicity in rats and mice, but not Syrian hamsters, and causes nasal damage upon inhalation. Nasal cavity inflammation, atrophy of olfactory epithelium and hyperplasia of the basal cells of the respiratory and olfactory epithelium were seen in Sprague-Dawley rats exposed to 5 ppm [23 mg/m^3] or more for 6 h per day on five days per week for three months. Haematological changes (reduced haemoglobin, erythrocyte count and haematocrit) and blood chemistry changes (reduced plasma protein and increased γ-glutamyltranspeptidase) were also observed.

4.3 Reproductive and developmental effects

No data were available to the Working Group on either substance.

4.4 Genetic and related effects

No data were available to the Working Group on either substance.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Little information was available to the Working Group regarding potential exposures to *N*-vinyl-2-pyrrolidone.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

N-Vinyl-2-pyrrolidone was tested for carcinogenicity in one experiment in rats by inhalation exposure. It produced adenomas and adenocarcinomas of the nasal cavity, squamous carcinomas of the larynx and hepatocellular carcinomas in both sexes.

Another 12-month inhalation experiment in rats of the same strain indicated occurrence of adenomas of the nasal cavity and foci of cellular alteration of the liver.

Polyvinyl pyrrolidone was tested for carcinogenicity in mice, rats and rabbits by several routes of administration, producing local tumours.

5.4 Other relevant data

N-Vinyl-2-pyrrolidone metabolites and polyvinyl pyrrolidone are excreted mainly in urine. Inhalation of low concentrations of *N*-vinyl-2-pyrrolidone by rats can cause nasal cavity inflammation, atrophy of olfactory epithelium and hyperplasia of the basal cells of the respiratory and olfactory epithelium. In humans and experimental animals, polyvinyl pyrrolidone accumulates in vacuoles of cells of many organs and, in humans, may be accompanied by pulmonary fibrosis and pneumonia. There have been no genetic toxicity studies with either compound.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of *N*-vinyl-2-pyrrolidone or polyvinyl pyrrolidone were available.

There is *limited evidence* for the carcinogenicity of *N*-vinyl-2-pyrrolidone in experimental animals.

There is *limited evidence* for the carcinogenicity of polyvinyl pyrrolidone in experimental animals.

Overall evaluation

N-Vinyl-2-pyrrolidone is *not classifiable as to its carcinogenicity to humans (Group 3).*

Polyvinyl pyrrolidone is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

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WHO (1993) *Guidelines for Drinking Water Quality*, 2nd Ed., Vol. 1, *Recommendations*, Geneva

XYLENES

Data were last evaluated in IARC (1989).

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

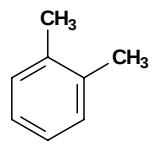
Chem. Abstr. Serv. Reg. No.: 1330-20-7
Chem. Abstr. Name: Dimethylbenzene
IUPAC Systematic Name: Xylene
Synonym: Xylol

Chem. Abstr. Serv. Reg. No.: 95-47-6
Chem. Abstr. Name: 1,2-Dimethylbenzene
IUPAC Systematic Name: *ortho*-Xylene
Synonym: *ortho*-Xylol

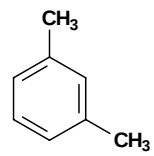
Chem. Abstr. Serv. Reg. No.: 108-38-3
Chem. Abstr. Name: 1,3-Dimethylbenzene
IUPAC Systematic Name: *meta*-Xylene
Synonym: *meta*-Xylol

Chem. Abstr. Serv. Reg. No.: 106-42-3
Chem. Abstr. Name: 1,4-Dimethylbenzene
IUPAC Systematic Name: *para*-Xylene
Synonym: *para*-Xylol

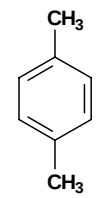
1.1.2 *Structural and molecular formulae and relative molecular mass*



o-Xylene



m-Xylene



p-Xylene

C_8H_{10} Relative molecular mass: 106.17

1.1.3 *Chemical and physical properties of the pure substances*

From American Conference of Governmental Industrial Hygienists (1992), unless otherwise noted.

(*a*) *Description*: Colourless liquids (Budavari, 1996)
(*b*) *Boiling-point*: 144.4°C (*ortho*); 139.1°C (*meta*); 138.3°C (*para*)
(*c*) *Melting-point*: –25.2°C (*ortho*); –47.9°C (*meta*); 13.3°C (*para*)
(*d*) *Solubility*: Insoluble in water; miscible with ethanol, diethyl ether and other organic solvents (*ortho*, *meta* and *para*)
(*e*) *Vapour pressure*: 904 Pa at 25°C (*ortho*); 1104 Pa at 25°C (*meta*); 1184 Pa at 25°C (*para*)
(*f*) *Flash point*: 17°C (*ortho*), closed cup; 25°C (*meta* and *para*), closed cup
(*g*) *Conversion factor*: $mg/m^3 = 4.34 \times ppm$

1.2 Production and use

World production of *para*-xylene in 1983 was 3900 thousand tonnes, of which the United States accounted for 48%, Europe 23% and Japan 16%; the world production of *ortho*-xylene in 1983 was 1300 thousand tonnes, of which western Europe produced 30% and the United States 18% (WHO, 1997). In 1993, United States production of *ortho*-xylene was reported to be about 377 thousand tonnes and of *para*-xylene was about 2600 thousand tonnes (United States International Trade Commission, 1994). United States production of mixed xylene and *para*-xylene in 1994 was approximately 4100 and 2800 thousand tonnes, respectively (WHO, 1997).

The major uses of mixed xylene are in aviation gasoline and protective coatings, and as a solvent for alkyd resins, lacquers, enamels and rubber cements. *meta*-Xylene is used as a solvent, as an intermediate for dyes and organic synthesis, especially isophthalic acid and insecticides, and in aviation fuel; *ortho*-xylene is used in manufacture of phthalic anhydride, vitamin and pharmaceutical synthesis, dyes, insecticides, motor fuels; *para*-xylene is used in synthesis of terephthalic acid for polyester resins and fibres, vitamin and pharmaceutical syntheses, and insecticides (Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES) (1997), approximately 1 106 800 workers in the United States were potentially exposed to xylene (see General Remarks).

Data on levels of occupational exposure to xylene are presented in a previous monograph (IARC, 1989).

1.3.2 *Environmental occurrence*

Commercial xylene is a mixture of the three xylene isomers in the following percentage ranges: *ortho*-xylene, 10–25%; *meta*-xylene, 45–70%; and *para*-xylene, 6–15%. Xylene may enter the atmosphere primarily from fuel emissions and exhausts, due to its

use in gasoline. Its production and use in petroleum products, as a solvent, and as an intermediate in organic synthesis may result in its release to the environment through various waste streams. Natural sources of xylene such as petroleum, forest fires and volatile substances in plants may also contribute to its presence in the environment (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 434 mg/m^3 as the threshold limit value for occupational exposures to xylene in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

The World Health Organization has established an international drinking-water guideline for xylene of 500 µg/L (WHO, 1993).

2. Studies of Cancer in Humans

2.1 Industry-based studies

In a case–control study within a cohort of 6678 rubber workers in the United States (IARC, 1987) (Wilcosky *et al*., 1984), one of the substances assessed was xylene, which was analysed as a potential risk factor in relation to each of five cancer types. There were somewhat increased odds ratios (OR) for prostate cancer (OR, 1.5; $n = 8$), lymphosarcoma (OR, 3.7; $n = 4$; $p < 0.05$) and lymphatic leukaemia (OR, 3.3; $n = 4$). [The Working Group noted that workers were typically exposed to multiple exposures.]

2.2 Community-based studies

Olsson and Brandt (1980) carried out a hospital-based case–control study of Hodgkin's disease and chemical exposures in Lund, Sweden. Twenty-five consecutive male cases aged 20–65 years were included. Two neighbourhood-matched controls were selected for each case from the Swedish population register. Interviews with study subjects focused on their detailed job history, and in particular exposure to solvents. Interview data were supplemented with visits to employers in some cases. Four of the cases and none of the controls had been exposed to xylene. All exposed cases were also exposed to other solvents. [The Working Group noted the opportunity for information bias, since the interviewer was not blind to disease status or to the study objectives.]

In the nested case–control study of Carpenter *et al*. (1988) (described in more detail in the monograph on toluene in this volume), there was a hint of excess risk of central nervous system cancer among workers exposed to toluene, xylene and methyl ethyl ketone (evaluated as one chemical group) at two nuclear facilities located in Tennessee (United States). The relative risk was 2.0 (95% confidence interval (CI), 0.7–5.5; $n = 28$) in comparison with unexposed workers. [The Working Group noted that no separate

analysis was reported for the three solvents, and that there were many concurrent exposures. Exposure levels were not quantified.]

Gérin *et al*. (1998) presented results concerning xylene from the population-based case–control study in Montreal, Canada (described in more detail in the monograph on toluene in this volume). Of the entire study population, 12.4% had been exposed to xylene at some time. Among the main occupations to which xylene exposure was attributed were vehicle mechanics and repairmen, painters (except construction) and shoemakers. For the following cancer sites, there was little indication of excess risk in relation to exposure to xylene (results for 'high' exposure): oesophagus (OR, 1.4; *n* = 5), stomach (OR, 1.8; *n* = 2), pancreas (OR, 1.1; *n* = 4), prostate (OR, 1.4; *n* = 6), urinary bladder (OR, 0.8; *n* = 3), kidney (OR, 1.0; *n* = 6), skin melanoma (ever exposed OR, 0.3; *n* = 3) and non-Hodgkin lymphoma (OR, 1.0; *n* = 6). For the following sites there was indication of excess risk: colon (OR, 5.8; 95% CI, 1.5–22.0; *n* = 8), rectum (OR, 2.7; 95% CI, 0.9–8.3; *n* = 5) and lung (OR, 1.6; 95% CI, 0.7–3.8; *n* = 16). [The Working Group noted that most workers exposed to xylene were also exposed to benzene, toluene and perhaps other substances. Exposure levels were not quantified.]

3. Studies of Cancer in Experimental Animals

Xylene (technical grade or mixed xylenes) was tested for carcinogenicity in one strain of mice and in two strains of rats by gavage. One study in rats with mixed xylenes was considered inadequate for evaluation. No increase in the incidence of tumours was observed in either mice or rats following administration of a technical-grade xylene. No data were available on the individual isomers (IARC, 1989).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

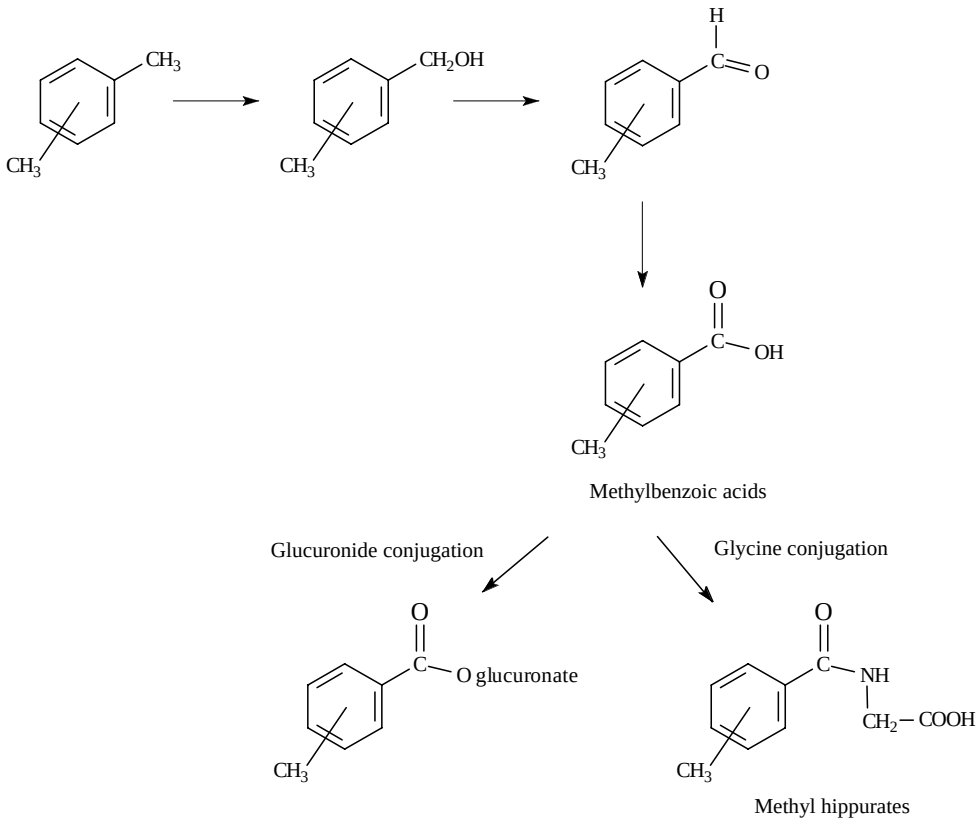
Xylenes are metabolized primarily by oxidation to the methylbenzyl alcohols, followed by further oxidation to the corresponding methylbenzoic acids (toluic acids). These can be conjugated with glycine to form methylhippurates, or with UDP-glucuronate to form acyl glucuronides (see Figure 1).

4.1.1 *Humans*

The pharmacokinetics and metabolism of the xylenes have been reviewed (Low *et al*., 1989; Langman, 1994).

In volunteers exposed by inhalation, lung retention of *meta*-xylene was about 60%. When volunteers immersed their hands in liquid *meta*-xylene, it was absorbed at 2 μg/cm^2

Figure 1. Metabolism of xylenes



Adapted from Low *et al.* (1989)

per min. The amount of *meta*-xylene absorbed after whole-body exposure of volunteers to 600 ppm [2600 mg/m^3] vapour, excluding inhalation, for 3.5 h was equivalent to the amount absorbed after inhalation exposure to 20 ppm [87 mg/m^3] for the same duration (IARC, 1989).

More than 70% of *meta*-xylene absorbed was excreted into the urine as metabolites. A minor portion was exhaled unchanged (IARC, 1989). Elimination is rapid, with a biological half-time of 1 h for a rapid phase after 6–16 h of exposure and of about 20 h for a slow phase. Removal of industrial xylene from subcutaneous adipose tissue, however, is slow, with a half-time of 25–128 h for the *meta* isomer (IARC, 1989).

Xylenes are metabolized in humans primarily to the corresponding methylhippuric acid (toluric acid); and glycine conjugation is considered to be a rate-limiting step. Only a small portion is excreted as dimethylphenol: 2,3-dimethylphenol and 3,4-dimethylphenol after exposure to *ortho*-xylene, 2,4-dimethylphenol after exposure to *meta*-xylene and 2,5-dimethylphenol after exposure to *para*-xylene (IARC, 1989).

When workers are exposed to xylenes at low exposure levels (up to 20 ppm [870 mg/m^3]), the end-of-shift urinary excretion of methylhippurates is a good indication of exposure because these methylhippurates are normally not present in urine (Jonai & Sato, 1988; Kawai *et al*., 1991).

4.1.2 *Experimental systems*

After inhalation exposure of mice to [^{14}C]-*para*-xylene, methylhippurate accumulated in nasal mucosa and the olfactory bulb, possibly due to axonal flow-mediated transport of the methylhippurate from the mucosa, where it is formed, to the olfactory lobe of the brain (Ghantous *et al*., 1990). Inhalation exposure of rats to *meta*-xylene with or without ethyl acetate showed that ethyl acetate caused a decrease in the blood concentration of *meta*-xylene (Freundt *et al*., 1989).

Tardif *et al*. (1997) developed a physiologically based pharmacokinetic model for *meta*-xylene in rats and humans. They also simulated interactions between *meta*-xylene, toluene and ethylbenzene, and showed that for exposures at air concentrations remaining within the permissible range for a mixture, biologically significant interactions at the pharmacokinetic level would not occur.

Exposure of Sprague-Dawley rats to 300 ppm [1300 mg/m^3] *meta*-xylene for 6 h inhibited several cytochrome P450 (CYP) isoenzymes in the lung (e.g., CYP2B1, 1A2, 2E1 and 4B1), but had little effect on the hepatic levels of these CYPs (Foy *et al*., 1996).

4.2 Toxic effects

The toxicity of xylene has been reviewed (Agency for Toxic Substances and Disease Registry, 1990).

4.2.1 *Humans*

Adverse effects on the kidney and liver have been observed in cases of accidental poisoning.

Local irritation and mild central nervous system symptoms were reported in a questionnaire survey, but no abnormalities were seen in a health examination, clinical chemistry, or haematological parameters among 175 xylene-exposed employees, whose exposure to xylene was on average 21 ppm [87 mg/m^3] (Uchida *et al*., 1993). Minor effects on body sway, reaction times or overnight sleep pattern were observed after experimental inhalation exposure to xylene (200 ppm [870 mg/m^3], 5 h per day for six days) (Laine *et al*., 1993).

4.2.2 *Experimental systems*

In experimental animals exposed to high doses of xylene, adverse effects have been observed in the kidney and liver (IARC, 1989).

Ninety-day gavage dosage of technical xylene (17.6% *ortho*-xylene, 62.3% *meta*- and *para*-xylene, 20% ethylbenzene; 150, 750 or 1500 mg/kg bw daily) induced no mortality in Sprague-Dawley rats (Condie *et al*., 1988). Slight increases in alanine aminotransferase

activity were observed in males (1500 mg/kg bw) and females (≥ 750 mg/kg bw), but not in other enzymes reflecting liver cell damage. No histological damage to the liver was noted, but liver weight was increased in males at all dose levels, and in females at levels ≥ 750 mg/kg bw per day. Minimal, but dose-dependent, nephropathy was observed in females.

Three-month inhalation exposure to *meta*-xylene (1000 ppm [4340 mg/m^3], 6 h per day on five days per week) of male Wistar rats had a very slight effect on the hepatocyte ultrastructure: limited proliferation of smooth endoplasmic reticulum and lysosomes was observed. Findings were similar after six months' exposure to 100 ppm [434 mg/m^3]. Simultaneous exposure to toluene made the proliferation of smooth endoplasmic reticulum more prominent (Rydzynski *et al*., 1992).

Exposure of male Fischer 344 rats to *para*-xylene (0–1600 ppm [0–6940 mg/m^3], 6 h per day for one or three days) had negligible effect on hepatic morphology and serum activities of alanine or aspartate aminotransferases, lactate dehydrogenase, ornithine carbamyl transferase, alkaline phosphatase or serum bilirubin concentration (Simmons *et al*., 1991). Liver size was increased and its cytochrome P450 content was elevated.

Treatment of male Charles-Foster rats with sublethal doses of xylene (0.2 mL of 5 mmol/L extra-pure xylene solution on alternate days for 30 days; isomeric composition not indicated) resulted in slight increases of serum aspartate and alanine aminotransferase and alkaline phosphatase activities and bilirubin concentration (Rana & Kumar, 1993). A slight increase in alanine aminotransferase activity was also observed after a 3.5-week treatment of male Wistar rats with *meta*-xylene (800 mg/kg bw per day on five days per week, by gavage) (Elovaara *et al*., 1989). Inhalation exposure of C3H/HeJ mice to *para*-xylene (1200 ppm [5200 mg/m^3], 6 h per day for four days) did not affect the serum alanine or aspartate aminotransferase or lactate dehydrogenase activities, or bilirubin level (Selgrade *et al*., 1993).

Inhalation and oral exposure to *meta*-xylene induced hepatic cytochrome P450 activities, notably that of CYP2B1, as well as the activities of UDP-glucuronosyltransferase, DT-diaphorase and glutathione *S*-transferase (Savolainen *et al*., 1978; Toftgård *et al*., 1983; Elovaara *et al*., 1989; Raunio *et al*., 1990; Gut *et al*., 1993). On the other hand, short-term inhalation exposure (≥ 75 ppm [325 mg/m^3], 24 h) led to a decrease in cytochrome P450 activity in rat lung (Elovaara *et al*., 1987). Inhalation exposure of C3H/HeJ mice to *para*-xylene (1200 ppm [5200 mg/m^3], 6 h per day for four days) slightly increased the hepatic cytochrome P450 content (Selgrade *et al*., 1993).

When *para*-xylene-exposed (1200 ppm [5200 mg/m^3], 6 h per day for four days) C3H/HeJ mice were infected intraperitoneally with murine cytomegalovirus (10^5 plaque-forming units after the first xylene exposure), 34% of the mice died, while none died after either exposure alone or after a similar exposure to xylene at 600 ppm [2600 mg/m^3], combined with exposure to the virus (Selgrade *et al*., 1993). Elevated mortality was not related to immune function or hepatic damage.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

In a case–control study of congenital malformations and spontaneous abortions within a cohort of workers who at some time of their career had had a biomonitoring measurement for occupational solvent exposure performed, data on medically diagnosed pregnancies were extracted from the hospital discharge registry, and data on abortions separately from polyclinics. Exposure of the father and mother of the cases and referents was collected by questionnaire (Taskinen *et al.*, 1989; Lindbohm *et al.*, 1990). An elevated odds ratio of spontaneous abortions for paternal exposure to xylene (37 cases; OR, 1.8; 95% CI, 1.1–3.2) was observed. However, most xylene-exposed fathers also had been exposed to other solvents; after adjustment for confounders, only paternal exposure to organic solvents as a group remained significant. Xylene exposure of women was not associated with spontaneous abortions; malformations were not associated with exposures of either men or women. In a similar case–control study on solvent exposure and pregnancy outcome among laboratory assistants (Taskinen *et al.*, 1994), the odds ratio of spontaneous abortion was increased among the women who were exposed to xylene for at least three days per week during the first trimester of pregnancy (OR, 3.1; 95% CI, 1.3–7.5). Simultaneous exposure to other solvents was common; two cases (out of 36) and two controls (out of 105) were exposed to xylene only. No elevated odds ratio for congenital malformations was observed for any solvent, but the power of the study was limited.

4.3.2 *Experimental systems*

Maternally toxic or near-toxic amounts of xylene have been associated with malformations in mice after oral administration and with embryotoxicity in rabbits, rats and mice after exposure by inhalation (IARC, 1989).

When female Wistar rats were exposed to technical xylene (200 ppm [870 mg/m^3], 6 h per day) on days 4 through 20 of gestation, no exposure-related embryo- or fetotoxicity or terata were observed, but the frequency of delayed ossification of maxillary bone was increased. Postnatal weight gain was faster in pups from exposed dams, and they also showed advanced development of some physical milestones (ear unfolding, eye opening) but a weaker performance on Rotarod (Hass & Jakobsen, 1993). Xylene did not induce malformations in explanted rat embryos at the highest concentrations tested (0.28–0.57 μL/mL), but retarded the growth and development of the embryos at the lowest concentration tested (0.15–0.45 μL/mL) (Brown-Woodman *et al.*, 1991). Male Sprague-Dawley rats exposed to xylene (1009 ± 47 ppm [4380 ± 204 mg/m^3], 18 h per day on seven days per week for 61 days) showed no evidence of histological damage to the testes (percentage of intact spermatozoa or of normal head and tail, testis weight, ventral prostate weight, noradrenaline content of vas deferens) two weeks or 10 months after the cessation of exposure (Nylén *et al.*, 1989).

4.4 Genetic and related effects

4.4.1 *Humans*

Sister chromatid exchanges were not induced in peripheral lymphocytes of workers in two studies (exposure to a variety of compounds) or in five healthy volunteers exposed for seven consecutive hours per day over three consecutive days to 40 ppm [174 mg/m^3] xylene either alone or in combination with 50 ppm [189 mg/m^3] toluene (Haglund *et al*., 1980; Pap & Varga, 1987; Richer *et al*., 1993).

4.4.2 *Experimental systems* (see Table 1 for references)

Technical grade xylene did not induce DNA damage in bacteria.

No gene mutations were induced by xylenes in *Salmonella typhimurium* strains or in *Escherichia coli* WP*uvr*A.

Xylene of unspecified grade did not induce morphological transformation in cultured Syrian hamster cells.

In vitro, mixtures of xylenes did not induce sister chromatid exchanges or chromosomal aberrations either in Chinese hamster ovary CHO cells or in human lymphocytes, in the absence of an exogenous metabolic system.

None of the three isomers of xylene induced micronuclei or chromosomal aberrations in mouse bone marrow *in vivo* (chromosomal aberrations were observed in mouse spleen lymphocytes *in vivo*).

5. Summary of Data Reported and Evaluation[1]

5.1 Exposure data

Exposure to xylenes may occur during their production and in the production of aviation gasoline and protective coatings, and during their use in petroleum products, e.g., solvents, and as intermediates in organic synthesis. Natural sources include petroleum, forest fires and volatile substances in plants.

5.2 Human carcinogenicity data

Xylene was mentioned as an exposure in four studies. Two were community-based case–control studies, one of which involved brain cancer and one involved several types of cancer. The two industry-based studies were configured as nested case–control studies, one of central nervous system tumours and one of several sites. In none of these studies was xylene the sole or predominant exposure. Cancers at most sites were not significantly associated with xylene exposure in any study. Incidence of colorectal cancer was significantly elevated in the Canadian case–control study, but no other study reported colorectal cancer results. Hodgkin's disease was elevated in one study; non-Hodgkin lymphoma was elevated in one study, but not in another. Most results were based on small numbers. In

[1] Summary (but not the evaluation) prepared by the Secretariat after the meeting.

Table 1. Genetic and related effects of xylenes

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
***meta*-Xylene**				
SA0, *Salmonella typhimurium* TA100, reverse mutation (spot test)	–	–	160	Florin *et al*. (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	250	Bos *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	16	Haworth *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Connor *et al*. (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation (spot test)	–	–	160	Florin *et al*. (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	250	Bos *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	16	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation (spot test)	–	–	160	Florin *et al*. (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	250	Bos *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	16	Haworth *et al*. (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	250	Bos *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation (spot test)	–	–	160	Florin *et al*. (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250	Bos *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	16	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Connor *et al*. (1985)
SAS, *Salmonella typhimurium* UTH 8414 and UTH 8413, reverse mutation	–	–	500	Connor *et al*. (1985)
MVM, Micronucleus test, NMRI mice *in vivo*	–		650 ip × 2	Mohtashamipur *et al*. (1985)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
***ortho*-Xylene**				
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	250	Bos *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	50	Haworth *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Connor *et al*. (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	250	Bos *et al*. (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	50	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	250	Bos *et al*. (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	50	Haworth *et al*. (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	250	Bos *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250	Bos *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	50	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Connor *et al*. (1985)
SAS, *Salmonella typhimurium* UTH 8414 and UTH 8413, reverse mutation	–	–	500	Connor *et al*. (1985)
MVM, Micronucleus test, NMRI mice *in vivo*	–		435 ip × 2	Mohtashamipur *et al*. (1985)
SPR, Sperm morphology, Sprague-Dawley rats *in vivo*	(+)		435 ip × 2	Washington *et al*. (19
***para*-Xylene**				
SA0, *Salmonella typhimurium* TA100, reverse mutation (spot test)	–	–	160	Florin *et al*. (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	250	Bos *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	50	Haworth *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Connor *et al*. (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	50	Shimizu *et al*. (1985)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
***para*-Xylene (contd)**				
SA5, *Salmonella typhimurium* TA1535, reverse mutation (spot test)	–	–	160	Florin *et al*. (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	250	Bos *et al*. (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	50	Haworth *et al*. (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	50	Shimizu *et al*. (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation (spot test)	–	–	160	Florin *et al*. (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	250	Bos *et al*. (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	50	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	50	Shimizu *et al*. (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	250	Bos *et al*. (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	50	Shimizu *et al*. (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation (spot test)	–	–	160	Florin *et al*. (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250	Bos *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	50	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Connor *et al*. (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	50	Shimizu *et al*. (1985)
SAS, *Salmonella typhimurium* UTH 8414 and UTH 8413, reverse mutation	–	–	500	Connor *et al*. (1985)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	–	50	Shimizu *et al*. (1985)
MVM, Micronucleus test, NMRI mice *in vivo*	–		650 ip × 2	Mohtashamipur *et al*. (1985)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Mixtures of xylenes				
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links	–	–	36	Nakamura *et al.* (1987)
ECL, *Escherichia coli pol* A/W3110-P3478, differential toxicity (liquid suspension)	–	–	10000	McCarroll *et al.* (1981a)
ERD, *Escherichia coli rec* strains, differential toxicity	–	–	10000	McCarroll *et al.* (1981a)
BSD, *Bacillus subtilis rec* strains, differential toxicity	–	–	100000	McCarroll *et al.* (1981b)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	250	Bos *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	16	Haworth *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	100	Haworth *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	166	Haworth *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Connor *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	50	Shimizu *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	100	Zeiger *et al.* (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	250	Bos *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	16	Haworth *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	100	Haworth *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	166	Haworth *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	50	Shimizu *et al.* (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	100	Zeiger *et al.* (1987)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	250	Bos *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	16	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	100	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	166	Haworth *et al.* (1983)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Mixtures of xylenes (contd)				
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	50	Shimizu *et al*. (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	250	Bos *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250	Bos *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	16	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	100	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	166	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Connor *et al*. (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	50	Shimizu *et al*. (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	100	Zeiger *et al*. (1987)
SAS, *Salmonella typhimurium* 4HT 8414 and 4HT 8413, reverse mutation	–	–	500	Connor *et al*. (1985)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	100	Zeiger *et al*. (1987)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	–	50	Shimizu *et al*. (1985)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	50	Anderson *et al.* (1990)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	100.5	Anderson *et al.* (1990)
T7S, Cell transformation, SA7/Syrian hamster embryo cells *in vitro*	–	NT	1000	Casto (1981)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	NT	1500	Gerner-Smidt & Friedrich (1978)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	–	NT	212	Richer *et al*. (1993)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	1500	Gerner-Smidt & Friedrich (1978)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Mixtures of xylenes (contd)				
MVM, Micronucleus test, NMRI mice *in vivo*	–		435 ip × 2	Mohtashamipur *et al*. (1985)
MVM, Micronucleus test, NMRI mice *in vivo*	–		650 ip × 2	Mohtashamipur *et al*. (1985)
SPR, Sperm morphology, Sprague-Dawley rats *in vivo*	(+)		435 ip × 2	Washington *et al*. (1983)
Mixtures of *para*-xylene, benzene, chloroprene and epichlorohydrin (2/2/2/1)				
SA0, *Salmonella typhimurium* TA100 (urine from treated mice), reverse mutation	–	–	9:10:11:5 ppm inh 6 wk	Au *et al*. (1988)
SA9, *Salmonella typhimurium* TA98 (urine from treated mice), reverse mutation	–	–	9:10:11:5 ppm inh 6 wk	Au *et al*. (1988)
MVM, Micronucleus test, CD-1 Swiss mouse bone marrow *in vivo*	–		9:10:11:5 ppm inh 6 wk	Au *et al*. (1988)
CBA, Chromosomal aberrations, CD-1 Swiss mouse bone marrow *in vivo*	–		9:10:11:5 ppm inh 6 w	Au *et al*. (1988)
CLA, Chromosomal aberrations, CD-1 Swiss mouse spleen lymphocytes *in vivo*	+		0.09:0.08:0.02:0.04 ppm inh 3 wk	Au *et al*. (1988)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; ip, intraperitoneal; inh, inhalation

view of the multiple exposure circumstances in most studies, the multiple inference context of these studies, and the weak consistency of the findings, these results are not strong enough to establish whether there is an association with xylene exposure.

5.3 Animal carcinogenicity data

Xylene (technical grade or mixed xylenes) was tested for carcinogenicity in one strain of mice and in two strains of rats by gavage. One study in rats with mixed xylenes was considered inadequate for evaluation. No increase in the incidence of tumours was observed in either mice or rats following the administration of a technical-grade xylene.

No data were available on the indidivual isomers.

5.4 Other relevant data

Xylenes are absorbed after inhalation and dermal exposure. Elimination after human exposure is rapid and mostly as urinary metabolites after oxidation to the methylbenzyl alcohols, methylbenzoic acids and their glycine and glucuronic acid conjugates. In mice inhaling *para*-xylene, methylhippurate accumulated in the nasal mucosa and olfactory bulb.

Renal and hepatic toxicity has been described following human accidental poisonings and experimental exposure of rats and mice. In rats, hepatic cytochrome P450 content, particularly of CYP2B1, and the activities of certain conjugation enzymes are increased upon inhalation exposure to *meta*-xylene. Although xylenes have been studied extensively, there is no confirmed evidence of genetic activity for any of the isomers.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of xylenes.

There is *inadequate evidence* in experimental animals for the carcinogenicity of xylenes.

Overall evaluation

Xylenes are *not classifiable as to their carcinogenicity to humans (Group 3)*.

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Part Three B
Few New Data

ACETAMIDE

Data were last reviewed in IARC (1974) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

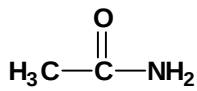
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 60-35-5

Systematic name: Acetamide

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_2H_5NO Relative molecular mass: 59.1

1.1.3 *Physical properties* (for details, see IARC, 1974)

(*a*) *Boiling-point*: 222°C

(*b*) *Melting-point*: 81°C

(*c*) *Conversion factor*: $mg/m^3 = 2.42 \times ppm$

1.2 Production and use

Acetamide has been produced commercially since the 1920s, but it is not certain that it is still in commercial use, although it was previously used as an intermediate in the synthesis of methylamine, thioacetamide, hypnotics, insecticides, medicinals and various plastics, a solvent, a soldering flux ingredient, a wetting agent and penetration accelerator for dyes, and as a plasticizer in leather, cloth and coatings (IARC, 1974).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Acetamide was tested for carcinogenicity by oral administration in rats, producing benign and malignant liver tumours (IARC, 1974).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female C57BL/6 mice were fed diets containing 1.18% or 2.36% acetamide for 12 months. In male mice treated with acetamide, an increase in the incidence of malignant lymphomas was observed (controls, 0/95; low-dose, 7/50; high-dose, 7/46; $p = 0.004$, Cochran-Armitage test for trend) (Fleischman *et al*., 1980).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats were fed 0 or 2.36% acetamide in the diet for 12 months. Neoplastic nodules were seen in the liver in 0/50 and 1/47 control and treated males and in 0/49 and 3/48 control and treated females, respectively. Hepatocellular carcinomas were found in 0/50, 41/47, 0/49, 33/48 male controls, male treated, female controls and female treated rats, respectively. The incidence, speed of onset and frequency of metastases were greater in males than in females (Fleischman *et al*., 1980).

In a study of hepatocellular changes induced by acetamide, 60 male Leeds strain rats were administered a diet containing 5.0% acetamide for up to 35 weeks and were then returned to a control diet for up to a further nine months. The experiment included a control group of 40 male rats. Subgroups of four rats from the treated and control groups were killed at nine days and four, 10, 26 and 35 weeks from the beginning of the experiment. Similar numbers from the treated and control groups were killed at one, four and six months and all survivors were killed at nine months after the end of treatment. All acetamide-treated rats killed at 26 weeks had neoplastic nodules of the liver. Hepatocellular carcinomas arose after cessation of treatment in 1/4 rats killed at one month, 5/10 rats killed at four months, 7/8 rats killed at six months and 4/4 rats killed at nine months (Flaks *et al*., 1983).

3.2 Administration with known carcinogens

Rat: Acetamide was studied for initiating activity in a modified Solt-Farber system at single intraperitoneal doses of 100 and 400 mg/kg bw administered to groups of 12 male Fischer 344 rats. Two weeks after injection, all of the rats were treated with 2-acetylaminofluorene (AAF), 2 mg/kg bw, every other day for two weeks. One week after commencing the AAF treatment, partial hepatectomies were performed. Two weeks after finishing the AAF treatment, the rats were fed a diet containing 0.05% phenobarbital for two months, after which they were killed and their livers examined. A negative control group of five rats and a positive control group of four rats were included in which acetamide treatment was replaced by saline and *N*-nitrosodiethylamine (100 mg/kg bw)

treatments, respectively. γ-Glutamyltranspeptidase (γ-GT)-positive foci occurred in 0/5, 5/12 and 12/12 rats of the negative control and the 100 and 400 mg/kg bw acetamide-dosed groups, respectively. Precise quantitation of the foci was not possible. Livers with γ-GT-positive foci showed basophilia, lipidosis and periportal hypertrophy, while γ-GT-negative livers had normal morphology. It was concluded that acetamide shows properties in this system consistent with initiator activity (Dybing *et al*., 1987).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

The half-life of radioactivity in blood after intravenous dosing of [^{14}C]acetamide to rats averaged 20.6 ± 0.3 h after a 10 mg/kg bw dose and 16.1 ± 1.6 h after a 50 mg/kg bw dose. The volume of distribution was about 1 mL/g, total body clearance was 0.27 mL/min and renal clearance was 0.19 mL/min. Approximately 64–72% of [^{14}C]acetamide was excreted in the urine, while only 0.5–0.8% appeared in exhaled air during the first 6 h after dosing. Thus, approximately 30% of the administered dose was not recovered and it was suggested that metabolized acetamide enters the acetate pool (Putcha *et al*., 1984).

Less than 0.07% of the recovered urinary radioactivity in rats given 100 or 1000 mg/kg bw [^{14}C]acetamide coeluted upon high-performance liquid chromatography with an *N*-hydroxyacetamide standard and this hydroxamic acid could not be detected after incubation of acetamide with rat liver microsomes and NADPH or in primary cultures of rat hepatocytes. [^{14}C]Acetamide does not bind covalently to proteins in the presence of rat liver microsomes and NADPH or cytosolic fraction, whereas hepatocyte cultures contained non-extractable radioactivity. This association was inhibited by cycloheximide to the same extent as [^{14}C]acetate incorporation into cellular proteins (Dybing *et al.*, 1987).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

During treatment with a diet containing 5.0% acetamide that led to a high incidence of hepatocellular carcinomas in male Leeds strain rats, neither necrosis nor cirrhosis occurred, in sharp contrast to the response to thioacetamide. Ultrastructural changes within hepatocytes of the acetamide-treated rats gave only minor indications of toxicity (Flaks *et al*.,

1983). The putative metabolite, *N*-hydroxyacetamide, did not cause necrosis within 24 h following a single intraperitoneal injection at doses of 100, 400, 500 and 1000 mg/kg bw, even when some rats in the latter two groups were also treated with 600 mg/kg bw of the glutathione-depleting agent, diethyl maleate, 30 min before *N*-hydroxyacetamide. Toxicity was assayed on the basis of liver morphology and plasma glutamic-oxaloacetic transaminase analysis. However, 2.5 mM *N*-hydroxyacetamide did not deplete cellular glutathione levels in primary cultures of rat hepatocytes (Dybing *et al*., 1987).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Tables 1 and 2 for references)

Acetamide does not cause differential toxicity in repair-deficient *Escherichia coli* strains, and is not mutagenic, with or without metabolic activation, in *Salmonella typhimurium* or *Saccharomyces cerevisiae* or, without metabolic activation, in *Aspergillus nidulans*. It is also not mutagenic in *Saccharomyces cerevisiae* in a host-mediated assay. In *Aspergillus nidulans*, it is not recombinogenic and does not induce chromosome malsegregation. It may weakly induce intrachromosomal recombination in *Saccharomyces cerevisiae*. In *Drosophila melanogaster*, it does not induce germ cell or somatic mutations in a number of loci. However, it weakly induces somatic mutations at the *zeste-white* locus and is weakly positive in the wing spot test. In cultured mammalian cells, it does not induce DNA strand breaks in rat hepatoma cells, gene amplification in Chinese hamster ovary CO361 cells or morphological transformation of C3H 10T½ mouse embryo cells, while it gave contradictory (probably negative) results for the induction of morphological transformation in Syrian hamster embryo cells. It does not inhibit gap-junctional communication in Chinese hamster lung V79 cells. It was marginally positive in the induction of bone-marrow micronuclei in male C57BL/6 mice in one study, but it was negative in another study at higher doses in the same species as well as in CBA male mice.

Genotoxicity studies with *N*-hydroxyacetamide, a possible metabolite of acetamide, have shown that this agent is weakly mutagenic in *Salmonella typhimurium* and induces DNA damage in a rat hepatoma cell line. However, it did not bind covalently to DNA *in vitro* and did not induce morphological transformation of Syrian hamster embryo cells *in vitro* or inhibit gap-junctional intercellular communication in Chinese hamster lung V79 cells.

Table 1. Genetic and related effects of acetamide

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, *Salmonella typhimurium* SOS induction (*umu* test)	–	–	1670	Nakamura *et al.*, 1987
ERD, *Escherichia coli rec A* strains, differential toxicity	–	–	63 800	Hellmér & Bocsfoldi (1992)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	125	Simmon (1979a)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	Haworth *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Dybing *et al*. (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	125	Simmon (1979a)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	125	Simmon (1979a)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Haworth *et al.* (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	125	Simmon (1979a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	125	Simmon (1979a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Dybing *et al*. (1987)
SAS, *Salmonella typhimurium* TA1536, reverse mutation	–	–	125	Simmon (1979a)
SCR, *Saccharomyces cerevisiae* C658-K42, reverse mutation	–	–	16000	Morita *et al*. (1989)
SCH, *Saccharomyces cerevisiae* D3, recombination	–	–	58000	Simmon (1979b)
SCH, *Saccharomyces cerevisiae* RS112, intrachromosomal recombination	(+)	NT	40000	Schiestl *et al*. (1989)
SCH, *Saccharomyces cerevisiae* RS112, interchromosomal recombination	–	NT	40000	Schiestl *et al*. (1989)
ANN, *Aspergillus nidulans*, chromosome malsegregation	–	NT	40000	Crebelli *et al*. (1986)
ANF, *Aspergillus nidulans*, forward mutation	–	NT	40000	Crebelli *et al*. (1986)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		50000 ppm feed	Valencia *et al*. (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		50000 ppm inj.	Valencia *et al*. (1985)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DMM, *Drosophila melanogaster*, somatic mutation/recombination $(w^i)_4$	–		2950 feed	Batiste-Alentorn *et al*. (1994)
DMM, *Drosophila melanogaster*, somatic mutation/recombination $(w^i)_4$	–		4720 feed	Consuegra *et al*. (1996)
DMM, *Drosophila melanogaster*, somatic mutation (*zeste-white* locus)	(+)		590 feed	Batiste-Alentorn *et al*. (1991)
DMM, *Drosophila melanogaster*, somatic mutation	–		4500 feed	Mitchell *et al*. (1981)
DMM, *Drosophila melanogaster*, somatic mutation	–		590 feed	Vogel & Nivard (1993)
DMM, *Drosophila melanogaster*, somatic mutation and recombination test (SMART), wing spot test	(+)		2950 feed	Batiste-Alentorn *et al*. (1995)
DIA, DNA strand breaks, rat hepatoma cells *in vitro*	–	NT	14 775	Dybing *et al*. (1987)
TCM, Cell transformation C3H 10T½ mouse embryo *in vitro*	–	NT	NG	Patierno *et al*. (1989)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay *in vitro*	(+)	NT	NG[c]	Pienta *et al*. (1977)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay *in vitro*	(+)	NT	1000[c]	Amacher & Zelljadt (1983)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay *in vitro*	–	NT	5000	Dybing *et al*. (1987)
ICR, Inhibition of intercellular communication, Chinese hamster lung V79 cells *in vitro*	–	NT	5910	Dybing *et al*. (1987)
SV40 DNA amplification, Chinese hamster ovary CO631 cells *in vitro*	+	NT	0.59	Fahrig & Steinkamp-Zucht (1996)
HMM, Host-mediated assay, *Saccharomyces cerevisiae* D3 in Swiss Webster mice, forward mutation	–		1000 ip × 1	Simmon *et al*. (1979)
MVM, Micronucleus test, male C57BL/6 mouse bone marrow *in vivo*	+		200 po × 2	Chieli *et al*. (1987)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVM, Micronucleus test, male CBA mouse bone marrow *in vivo*	–		5000 po × 1	Mirkova (1996)
MVM, Micronucleus test, male and female C57BL/6 mouse bone marrow *in vivo*	–		5000 po × 1	Mirkova (1996)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; inj, injection; ip, peritoneal; po, oral
[c] No indication of the dose–response

Table 2. Genetic and related effects of *N*-hydroxyacetamide

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	(+)	1000	Dybing *et al.* (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	(+)	(+)	500	Dybing *et al.* (1987)
DIA, DNA strand breaks, cross-links or related damage, Reuber rat hepatoma cells *in vitro*	(+)	NT	750	Dybing *et al.* (1987)
TIH, Cell transformation, Syrian hamster embryo cells *in vitro*	–	–	50	Dybing *et al.* (1987)
ICR, Inhibition of intercellular communication, Chinese hamster V79 cells *in vitro*	–	–	225	Dybing *et al.* (1987)

[a] (+), weakly positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL

5. Evaluation

No epidemiological data relevant to the carcinogenicity of acetamide were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of acetamide.

Overall evaluation

Acetamide is *possibly carcinogenic to humans (Group 2B)*.

6. References

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ACRYLIC ACID

Data were last reviewed in IARC (1979) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

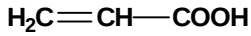
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 79-10-7

Systematic name: 2-Propenoic acid

1.1.2 *Structural and molecular formula and relative molecular mass*



$C_3H_4O_2$ Relative molecular mass: 72.06

1.1.3 *Physical properties* (for details, see IARC, 1979)

(*a*) *Boiling-point*: 141.0°C

(*b*) *Melting-point*: 14°C

(*c*) *Conversion factor*: mg/m^3 = 2.94 × ppm

1.2 Production, use and human exposure

Acrylic acid is used primarily as an intermediate in the production of acrylates, which, in turn, are used in the production of polymers for coatings, paints, adhesives, paper and textiles. Exposure to unreacted acrylic acid may occur among consumers. The present recommendation by the American Conference of Governmental Industrial Hygienists (ACGIH) for the threshold limit value (TLV) is 5.9 mg/m^3 in workplace air. The previous TLV, before 1990, was 30 mg/m^3.

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

No data were available to the Working Group.

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

[1-^{14}C]Acrylic acid administered orally by gavage to rats (400 mg/kg) was well absorbed and rapidly and extensively metabolized, principally to exhaled $^{14}CO_2$, with 83% excreted in this form in 24 h. About 5% of the radioactivity was recovered in the urine and 9% in the faeces with 1.3% remaining in the tissues after 72 h (Winter & Sipes, 1993). Black *et al*. (1995) gave Fischer 344 rats 40 and 150 mg/kg bw [1-^{14}C]acrylic acid by gavage and obtained similar findings. Winter *et al*. (1992) gave 400 mg/kg bw [2,3-^{14}C]acrylic acid by gavage and, in comparison with their results with [1-^{14}C]acrylic acid, recovered 82% of the dose as $^{14}CO_2$, 5% in urine and 1% faeces, while 10% remained in the tissues after 72 h. In marked contrast, when DeBethizy *et al*. (1987) treated rats with 4, 40 or 400 mg/kg bw [2,3-^{14}C]acrylic acid by gavage, they recovered less as $^{14}CO_2$, approximately the same amounts in urine and faeces, and more in the tissues. The disposition of ^{14}C was a function of dose: as the dose increased, less was excreted, notably as $^{14}CO_2$ (65% of the dose at 4 mg/kg and 44% at 400 mg/kg) and more was retained in the tissues after 72 h (19% at 4 mg/kg and 25% at 400 mg/kg). The higher tissue retention of radioactivity from [2,3-^{14}C]acrylic acid is explicable by the entry of carbon atoms 2 and 3 into the tricarboxylic acid cycle, whereas carbon 1 can be oxidized immediately to CO_2.

The absorption and elimination patterns of orally administered acrylic acid (40 and 150 mg/kg bw) in mice were similar to those seen in rats (Black *et al*., 1995).

The percutaneous absorption of acrylic acid has also been examined in rats and mice. After application to the skin, approximately 73% of a dose of approximately 17 mg/kg bw (501 μg/cm^2) was lost by evaporation (Winter & Sipes, 1993). Of the remainder, 6% of dose was retained on or in the skin from the site of application and 16% was exhaled as $^{14}CO_2$. Urinary and faecal excretions were very minor routes (less than 1% and 2–4% of the dose, respectively).

These findings were confirmed and extended in rats and mice given doses of 10 and 40 mg/kg bw by Black *et al*. (1995), whose studies did not account for the complete balance of ^{14}C after dermal application (52–61% of dose, compared with 96% by Winter & Sipes, 1993).

The absorption of [^{11}C]acrylic acid after a 1 min inhalation exposure was studied in rat (Kutzman *et al*., 1982). After 1.5 min, 28% of the label was present in the snout of the animal and the major site of absorption was the gastrointestinal tract. Parallel studies of oral administration showed rapid and extensive absorption from the stomach and rapid metabolism and elimination as $^{11}CO_2$, which accounted for 60% of the dose within 1 h of dosing.

As stated above, various authors have confirmed the extensive conversion of acrylic acid to carbon dioxide in rats and mice treated orally or topically. In addition, urinary metabolites include 3-hydroxypropionic acid and the mercapturic acid *N*-acetyl-*S*-(2-carboxyethyl)cysteine and its *S*-oxide (DeBethizy *et al*., 1987; Winter *et al*., 1993).

The oxidation of acrylic acid can be rationalized in terms of the endogenous catabolism of propionic acid, in which acrylyl coenzyme A is an intermediate. This pathway is analogous with fatty acid β-oxidation, common to all species and, unlike the corresponding pathway in plants, does not involve vitamin B_{12}. 3-Hydroxypropionic acid has been found as an intermediate in the metabolism of acrylic acid *in vitro* in rat liver and mitochondria (Finch & Frederick, 1992). The CO_2 excreted derives from the carboxyl carbon, while carbon atoms 2 and 3 are converted to acetyl coenzyme A, which participates in a variety of reactions. The oxidation of acrylic acid is catalysed by enzymes in a variety of tissues (Black & Finch, 1995). In mice, the greatest activity was found in kidney, which was five times more active than liver and 50 times more active than skin (Black *et al*., 1993).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

In a study by Hellwig *et al*. (1993), Wistar rats of each sex were administered 150 or 375 mg/kg bw acrylic acid by gavage five times a week over three months. Fifty per cent of the animals in the low-dose group and 60% of the male and 90% of the female animals in the high-dose group died during the experiment. Pathological examination revealed a dose-dependent pronounced irritation of the forestomach and glandular stomach, purulent rhinitides and tubular necrosis of the kidneys.

After dermal exposure, 4% acrylic acid resulted in marked skin irritation in three strains of mice (McLaughlin *et al*., 1995), while 1% in acetone was tolerated, i.e. a less pronounced irritative effect was observed. In a commercial acrylic acid sample, α,β-diacryloxypropionic acid was identified as a strongly contact sensitizing constituent in guinea-pigs (Waegemaekers & van der Walle, 1984).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Klimisch and Hellwig (1991) exposed pregnant Sprague-Dawley rats by inhalation to 0, 40, 120 or 360 ppm [0, 118, 354 or 1060 mg/m^3] acrylic acid for 6 h per day during days 6–15 of gestation. At 360 ppm, eye and nose irritation, reduced body weight gain and reduced food consumption were observed in the animals. A slight effect on body weight gain was already observed at 40 ppm. No effects on the number of preimplantation losses, live fetuses or resorptions, and no indications for abnormalities or retardations in the fetuses above the background level were obtained.

Slott and Hales (1985) laparotomized pregnant Sprague-Dawley rats on day 13 of gestation and the uterus was exposed. Each embryo in one uterine horn received an intraamniotic injection of acrylic acid in 0.9% NaCl at doses of up to 1000 μg per fetus. The contralateral embryos received equivalent volumes of saline. The uterus was repositioned in the dam and the incision sutured. Dams were sacrificed on day 20 of gestation and the fetuses scored for survival, resorptions and external malformations. No significant increase in fetal malformations was observed, although a dose of 1000 (but not 100) μg per fetus enhanced the number of dead or resorbed fetuses significantly.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Acrylic acid did not induce mutations in bacteria. It formed DNA adducts *in vitro*. It did not induce unscheduled DNA synthesis or cell transformation in rodent cells *in vitro*, or sex-linked recessive lethal mutations in *Drosophila*. It induced gene mutations and chromosomal aberrations in rodent cells *in vitro*. In single studies, acrylic acid given *in vivo* did not induce dominant lethal mutations in mice or chromosomal aberrations in rat bone marrow.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of acrylic acid were available.

No experimental data relevant to the carcinogenicity of acrylic acid were available.

Overall evaluation

Acrylic acid is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

Black, K.A. & Finch, L. (1995) Acrylic acid oxidation and tissue-to-blood partition coefficients in rat tissues. *Toxicol. Lett.*, **78**, 75–78

Table 1. Genetic and related effects of acrylic acid

Test system	Result[a]		Dose[b] (LEDor HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Lijinsky & Andrews (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Zeiger *et al.* (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500	Cameron *et al.* (1991)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Lijinsky & Andrews (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Zeiger *et al.* (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500	Cameron *et al.* (1991)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Lijinsky & Andrews (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Zeiger *et al.* (1987)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500	Cameron *et al.* (1991)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	500	Lijinsky & Andrews (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Lijinsky & Andrews (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Zeiger *et al.* (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	Cameron *et al.* (1991)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		2% feed	McCarthy *et al.* (1992)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		2% inj	McCarthy *et al.* (1992)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	630	McCarthy *et al.* (1992)
GCO, Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	–	–	2000	McCarthy *et al.* (1992)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LEDor HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	300	Moore *et al.* (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	200	Cameron *et al.* (1991)
MIA, Micronucleus test, Syrian hamster embryo cells *in vitro*	–	NT	10	Wiegand *et al.* (1989)
CIM, Chromosomal aberrations, mouse lymphoma L5178Y cells *in vitro*	+	NT	450	Moore *et al.* (1988)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	+	1680	McCarthy *et al.* (1992)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	–	NT	25	Wiegand *et al.* (1989)
CBA, Chromosomal aberrations, rat bone-marrow cells *in vivo*	–[c]		1000 po × 1	McCarthy *et al.* (1992)
DLM, Dominant lethal test, CD-1 mice *in vivo*	–[d]		324 po × 1	McCarthy *et al.* (1992)
BID, Binding (covalent) to calf thymus DNA *in vitro*	+	NT	100 000	Segal *et al.* (1987)

[a] +, positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; inj, injection; po, oral

[c] Results were also negative for rats treated with 5000 ppm in the drinking-water for five days.

[d] Results were also negative for mice treated with 162 mg/kg/bw by gavage for five days.

Black, K.A., Finch, L. & Frederick, C.B. (1993) Metabolism of acrylic acid to carbon dioxide in mouse tissues. *Fundam. appl. Toxicol.*, **21**, 97–104

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Cameron, T.P., Rogers-Back, A.M., Lawlor, T.E., Harbell, J.W., Seifried, H.E. & Dunkel, V.C. (1991) Genotoxicity of multifunctional acrylates in the *Salmonella*/mammalian-microsome assay and mouse lymphoma $TK^{+/-}$ assay. *Environ. mol. Mutag.,* **17,** 264–271

DeBethizy, J.D., Udinsky, J.R., Scribner, H.E. & Frederick, C.B. (1987) The disposition and metabolism of acrylic acid and ethyl acrylate in male Sprague-Dawley rats. *Fundam. appl. Toxicol.*, **8**, 549–561

Finch, L. & Frederick, C.B. (1992) Rate and route of oxidation of acrylic acid to carbon dioxide in rat liver. *Fundam. appl. Toxicol.*, **19**, 498–505

Hellwig, J., Deckardt, K. & Freisberg, K.O. (1993) Subchronic and chronic studies of the effects of oral administration of acrylic acid to rats. *Food chem. Toxicol.*, **31**, 1–18

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Klimisch, H.-J. & Hellwig, J. (1991) The prenatal inhalation toxicity of acrylic acid in rats. *Fundam. appl. Toxicol.*, **16**, 656–666

Kutzman, R.S., Meyer, G.-J. & Wolf, A.P. (1982) The biodistribution and metabolic fate of [^{11}C]acrylic acid in the rat after acute inhalation exposure or stomach intubation. *J. Toxicol. environ. Health*, **10**, 969–979

Lijinsky, W. & Andrews, A.W. (1980) Mutagenicity of vinyl compounds in *Salmonella typhimurium*. *Teratog. Carcinog. Mutag.,* **1,** 259–267

McCarthy, K.L., Thomas, W.C., Aardema, M.J., Seymour, J.L., Putman, D.L., Yang, L.L., Curren, R.D. & Valencia, R. (1992) Genetic toxicology of acrylic acid. *Food chem. Toxicol.,* **30,** 505–515

McLaughlin, J.E., Parno, J., Garner, F.M., Clary, J.J., Thomas, W.C. & Murphy, S.R. (1995) Comparison of the maximum tolerated dose (MTD) dermal response in three strains of mice following repeated exposure to acrylic acid. *Food chem. Toxicol.*, **33**, 507–513

Moore, M.M., Amtower, A., Doerr, C.L., Brock, K.H. & Dearfield, K.L. (1988) Genotoxicity of acrylic acid, methyl acrylate, ethyl acrylate, methyl methacrylate, and ethyl methacrylate in L5178Y mouse lymphoma cells. *Environ. mol. Mutag.,* **11,** 49–63

Segal, A., Fedyk, J., Melchionne, S. & Seidman, I. (1987) The isolation and characterization of 2-carboxyethyl adducts following in vitro reaction of acrylic acid with calf thymus DNA and bioassay of acrylic acid in female Hsd:(ICR)Br mice. *Chem.-biol. Interact.,* **61,** 189–197

Slott, V.L. & Hales, B.F. (1985) Teratogenicity and embryolethality of acrolein and structurally related compounds in rats. *Teratology*, **32**, 65–72

Waegemaekers, T.H.J.M. & van der Walle, H.B. (1984) α,β-Diacryloxypropionic acid, a sensitizing impurity in commercial acrylic acid. *Derm. Beruf Umwelt*, **32**, 55–58

Wiegand, H.J., Schiffmann, D. & Henschler, D. (1989) Non-genotoxicity of acrylic acid and *n*-butyl acrylate in a mammalian cell system (SHE cells). *Arch. Toxicol.,* **63,** 250–251

Winter, S.M. & Sipes, I.G. (1993) The disposition of acrylic acid in the male Sprague-Dawley rat following oral or topical administration. *Food chem. Toxicol.*, **31**, 615–621

Winter, S.M., Weber, G.L., Gooley, P.R., Mackenzie, N.E. & Sipes, I.G. (1992) Identification and comparison of the urinary metabolites of [1,2,3-$^{13}C_3$]acrylic acid and [1,2,3-$^{13}C_3$]propionic acid in the rat by homonuclear ^{13}C nuclear magnetic resonance spectroscopy. *Drug Metab. Disp.*, **20**, 665–672

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K. & Speck, W. (1987) *Salmonella* mutagenicity tests: III. Results from the testing of 255 chemicals. *Environ. Mutag.*, **9** (Suppl. 9), 1–110

ALLYL CHLORIDE

Data were last reviewed in IARC (1985) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

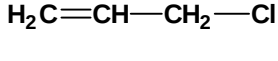
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 107-05-1
Chem. Abstr. Name: 3-Chloro-1-propene
IUPAC Systematic Name: 3-Chloropropene
Synonyms: 3-Chloropropylene; 2-propenyl chloride

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_3H_5Cl Relative molecular mass: 76.53

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless to pale yellow liquid with an unpleasant pungent odour (Budavari, 1996)
(*b*) *Boiling-point*: 45.1°C (Lide, 1997)
(*c*) *Melting-point*: –134.5°C (Lide, 1997)
(*d*) *Solubility*: Slightly soluble in water (0.36 g/100 g at 20°C); miscible with ethanol, diethyl ether, chloroform and petroleum ether (American Conference of Governmental Industrial Hygienists, 1991; Budavari, 1996)
(*e*) *Vapour pressure:* 45 kPa at 20°C; relative vapour density (air = 1), 2.64 (Verschueren, 1996)
(*f*) *Flash point*: –31°C, closed cup (Budavari, 1996)
(*g*) *Explosive limits*: Upper, 11.1%; lower, 3.3% by volume in air (American Conference of Governmental Industrial Hygienists, 1991)
(*h*) *Reactivity*: Highly reactive and flammable (American Conference of Governmental Industrial Hygienists, 1991)
(*i*) *Conversion factor*: $mg/m^3 = 3.13 \times ppm$

1.2 Production and use

World production of allyl chloride in 1989–90 was estimated to be 500–600 thousand tonnes per year. Production facilities in 1990 were reported in Brazil, China, the Czech Republic, France, Germany, Japan, the Netherlands, Poland and the United States (Kneupper & Saathoff, 1993).

Allyl chloride is used to make intermediates for downstream derivatives such as resins and polymers. Approximately 90% of allyl chloride production is used to synthesize epichlorohydrin, which is used as a basic building block for epoxy resins and in glycerol synthesis. Allyl chloride is also a starting material for allyl ethers of phenols, bisphenol A and phenolic resins, and for some allyl esters. Other compounds made from allyl chloride are quaternary amines used in chelating agents and quaternary ammonium salts, which are used in water clarification and sewage sludge flocculation (Kneupper & Saathoff, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 3000 workers in the United States were potentially exposed to allyl chloride (see General Remarks). Occupational exposure to allyl chloride may occur in its manufacture and in the production of epichlorohydrin, glycerol and a wide range of other chemical products.

1.3.2 *Environmental occurrence*

Although the production and use of allyl chloride may result in its release to the environment, few data are available (United States National Library of Medicine, 1998).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 3 mg/m^3 as the 8-h time-weighted average threshold limit value for occupational exposures to allyl chloride in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for allyl chloride in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

Olsen *et al*. (1994) reported on the results of a retrospective cohort mortality study of workers in Texas, United States, with potential exposure to epichlorohydrin (see this volume) and allyl chloride. The cohort consisted of 1064 men employed in the epoxy resin, glycerine and allyl chloride/epichlorohydrin production areas of a large chemical facility between 1957 and 1986. Follow-up was carried out until 1989. Mortality was

compared with national rates and company rates for other facilities. There was no exposure to allyl chloride in the epoxy resin area. Exposures to allyl chloride were estimated to be between 1 and 5 ppm [3.1 and 15.7 mg/m^3] in the glycerine area before 1970 and occasionally in some jobs in the allyl chloride/epichlorohydrin area, although respiratory protection may have been worn by these workers. There were 66 deaths (standardized mortality ratio (SMR), 0.8; 95% confidence interval (CI), 0.6–1.0). Ten cancers were observed (SMR, 0.5; 95% CI, 0.2–0.9, compared with national rates) in the entire cohort and no association between site-specific cancer risks and exposure to allyl chloride was observed.

3. Studies of Cancer in Experimental Animals

Allyl chloride was tested for carcinogenicity by gavage in mice and rats, by skin application in mice, both by repeated application and in a two-stage assay, and by intraperitoneal injection in mice. Following its oral administration to mice in an experiment that was compromised by high mortality in males, a nonsignificant increase in the incidence of squamous cell papillomas and carcinomas of the forestomach was observed in both sexes; the experiment in rats was inadequate for evaluation. No skin tumours were observed in mice following repeated skin applications; however, a single application followed by treatment with 12-*O*-tetradecanoylphorbol 13-acetate (TPA) increased the incidence of tumour-bearing mice from 6/90 in the TPA controls to 7/30 in treated mice ($p < 0.025$) and reduced the time to first papilloma from 449 days to 197 days. Following intraperitoneal injection in strain A mice, there was a marginal increase in the multiplicity of lung adenomas (IARC, 1985).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Allyl chloride is presumed to be metabolized to allyl alcohol, which could then be further metabolized via two pathways to form either acrolein or glycidol, from which a variety of metabolites could result. Metabolites identified in rat urine are 3-hydroxypropylmercapturic acid and allyl mercapturic acid and its sulfoxide. Allyl glutathione and *S*-allyl-L-cysteine have been detected in the bile of dosed rats. In-vitro metabolism of allyl chloride results in haem destruction in microsomal cytochrome P450 (IARC, 1985).

Activation of allyl chloride to genotoxic substances appears to involve aldehydes, since inhibition of aldehyde dehydrogenase by cyanamide increases the mutagenic activity in *Salmonella typhimurium* TA100; on the other hand neither SKF525 nor 1,1,1-trichloropropene-2,3-oxide affect the mutagenicity, so that metabolic activation via an epoxide is unlikely (Neudecker & Henschler, 1986).

4.2 Toxic effects

4.2.1 *Humans*

Workers exposed to allyl chloride at concentrations ranging from 1 to 113 ppm [3–350 mg/m^3] for 16 months showed enzymatic evidence of liver damage, which was reversible. One report described impaired kidney function in workers exposed to unknown concentrations. A reversible polyneuropathy was also described (IARC, 1985). A later study in China indicated that two-thirds of 26 workers in one factory exposed to allyl chloride at concentrations of 2.6–6650 mg/m^3 for 2.5–6 years during the production of allyl sulfonate from allyl chloride and sodium sulfite developed symptoms and signs of polyneuropathy. Thirteen of 27 workers in another factory in China who were exposed to allyl chloride at concentrations in the range of 0.2–25.1 mg/m^3 for 1–4.5 years showed similar but much milder symptoms of neuropathy, without evident neurological signs (He & Zhang, 1985; He *et al*., 1985).

No evidence of liver or kidney dysfunction was found in 73 male workers employed for an average of 8.2 years (range, 0.5–23 years) in a plant in the Netherlands producing allyl chloride, 1,3-dichloropropene, epichlorohydrin and hexachlorocyclopentadiene. Mean allyl chloride concentrations ranged from 0.21 to 2.89 mg/m^3; the values for hexachlorocyclopentadiene ranged from 0.01 to 0.23 mg/m^3. Exposures to 1,3-dichloropropene and epichlorohydrin were well below the current maximum allowable concentrations. The results of the liver and kidney function tests were compared with those of 35 men in the same plant who were not occupationally exposed to these chemicals (Boogaard *et al*., 1993).

It has been suggested that co-exposure to allyl chloride and epichlorohydrin may increase the risk of heart disease mortality. A study population of workers at a plant in Texas, United States, was divided into groups with a likelihood of exposure to allyl chloride in conjunction with high, moderate, low or nil exposure to epichlorohydrin. The SMR for heart disease mortality was 1.2 for 160 workers for whom exposure to allyl chloride and high/moderate exposure to epichlorohydrin had occurred, compared with 0.6 for 35 workers with low/nil co-exposure to epichlorohydrin, 0.7 for 88 workers who were probably not exposed to allyl chloride but who had potentially been heavily exposed to epichlorohydrin and 0.5 for 116 workers who also were probably not exposed to allyl chloride but had had light exposure to epichlorohydrin (Enterline *et al*., 1990). It has been argued that important confounders and specific exposure data were not considered (Ross, 1990) and the need for further research on this apparent association has been stressed (Enterline, 1990).

A retrospective cohort mortality study in Texas, United States (see also Section 2) failed to confirm an effect of co-exposure upon mortality (Olsen *et al*., 1994). However,

the authors noted that the results were limited by the cohort size, duration of follow-up, relatively small numbers of observed and expected deaths and the level of potential exposure to epichlorohydrin.

4.2.2 *Experimental systems*

Following inhalation exposure to allyl chloride, necrosis in the respiratory tract was the usual cause of death in rats and guinea-pigs. Major systemic effects were degenerative changes in kidney and liver in rats, guinea-pigs and rabbits inhaling 8 ppm [25 mg/m^3] for 7 h per day on five days per week for five weeks, whereas 3 ppm [9.3 mg/m^3] for six months under the same conditions produced no observed effect in these species or in dogs. Exposure of rabbits and cats to allyl chloride atmospheres of 206 mg/m^3 for 6 h per day on six days per week for three months resulted in partially reversible flaccid paralysis with muscular atrophy in rabbits; cats were less affected. Exposure to 17.5 mg/m^3 had no effect in either species. Subcutaneous injection of 50 mg/kg bw allyl chloride also resulted in peripheral neuropathy in rabbits (IARC, 1985).

Mice treated orally with 300 or 500 mg/kg bw allyl chloride on three days per week for two to 17 weeks developed focal kidney damage and degeneration in many peripheral nerves and roots (He *et al*., 1981), confirming earlier observations in other species. Peripheral nerve fibre degeneration was also confirmed in rabbits treated by subcutaneous injection (He *et al*., 1985).

Male ICR mice were given single subcutaneous injections of allyl chloride at dose levels of 496–1037 mg/kg bw (LD_{50} by this route being 621 mg/kg bw; 95% CI, 522–739 mg/kg bw). Mice dying within 24 h showed consistent, severe haemorrhage and oedema of the lung and inconsistent, non-dose-related damage to the liver and kidney. The latter was not observed in mice killed on day 7, when histopathological changes were confined to the testes (Omura *et al*., 1993), a novel finding.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Inhalation exposure to allyl chloride of high purity was not teratogenic to either rats or rabbits (IARC, 1985). However, intraperitoneal administration of allyl chloride (80 mg/kg bw) to pregnant rats on gestation days 1–15 was reported to increase the frequencies of resorptions and of fetuses with short snout and protruding tongue. The authors intended to investigate possible reasons for the differences between the results from the two studies (Hardin *et al*., 1981).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Allyl chloride caused DNA damage in bacteria, was mutagenic to bacteria and fungi and induced gene conversion in yeast. It also induced chromosomal aberrations in cultured Chinese hamster lung cells, this activity being greater in the absence of an exogenous activation system. Allyl chloride can bind to isolated DNA, although it is a weak alkylating agent. Five alkylated bases have been identified: *N*3-allyladenine, N^6-allyladenine, N^2-allylguanine, *N*7-allylguanine and O^6-allylguanine (Eder *et al*., 1987).

5. Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of allyl chloride.

There is *inadequate evidence* in experimental animals for the carcinogenicity of allyl chloride.

Overall evaluation

Allyl chloride is *not classifiable as to its carcinogenicity to humans (Group 3).*

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Table 1. Genetic and related effects of allyl chloride

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ECD, *Escherichia coli pol* A^+/*pol* A^- differential toxicity (spot test)	+	NT	9400	McCoy *et al*. (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	9400/disk	McCoy *et al*. (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	(+)	2350	Bignami *et al*. (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	(+)	1150	Eder *et al*. (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	0.02	Norpoth *et al*. (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	0.05[c]	Simmon (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	(+)	NG	Eder *et al*. (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	+	250	Neudecker & Henschler (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	+	235	Neudecker & Henschler (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	+	940/disk	McCoy *et al*. (1978)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	2350	Bignami *et al*. (1980)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	9400/disk	McCoy *et al*. (1978)
SCG, *Saccharomyces cerevisiae* D4, gene conversion	+	NT	14	McCoy *et al*. (1978)
STF, *Streptomyces coelicolor*, forward mutation	+	NT	4700	Bignami *et al*. (1980)
STR, *Streptomyces coelicolor*, reverse mutation	+	NT	4700	Bignami *et al*. (1980)
ANF, *Aspergillus nidulans*, forward mutation	–	NT	18800	Bignami *et al*. (1980)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CIC, Chromosomal aberrations, Chinese hamster lung CHL cells *in vitro*	+	+	400	JETOC (1997)
BID, Binding (covalent) to DNA *in vitro*	(+)	NT	9000	Eder *et al*. (1987)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; NG, not given
[c] Cells were exposed to allyl chloride vapour; dose = µg/mL in air.

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ALLYL ISOVALERATE

Data were last reviewed in IARC (1985) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

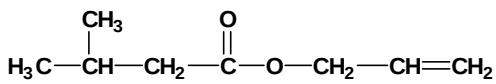
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 2835-39-4

Systematic name: Butanoic acid, 3-methyl-, 2-propenyl ester

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_8H_{14}O_2$ Relative molecular mass: 142.2

1.1.3 *Physical properties* (for details, see IARC, 1985)

(*a*) *Boiling-point*: 89–90°C

(*b*) *Conversion factor*: $mg/m^3 = 5.82 \times ppm$

1.2 Production and use

Allyl isovalerate has been used since the 1950s as a raw material for fragrances in cosmetics, lotions and perfumes and in certain food products, although it is not known whether it is currently used in this way (IARC, 1985).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Allyl isovalerate was tested for carcinogenicity by gavage in mice and rats. In mice, it induced squamous-cell papillomas of the forestomach in males and increased the

incidence of lymphomas in females. In rats of both sexes, increases in the incidence of mononuclear-cell leukaemia were observed (IARC, 1985).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Allyl isovalerate is metabolized to isovaleric acid, which can conjugate with glycine, and allyl alcohol, which could then be further metabolized via two pathways to form either acrolein or glycidol, from which a variety of metabolites could result (IARC, 1985).

4.2 Toxic effects

4.2.1 *Humans*

Allyl isovalerate has low irritancy potential. It is deduced that one of its metabolites, isovaleric acid, is toxic, based upon the effects of an inborn error of leucine metabolism caused by isovaleryl-coenzyme A dehydrogenase deficiency. This is a syndrome of neonatal vomiting and lethargy progressing to coma, pancytopenia and ketoacidosis that can be alleviated by glycine treatment, which enhances the synthesis and excretion of isovalerylglycine (Cohn *et al*., 1978; IARC, 1985).

4.2.2 *Experimental systems*

Allyl isovalerate can cause liver-cell necrosis in orally dosed rats (IARC, 1985). This was substantiated in a 13-week dose ranging study in which Fischer 344/N rats dosed by gavage at 250 mg/kg bw developed hepatic multifocal coagulative necrosis, cholangiofibrosis, nodular hyperplasia and bile-duct hyperplasia in both sexes and cytoplasmic vacuolization in males. As part of the same study, some B6C3F_1 mice of each sex also developed hepatic coagulative necrosis at the same dose level; other lesions observed in the mice included ulcerative inflammation of the stomach, thickening of the stomach mucosa and thickening of the urinary bladder wall (United States National Toxicology Program, 1983). Further studies in the same strains of rats and mice administered doses of 250 mg/kg bw to rats and 125 mg/kg bw to mice by gavage on five days a week for two weeks showed that allyl isovalerate had no effect upon haematology or bone marrow cellularity, but there were subtle myelotoxic effects in mice and hepatotoxicity in rats (Hong *et al*., 1988).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Allyl isovalerate was not mutagenic in bacteria, but did induce sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary CHO cells.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of allyl isovalerate were available.

There is *limited evidence* in experimental animals for the carcinogenicity of allyl isovalerate.

Overall evaluation

Allyl isovalerate is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

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Table 1. Genetic and related effects of allyl isovalerate

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	166	US National Toxicology Program (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	166	US National Toxicology Program (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	US National Toxicology Program (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	US National Toxicology Program (1983)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	(+)	250	Gulati *et al.* (1989)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	+	300	Gulati *et al.* (1989)

[a] +, positive; (+), weak positive; –, negative
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL

1,4-BENZOQUINONE (*para*-QUINONE)

Data were last reviewed in IARC (1977) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

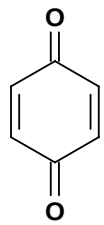
1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 106-51-4

Systematic name: 2,5-Cyclohexadiene-1,4-dione

Synonym: *para*-Benzoquinone

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_6H_4O_2$ Relative molecular mass: 108.09

1.1.3 *Physical properties* (for details, see IARC, 1977)

(*a*) *Melting-point*: 115.7°C

(*b*) *Conversion factor*: $mg/m^3 = 4.73 \times ppm$

1.2 Production and use

1,4-Benzoquinone was first produced commercially in 1919, and has since been manufactured in several European countries, Japan and the United States. Its major use is in hydroquinone production, but it is also used as a polymerization inhibitor and as an intermediate in the production of a variety of substances, including rubber accelerators and oxidizing agents (IARC, 1977).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

1,4-Benzoquinone was tested for carcinogenicity in mice by skin application and inhalation and in rats by subcutaneous injection. The available data are insufficient to evaluate the carcinogenicity of this compound (IARC, 1977).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

1,4-Benzoquinone is readily absorbed from the gastrointestinal tract and subcutaneous tissue [species not specified]. It is excreted partly unchanged and partly as hydroquinone, the major proportion of which is eliminated as acid conjugates (IARC, 1977).

4.2 Toxic effects

4.2.1 *Humans*

Application of 1,4-benzoquinone causes local skin changes including discoloration, erythema and the appearance of papules; necrosis can occur. Exposure to vapours induces serious vision disturbances; injury extends through the entire conjunctiva and cornea (IARC, 1977).

4.2.2 *Experimental systems*

1,4-Benzoquinone depresses respiration in tissue preparations. Large doses induce local irritation, clonic convulsions, decreased blood pressure and death due to paralysis of the medullary centres. Signs of kidney damage were observed in severely poisoned animals (IARC, 1977).

1,4-Benzoquinone is a metabolite of benzene. Exposure to 1,4-benzoquinone of cultured murine peritoneal macrophages for 10 min at 12.5 μM inhibited Fc and complement receptor-mediated phagocytosis by ≥ 90%, although macrophage viability was unaffected. In this comparative study, 1,4-benzoquinone was the most potent of the benzene metabolites tested, and Fc receptor-mediated phagocytosis was not regained after overnight incubation in the presence of 1,4-benzoquinone. There was little effect upon Fc receptor-binding of target cells, whereas there was a marked decrease in the filamentous actin content of the macrophages. 1,4-Benzoquinone bound in only low amounts to purified actin and did not affect its assembly; thus disruption of filamentous actin occurs by some mechanism other than direct alkylation (Manning *et al*., 1994).

The development of colony-forming unit-erythroid of bone marrow from male Swiss-Webster and C57BL/6J mice was reduced upon exposure to 1,4-benzoquinone in a dose-dependent manner from 10 μM, the lowest concentration tested (Neun *et al*., 1992).

At a concentration of 3 μM, 1,4-benzoquinone caused 50% inhibition of CPP32, an interleukin-1β-enzyme/Ced-3 cysteine protease involved in the implementation of apoptosis and which is present in myeloid cells (Hazel *et al*., 1996).

1,4-Benzoquinone was reported to be dysmorphogenic to rat embryos in an in-vitro system at a concentration of 10 μM but not at 50 μM for 30 h. It was lethal at 100 μM (Chapman *et al*., 1994).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Results of mutation tests with *Salmonella typhimurium* are inconclusive, but 1,4-benzoquinone does not cause mutations in *Neurospora crassa*. In cultured mammalian cells, it induced DNA strand breakage, mutation at the *hprt* locus and micronuclei. It also induced micronuclei in the bone-marrow cells of mice treated *in vivo*. Dominant lethal effects were not induced in male mice by a single low dose.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of 1,4-benzoquinone were available.

There is *inadequate evidence* in experimental animals for the carcinogenicity of 1,4-benzoquinone.

Overall evaluation

1,4-Benzoquinone is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

Anderson, D., Yu, T.-W. & Schmezer, P. (1995) An investigation of the DNA-damaging ability of benzene and its metabolites in human lymphocytes, using the comet assay. *Environ. mol. Mutag*., **26**, 305–314

Chapman, D.E., Namkung, M.J. & Juchau, M.R. (1994) Benzene and benzene metabolites as embryotoxic agents: effects on cultured rat embryos. *Toxicol. appl. Pharmacol*., **128**, 129–137

Table 1. Genetic and related effects of 1,4-benzoquinone

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	5.0	Nazar *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	16.5	Mortelmans *et al*. (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	16.5	Mortelmans *et al*. (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	16.5	Mortelmans *et al*. (1986)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	16.5	Mortelmans *et al*. (1986)
NCF, *Neurospora crassa*, forward mutation to pyrimidine dependence	–	NT	NG	Reissig (1963)
NCR, *Neurospora crassa*, reverse mutation to *arg*$^+$	–	NT	NG	Reissig (1963)
DIA, DNA strand breaks, mouse lymphoma L5178YS cells *in vitro*	+	NT	0.11	Pellack-Walker & Blumer (1986)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	+	NT	0.54	Ludewig *et al*. (1989)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	–	NT	11	Ludewig *et al*. (1989)
MIA, Micronucleus test, Chinese hamster lung V79 cells *in vitro*	+	NT	5.4	Ludewig *et al*. (1989)
MIA, Micronucleus test, animal cell lines (V79, IEC-17 and 18) *in vitro*	+	NT	0.01	Glatt *et al*. (1990)
DIH, DNA strand breaks, cross-links or related damage, human lymphocytes *in vitro* (comet assay)	–	+	11	Anderson *et al*. (1995)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.55	Erexson *et al*. (1985)
MIH, Micronucleus test, HuFoe-15 embryonal human liver cells *in vitro*	+	NT	0.01	Glatt *et al*. (1990)
MIH, Micronucleus test, human lymphocytes *in vitro*	+	NT	0.275	Yager *et al*. (1990)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVM, Micronucleus test, pregnant CD-1 mouse bone-marrow cells *in vivo*	(+)		20 po × 1	Ciranni *et al*. (1988a)
MVM, Micronucleus test, fetal CD-1 mouse liver cells *in utero*	(+)		20 (to dam) × 1	Ciranni *et al*. (1988a)
MVM, Micronucleus test, CD-1 mice *in vivo*	(+)[c]		20 po × 1	Ciranni *et al*. (1988b)
DLM, Dominant lethal test, male C3H and (C3H × 101)F_1 mice *in vivo*	–		6.25 ip × 1	Röhrborn & Vogel (1967)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; po, oral; ip, intraperitoneal

[c] Negative if 5 mg/kg bw is given by the intraperitoneal route, which causes greater toxicity.

Ciranni, R., Barale, R., Marrazzini, A. & Loprieno, N. (1988a) Benzene and the genotoxicity of its metabolites. I. Transplacental activity in mouse fetuses and in their dams. *Mutat. Res.*, **208**, 61–67

Ciranni, R., Barale, R., Ghelardini, G. & Loprieno, N. (1988b) Benzene and the genotoxicity of its metabolites. II. The effect of the route of administration on the micronuclei and bone marrow depression in mouse bone marrow cells. *Mutat. Res.*, **209**, 23–28

Erexson, G.L., Wilmer, J.L. & Kligerman, A.D. (1985) Sister chromatid exchange induction in human lymphocytes exposed to benzene and its metabolites *in vitro*. *Cancer Res.*, **45**, 2471–2477

Glatt, H., Gemperlein, I., Setiabudi, F., Platt, K.L. & Oesch, F. (1990) Expression of xenobiotic-metabolizing enzymes in propagatable cell cultures and induction of micronuclei by 13 compounds. *Mutagenesis*, **5**, 241–249

Hazel, B.A., Baum, C. & Kalf, F.G. (1996) Hydroquinone, a bioreactive metabolite of benzene, inhibits apoptosis in myeloblasts. *Stem Cells*, **14**, 730–742

IARC (1977) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*, Vol. 15, *Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals*, Lyon, pp. 255–264

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 71

Ludewig, G., Dogra, S. & Glatt, H. (1989) Genotoxicity of 1,4-benzoquinone and 1,4-naphthoquinone in relation to effects on glutathione and NAD(P)H levels in V79 cells. *Environ. Health Perspect.*, **82**, 223–228

Manning, B.W., Adams, D.O. & Lewis, J.G. (1994) Effects of benzene metabolites on receptor-mediated phagocytosis and cytoskeletal integrity in mouse peritoneal macrophages. *Toxicol. appl. Pharmacol*., **126**, 214–223

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B. & Zeiger, E. (1986) *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ. Mutag.*, **8** (Suppl. 7), 1–119

Nazar, M.A., Rapson, W.H., Brook, M.A., May, S. & Tarhanen, J. (1981) Mutagenic reaction products of aqueous chlorination of catechol. *Mutat. Res.*, **89**, 45–55

Neun, D.J., Penn, A. & Snyder, C.A. (1992) Evidence for strain-specific differences in benzene toxicity as a function of host target cell susceptibility. *Arch. Toxicol*., **66**, 11–17

Pellack-Walker, P. & Blumer, J.L. (1986) DNA damage in L5178YS cells following exposure to benzene metabolites. *Mol. Pharmacol.*, **30**, 42–47

Reissig, J.L. (1963) Induction of forward mutants in the *pyr*-3 region of *Neurospora*. *J. gen. Microbiol*., **30**, 317–325

Röhrborn, G. & Vogel, F. (1967) Chemically induced mutation in mammals and humans. 2. Genetic examination in mice. *Dtsch. med. Wschr*., **92**, 2315–2321

Yager, J.W., Eastmond, D.A., Robertson, M.L., Paradisin, W.M. & Smith, M.T. (1990) Characterization of micronuclei induced in human lymphocytes by benzene metabolites. *Cancer Res.*, **50**, 393–399

1,4-BENZOQUINONE DIOXIME

Data were last reviewed in IARC (1982) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

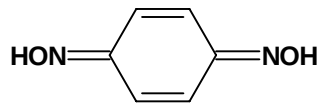
1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 105-11-3

Systematic name: 2,5-Cyclohexadiene-1,4-dione, dioxime

Synonym: *para*-Benzoquinone dioxime

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_6H_6N_2O_2$ Relative molecular mass: 138.1

1.1.3 *Physical properties* (for details, see IARC, 1982)

(*a*) *Melting-point*: Decomposes at 240°C

(*b*) *Conversion factor*: $mg/m^3 = 5.65 \times ppm$

1.2 Production, use and human exposure

Occupational exposure to 1,4-benzoquinone dioxime probably occurs during its manufacture, its use as a rubber vulcanizing agent and its conversion to chemical derivatives (IARC, 1982).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

1,4-Benzoquinone dioxime was tested for carcinogenicity in mice and rats by oral administration in the diet. No significant increase in the number of neoplasms was observed in male rats, but in females in the highest-dose group there was an increase in the number of transitional cell papillomas and carcinomas of the urinary bladder. In mice, no carcinogenic effect was observed (IARC, 1982).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Slight increases in chronic inflammation and epithelial hyperplasia in the kidney in mice and rats and haemosiderosis of the spleen in rats of both sexes were observed in the carcinogenicity studies (IARC, 1982).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

1,4-Benzoquinone dioxime induced mutations in bacteria and in cultured mouse lymphoma L5178Y cells, but not in *Drosophila melanogaster*. It gave inconclusive results for the frequency of transformed C3H 10T½ cells. In female rats treated *in vivo*, 1,4-benzoquinone dioxime did not induce either unscheduled DNA synthesis in hepatocytes or micronuclei in bone-marrow cells.

Table 1. Genetic and related effects of 1,4-benzoquinone dioxime

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	(+)	167	Haworth *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	?	167	Dunkel *et al.* (1985)[c]
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	250	Haworth *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1667	Dunkel *et al.* (1985)[c]
SA7, *Salmonella typhimurium* TA1537, reverse mutation	(+)	–	167	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	+	+	16.7	Dunkel *et al.* (1985)[c]
SA8, *Salmonella typhimurium* TA1538, reverse mutation	+	+	5	Dunkel *et al.* (1985)[c]
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	5	Haworth *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	16.7	Dunkel *et al.* (1985)[c]
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	1.5	Westmoreland *et al.* (1992)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	–	1667	Dunkel *et al.* (1985)[c]
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		3000 feed	Yoon *et al.* (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	25	McGregor *et al.* (1988)
TCM, Cell transformation, C3H 10T½ mouse embryo cells *in vitro*	–	NT	5	Schechtman *et al.* (1987)
TCM, Cell transformation, C3H 10T½ mouse embryo cells *in vitro*	?	NT	5	Dunkel *et al.* (1988)[d]
UPR, Unscheduled DNA synthesis, female PVG rat hepatocytes *in vivo*	–		250 po × 1	Westmoreland *et al.* (1992)
MVM, Micronucleus test, female Fischer 344 rat bone-marrow cells *in vivo*	–		500 po × 1	Westmoreland *et al.* (1992)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; po, oral
[c] Independent testing in four laboratories
[d] Independent testing in two laboratories

5. Evaluation

No epidemiological data relevant to the carcinogenicity of 1,4-benzoquinone dioxime were available.

There is *limited evidence* in experimental animals for the carcinogenicity of 1,4-benzoquinone dioxime.

Overall evaluation

1,4-Benzoquinone dioxime is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

Dunkel, V.C., Zeiger, E., Brusick, D., McCoy, E., McGregor, D., Mortelmans, K., Rosenkranz, H.S. & Simmon, V.F. (1985) Reproducibility of microbial mutagenicity assays: II. Testing of carcinogens and noncarcinogens in *Salmonella typhimurium* and *Escherichia coli*. *Environ. Mutag*., **7** (Suppl. 5), 1–248

Dunkel, V.C., Schechtman, L.M., Tu, A.S., Sivak, A., Lubet, R.A. & Cameron, T.P. (1988) Interlaboratory evaluation of the C3H/10T1/2 cell transformation assay. *Environ. mol. Mutag*., **12**, 21–31

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W. & Zeiger, E. (1983) *Salmonella* mutagenicity test results for 250 chemicals. *Environ. Mutag*., **5** (Suppl. 1), 3–142

IARC (1982) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 29, *Some Industrial Chemicals and Dyestuffs*, Lyon, pp. 185–191

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 58

McGregor, D.B., Brown, A., Cattanach, P., Edwards, I., McBride, D., Riach, C. & Caspary, W.J. (1988) Responses of the L5178Y tk^{+}/tk^{-} mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. *Environ. mol. Mutag.*, **12**, 85–154

Schechtman, L.M., Kiss, E., McCarvill, J., Nims, R., Kouri, R.E. & Lubet, R.A. (1987) A method for the amplification of chemically induced transformation in C3H/10T1/2 clone 8 cells: its use as a potential screening assay *J. natl Cancer Inst*., **79**, 487–498

Westmoreland, C., George, E., York, M. & Gatehouse, D. (1992) In vivo genotoxicity studies with *p*-benzoquinone dioxime. *Environ. mol. Mutag*., **19**, 71–76

Yoon, J.S., Mason, J.M., Valencia, R., Woodruff, R.C. & Zimmering, S. (1985) Chemical mutagenesis testing in *Drosophila*. IV. Results of 45 coded compounds tested for the National Toxicology Program. *Environ. Mutag*., **7**, 349–367

BENZYL ACETATE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

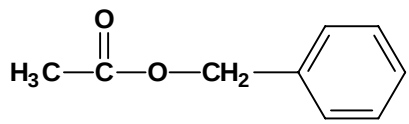
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 140-11-4

Systematic name: Acetic acid, phenylmethyl ester

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_9H_{10}O_2$ Relative molecular mass: 150.18

1.1.3 *Physical properties* (for details, see IARC, 1986)

(*a*) *Boiling-point*: 215.5° C

(*b*) *Melting-point*: –51.3°C

(*c*) *Conversion factor*: $mg/m^3 = 6.14 \times ppm$

1.2 Occurrence, use and human exposure

Benzyl acetate has been identified in several fruits, such as bael fruit (from the *Aegle marmelos* tree) and quince (*Cydonia vulgaris*), and in a mushroom (*Agaricus* species). It is a major volatile constituent of the flowers of a number of plants, including jasmine (*Jasminium grandiflorum* L.), hyacinth (*Hyacinthus orientalis*), gardenia (*Gardenia jasminoides*), ylang-ylang (*Cananga odorata*), alfalfa (*Medicago sativa* L.) and others. It has been used as a food additive in fruit flavours and as a component of perfumes since the early 1990s and is widely used as a fragrance in soaps, detergents and incense. There is widespread human exposure to benzyl acetate by ingestion, skin application and inhalation.

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Benzyl acetate was tested for carcinogenicity by gavage in one experiment in mice of both sexes and in one experiment in rats of both sexes. In the study in mice, increased incidences of liver adenomas and of combined liver adenomas and carcinomas were observed in animals of each sex; the incidence of carcinomas of the liver alone was not significantly increased in animals of either sex. An increased incidence of forestomach tumours was observed in mice of each sex. An increased incidence of acinar-cell adenomas of the pancreas was observed in male rats (IARC, 1986).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 60 male and 60 female B6C3F_1 mice, six weeks of age, were administered benzyl acetate (purity, > 98%) in the diet at concentrations of 0, 330, 1000 or 3000 mg/kg diet (ppm) for two years. High-dose females had prolonged survival. The mean body weights of the two high-dose groups of both sexes were reduced. No increase in neoplasms was found in exposed mice (United States National Toxicology Program, 1993).

3.1.2 *Rat*

Groups of 49 or 38 male Fischer 344 rats, four weeks of age, were administered benzyl acetate [purity not specified] in the diet at 0 or 0.8%, respectively, for two years. Benzyl acetate had no significant adverse effect on growth or survival. The incidence of pancreatic adenomas was 10/49 (20%) in controls and 8/38 (21%) in the exposed group. Carcinoma *in situ* was found in 3/38 rats ($p = 0.0428$, chi-square test) compared to 0/49 controls [no other tissue was reported] (Longnecker *et al*., 1990).

Groups of 60 male and 60 female Fischer 344/N rats, six weeks of age, were administered benzyl acetate (purity, > 98%) in the diet at concentrations of 0, 3000, 6000 or 12 000 mg/kg diet (ppm) for two years. The mean body weights of both sexes given the high dose were slightly reduced. No increase in neoplasms was found in exposed rats (United States National Toxicology Program, 1993).

3.2 Administration with known carcinogens

Rat: Groups of four to five weanling male Lewis or Fischer 344 rats were administered daily 500 mg/kg bw benzyl acetate [purity unspecified] by gavage on five days per week or 0.9% in the diet for four months alone or following injection with 30 mg/kg bw azaserine at 14 days of age to initiate pancreatic carcinogenesis. Benzyl acetate alone

induced no pancreatic foci. When given after azaserine, no increase in pancreatic foci was produced (Longnecker *et al*., 1986).

Groups of 20 male Fischer 344 rats [age unspecified] were administered benzyl acetate [purity unspecified] at 0, 0.4 or 0.8% in the diet for 6–12 months either alone or following injection with azaserine to initiate pancreatic carcinogenesis. At six months, there were fewer pancreatic acidophilic foci in the groups fed benzyl acetate than in controls, but their mean diameter was increased. No effect on basophilic foci was found. In the groups observed for up to 12 months, survival was decreased in those given benzyl acetate. However, the incidence of carcinomas of the pancreas was decreased in a dose-related manner, i.e. 12% in controls, 8% in low-dose and 0% in high-dose rats (Longnecker *et al*., 1990).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

The metabolism of benzyl acetate involves very rapid hydrolysis to acetate and benzyl alcohol. The latter is subsequently mainly oxidized to benzaldehyde and benzoic acid. A small part of the benzyl alcohol may be conjugated with sulfate, leading, ultimately, to formation of a glutathione conjugate that is excreted as mercapturate in urine. The benzoic acid is excreted mainly as hippurate and, to a lesser extent, as acyl glucuronide (see Figure 1).

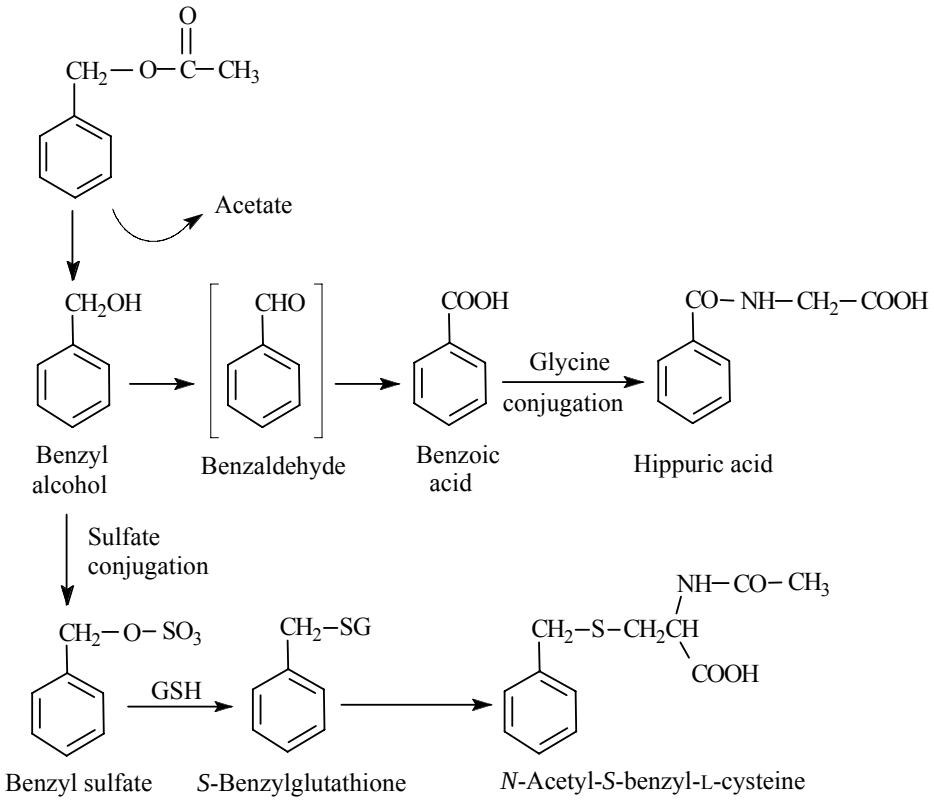
4.1.1 *Humans*

No recent data on humans are available, except for some very limited data on rate of skin penetration *in vitro* (see Section 4.1.2).

4.1.2 *Experimental systems*

Benzyl acetate is quite soluble in lipids and therefore readily absorbed from the gastrointestinal tract and lung, as well as through the skin, in the species investigated. The absorption after oral administration in the rat was delayed if it was administered in corn oil or propylene glycol as compared to neat [*methylene*-^{14}C]benzyl acetate (Chidgey & Caldwell, 1986): the peak plasma concentration of benzyl acetate-derived radioactivity occurred later (T_{max} after 1 h versus 4–6 h) and was lower at a 500 mg/kg benzyl acetate dose; at 5 mg/kg benzyl acetate, there was no difference. The urinary excretion of the metabolites was also delayed by corn oil, but the extent of absorption seemed not to be affected: more than 80% was absorbed and excreted within 24 h, mainly in urine and, ultimately, less than 5% in faeces. In plasma and urine, no intact benzyl acetate was detected at any time; only its metabolites were present (Chidgey & Caldwell, 1986). Benzyl acetate is rapidly hydrolysed by esterases to benzyl alcohol and acetate (Yuan *et al*., 1995). These esterases are present in plasma and probably also in the tissues; it is

Figure 1. Metabolism of benzyl acetate



Redrawn from Chidgey *et al.* (1986)

possible that during absorption benzyl acetate is already hydrolysed, so that little if any of the intact compound reaches the general circulation (Hotchkiss *et al.*, 1992). The peak plasma levels of the metabolite benzoic acid in rats and mice fed benzyl acetate in the diet were much lower than after gavage of roughly the same dose, whereas the levels of hippurate were very similar (Yuan *et al.*, 1995). The explanation may be that glycine conjugation of benzoic acid becomes saturated after the high, acute gavage dose, while during feeding the dose is more slowly taken up. Yuan *et al.* (1995) provide a pharmacokinetic model of benzyl acetate in rats and mice. Extensive absorption after dermal application of 100–500 mg/kg to rats was observed: 35–55% of the dose was recovered as metabolites in urine within 24 h (Chidgey *et al.*, 1987). The metabolic profile was the same as after oral or intravenous administration. Some data on organ distribution of radioactivity were provided. This percutaneous absorption was confirmed *in vitro* with rat and human skin (Hotchkiss *et al.*, 1990, 1992; Garnett *et al.*, 1994). Dimethylsulfoxide had a minor enhancing effect on skin absorption. The absorption rate through rat skin was approximately

six-fold that through human skin. The identity of the absorbed radioactivity was not determined, but most likely represented benzyl acetate metabolites.

No unchanged benzyl acetate was detected in rat or mouse plasma. The plasma concentrations of metabolites after intravenous administration of 5 mg/kg bw benzyl acetate (McMahon *et al*., 1989) showed only minor changes with age (3–4, 9 and 25 months) in Fischer 344 rats and B6C3F_1 or C57BL/6N mice. In urine, by far the major metabolite is the hippurate at all doses and in all species (Abdo *et al*., 1985; Chidgey & Caldwell, 1986; McMahon *et al*., 1989). The acyl glucuronide of benzoic acid comprised from 2 to 12% of the urinary metabolites (low versus high dose) and benzyl mercapturate 1–2% of the dose (Chidgey & Caldwell, 1986). This mercapturate is probably formed from benzyl alcohol through its (reactive) sulfate ester, since prevention of oxidation by pyrazole increases the mercapturate from 1.1 to 12% of the dose, while the sulfation inhibitor pentachlorophenol subsequently decreases it again to 2% (Chidgey *et al*., 1986).

4.1.3 *Comparison of human and rodent data*

Since no data are available on humans except for skin penetration data *in vitro*, no direct comparison can be made. Because in humans most of the dose seems to be excreted as hippurate (IARC, 1986) and because the metabolism of the primary hydrolysis product of benzyl acetate, benzyl alcohol, is very similar in rodents and humans (see, e.g., the monograph on toluene (this volume)), it is to be expected that the fate of benzyl acetate in humans is very similar to that in rodents.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

In a study in male and female mice, the survival of the animals was not decreased at any dose level (IARC, 1986).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Benzyl acetate gave negative results in the *Bacillus subtilis rec* assay. It was not mutagenic to *Salmonella typhimurium* in the presence or absence of exogenous metabolic activation nor did it induce sex-linked recessive lethal mutations in *Drosophila melanogaster* following exposure by injection.

Table 1. Genetic and related effects of benzyl acetate

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
BSD, *Bacillus subtilis rec*, differential toxicity	–	NT	21	Oda *et al*. (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	450	Florin *et al*. (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1667	Mortelmans *et al*. (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	450	Florin *et al*. (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1667	Mortelmans *et al*. (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	450	Florin *et al*. (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Mortelmans *et al*. (1986)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	450	Florin *et al*. (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Mortelmans *et al*. (1986)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		20000 inj	US National Toxicology Program (1993)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	900	McGregor *et al*. (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	+	750	Caspary *et al*. (1988)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	5000	Galloway *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	5000	Galloway *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster lung CHL fibroblasts *in vitro*	–	–	300	Matsuoka *et al*. (1996)
AIA, Aneuploidy, Chinese hamster lung CHL fibroblasts *in vitro*	–	–	300	Matsuoka *et al*. (1996)
DNA strand breaks and related damage (alkaline elution assay) in rat pancreas *in vivo*	–		1500 ip × 1	Longnecker *et al*. (1990)
UPR, Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	–		1000 po × 1	Mirsalis *et al*. (1989)

Table 1 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SVA, Sister chromatid exchange, B6C3F$_1$ mouse bone marrow *in vivo*	–		1700 ip × 1	US National Toxicology Program (1993)
MVM, Micronucleus test, B6C3F$_1$ mouse bone marrow *in vivo*	–		1250 ip × 3	US National Toxicology Program (1993)
MVM, Micronucleus test, B6C3F$_1$ mouse peripheral blood lymphocytes *in vivo*	–		6000 feed 13 wk	US National Toxicology Program (1993)
CBA, Chromosomal aberrations, B6C3F$_1$ mouse bone marrow *in vivo*	–		1700 ip × 1	US National Toxicology Program (1993)

[a] +, positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; inj, injection; ip, intraperitoneal; po, oral

Benzyl acetate did not induce unscheduled DNA synthesis in rat primary hepatocytes *in vitro* in a single study, as reported in an abstract (Mirsalis *et al.*, 1983). It was mutagenic in mouse lymphoma L5178Y cells at the *tk* locus with or without the addition of exogenous metabolic activation. It did not induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells nor chromosomal aberrations or aneuploidy in Chinese hamster lung fibroblasts in the presence or absence of exogenous metabolic activation.

Benzyl acetate did not induce unscheduled DNA synthesis in hepatocytes of rats following gavage treatment and a single intraperitoneal dose did not induce DNA damage in the pancreas of rats. It did not increase the frequency of sister chromatid exchanges, chromosomal aberrations or micronuclei in the bone marrow of $B6C3F_1$ mice *in vivo*, nor did it induce micronuclei in peripheral blood lymphocytes of mice given benzyl acetate for 13 weeks in their diet.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

There is widespread human exposure to benzyl acetate by ingestion, skin application and inhalation.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Benzyl acetate was tested for carcinogenicity by gavage in one experiment in mice and in one experiment in rats, and by administration in the diet in two studies in rats and in one study in mice. In the gavage study in mice, increased incidences of liver adenomas and of combined liver adenomas and carcinomas were oberved in animals of each sex. An increased incidence of forestomach tumours was observed in mice of each sex. An increased incidence of acinar-cell adenomas of the pancreas was observed in male rats administered benzyl acetate by gavage. Benzyl acetate did not increase the incidence of tumours in either mice or rats when administered in the diet. A low incidence of pancreatic carcinomas *in situ* was reported in one study.

Benzyl acetate was tested in two studies for promotion of pancreatic carcinogenesis in rats and was found to be inactive.

5.4 Other relevant data

Benzyl acetate is hydrolysed to benzoic acid and acetate. It is metabolized similarly by humans and rodents. Except for one positive result *in vitro*, findings on genotoxicity *in vitro* and *in vivo* were negative.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of benzyl acetate were available.

There is *limited evidence* in experimental animals for the carcinogenicity of benzyl acetate.

Overall evaluation

Benzyl acetate is *not classifiable as to its carcinogenicity to humans (Group 3)*.

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BIS(2-CHLOROETHYL)ETHER

Data were last reviewed in IARC (1975) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

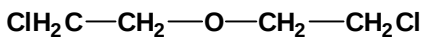
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 111-44-4

Systematic name: 1,1′-Oxybis(2-choro)ethane

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_4H_8Cl_2O$ Relative molecular mass: 143.02

1.1.3 *Physical properties* (for details, see IARC, 1975)

(*a*) *Boiling-point*: 178°C

(*b*) *Melting-point*: –50°C (Budavari, 1989)

(*c*) *Conversion factor*: $mg/m^3 = 5.85 \times ppm$

1.2 Production and use

It is not clear whether bis(2-chloroethyl)ether is still produced commercially. It has been used as a solvent, a chemical intermediate and as a soil fumigant (IARC, 1975; WHO, 1998).

2. Studies of Cancer in Humans

Two studies have examined the risk of cancer among chlorohydrin production workers potentially exposed to bis(2-chloroethyl)ether as well as 1,2-dichloroethane and ethylene chlorohydrin (see the monograph on 1,2-dichloroethane in this volume for a detailed description of the study methods). In one study there was an excess of pancreatic, lymphatic and haematopoietic cancers (Benson & Teta, 1993), while in the other there was not (Olsen *et al*., 1997). In neither study was it possible to link mortality to any particular chemical exposure.

3. Studies of Cancer in Experimental Animals

Bis(2-chloroethyl)ether produced an increased incidence of hepatomas in male mice of two strains following its oral administration at a dose of 100 mg/kg bw per day in a screening study. The hepatoma incidences were: (C57BL/6 × C3H/Anf)F_1 strain males, 8/79 in the control group and 14/16 in the treated group; (C57BL/6 × AKR)F_1 males, 5/90 in the control group and 9/17 in the treated group. Subcutaneous administration to mice produced a low incidence of sarcomas at the injection site (IARC, 1975).

3.1 Oral administration

Bis(2-chloroethyl)ether (purity 100% according to nuclear magnetic resonance analysis) was administered orally by gavage to groups of 26 male and female Sprague-Dawley rats at doses of 25 and 50 mg/kg bw per day twice a week for 78 weeks and then observed without further dosing up to 104 weeks. The test was conducted concurrently with several other chemicals, for which a common vehicle control group of 58 rats of each sex was used for comparison. Survival at 52 weeks among male and female rats was 96% and 96%, respectively, in the 25 mg/kg bw group and 100% and 65%, respectively, in the 50 mg/kg bw group. Apart from the high-dose female group, at least 81% of the rats were alive at 78 weeks. There were no increases in the incidence of tumours in either male or female rats (Weisburger *et al*., 1981). [Tumour incidence data for the treatment groups were not presented; the group sizes were smaller than contemporary recommendations. The Working Group noted the short duration of dosing.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Bis(2-chloroethyl)ether is rapidly absorbed through the skin of rabbits (IARC, 1975).

Young adult male Sprague-Dawley rats were given single oral doses of bis(2-chloro-[1-^{14}C]ethyl)ether (40 mg/kg bw) and the excretion of $^{14}CO_2$ and urinary ^{14}C was followed for 48 h. Half of the administered radioactivity was eliminated within 12 h. Recoveries of the administered radioactivity from the expired air, urine, faeces and body organs at 48 h were 11.5%, 64.7%, 2.4% and 2.3%, respectively. Unchanged bis(2-chloroethyl)ether would have contributed no more than 2% of the dose. The main body depots of radioactivity were blood (0.49%), liver (0.19%), kidney (0.56%) and muscle (0.96%). The major

urinary metabolite was thiodiglycolic acid, which accounted for about 75% of the radioactivity. Minor urinary metabolites were 2-chloroethoxyacetic acid (5%) and *N*-acetyl-*S*-[2-(2-chloroethoxy)ethyl]-L-cysteine (7%). The authors proposed that facile in-vivo cleavage at the ether linkage to form active metabolites may be important for adverse responses to the compound (Lingg *et al*., 1979, 1982).

4.2 Toxic effects

4.2.1 *Humans*

Brief exposure of volunteers to 550 ppm [3220 mg/m^3] bis(2-chloroethyl)ether was intolerably irritating to the eyes and nasal passages (IARC, 1975).

4.2.2 *Experimental systems*

Bis(2-chloroethyl)ether is rapidly lethal to rabbits upon skin application and to guinea-pigs upon inhalation (IARC, 1975).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Bis(2-chloroethyl)ether is weakly mutagenic in *Salmonella typhimurium* TA1535. In one study, it produced sex-linked recessive lethal mutations in *Drosophila melanogaster* when administered by injection. It did not induce heritable translocations in *D. melanogaster* or mice. In rats, it formed covalent complexes with protein but not with DNA.

5. Evaluation

There is *inadequate evidence* for the carcinogenicity of bis(2-chloroethyl)ether in humans.

There is *limited evidence* in experimental animals for the carcinogenicity of bis(2-chloroethyl)ether.

Overall evaluation

Bis(2-chloroethyl)ether is *not classifiable as to its carcinogenicity to humans (Group 3)*.

Table 1. Genetic and related effects of bis(2-chloroethyl)ether

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, *Escherichia coli*, SOS induction	–	NT	NG	Quinto & Radman (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	?	5000	Mortelmans *et al*. (1986)[c]
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1250	JETOC (1997)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	(+)	5000	Mortelmans *et al*. (1986)[c]
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	(+)	1250	JETOC (1997)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Mortelmans *et al*. (1986)[c]
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1875	JETOC (1997)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Mortelmans *et al*. (1986)[c]
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1875	JETOC (1997)
ECF, *Escherichia coli*, forward mutation	–	NT	NG	Quinto & Radman (1987)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation	–	–	2500	JETOC (1997)
ECB, *Escherichia coli*, recombination	–	NT	NG	Quinto & Radman (1987)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	(+)		10 500 ppm inj	Foureman *et al*. (1994)
DMH, *Drosophila melanogaster*, heritable translocation test	–		13 000 ppm inj	Foureman *et al*. (1994)
MHT, Mouse heritable translocation test *in vivo*	–		100 po × 1, 8 wk	Jorgenson & Rushbrook (1977)
BIP, Binding (covalent) to RNA *in vitro*	–	NT	7150	Shooter (1975)
BVP, Protein binding, Wistar rat liver *in vivo*	+		125 inh 24 h	Gwinner *et al*. (1983)
BVD, Binding (covalent) to DNA, Wistar rat liver *in vivo*	–		125 inh 24 h	Gwinner *et al*. (1983)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; NG, not given; inj, injection; po, oral; inh, inhalation

[c] Tested in two laboratories; results shown are for the one testing to higher doses; all results from the other laboratory were negative.

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1,2-BIS(CHLOROMETHOXY)ETHANE

Data were last reviewed in IARC (1977) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

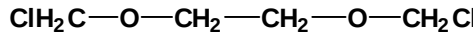
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 13483-18-6

Systematic name: 1,2-Bis(chloromethoxy)ethane

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_4H_8Cl_2O_2$ Relative molecular mass: 159.0

1.1.3 *Physical properties* (for details, see IARC, 1977)

(*a*) *Boiling-point*: 97–99°C at 1.7 kPa; 99–100°C at 2.9 kPa

(*b*) *Conversion factor*: $mg/m^3 = 6.50 \times ppm$

1.2 Production and use

1,2-Bis(chloromethoxy)ethane may never have been produced in commercial quantities, although its use in various situations has been investigated (IARC, 1977).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

1,2-Bis(chloromethoxy)ethane was tested for carcinogenicity in mice by application to the skin or by subcutaneous or intraperitoneal injection; it produced malignant tumours at the sites of application (IARC, 1977).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

No data were available to the Working Group.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of 1,2-bis(chloromethoxy)-ethane were available.

There is *limited evidence* in experimental animals for the carcinogenicity of 1,2-bis(chloromethoxy)ethane.

Overall evaluation

1,2-Bis(chloromethoxy)ethane is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

IARC (1977) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*, Vol. 15, *Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzo-dioxins and Miscellaneous Industrial Chemicals*, Lyon, pp. 31–35

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 58

1,4-BIS(CHLOROMETHOXYMETHYL)BENZENE

Data were last reviewed in IARC (1977) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

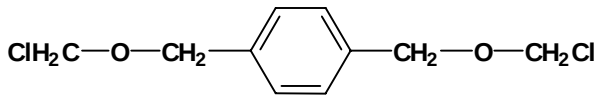
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 56894-91-8

Systematic name: 1,4-Bis(chloromethoxymethyl)benzene

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{10}H_{12}Cl_2O_2$ Relative molecular mass: 235.1

1.2 Production and use

1,4-Bis(chloromethoxymethyl)benzene was investigated in the United States for use in the production of ion-exchange resins (IARC, 1977).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

1,4-Bis(chloromethoxymethyl)benzene was tested for carcinogenicity in mice by application to the skin or subcutaneous injection; it produced malignant tumours at the sites of application (IARC, 1977).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

No data were available to the Working Group.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of 1,4-bis(chloromethoxymethyl)benzene were available.

There is *limited evidence* in experimental animals for the carcinogenicity of 1,4-bis(chloromethoxymethyl)benzene.

Overall evaluation

1,4-Bis(chloromethoxymethyl)benzene is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

IARC (1977) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*, Vol. 15, *Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals*, Lyon, pp. 37–39

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 58

BIS(2-CHLORO-1-METHYLETHYL)ETHER

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

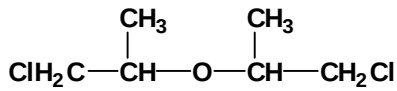
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 108-60-1

Systematic name: 2,2′-Oxybis(1-chloropropane)

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_6H_{12}Cl_2O$ Relative molecular mass: 171.7

1.1.3 *Physical properties* (for details, see IARC, 1986)

(*a*) *Boiling-point*: 187°C

(*b*) *Melting-point*: –100°C

(*c*) *Conversion factor*: $mg/m^3 = 7.02 \times ppm$

1.2 Production, use and human exposure

Bis(2-chloro-1-methylethyl)ether has been produced as a solvent and soil fumigant and is also formed in large quantities as a by-product in some propylene oxide/propylene glycol production processes. Low levels have been found in water. Thus, both occupational and environmental exposures may occur (IARC, 1986).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Bis(2-chloro-1-methylethyl)ether, containing 2-chloro-1-methylethyl(2-chloro-*n*-propyl)ether and bis(2-chloro-*n*-propyl)ether, was tested for carcinogenicity orally by gavage in one experiment in mice and in one experiment in rats. In mice, increased incidences of lung adenomas in males and females and of hepatocellular carcinomas in males were observed. In rats, no increase in tumour incidence was observed (IARC, 1986).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

In rats receiving a single oral dose of bis(2-chloro-1-methylethyl)ether of 0.0002–300 mg/kg bw, peak blood levels of radioactivity were reached at about 2–4 h. Following administration of a dose of 30 mg/kg bw, elimination was biphasic in rhesus monkeys, with half-lives of 5 h and two days, and monophasic in rats, with a half-life of two days. In rats, total recovery of radioactivity was 75% of an oral dose of the 1-^{14}C-labelled compound and 90% after an intraperitoneal dose with the 2-^{14}C-labelled compound; approximately 20% of the oral dose was exhaled as $^{14}CO_2$ in 48 h. Also in rats, urinary excretion of radioactivity accounted for 48% of a 90 mg/kg bw oral dose of the 1-^{14}C-labelled compound within 48 h and for 60% of a 30 mg/kg bw intraperitoneal dose of the 2-^{14}C-labelled compound within 24 h. Urinary metabolites identified after administration of an oral dose of 90 mg/kg bw of the 1-^{14}C-labelled compound to rats were 2-(2-chloro-1-methylethoxy)propanoic acid (17% of the dose) and *N*-acetyl-*S*-(2-hydroxypropyl)-cysteine (approximately 9% of the dose); following an intraperitoneal dose of the 2-^{14}C-labelled compound, metabolites identified were 1-chloropropan-2-ol, propylene oxide and 2-(2-chloro-1-methylethoxy)propanoic acid (IARC, 1986).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Inhalation exposure of rats to 350 ppm [2450 mg/m^3] for 5 h per day on eight consecutive days caused respiratory distress, reduced body weight gain, irritation to the

eyes, nose and lung and hepatic and renal injury. Dietary exposure of mice also caused anaemia (IARC, 1986).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

[Some experiments reported in the previous monograph (IARC, 1986) were not, in fact, done with this compound.]

Bis(2-chloro-1-methylethyl)ether is weakly mutagenic in *Salmonella typhimurium* TA1535 in the presence of an exogenous metabolic system. It induced mutations at the *tk* locus in mouse lymphoma cells. It did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster* (as reported in an abstract: Mirsalis *et al.*, 1985).

5. Evaluation

No epidemiological data relevant to the carcinogenicity of bis(2-chloro-1-methylethyl)ether were available.

There is *limited evidence* in experimental animals for the carcinogenicity of bis(2-chloro-1-methylethyl)ether.

Overall evaluation

Bis(2-chloro-1-methylethyl)ether is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

IARC (1986) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 41, *Some Halogenated and Pesticide Exposures*, Lyon, pp. 149–160

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 59

McGregor, D.B., Brown, A., Cattanach, P., Edwards, I., McBride, D., Riach, C. & Caspary, W.J. (1988) Responses of the L5178Y $tk^{+/-}$ mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. *Environ. mol. Mutag.*, **12**, 85–154

Table 1. Genetic and related effects of bis(2-chloro-1-methylethyl)ether

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Mortelmans *et al.* (1986)[c]
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	(+)	166	Mortelmans *et al.* (1986)[c]
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Mortelmans *et al.* (1986)[c]
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	166	Mortelmans *et al.* (1986)[c]
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	166	Mortelmans *et al.* (1986)[c]
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		1600 ppm inj[d]	Valencia *et al.* (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	250	McGregor *et al.* (1988)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; inj, injection

[c] Results from two independent laboratories

[d] Negative also when exposed to 283 ppm in the diet

Mirsalis, J., Tyson, K., Loh, E., Baake, J., Hamilton, C., Spak, D., Steinmetz, K. & Spalding, J. (1985) Induction of unscheduled DNA synthesis (UDS) and cell proliferation in mouse and rat hepatocytes following in vivo treatment (Abstract). *Environ. Mutag.*, **7** (Suppl. 3), 73

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B. & Zeiger, E. (1986) *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ. Mutag.*, **8** (Suppl. 7), 1–119

Valencia, R., Mason, J.M., Woodruff, R.C. & Zimmering, S. (1985) Chemical mutagenesis testing in *Drosophila*. III. Results of 48 coded compounds tested for the National Toxicology Program. *Environ. Mutag.*, **7**, 325–348

BIS(2,3-EPOXYCYCLOPENTYL)ETHER

Data were last evaluated in IARC (1989).

1. Exposure Data

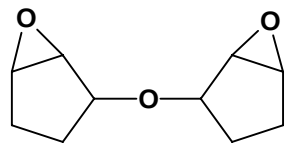
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 2386-90-5

Systematic name: 2,2′-Oxybis(6-oxabicyclo[3.1.0]hexane)

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{10}H_{14}O_3$ Relative molecular mass: 182.22

1.1.3 *Physical properties* (for details, see IARC, 1989)

(*a*) *Boiling-point*: 203°C at 13 kPa

(*b*) *Melting-point*: 29.7°C

(*c*) *Conversion factor*: $mg/m^3 = 7.45 \times ppm$

1.2 Production, use and human exposure

Bis(2,3-epoxycyclopentyl)ether is a synthetic organic liquid which has been used as a component and modifier of epoxy resins. Measurements of occupational exposure levels have not been reported (IARC, 1989).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Bis(2,3-epoxycyclopentyl)ether was tested for carcinogenicity by skin application in one experiment in two strains of mice. A small number of skin tumours was observed in both strains and an increased incidence of lung tumours in females of one strain. Another experiment with skin application in mice was inadequate for evaluation (IARC, 1989).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Bis(2,3-epoxycyclopentyl)ether was a mild irritant to rabbit skin and produced moderate corneal injury in rabbits. It did not induce sensitization reactions in guinea-pigs (IARC, 1989).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

In a single study, bis(2,3-epoxycyclopentyl)ether was mutagenic to bacteria, and produced sister chromatid exchanges in cultured human lymphocytes and micronuclei in the bone-marrow cells of mice treated *in vivo* (IARC, 1989).

5. Evaluation

No epidemiological data relevant to the carcinogenicity of bis(2,3-epoxycyclopentyl)ether were available.

There is *limited evidence* in experimental animals for the carcinogenicity of bis(2,3-epoxycyclopentyl)ether.

Overall evaluation

Bis(2,3-epoxycyclopentyl)ether is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. Reference

IARC (1989) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 47, *Some Organic Solvents, Resin Monomers and Related Compounds, Pigments and Occupational Exposures in Paint Manufacture and Painting*, Lyon, pp. 231–236

BISPHENOL A DIGLYCIDYL ETHER

Data were last evaluated in IARC (1989).

1. Exposure Data

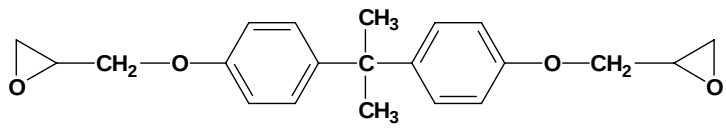
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 1675-54-3

Systematic name: 2,2′-[(1-Methylethylidene)bis(4,1-phenyleneoxymethylene)]bis-(oxirane)

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{21}H_{24}O_4$ Relative molecular mass: 340.42

1.2 Production, use and human exposure

Glycidyl ethers are basic components of epoxy resins which have been commercially available since the late 1940s. Bisphenol A diglycidyl ether and its oligomers are major components of epoxy resins. Other glycidyl ethers, including phenyl glycidyl ether, are frequently incorporated into epoxy resin systems as reactive modifiers. Epoxy resins based on bisphenol A diglycidyl ether are widely used in protective coatings, including paints, in reinforced plastic laminates and composites, in tooling, casting and moulding resins, in bonding materials and adhesives, and in floorings and aggregates. Occupational exposure to bisphenol A diglycidyl ether may occur during its production, during the production of epoxy products and during various uses of epoxy products, but data on exposure levels are sparse (IARC, 1989).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Bisphenol A diglycidyl ether of various technical grades was tested for carcinogenicity by skin application in mice in five studies. In one of the studies, an increased incidence of epidermal tumours was found in C57BL/6 mice, but not in C3H mice. In a study with CF1 mice, a small increase in the incidence of epidermal tumours and small increases in the incidences of kidney tumours in male mice and of lymphoreticular/haematopoietic tumours in female mice were observed. No increase in the incidence of skin tumours was observed in two further studies, one with CF1 mice, the other with C3H mice and the remaining study with C3H mice was inadequate for evaluation. Following subcutaneous injection of technical grade bisphenol A diglycidyl ether to male Long-Evans rats, a small number of local fibrosarcomas was observed. Following application of technical grade bisphenol A diglycidyl ether to the skin of albino rabbits, no skin tumour was observed.

Pure ('analytical grade') bisphenol A diglycidyl ether was tested in one experiment by skin application in CF1 mice; no epidermal, but a few dermal tumours were observed in males and there was a small increase in the incidence of lymphoreticular/haematopoietic tumours in females (IARC, 1989). No subsequent studies were available to the Working Group.

It was noted that glycidaldehyde, a metabolite of bisphenol A diglycidyl ether, is carcinogenic to experimental animals and classified as *possibly carcinogenic* to humans (*group 2B*) (see this volume).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

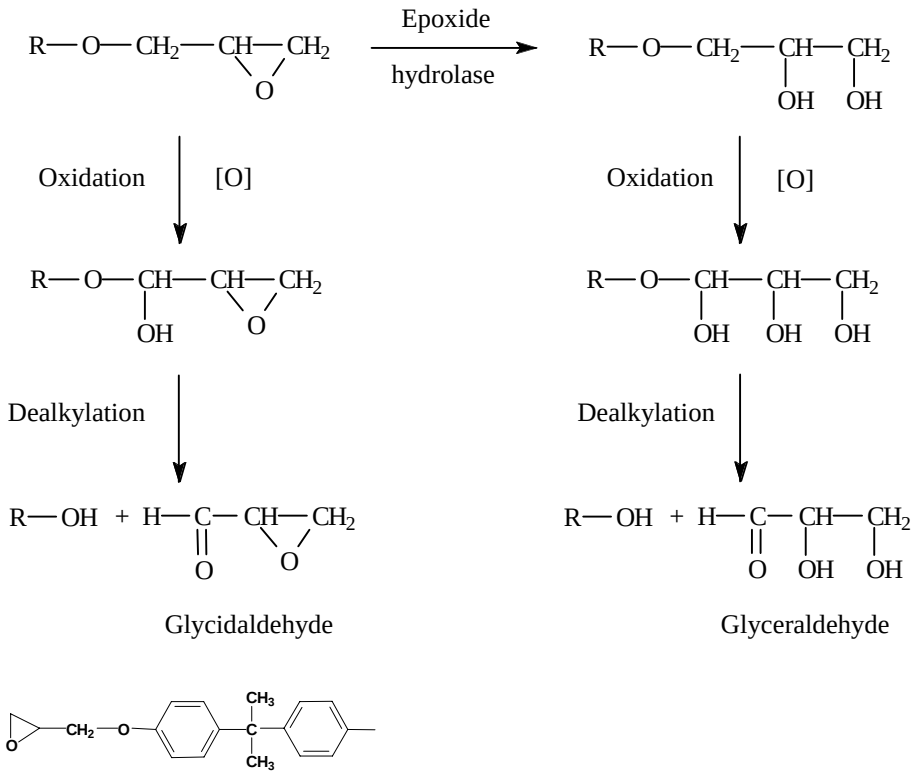
No data were available to the Working Group.

4.1.2 *Experimental systems*

A dose of approximately 55 mg/kg bw [^{14}C]bisphenol A diglycidyl ether to mice was well absorbed after administration by either the oral or dermal route. Most of the orally administered material was excreted within 24 h. After eight days, 79% was recovered from faeces and 10% from urine following oral dosing. Following dermal dosing, 67% and 11% of the radioactivity could be recovered from the application site after 24 h and 8 days, respectively. Bisphenol A diglycidyl ether is rapidly metabolized in mice, the major route involving hydration to the corresponding bis-diol, which occurs both enzymatically, through the epoxide hydrolase, and nonenzymatically. This hydration is followed by mono-

oxygenase-mediated dealkylation to form a phenol and glyceraldehyde. It also appears that bisphenol A diglycidyl ether may be directly oxidized with the release of glycidaldehyde (Figure 1). Urinary and faecal metabolites include glucuronides and sulfates of the bis-diol and corresponding carboxylic acids (IARC, 1989).

Figure 1. Two possible routes of oxidative dealkylation of the glycidyl moiety of bisphenol A diglycidyl ether



R,

From Climie *et al.* (1981)

4.2 Toxic effects

4.2.1 *Humans*

Bisphenol A diglycidyl ether is a contact allergen among people who have worked with low-molecular-weight epoxy resins (IARC, 1989).

4.2.2 *Experimental systems*

Bisphenol A diglycidyl ether has low oral and dermal lethal toxicity in rats and rabbits, respectively, but it is irritant to rabbit skin. It is a highly reactive allergen, according to a guinea-pig skin maximization test (IARC, 1989).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Bisphenol A diglycidyl ether applied to the skin of pregnant rabbits had no effect upon fetal development (IARC, 1989). Daily oral administration by gavage during gestational days 6–15 of up to 640 mg/kg bw per day to rats and 1250 mg/kg bw per day to mice had no effect upon the incidence of malformations in either species. These maternally toxic doses produced fetal toxicity in mice, but not in rats (Morrissey *et al*., 1987).

4.4 Genetic and related effects

4.4.1 *Humans*

One study of workers exposed to bisphenol A diglycidyl ether showed no increase in chromosomal aberrations in peripheral blood lymphocytes (IARC, 1989).

4.4.2 *Experimental systems*

The compound is mutagenic to bacteria (IARC, 1989).

A single major DNA adduct has been observed in the skin of C3H mice treated cutaneously with [^{14}C]bisphenol A diglycidyl ether. Initially it was proposed that this was a reaction product of glycidaldehyde and deoxyguanosine, based upon co-chromatography on an XAD-resin (Bentley *et al*., 1989). Later studies with higher resolution high-performance liquid chromatography on a C-18 column demonstrated that the adducts of bisphenol A diglycidyl ether and glycidaldehyde with DNA are indeed identical, but that the mouse skin adduct is probably hydroxymethylethenodeoxyadenosine-3′-monophosphate, by comparison with a synthetic reference standard. The alkylation frequency was 0.1–0.8 adducts/10^6 nucleotides following dosing with 2 mg bisphenol A diglycidyl ether per mouse and 166 adducts/10^6 nucleotides after a similar dose of glycidaldehyde (Steiner *et al*., 1992a,b).

5. Evaluation

No epidemiological data relevant to the carcinogenicity of bisphenol A diglycidyl ether were available.

There is *limited evidence* in experimental animals for the carcinogenicity of bisphenol A diglycidyl ether.

Overall evaluation

Bisphenol A diglycidyl ether is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

Bentley, P., Bieri, F., Kuster, H., Muakkassah-Kelly, S., Sagelsdorff, P., Stäubli, W. & Waechter, F. (1989) Hydrolysis of bisphenol A diglycidylether by epoxide hydrolases in cytosolic and microsomal fractions of mouse liver and skin: inhibition by bis epoxycyclopentylether and the effects upon the covalent binding to mouse skin DNA. *Carcinogenesis*, **10**, 321–327

Climie, I.J., Hutson, D.H. & Stoydin, G. (1981) Metabolism of the epoxy resin component 2,2-bis[4-(2,3-epoxypropoxy)phenyl]propane, the diglycidyl ether of bisphenol A (DGEBPA) in the mouse. Part II. Identification of metabolites in urine and faeces following a single oral dose of ^{14}C-DGEBPA. *Xenobiotica*, **11**, 401–424

IARC (1976) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 11, *Cadmium, Nickel, Some Epoxides, Miscellaneous Industrial Chemicals and General Considerations on Volatile Anaesthetics*, Lyon, pp. 175–181

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 64

IARC (1989) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 47, *Some Organic Solvents, Resin Monomers and Related Compounds, Pigments and Occupational Exposures in Paint Manufacture and Painting*, Lyon, pp. 237–261

Morrissey, R.E., George, J.D., Price, C.J., Tyl, R.W., Marr, M.C. & Kimmel, C.A. (1987) The developmental toxicity of bisphenol A diglycidyl ether in rats and mice. *Fundam. appl. Toxicol*., **8**, 571–582

Steiner, S., Crane, A.E. & Watson, W.P. (1992a) Molecular dosimetry of DNA adducts in C3H mice treated with glycidaldehyde. *Carcinogenesis*, **13**, 119–124

Steiner, S., Hönger, G. & Sagelsdorff, P. (1992b) Molecular dosimetry of DNA adducts in C3H mice treated with bisphenol A diglycidylether. *Carcinogenesis*, **13**, 969–972

BROMOCHLOROACETONITRILE

Data were last evaluated in IARC (1991).

1. Exposure Data

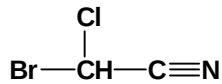
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 83463-62-1

Systematic name: Bromochloroacetonitrile

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2HBrClN$ Relative molecular mass: 154.39

1.1.3 *Physical properties* (for details, see IARC, 1991)

(*a*) *Boiling-point*: 138–140°C

(*b*) *Conversion factor*: $mg/m^3 = 6.31 \times ppm$

1.2 Production, use and human exposure

Halogenated acetonitriles are not produced on an industrial scale. Several halogenated acetonitriles have been detected in chlorinated drinking-water in a number of countries as a consequence of the reaction of chlorine with natural organic substances and bromide present in untreated water. The only known route of human exposure is through chlorinated drinking-water (IARC, 1991).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Bromochloroacetonitrile was tested in a limited carcinogenicity study in female SEN mice by skin application, in an initiation/promotion study in female SEN mice by skin application and in a screening assay for lung tumours in female strain A/J mice by oral administration. No skin tumour was produced after skin application in mice. In the initiation/promotion study, in which bromochloroacetonitrile was applied topically as six equal doses over a two-week period, followed by repeated doses of 12-*O*-tetradecanoylphorbol 13-acetate for 20 weeks, there was a small increase in the number of mice with tumours in the highest dose group: control, 9/105; intermediate dose, 7/37 (non-significant); highest dose (total dose 4800 mg/kg bw), 8/37 ($p < 0.05$). In orally treated mice, an increase in the proportion of animals with lung tumours and number of tumours per mouse was observed: control, 3/31 and 0.1; treated group (10 mg/kg bw, three times per week, eight weeks), 10/32 and 0.34 ($p < 0.05$) (IARC, 1991).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Approximately 13% of a single oral dose to rats of 116 mg/kg bw of bromochloroacetonitrile was excreted in urine within 24 h as thiocyanate, the product of released cyanide metabolized by rhodanese (IARC, 1991).

4.2 Toxic effects

No data were available to the Working Group.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

There were slight decreases in early postnatal body weights of pups born to rats given bromochloroacetonitrile orally at a dose of 55 mg/kg bw per day on gestation days 7–21 (IARC, 1991).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Bromochloroacetonitrile induced DNA damage and mutation in bacteria. It also induced sister chromatid exchanges and DNA strand breaks in mammalian cell lines. Micronuclei were induced in the erythrocytes of newt (*Pleurodeles waltl*) larvae exposed for 12 days, but in mice dosed orally for five days, neither micronuclei in bone marrow nor abnormal sperm morphology were induced.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of bromochloroacetonitrile were available.

There is *inadequate evidence* in experimental animals for the carcinogenicity of bromochloroacetonitrile.

Overall evaluation

Bromochloroacetonitrile is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

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Table 1. Genetic and related effects of bromochloroacetonitrile

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS chromotest, *Escherichia coli* PQ37	+	–	5	Le Curieux *et al.* (1995)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	13	Bull *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation (fluctuation test)	+	–	0.6	Le Curieux *et al.* (1995)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	26	Bull *et al.* (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Bull *et al.* (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	NG	Bull *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	105	Bull *et al.* (1985)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	0.65	Bull *et al.* (1985)
DIH, DNA strand breaks, human lymphoblast cell line *in vitro*	+	NT	NG	Daniel *et al.* (1986)
Micronucleus test, *Pleurodeles waltl* erythrocytes *in vivo*	(+)		0.125	Le Curieux *et al.* (1995)
MVM, Micronucleus test, CD-1 mouse bone-marrow cells *in vivo*	–		50 po × 5	Bull *et al.* (1985)
SPF, Sperm morphology, B6C3F_1 mice *in vivo*	–		50 po × 5	Meier *et al.* (1985)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; po, oral

BROMODICHLOROMETHANE

Data were last evaluated in IARC (1991).

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 75-27-4

Systematic name: Bromodichloromethane

1.1.2 *Structural and molecular formulae and relative molecular mass*

$CHBrCl_2$

$CHBrCl_2$ Relative molecular mass: 163.83

1.1.3 *Physical properties* (for details, see IARC, 1991)

(*a*) *Boiling-point*: 90.1°C

(*b*) *Melting-point*: –57.1°C

(*c*) *Conversion factor*: $mg/m^3 = 6.70 \times ppm$

1.2 Production, use and human exposure

Bromodichloromethane is found in chlorinated drinking-water as a consequence of the reaction between chlorine, added during water treatment, and natural organic substances in the presence of bromide. The major route of human exposure is via drinking-water. Bromodichloromethane has also been detected in some untreated waters, but at much lower levels. It is a major component of the organohalides produced by marine algae (IARC, 1991).

2. Studies of Cancer in Humans

No data were available to the Working Group regarding exposure to bromodichloromethane alone. Exposure to this compound as a contaminant of drinking-water is treated in Volume 52 of the *IARC Monographs* (IARC, 1991).

3. Studies of Cancer in Experimental Animals

Bromodichloromethane was tested for carcinogenicity in two-year studies in male and female Fischer 344 rats and B6C3F_1 mice by oral gavage, in life-span studies in male and female Wistar rats and in CBA × C57BL/6 hybrid mice by administration in drinking-water. In the gavage studies, bromodichloromethane increased the incidences of adenomatous polyps and adenocarcinomas of the large intestine and of tubule-cell adenomas and adenocarcinomas of the kidney in male and female rats, of tubule-cell adenomas and adenocarcinomas of the kidney in male mice and of hepatocellular adenomas and carcinomas in female mice. When administered in drinking-water, it induced neoplastic nodules and adenofibrosis of the liver in rats; no increase in tumour incidence was seen in mice. In a screening test for lung adenomas by intraperitoneal injection, bromodichloromethane did not increase the incidence of lung tumours in strain A mice (IARC, 1991).

3.1 Oral administration

Rat: Groups of 40 male and 40 female Wistar rats, five weeks of age, were administered microencapsulated bromodichloromethane (purity, 98%) at dietary concentrations of 0.014, 0.055 or 0.22% (equivalent to 6.1, 25.5 or 138 mg/kg bw per day for males and 8.0, 31.7 or 168.4 mg/kg bw per day for females) for 24 months. A concurrent control group of 70 male and 70 female rats received the same diet containing the starch-gelatin microcapsules alone. The microencapsulated diets were prepared every four months and the bromodichloromethane concentrations were verified. Ten rats of each sex from the control group and six rats of each sex from each of the dosed groups were killed at six months, while nine rats of each sex from the control group and five rats of each sex of the dosed groups were killed at 12 and 18 months. The remaining survivors were killed at 24 months. Body weight gain was suppressed in the 0.22% group for both males and females, while absolute liver weights were increased in the same groups. Dose-related changes included fatty degeneration in the livers of the 0.014% or higher-dose male groups, and fatty degeneration and granuloma in the 0.055 and 0.22% group females, as well as bile duct proliferation and cholangiofibrosis in the 0.22% group for both males and females. No significant differences in incidences or numbers of neoplastic changes were seen between the controls and any of the treatment groups. The numbers of cholangiocarcinomas of the liver in the controls and the low-, medium- and high-dose groups, respectively, were, for males, 0, 0, 0, 1 and, for females, 0, 0, 0, 3 (Aida *et al.*, 1992a; Yasuhara *et al.*, 1995). [The Working Group noted that a maximum of 24 rats per sex per group were permitted to remain on the experiment beyond 18 months.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

After oral administration to rats, bromodichloromethane is rapidly absorbed; part of the dose is exhaled as unchanged bromodichloromethane and as CO_2, and only minor fractions of the dose are found in the urine. Absorbed bromodichloromethane is rapidly eliminated from experimental animals, mainly by exhalation (IARC, 1991).

The disposition of bromodichloro[^{14}C]methane was studied in male Fischer rats after single oral doses of 1, 10, 32 or 100 mg/kg bw and oral dosing of 10 or 100 mg/kg bw every day for 10 days. Bromodichloromethane was extensively (approximately 80–90%) metabolized within 24 h after dosing, with approximately 70–80% of the administered dose appearing as $^{14}CO_2$ and approximately 3–5% as ^{14}CO. At all dose levels, urinary and faecal elimination of ^{14}C accounted for only 4% and 1–3% of the dose, respectively. Oral administration of bromodichloromethane at a concentration of 10 mg/kg bw per day for 10 days did not result in bioaccumulation or altered disposition of the test chemical, but during the course of the repeated dosing at 100 mg/kg bw per day, the rate of production of $^{14}CO_2$ increased, suggesting that this dose of bromodichloromethane induced its own metabolism. Persistence of radiolabelled residues in tissues collected 24 h after single-dose administration was low (3–4% of dose), with the most marked accumulation (1–3% of dose) in liver. Kidney tissue, particularly the cortical region, also contained significant concentrations of residues (Mathews *et al.*, 1990).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Repeated exposure of rats and mice to bromodichloromethane resulted in toxic effects in several organs, including the liver and kidney (IARC, 1991).

Biochemical and histopathological signs of hepatic damage were observed in rats at dose levels of 150–300 mg/kg bw per day after five days; in mice serum alanine aminotransferase and sorbitol dehydrogenase activities were elevated at a dose level of 150 mg/kg, but no histopathological lesions were observed. In rats, but not in mice, the total hepatic cytochrome P450 levels were decreased. As a sign of kidney damage, serum creatinine and blood urea nitrogen were elevated in rats dosed with 300 mg/kg but not in mice (Thornton-Manning *et al*., 1994). Histopathological liver damage was observed in

female and male rats after dietary administration of 0.014% or more of bromodichloromethane; the changes were observed after six months and were accentuated after 12 and 18 months of administration. No histopathological changes were observed in the kidneys. A dose-dependent increase in the frequency of fatty degeneration and increased frequency of bile duct proliferation and cholangiofibrosis at the highest dose level were observed (Aida *et al.*, 1992a). In a similar one-month experiment, hepatic—but not renal—damage was observed in males at a dose level of 0.215% and in females at a dose level of 0.076% or higher (Aida *et al.*, 1992b). Pretreatment of Fischer 344 rats with buthionine sulfoximine, which decreased the hepatic glutathione content by 86%, markedly accentuated the hepatic and renal toxicity of bromodichloromethane administered as a single dose (400 mg/kg bw) by gavage (Gao *et al*., 1996).

In a carcinogenicity study, liver cell proliferation in Wistar rats measured using immunostaining with a monoclonal antibody to proliferating cell nuclear antigen in the highest-dose group was maximal at six months of treatment (Yasuhara *et al.*, 1995).

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

In rats given bromodichloromethane throughout the period of major organogenesis, skeletal variations were observed in the presence of maternal toxicity, but no teratogenic effect was seen (IARC, 1991).

Bromodichloromethane, given in the drinking-water to Fischer 344 rats for one year (39 mg/kg bw per day), decreased the velocity of epididymal sperm, but had no effect on testicular morphology (Klinefelter *et al*., 1995).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Bromodichloromethane induced gene mutations in most studies with bacteria, which included various *Salmonella typhimurium* strains and *Escherichia coli* PQ 37 (SOS chromotest). Gene mutations were obtained with mouse lymphoma L5178Y cells in the presence of an exogenous metabolic system in one study. Sister chromatid exchanges were observed in rat erythroblastic leukaemia cells and human lymphocytes *in vitro* but not in a Chinese hamster cell line *in vitro*.

Chromosomal aberrations were observed *in vitro* except in two studies with Chinese hamster ovary cells. The single study on polyploidy induction in Chinese hamster lung fibroblasts reported no effect.

Table 1. Genetic and related effects of bromodichloromethane

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, *Escherichia coli* PQ 37, SOS chromotest	(+)	+	3	Le Curieux *et al*. (1995)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	NG	Simmon *et al*. (1977)[c,d]
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Mortelmans *et al*. (1986)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Khudoley *et al*. (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	5	Strobel & Grummt (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	170	Varma *et al*. (1988)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	NG	Mersch-Sunderman (1989)[e]
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	+	NG	Khudoley *et al*. (1989)[c]
SA0, *Salmonella typhimurium* TA100, reverse mutation, fluctuation test	–	–	3000	Le Curieux *et al*. (1995)
SA2, *Salmonella typhimurium* TA102, reverse mutation	–	–	NG	Mersch-Sunderman (1989)
SA4, *Salmonella typhimurium* TA104, reverse mutation	(+)	(+)	125	Strobel & Grummt (1987)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Mortelmans *et al*. (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	170	Varma *et al*. (1988)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)[f]	NT	110	Pegram *et al*. (1997)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Mortelmans *et al*. (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	+	–	130	Varma *et al*. (1988)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Mortelmans *et al*. (1986)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Khudoley *et al*. (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	(+)	500	Strobel & Grummt (1987)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	170	Varma *et al*. (1988)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	NG	Mersch-Sunderman (1989)[e]
SA9, *Salmonella typhimurium* TA98, reverse mutation	NT	+	NG	Khudoley *et al*. (1989)[c]
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	+	5	Strobel & Grummt (1987)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	+	NG	Mersch-Sunderman (1989)[e]

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SZG, *Saccharomyces cerevisiae* D7, gene conversion	(+)		10	Nestmann & Lee (1985)
SGR, *Saccharomyces cerevisiae* XV185-14C, reverse mutation	(+)		20	Nestmann & Lee (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	+	180	McGregor *et al*. (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	?	1000	Sofuni *et al*. (1996)
SIC, Sister chromatid exchange, Chinese hamster FAF cell line *in vitro*	–	NT	8	Strobel & Grummt (1987)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	5000	US National Toxicology Program (1987)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	?	5000	Anderson *et al*. (1990)
SIR, Sister chromatid exchange, rat erythroblastic leukaemia K_3D cells *in vitro*	+	+	33	Fujie *et al*. (1993)
CIC, Chromosomal aberrations, Chinese hamster FAF cell line *in vitro*	+	NT	8	Strobel & Grummt (1987)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	5000	US National Toxicology Program (1987)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	(+)	+	240	Ishidate (1987)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	5000	Anderson *et al*. (1995)
CIC, Chromosomal aberrations, Chinese hamster lung fibroblast (CHL/IU) cells *in vitro*	(+)	+	1250	Matsuoka *et al*. (1996)
AIA, Aneuploidy, Chinese hamster lung fibroblast (CHL/IU) cells, polyploidy *in vitro*	–	–	2500	Matsuoka *et al*. (1996)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	65	Morimoto & Koizumi (1983)
UPR, Unscheduled DNA synthesis, CD-1 rat hepatocytes *in vivo*	–		450 po × 1	Stocker *et al*. (1997)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SVA, Sister chromatid exchange, male ICR/SJ mouse bone-marrow cells *in vivo*	+		50 po × 4	Morimoto & Koizumi (1983)
MVM, Micronucleus test, ddY mouse bone-marrow cells *in vivo*	?		500 ip × 1	Hayashi *et al*. (1988)
MVM, Micronucleus test, ddY mouse bone-marrow cells *in vivo*	–		200 ip × 4	Hayashi *et al*. (1988)
Micronucleus test, *Pleurodeles waltl* larvae *in vivo*	(+)		50 feed	Le Curieux *et al*. (1995)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; po, oral; ip, intraperitoneal

[c] Closed container

[d] Closed container +, standard –

[e] Spot test +, standard –

[f] *Salmonella typhimurium* transfected with rat θ-class GST T1-1 positive at 4.6 µg/mL

A micronucleus induction study gave inconclusive or negative results in the bone-marrow cells of mice treated *in vivo* and weakly positive results in *Pleurodeles waltl*. Bromodichloromethane did not induce unscheduled DNA synthesis in rat hepatocytes *in vivo*, while sister chromatid exchanges were increased in the bone marow of mice dosed *in vivo*.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of bromodichloromethane were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of bromodichloromethane.

Overall evaluation

Bromodichloromethane is *possibly carcinogenic to humans (Group 2B).*

6. References

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BROMOETHANE

Data were last evaluated in IARC (1991).

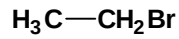
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 74-96-4
Systematic name: Bromoethane
Synonym: Ethyl bromide

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_2H_5Br Relative molecular mass: 108.97

1.1.3 *Physical properties* (for details, see IARC, 1991)

(*a*) *Boiling-point*: 38.4°C
(*b*) *Melting-point*: –118.6°C
(*c*) *Conversion factor*: $mg/m^3 = 4.46 \times ppm$

1.2 Production, use and human exposure

Bromoethane has limited commercial use, including that as an ethylating agent. It has been detected in ocean air as a result of its formation by marine algae (IARC, 1991).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Bromoethane was tested for carcinogenicity in a two-year study in male and female Fischer 344 rats and $B6C3F_1$ mice by inhalation. In male rats, there was a significant increase in the incidence of phaeochromocytomas, which was not dose-related. A marginal

increase in the incidence of glial tumours of the brain occurred in females. In mice, bromoethane induced neoplasms of the endometrium; a marginal increase in the incidence of lung tumours was observed in males. In a screening study by intraperitoneal injection, bromoethane did not increase the incidence of lung tumours in strain A mice (IARC, 1991).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Unchanged bromoethane accounted for approximately 70% of the dose in the expired air of rats dosed orally by gavage (IARC, 1991).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

In 14-week studies, male and female Fischer 344/N rats and B6C3F_1 mice were exposed to 100–1600 ppm [446–1780 mg/m^3] bromoethane by inhalation for 6 h per day on five days per week. In rats, tremors, paresis, mineralization and degeneration in the brain, atrophy of the testes, haemosiderosis of the spleen and some depletion of haematopoietic cells in the bone marrow were observed. Involution of the ovary was observed in mice in the 800 and 1600 ppm dose groups (IARC, 1991).

In response to the unusual observation of an increased incidence of uterine tumours in mice (see above), possible changes in blood concentrations of sex hormones were investigated. Female B6C3F_1 mice (11–12 weeks of age) were exposed by inhalation to 400 ppm [1780 mg/m^3] for 6 h per day for 21 days. No consistent changes were found in oestrous cyclicity or in serum concentrations of oestradiol and progesterone. Thus, none of the measured parameters emerged as a mechanistic factor that might contribute to the high incidence of endometrial tumours (Bucher *et al*., 1995).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

Bromoethane was mutagenic to bacteria, but not to *Drosophila melanogaster* in a single study. In another single study, bromoethane increased the incidence of sister chromatid exchanges, but not of chromosomal aberrations in cultured mammalian cells (IARC, 1991).

5. Evaluation

No epidemiological data relevant to the carcinogenicity of bromoethane were available.

There is *limited evidence* in experimental animals for the carcinogenicity of bromoethane.

Overall evaluation

Bromoethane is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

Bucher, J.R., Morgan, D.L., Adkins, B., Jr, Travlos, G.S., Davis, B.J., Morris, R. & Elwell, M.R. (1995) Early changes in sex hormones are not evident in mice exposed to the uterine carcinogens chloroethane or bromoethane. *Toxicol. appl. Pharmacol.*, **130**, 169–173

IARC (1991) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 52, *Chlorinated Drinking-water; Chlorination By-products; Some Other Halogenated Compounds; Cobalt and Cobalt Compounds*, Lyon, pp. 299–314

BROMOFORM

Data were last evaluated in IARC (1991).

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 75-25-2
Systematic name: Tribromomethane

1.1.2 *Structural and molecular formulae and relative molecular mass*

$CHBr_3$

$CHBr_3$ Relative molecular mass: 252.75

1.1.3 *Physical properties* (for details, see IARC, 1991)

(*a*) *Boiling-point*: 149.5°C
(*b*) *Melting-point*: 8.3°C
(*c*) *Conversion factor*: $mg/m^3 = 10.34 \times ppm$

1.2 Production, use and human exposure

Bromoform has a limited number of industrial uses. It is found in chlorinated drinking-water as a consequence of the reaction between chlorine, added during water treatment, and natural organic substances in the presence of bromide ion. It has also been detected in untreated water, but at lower levels. Bromoform is the major organohalide produced by chlorination of seawater during desalination. It is a major component of the organohalides produced by marine algae (IARC, 1991).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Bromoform was tested for carcinogenicity in two-year studies by oral gavage in male and female B6C3F$_1$ mice and Fischer 344 rats. It induced a low incidence of adenomatous polyps and adenocarcinomas of the large intestine in male and female rats. Bromoform did not increase the proportion of mice with tumours. In a screening study by intraperitoneal injection, there was no dose-related increase in the average number of lung tumours in strain A mice given bromoform (IARC, 1991).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Bromoform administered in corn oil orally by gavage to rats is rapidly absorbed and distributed to the liver, brain, kidney and adipose tissue. In the expired air of dosed rats, unchanged bromoform accounted for approximately 67% of the dose and CO_2 for 4% within 8 h. Only 2% appeared in urine and 2% was retained in tissues. In contrast, in mice that were dosed orally, unchanged bromoform in expired air accounted for about 6% of the dose and CO_2 for about 40% within 8 h. About 5% of the dose was excreted in urine and 12% was retained in tissues. Bromoform is metabolized to carbon monoxide and dibromocarbonyl, the bromine analogue of phosgene (IARC, 1991).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

The liver is the primary target organ in rats and mice for bromoform toxicity following oral gavage dosing; hepatic fatty changes were observed in both short-term studies and carcinogenicity studies. A single dose of bromoform did not increase the incidence of γ-glutamyltranspeptidase-positive foci in rat liver in a two-stage carcinogenicity model experiment (IARC, 1991). However, bromoform induces S-phase DNA synthesis in male mouse liver at 48 h following a single oral dose of 600 mg/kg bw (Mirsalis *et al*., 1989).

In mice, bromoform has been observed to produce tubular hyperplasia and glomerular degeneration after an oral dose of 289 mg/kg bw daily for 14 days (IARC, 1991). A single

intraperitoneal dose of 3 mmol/kg bw given to Sprague-Dawley rats has also been shown to produce renal dysfunction, characterized by a reduction in glomerular filtration rate, reduced renal concentrating ability and elevated blood urea nitrogen levels (Kroll *et al*., 1994).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

There was some evidence that bromomethane can cause developmental toxicity in the absence of maternal toxicity in orally dosed rats (IARC, 1991).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Bromoform induced DNA damage and mutation in bacteria, particularly if exposures were in closed containers. In single studies, it induced aneuploidy in fungi, sex-linked recessive lethal mutations, but not heritable translocations, in *Drosophila melanogaster*, and micronuclei in the peripheral erythrocytes of larvae of the newt, *Pleurodeles waltl*. Results were inconsistent from tests in mammalian cell lines for the induction of chromosomal aberrations and sister chromatid exchanges, while mutations were induced in a single study with mouse lymphoma L5178Y cells. Sister chromatid exchanges were induced in cultured human lymphocytes and in mice treated *in vivo*. Micronuclei were increased in one of three studies with mice treated *in vivo*. Unscheduled DNA synthesis was not induced in hepatocytes and binding to DNA was not observed in the liver and kidney of rats treated *in vivo*.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of bromoform were available.

There is *limited evidence* in experimental animals for the carcinogenicity of bromoform.

Overall evaluation

Bromoform is *not classifiable as to its carcinogenicity to humans (Group 3)*.

Table 1. Genetic and related effects of bromoform

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS chromotest, *Escherichia coli* PQ37	+	+	10	Le Curieux *et al.* (1995)
SAF, *Salmonella typhimurium* BA13/BAL13, forward mutation, arabinose resistance test (Ara test)	?	–	353	Roldán-Arjona & Pueyo (1993)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	NG	Simmon *et al.* (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	–	300	Haworth *et al*. (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1667	US National Toxicology Program (1989)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	NT	500	Rapson *et al.* (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	–	250	Varma *et al.* (1988)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Mersch-Sundermann (1989)[c]
SA0, *Salmonella typhimurium* TA100, reverse mutation (fluctuation test)	+	–	300	Le Curieux *et al.* (1995)
SA2, *Salmonella typhimurium* TA102, reverse mutation	–	–	NG	Mersch-Sundermann (1989)[c]
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	NG	Simmon *et al.* (1977)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	300	Haworth *et al*. (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1667	US National Toxicology Program (1989)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	NG	Varma *et al.* (1988)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	300	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1667	US National Toxicology Program (1989)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Varma *et al.* (1988)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	300	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	US National Toxicology Program (1989)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Varma *et al.* (1988)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	–	NG	Mersch-Sundermann (1989)[c]
SAS, *Salmonella typhimurium* TA97, reverse mutation	+	–	NG	Mersch-Sundermann (1989)[c]
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	1667	US National Toxicology Program (1989)
ANN, *Aspergillus nidulans*, aneuploidy	+	NT	870	Benigni *et al.* (1993)
ACC, *Allium cepa*, c-mitosis	+	NT	250	Östergren (1944)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		3000 feed	Woodruff *et al.* (1985)
DMH, *Drosophila melanogaster*, heritable translocations	–		3000 feed	Woodruff *et al.* (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	70	US National Toxicology Program (1989)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–[d]	–	968	Galloway *et al.* (1985)
SIR, Sister chromatid exchange, rat erythroblastic leukaemia K_3D cells *in vitro*	+	+	5.1	Fujie *et al.* (1993)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	(+)	+	116	Ishidate (1988)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–[d]	–	1600	Galloway *et al.* (1985)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	20	Morimoto & Koizumi (1983)
UPR, Unscheduled DNA synthesis, male Sprague-Dawley rat hepatocytes *in vivo*	–		1080 po × 1	Stocker *et al.* (1997)
SVA, Sister chromatid exchange, ICR/SJ mouse bone-marrow cells *in vivo*	+		25 po × 4	Morimoto & Koizumi (1983)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SVA, Sister chromatid exchange, $B6C3F_1$ mouse bone-marrow cells *in vivo*	+		800 ip × 1	US National Toxicology Program (1989)
Micronucleus test, *Pleurodeles waltl* erythrocytes *in vivo*	+		2.5	Le Curieux *et al.* (1995)
MVM, Micronucleus test, ddY mouse bone-marrow cells *in vivo*	–		1400 ip × 1	Hayashi *et al.* (1988)
MVM, Micronucleus test, $B6C3F_1$ mouse *in vivo*	+		800 ip × 2	US National Toxicology Program (1989)
MVM, Micronucleus test, Swiss CD-1 mouse bone-marrow cells *in vivo*	–		1000 po × 1	Stocker *et al.* (1997)
CBA, Chromosomal aberrations, $B6C3F_1$ mouse bone-marrow cells *in vivo*	–		800 ip × 1	US National Toxicology Program (1989)
BVD, DNA binding, Sprague-Dawley rat liver and kidney *in vivo*	–		380 po × 1	Pereira *et al.* (1982)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; NG, not given; po, oral; ip, intraperitoneal

[c] Standard assay, closed container or spot test

[d] Weak positive response in one or two laboratories at a single dose

6. References

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IARC (1991) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 52, *Chlorinated Drinking-Water; Chlorination By-Products; Some Other Halogenated Compounds; Cobalt and Cobalt Compounds*, Lyon, pp. 213–242

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β-BUTYROLACTONE

Data were last reviewed in IARC (1976) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

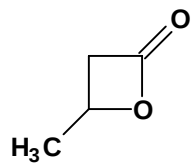
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 3068-88-0

Systematic name: 4-Methyl-2-oxetanone

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_4H_6O_2$ Relative molecular mass: 86.1

1.1.3 *Physical properties* (for details, see IARC, 1976)

(*a*) *Boiling-point*: 54–56°C at 1.3 kPa; 110–118°C at 24 kPa

(*b*) *Conversion factor*: $mg/m^3 = 3.52 \times ppm$

1.2 Production and use

β-Butyrolactone has been used in the preparation of β-oxybutyryl-*para*-phenetidine (IARC, 1976).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

β-Butyrolactone was tested for carcinogenicity in mice by skin application and by subcutaneous injection and in rats by oral administration and by subcutaneous injection. It produced tumours at the site of administration in both species (IARC, 1976).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

No data were available to the Working Group.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of β-butyrolactone were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of β-butyrolactone.

Overall evaluation

β-Butyrolactone is *possibly carcinogenic to humans (Group 2B).*

6. References

IARC (1976) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 11, *Cadmium, Nickel, Some Epoxides, Miscellaneous Industrial Chemicals and General Considerations on Volatile Anaesthetics*, Lyon, pp. 225–229

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs Volumes 1 to 42, Lyon, p. 59

CARBAZOLE

Data were last reviewed in IARC (1983) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

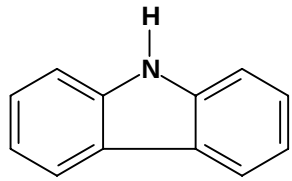
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 86-74-8

Systematic name: 9H-Carbazole

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{12}H_9N$ Relative molecular mass: 167.2

1.1.3 *Physical properties* (for details, see IARC, 1983)

(*a*) *Boiling-point*: 355°C

(*b*) *Melting-point*: 247–248°C

(*c*) *Conversion factor*: $mg/m^3 = 6.84 \times ppm$

1.2 Production, use and human exposure

Carbazole occurs in the products of incomplete combustion of nitrogen-containing organic matter, e.g., tobacco. It in an important dye intermediate (IARC, 1983).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Carbazole was tested for carcinogenicity in mice by administration in the diet, by skin application and by subcutaneous injection. In the study by oral administration, a dose-dependent increase in the incidence of liver neoplastic nodules and hepatocellular carcinomas was observed. Papillomas and carcinomas of the forestomach occurred in animals that received the highest dose. The other studies in mice were considered inadequate for evaluation (IARC, 1983).

3.1 Intraperitoneal administration

Mouse: CD-1 mouse pups born to untreated dams were administered intraperitoneal doses of 5, 10 and 20 µL of either dimethyl sulfoxide (DMSO) or a 50 mM solution of carbazole in DMSO on days 1, 8 and 15 of postnatal life, respectively. The total dose of carbazole was 1.75 µmol per mouse. The effective numbers of mice (i.e., those alive at two months of age) were: DMSO control, 46 females, 38 males; carbazole-treated, 42 females, 34 males. All of the mice were killed at 52 weeks and examined for gross lesions. The liver, lungs and any gross lesions in other tissues were examined histologically. No increase in neoplasms was found (Weyand *et al*., 1993). [The Working Group noted the limited exposure.]

3.2 Administration with known carcinogens

3.2.1 *Rat*

In a screening assay for promoters of urinary bladder carcinogenesis, groups of male Fischer 344 rats, six weeks of age, were given drinking-water either alone or containing 0.05% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) for two weeks. Each of these groups was then subdivided into two groups, which received basal diet either alone or containing 0.6% carbazole for 22 weeks. On day 22 of the experiment, the left ureter of all rats was ligated. The rats were killed at week 24 and autopsied. The urinary bladder, kidneys, ureter and liver were examined histologically. No neoplasia or hyperplasia was observed in liver, kidneys or ureter. The proportions of the groups with papillary or nodular hyperplasia or with papilloma of the urinary bladder, respectively, were: BBN alone, 3/44 (7%) and 0/44; BBN and carbazole diet, 5/14 (36%) ($p < 0.05$) and 2/14 (14%); carbazole diet alone, 0/15 and 0/15 (Miyata *et al*., 1985).

Male Fischer 344 rats, six weeks of age, were given drinking-water either alone or containing 0.2% *N*-bis(2-hydroxypropyl)nitrosamine (DHPN) for one week. One week later, each of these groups was subdivided into two groups, which received basal diet either alone or containing 0.6% carbazole for 50 weeks. All rats were killed at the end of week 52 and autopsied. The urinary bladder, kidneys, liver, thyroid and lungs and any organ with gross abnormalities were examined histologically. The proportions of tumour-bearing rats and the multiplicity of certain tumours are shown in Table 1. Carbazole showed no promoting effect in liver, lung or thyroid and did not induce tumours in the

Table 1. Incidence and multiplicity of certain tumours in rats treated with carbazole in an initiation-promotion model

Treatment		DHPN	DHPN–Carbazole	Carbazole
Number of rats		20	19	20
Lung	Adenoma, no. (%)	18 (90)	17 (89)	Not
	No. per rat	2.25 ± 1.41	2.05 ± 1.18	recorded
	Carcinoma, no. (%)	16 (80)	11 (58)	
	No. per rat	1.60 ± 1.29	1.00 ± 0.88	
Thyroid	Adenoma, no. (%)	7 (35)	8 (42)	Not
	Carcinoma, no. (%)	14 (70)	15 (79)	recorded
	No. per rat (adenoma + carcinoma)	1.56 ± 1.31	1.67 ± 0.79	
Kidney	Renal cell, no. (%)	6 (30)	4 (21)	Not
	Nephroblastoma, no. (%)	2 (10)	4 (21)	recorded
	Pelvic papilloma, no. (%)	0	5 (26) ($p < 0.05$)	
	Pelvic carcinoma, no. (%)	4 (21)	7 (37)	
	Pelvic papilloma + carcinoma, no. (%)	4 (21)	11 (58) ($p < 0.05$)	
Bladder	Papilloma (%)	2 (11)	4 (21)	Not
	Carcinoma (%)	1 (5)	3 (16)	recorded
	Papilloma + carcinoma (%)	3 (15)	7 (37)	
Liver	Foci (%)	5 (25)	11 (58)	9 (45)
	Hyperplastic nodules (%)	1 (5)	0	1 (5)

From Shirai *et al*. (1988)

lung. It increased the incidence of transitional-cell tumours in the renal pelvis significantly and the incidence of urinary bladder tumours slightly, but not significantly (Shirai *et al*., 1988).

Male Fischer 344 rats [age unspecified] were given a single intraperitoneal injection of 200 mg/kg bw *N*-nitrosodiethylamine and then, two weeks later, they were divided into three equal groups and maintained for six weeks on basal diet, either alone (one group) or containing 33 ppm (mg/kg) carbazole (two groups). One week into this period, all rats were subjected to partial hepatectomy. All rats were killed eight weeks from the beginning of the experiment and their livers were assessed for development of glutathione-*S*-transferase placental form (GST-P)-positive foci by immunohistochemical staining and image analysis. There was no difference among the *N*-nitrosodiethylamine-treated rats in the area of liver foci between the groups on control and carbazole-containing diet (Hasegawa *et al*., 1989).

3.2.1 *Hamster*

A group of 40 Syrian golden hamsters [sex unspecified], six weeks of age, was given a single intraperitoneal injection of 20 mg/kg bw 2,2′-dioxo-*N*-nitrosodipropylamine

(DOPN), while another group of 80 animals was left untreated. Beginning one week later, half of each group continued to receive basal diet alone, while the other half received basal diet containing 0.2% carbazole until they were killed at week 40. The numbers of GST-P-positive foci, expressed as foci/cm^2, were: basal diet, 0; carbazole diet, 3.6 ± 1.3 ($p < 0.001$); DOPN + basal diet, 9.2 ± 4.1; DOPN + carbazole diet, 19.0 ± 7.6 ($p < 0.001$) (Moore *et al*., 1987).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

3-Hydroxycarbazole has been reported to be a urinary metabolite of carbazole in rats and rabbits (IARC, 1983).

4.2 Toxic effects

No data were available to the Working Group.

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

Carbazole was not mutagenic to *Salmonella typhimurium* (IARC, 1983).

5. Evaluation

No epidemiological data relevant to the carcinogenicity of carbazole were available.

There is *limited evidence* in experimental animals for the carcinogenicity of carbazole.

Overall evaluation

Carbazole is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

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Miyata, Y., Fukushima, S., Hirose, M., Masui, T. & Ito, N. (1985) Short-term screening of promoters of bladder carcinogenesis in *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine-initiated, unilaterally ureter-ligated rats. *Jpn. J. Cancer Res.,* **76,** 828–834

Moore, M.A., Tsuda, H., Thamavit, W., Masui, T. & Ito, N. (1987) Differential modification of development of preneoplastic lesions in the Syrian golden hamster initiated with a single dose of 2,2′-dioxo-*N*-nitrosodipropylamine: influence of subsequent butylated hydroxyanisole, alpha-tocopherol, or carbazole. *J. natl Cancer Inst.,* **78,** 289–293

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Weyand, E.H., Defauw, J., McQueen, C.A., Meschter, C.L., Meegalla, S.K. & LaVoie, E.J. (1993) Bioassay of quinoline, 5-fluoroquinoline, carbazole, 9-methylcarbazole and 9-ethylcarbazole in newborn mice. *Food chem. Toxicol.,* **31,** 707–715

CHLOROACETONITRILE

Data were last evaluated in IARC (1991)

1. Exposure Data

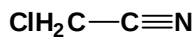
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 107-14-2

Systematic name: Chloroacetonitrile

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_2H_2ClN Relative molecular mass: 75.50

1.1.3 *Physical properties* (for details, see IARC, 1991)

(*a*) *Boiling-point*: 126–127°C

(*b*) *Conversion factor*: $mg/m^3 = 3.09 \times ppm$

1.2 Production, use and human exposure

Halogenated acetonitriles are not produced on an industrial scale. Chloroacetonitrile has been used on a limited basis in the past as a pesticide. Several halogenated acetonitriles have been detected in chlorinated drinking-water in a number of countries as a consequence of the reaction of chlorine with natural organic substances present in untreated water. The only known route of human exposure is through chlorinated drinking-water (IARC, 1991).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Chloroacetonitrile was tested in a limited carcinogenicity study in female Sencar mice by skin application, in an initiation/promotion study in female Sencar mice by skin

application and in a screening assay for lung tumours in female strain A mice by oral administration. No skin tumour was produced after skin application in mice or in the initiation/promotion study, in which chloroacetonitrile was applied topically as six equal doses over a two-week period, followed by repeated doses of 12-*O*-tetradecanoylphorbol 13-acetate for 20 weeks. After oral administration, a small, significant increase in the proportion of mice with lung tumours and number of tumours per mouse was observed: control, 3/31 and 0.1; treated group (10 mg/kg bw, three times per week, eight weeks), 9/28 and 0.43 ($p < 0.05$) (IARC, 1991).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Approximately 14% of a single oral dose to rats of 57 mg/kg bw of chloroacetonitrile was excreted in urine within 24 h as thiocyanate, the product of released cyanide metabolized by rhodanese (IARC, 1991).

Male Sprague-Dawley rats given intravenous injections of [2-^{14}C]chloroacetonitrile excreted in urine, faeces and expired air as $^{14}CO_2$, respectively, 51%, 2.7% and 12% of the dose in 12 h. Only 0.8% of the dose was exhaled as unchanged chloroacetonitrile. Computer-assisted image analysis of whole-body autoradiographs at various times up to 48 h indicated high, persistent levels of radioactivity in the thyroid, gastrointestinal tract, testes, brain and eye. Metabolic pathways were not studied in detail, but only 11% of the dose was excreted as CO_2 and no chloroacetonitrile was detected in the urine (Ahmed *et al*., 1991). In-vivo and in-vitro studies indicate that chloroacetonitrile reacts extensively with glutathione and causes significant decreases in glutathione levels in treated rats (Ahmed & Hussein, 1987; Lin & Guion, 1989).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Chloroacetonitrile did not induce γ-glutamyltranspeptidase-positive foci in rat liver (IARC, 1991).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

There were slight decreases in maternal weight gain during the treatment period and in the birth weights of the pups born to rats given chloroacetonitrile orally at a dose of 55 mg/kg bw daily on gestation days 7–21 (IARC, 1991).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Chloroacetonitrile did not induce DNA damage or mutation in bacteria, whereas it induced sister chromatid exchanges and, weakly, DNA strand breaks in mammalian cell lines. Micronuclei were induced in the erythrocytes of newt (*Pleurodeles waltl*) larvae exposed for 12 days, but in mice dosed for five days, neither micronuclei in bone marrow nor abnormal sperm morphology were induced.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of chloroacetonitrile were available.

There is *inadequate evidence* in experimental animals for the carcinogenicity of chloroacetonitrile.

Overall evaluation

Chloroacetonitrile is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

Ahmed, A.E. & Hussein, G.I. (1987) Studies on the mechanism of haloacetonitriles acute toxicity. Interactions of dibromoacetonitrile (DBAN) with glutathione (GSH) and glutathione-*S*-transferase (GSHT) in rats (Abstract). *Toxicologist*, **7**, 452

Ahmed, A.E., Jacob, S. & Loh, J.P. (1991) Studies on the mechanism of haloacetonitriles toxicity: quantitative whole body autoradiographic distribution of [2-^{14}C]chloroacetonitrile in rats. *Toxicology,* **67,** 279–302

Bull, R.J., Meier, J.R., Robinson, M., Ringhand, H.P., Laurie, R.D. & Stober, J.A. (1985) Evaluation of mutagenic and carcinogenic properties of brominated and chlorinated acetonitriles: by-products of chlorination. *Fundam. appl. Toxicol.,* **5,** 1065–1074

Table 1. Genetic and related effects of chloroacetonitrile

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS chromotest, *Escherichia coli* PQ37	–	–	1000	Le Curieux *et al.* (1995)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1500	Bull *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation (fluctuation test)	–	+	30	Le Curieux *et al.* (1995)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1500	Bull *et al.* (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Bull *et al.* (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	NG	Bull *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1500	Bull *et al.* (1985)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	4	Bull *et al.* (1985)
DIH, DNA strand breaks, human lymphoblastic cell line *in vitro*	(+)	NT	NG	Daniel *et al.* (1986)
Micronucleus test, *Pleurodeles waltl* erythrocytes *in vivo*	+		1.25	Le Curieux *et al.* (1995)
MVM, Micronucleus test, CD-1 mouse bone-marrow cells *in vivo*	–		50 po × 5	Bull *et al.* (1985)
SPM, Sperm morphology, B6C3F$_1$ mice *in vivo*	–		50 po × 5	Meier *et al.* (1985)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; po, oral

Daniel, F.B., Schenck, K.M., Mattox, J.K., Lin, E.L., Haas, D.L. & Pereira, M.A. (1986) Genotoxic properties of haloacetonitriles: drinking water by-products of chlorine disinfection. *Fundam. appl. Toxicol.,* **6,** 447–453

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Le Curieux, F., Giller, S., Gauthier, L., Erb, F. & Marzin, D. (1995) Study of the genotoxic activity of six halogenated acetonitriles, using the SOS chromotest, the Ames-fluctuation test and the newt micronucleus test. *Mutat. Res.*, **341**, 289–302

Lin, E.L.C. & Guion, C.W. (1989) Interactions of haloacetonitriles with GSH and GST. *Biochem. Pharmacol.*, **38,** 685–686

Meier, J.R., Bull, R.J., Stober, J.A. & Cimino, M.C. (1985) Evaluation of chemicals used for drinking water disinfection for production of chromosomal damage and sperm-head abnormalities in mice. *Environ. Mutag.,* **7,** 201–211

CHLORODIBROMOMETHANE

Data were last evaluated in IARC (1991).

1. Exposure Data

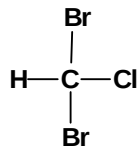
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 124-48-1

Systematic name: Chlorodibromomethane

1.1.2 *Structural and molecular formulae and relative molecular mass*



$CHBr_2Cl$ Relative molecular mass: 208.29

1.1.3 *Physical properties* (for details, see IARC, 1991)

(*a*) *Boiling-point*: 119–200°C at 99 kPa

(*b*) *Melting-point*: < –20°C

(*c*) *Conversion factor*: $mg/m^3 = 8.52 \times ppm$

1.2 Production, use and human exposure

Chlorodibromomethane has limited commercial use but is used industrially as a chemical intermediate. It is found in chlorinated drinking-water as a consequence of the reaction between chlorine, added during drinking-water treatment, and natural organic substances in the presence of bromide. The major route of human exposure is via drinking-water. Chlorodibromomethane is not normally present in untreated water. It is a major component of organohalide emissions from marine algae (IARC, 1991).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Chlorodibromomethane was tested for carcinogenicity in two-year studies by oral gavage in male and female B6C3F_1 mice and Fischer 344 rats and in a lifetime study in CBA × C57BL/6 hybrid mice by administration in the drinking-water. In B6C3F_1 mice, it produced a significant increase in the incidence of hepatocellular neoplasms in females and a marginal increase in males. Chlorodibromomethane did not increase the proportion of rats with tumours at any site relative to that in controls. There was no increase in tumour incidence in CBA × C57BL/6 hybrid mice given chlorodibromomethane in the drinking-water (IARC, 1991).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Chlorodibromomethane administered in corn oil orally by gavage to rats was eliminated in the expired air as unchanged chlorodibromomethane (approx. 48% of the dose) and CO_2 (18%) within 8 h. Only 1% appeared in urine and about 1% was retained in tissues. In contrast, after oral dosing to mice, unchanged chlorodibromomethane in expired air accounted for approximately 12% of the dose and CO_2 for approximately 72% within 8 h. About 2% of the dose was excreted in urine and 5% was retained in tissues (IARC, 1991).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Liver and kidney are target organs in rats and mice for chlorodibromomethane toxicity following oral gavage dosing. In a 13-week study, male and female Fischer 344/N rats and B6C3F_1 mice were administered 15–250 mg/kg bw chlorodibromomethane by gavage on five days per week. The highest dose was lethal for 9/10 male and 9/10 female rats and resulted in fatty changes and centrilobular necrosis in the liver and proximal tubule-cell degeneration and regeneration in the kidney. Inflammation and squamous metaplasia were observed in the salivary glands. Mice were less sensitive. Fatty changes of the liver and

tubule degeneration of the kidney were observed in 5/10 of the males but not in the females of the highest dose group. In the subsequent carcinogenicity study in which the doses used were 40 or 80 mg/kg bw for rats and 50 or 100 mg/ kg bw for mice, fatty changes occurred in the liver of all dose groups, whereas cytomegaly and necrosis of the liver were observed only in the high-dose male mice. Nephrosis was observed in female rats and male mice and follicular-cell hyperplasia of the thyroid gland was observed in female mice in all dose groups (IARC, 1991).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Chlorodibromomethane can cause maternal toxicity in the absence of fetal or embryo toxicity in orally dosed rats. No teratogenic effects have been observed (IARC, 1991).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Chlorodibromomethane induced DNA damage and mutation in bacteria. In single studies, it induced gene conversion and aneuploidy, but not mutation, in fungi. It induced chromosomal aberrations, sister chromatid exchanges and mutations in mammalian cell lines and sister chromatid exchanges in cultured human lymphocytes. Sister chromatid exchanges, but not micronuclei, were increased in mice treated *in vivo*. Unscheduled DNA synthesis was not induced in hepatocytes from rats treated *in vivo*.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of chlorodibromomethane were available.

There is *limited evidence* in experimental animals for the carcinogenicity of chlorodibromomethane.

Overall evaluation

Chlorodibromomethane is *not classifiable as to its carcinogenicity to humans (Group 3)*.

Table 1. Genetic and related effects of chlorodibromomethane

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS chromotest, *Escherichia coli* PQ37	+	+	10	Le Curieux *et al.* (1995)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	NG	Simmon *et al.* (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	US National Toxicology Program (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Khudoley *et al.* (1987)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	8	Varma *et al.* (1988)
SA0, *Salmonella typhimurium* TA100, reverse mutation	NT	+	NG	Khudoley *et al.* (1989)[c]
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Mersch-Sundermann (1989)[d]
SA0, *Salmonella typhimurium* TA100, reverse mutation (fluctuation test)	–	–	3000	Le Curieux *et al.* (1995)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	US National Toxicology Program (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	8	Varma *et al.* (1988)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1667	US National Toxicology Program (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	+	+	8	Varma *et al.* (1988)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1667	US National Toxicology Program (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	8	Varma *et al.* (1988)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Khudoley *et al.* (1989)
SA9, *Salmonella typhimurium* TA98, reverse mutation	NT	+	NG	Khudoley *et al.* (1989)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Mersch-Sundermann (1989)[d]
SA2, *Salmonella typhimurium* TA102, reverse mutation	–	–	NG	Mersch-Sundermann (1989)[d]

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	NG	Mersch-Sundermann (1989)[d]
SCG, *Saccharomyces cerevisiae* D7, gene conversion	+	(+)	245	Nestmann & Lee (1985)
SCR, *Saccharomyces cerevisiae* XVI85-14C, reverse mutation	–	–	1225	Nestmann & Lee (1985)
ANN, *Aspergillus nidulans*, aneuploidy	+	NT	360	Benigni *et al.* (1993)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–[e]		2000 ppm inj.	Foureman *et al.* (1994)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	100	McGregor *et al.* (1991)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	(+)[f]	+[f]	25	Sofuni *et al.* (1996)
SIR, Sister chromatid exchange, rat erythroblastic leukaemia K_3D cells *in vitro*	+	+	420	Fujie *et al.* (1993)
CIC, Chromosomal aberrations, Chinese hamster lung (CHL)cells *in vitro*	–	+	100	Ishidate (1988)
CIC, Chromosomal aberrations, Chinese hamster lung fibroblast (CHL/IU) cells *in vitro*	–	(+)	1680	Matsuoka *et al.* (1996)
AIA, Aneuploidy, Chinese hamster lung fibroblast (CHL/IU) cells, polyploidy *in vitro*	+	–	720	Matsuoka *et al.* (1996)
SHL, Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	80	Morimoto & Koizumi (1983)
UPR, Unscheduled DNA synthesis, male Sprague-Dawley rat hepatocytes *in vivo*	–		2000 po × 1	Stocker *et al.* (1997)
SVA, Sister chromatid exchange, ICR/SJ mouse bone-marrow cells *in vivo*	+		25 po × 4	Morimoto & Koizumi (1983)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus test, *Pleurodeles waltl* erythrocytes *in vivo*	–		50	Le Curieux *et al.* (1995)
MVM, Micronucleus test, ddY mouse bone-marrow cells *in vivo*	–		1000 ip × 1	Hayashi *et al.* (1988)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; inj., injection; po, oral; ip, intraperitoneal
[c] Closed container
[d] Standard assay, closed container or spot test
[e] Sex-linked recessive lethal mutations also negative with 1500 ppm chlorodibromomethane in the diet
[f] One of two laboratories reported positive results

6. References

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CHLORODIFLUOROMETHANE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987a).

1. Exposure Data

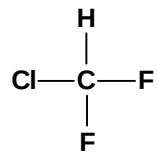
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 75-45-6

Systematic name: Chlorodifluoromethane

1.1.2 *Structural and molecular formulae and relative molecular mass*



$CHClF_2$ Relative molecular mass: 86.47

1.1.3 *Physical properties* (for details, see IARC, 1986)

(*a*) *Boiling-point*: –40.8°C

(*b*) *Melting-point*: –146°C

(*c*) *Conversion factor*: $mg/m^3 = 3.54 \times ppm$

1.2 Production, use and human exposure

Chlorodifluoromethane is produced extensively for use in refrigeration and air conditioning; significant quantities are subsequently released into the atmosphere, resulting in widespread, low-level human exposure. Occupational exposure to chlorodifluoromethane occurs during its production and use (IARC, 1986).

2. Studies of Cancer in Humans

A small study of workers exposed to a mixture of chlorofluorocarbons, including chlorodifluoromethane, was uninformative with regard to the carcinogenic hazard of this chemical due to small numbers of individuals studied (IARC, 1986, 1987a).

3. Studies of Cancer in Experimental Animals

Chlorodifluoromethane was tested for carcinogenicity in one experiment in rats by oral administration and in experiments in rats and mice by inhalation exposure. No increase in tumour incidence was observed in rats after oral administration. The inhalation study in mice was inconclusive for males, and negative results were obtained for females. In the inhalation study in rats, males receiving the high dose had increased incidences of fibrosarcomas and Zymbal gland tumours; negative results were obtained for female rats (IARC, 1986).

3.1 Inhalation exposure

3.1.1 *Mouse*

Groups of 60 male and 60 female Swiss mice, nine weeks of age, were exposed by inhalation to 0, 1000 or 5000 ppm [0, 3540 or 17 700 mg/m^3] chlorodifluoromethane (FC 22; purity, 99.98%) for 4 h per day on five days per week for 78 weeks. The animals were kept under observation until spontaneous death [survival unspecified]. Full necropsy was performed on all animals. No effects were found on survival or body weight. No difference related to treatment was found in the incidence of benign or malignant tumours (Maltoni *et al*., 1988).

3.1.2 *Rat*

Groups of 60 male and 60 female Sprague-Dawley rats, eight weeks of age, were exposed by inhalation to 0, 1000 or 5000 ppm [0, 3540 or 17 700 mg/m^3] chlorodifluoromethane (FC 22; purity, 99.98%) for 4 h per day on five days per week for 104 weeks. Full necropsy was performed on all animals. No effects were found on survival or body weight. No difference related to treatment was found in the incidence of benign or malignant tumours (Maltoni *et al*., 1988).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Two groups of three male volunteers were exposed by inhalation to chlorodifluoromethane at either 327 or 1833 mg/m^3 for 4 h. The average maximal blood concentrations were 0.25 and 1.36 μg/mL, respectively, and were achieved within 1 h of the beginning of exposure. The average blood/air partition coefficients for chlorodifluoromethane towards the end of the exposure period were 0.82 and 0.76, respectively, and the fat/air partition coefficients were 7.7 and 8.1. Thus, the fat/blood partition coefficient was estimated to be

approximately 10. Three phases for the elimination of chlorodifluoromethane in breath were identified, with estimated half-lives of 0.005, 0.2 and 2.6 h. An average of 2.1% of the inhaled chlorodifluoromethane was recovered from the exhaled air over 26 h and minimal amounts were found in urine. Minimal changes in the fluoride concentration in urine were observed, which is consistent with a low degree of metabolism (Woollen *et al*., 1992).

Two simultaneous accidental, lethal exposures to chlorodifluoromethane alone were associated with concentrations (in μL/mL) of the chemical in body fluid samples taken 16 h after death of: blood, 37.1 and 26.0; urine, 1.7 and 0.9; vitreous humor, 1.0 and 0.7 (Kintz *et al*., 1996).

4.1.2 *Experimental systems*

The metabolism of chlorodifluoromethane has been briefly reviewed (Anders, 1991).

Chlorodifluoromethane is very rapidly cleared from the blood of rats and rabbits exposed by inhalation. Little distribution to tissues or metabolism occurs in rats, with recoveries from expired air as CO_2 in 15–24 h and in the urine, being about 0.1% and 0.02% of the dose, respectively (IARC, 1986).

Chlorodifluoromethane inhaled by male Wistar rats at a concentration of 160 ppm [566 mg/m^3] underwent no detectable metabolism and prior treatment of the rats with either DDT or phenobarbital did not stimulate its metabolic transformation (Peter *et al*., 1986).

4.2 Toxic effects

4.2.1 *Humans*

Although chlorodifluoromethane has low toxicity, it is capable of producing rapid death in people abusing it for euphoric effects (Fitzgerald *et al*., 1993) or in situations of accidental high exposure in occupational circumstances (Kintz *et al*., 1996). Cardiac arrhythmias have been studied in refrigerator repair men who were exposed to chlorodifluoromethane during their work and a comparison group of plumbers. Peak concentrations of chlorodifluoromethane measured during the repair work were 1300–10 000 cm^3/m^3 for times of 2–35 min. No clear connection between exposure and cardiac arrhythmia was found, although one subject had several ventricular ectopic beats that may have been connected with exposure (Antti-Poika *et al*., 1990). There appear to have been no reports of Freons including chlorodifluoromethane causing secondary arterial hypertension prior to two case reports where one of the subjects received an acute, massive, accidental exposure to a mixture of chlorodifluoromethane and dichlorodifluoromethane (Voge, 1996).

4.2.2 *Experimental systems*

Chlorodifluoromethane has low acute toxicity; concentrations of 20% were not lethal to rodents, rabbits or dogs. Exposure to high concentrations causes central nervous

system and myocardial depression. No effect was observed on mortality, haematology or biochemistry in mice or rats exposed to 1000–50 000 ppm [3540–177 000 mg/m^3] for 5 h per day on five days per week for up to 94 weeks (mice) or 131 weeks (rats) (IARC, 1986).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Chlorodifluoromethane causes malformations of the eyes of fetal rats, but has no reproductive effect in male rats and does not cause prenatal toxicity in rabbits following exposure by inhalation (IARC, 1986).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

Chlorodifluoromethane is mutagenic to *Salmonella typhimurium* but it did not induce either mutation or gene conversion in *Saccharomyces cerevisiae*. Chlorodifluoromethane did not induce mutations at the *hprt* locus or unscheduled DNA synthesis in mammalian cell lines in the presence or absence of an exogenous metabolic activation system. *In vivo*, it did not induce chromosomal aberrations in bone-marrow cells or dominant lethal effects (IARC, 1987b). These conclusions are supported by a more recent review (WHO, 1991).

5. Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of chlorodifluoromethane.

There is *limited evidence* in experimental animals for the carcinogenicity of chlorodifluoromethane.

Overall evaluation

Chlorodifluoromethane is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

Anders, M.W. (1991) Metabolism and toxicity of hydrochlorofluorocarbons: current knowledge and needs for the future. *Environ. Health Perspect.*, **96**, 185–191

Antti-Poika, M., Heikkilä, J. & Saarinen, L. (1990) Cardiac arrhythmias during occupational exposure to fluorinated hydrocarbons. *Br. J. ind. Med.*, **47**, 138–140

Fitzgerald, R.L., Fishel, C.E. & Bush, L.L.E. (1993) Fatality due to recreational use of chlorodifluoromethane and chloropentafluoroethane. *J. forens. Sci.*, **38**, 477–483

IARC (1986) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 41, *Some Halogenated Hydrocarbons and Pesticide Exposures*, Lyon, pp. 237–252

IARC (1987a) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, pp. 149–150

IARC (1987b) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 6, *Genetic and Related Effects: An Updating of Selected* IARC Monographs *from Volumes 1 to 42*, Lyon, pp. 150–151

Kintz, P., Baccino, E., Tracqui, A. & Mangin, P. (1996) Headspace GC/MS testing for chlorodifluoromethane in two fatal cases. *Forensic Sci. int.*, **82**, 171–175

Maltoni, C., Lefemine, G., Tovoli, D. & Perino, G. (1988) Long-term carcinogenicity bioassays on three chlorofluorocarbons (trichlorofluoromethane, dichlorodifluoromethane, chlorodifluoromethane) administered by inhalation to Sprague-Dawley rats and Swiss mice. *Ann. N.Y. Acad. Sci.*, **534**, 261–283

Peter, H., Filser, J.G., von Szentpály, L. & Wiegand, H.J. (1986) Different pharmacokinetics of dichlorofluoromethane (CFC 21) and chlorodifluoromethane (CFC 22) (Letter to the Editor). *Arch. Toxicol.*, **58**, 282–283

Voge, V.M. (1996) Secondary arterial hypertension linked to Freon exposure. *South. med. J.*, **89**, 516–518

WHO (1991) *Partially Halogenated Chlorofluorocarbons (Methane Derivatives)* (Environmental Health Criteria No. 126), International Programme on Chemical Safety, Geneva

Woollen, B.H., Marsh, J.R., Mahler, J.D., Auton, T.R., Makepeace, D., Cocker, J. & Blain, P.G. (1992) Human inhalation pharmacokinetics of chlorodifluoromethane (HCFC22). *Int. Arch. occup. environ. Health*, **64**, 383–387

CHLOROETHANE

Data were last evaluated in IARC (1991).

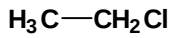
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 75-00-3
Systematic name: Chloroethane
Synonym: Ethyl chloride

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_2H_5Cl Relative molecular mass: 64.52

1.1.3 *Physical properties* (for details, see IARC, 1991)

(*a*) *Melting-point*: –136.4°C
(*b*) *Boiling-point*: 12.3°C
(*c*) *Conversion factor*: $mg/m^3 = 2.64 \times ppm$

1.2 Production, use and human exposure

Chloroethane is produced by the hydrochlorination of ethylene. It is used in the manufacture of tetraethyllead, as an industrial ethylating agent, as a blowing agent in the production of polystyrene foam and as a local anaesthetic. Occupational exposure occurs during the production of tetraethyllead, and industrial emissions have led to detectable levels of chloroethane in ambient air (IARC, 1991).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Chloroethane was tested for carcinogenicity in a two-year study in male and female Fischer 344 rats and B6C3F$_1$ mice by inhalation at a single concentration of 15 000 ppm [39 600 mg/m^3]. It induced uterine carcinomas in mice; marginal increases occurred in the incidence of hepatocellular tumours in female mice and in the incidence of alveolar/bronchiolar tumours in male mice. There was a marginal increase in the incidence of skin tumours in male rats, and a few uncommon glial cell tumours occurred in female rats (IARC, 1991).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Human volunteers exhaled approximately 30% of an inhaled dose within 1 h (IARC, 1991).

4.1.2 *Experimental systems*

There was little dechlorination (< 0.5%) of chloroethane when it was incubated with rat hepatic microsomes and NADPH (IARC, 1991).

Cytochrome P450-dependent metabolism was examined in microsomal preparations from male and female Fischer 344 rats and B6C3F$_1$ mice exposed to 15 000 ppm [39 600 mg/m^3] chloroethane for 6 h per day for five days. Chloroethane is oxidatively dechlorinated in an NADPH- and oxygen-dependent reaction, yielding acetaldehyde. The involvement of CYP2E1 is indicated by its inhibition by 3-amino-1,2,4-triazole. This activity is inducible by chloroethane itself in mice and female rats and correlates with increased *para*-nitrophenol hydroxylation, an indicator of CYP2E1 metabolism (Fedtke *et al*., 1994a). Chloroethane is also conjugated with glutathione in hepatic cytosolic preparations and generally to a higher extent in mouse than in rat. Glutathione was depleted in the lungs and uterus of both species after exposure, but not in the liver and kidneys. The initial conjugate *S*-ethylglutathione was excreted as the mercapturic acid, *S*-ethyl-*N*-acetyl-L-cysteine, in the urine of both species. *S*-Ethyl-L-cysteine was also excreted in the urine of mice, but not rats. The combined quantities of these metabolites excreted in five days were up to five-fold higher for mice than for rats. Excretion of *S*-ethyl-*N*-acetyl-L-cysteine occurred mainly during the exposure period for mice, but after the exposure period for rats (Fedtke *et al*., 1994b).

4.2 Toxic effects

4.2.1 *Humans*

Allergic sensitization to chloroethane can occur as a consequence of its use as a local anaesthetic in medical practice (Aberer & Zonzits, 1989; Bircher *et al*., 1994). Chloro-

ethane has also been observed to produce severe neurological impairment, including hallucinations and ataxia after direct inhalation two to three times per week over a four-month period of abuse of this specific solvent by the patient (Soult & Walker, 1993).

4.2.2 *Experimental systems*

In 13-week studies, male and female Fischer 344 rats and $B6C3F_1$ mice were exposed to 2500–19 000 ppm [6600–50 200 mg/m^3] chloroethane for 6 h per day on five days per week. In rats and mice, no adverse effects except for reduced body weight gain were observed. Increases in liver weight were observed in male rats and female mice exposed to 19 000 ppm (IARC, 1991).

In response to the unusual observation of increased uterine tumours in mice (see above), possible changes in blood concentrations of sex hormones were investigated. Female $B6C3F_1$ mice (77–83 days of age) were exposed to 15 000 ppm [39 600 mg/m^3] chloroethane for 6 h per day for 21 days. No consistent changes were found in oestrous cyclicity or in serum concentrations of oestradiol and progesterone. Thus, none of the measured parameters emerged as a mechanistic factor that might contribute to the high incidence of endometrial tumours (Bucher *et al.*, 1995).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Chloroethane was mutagenic to bacteria and at the *hprt* locus in a study with the Chinese hamster ovary cell line, but not did not induce transformation in BALB/c 3T3 cells. In $B6C3F_1$ mice exposed by inhalation, it did not induce either unscheduled DNA synthesis in hepatocytes or micronuclei in bone-marrow cells.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of chloroethane were available.

There is *limited evidence* in experimental animals for the carcinogenicity of chloroethane.

Overall evaluation

Chloroethane is *not classifiable as to its carcinogenicity to humans (Group 3).*

Table 1. Genetic and related effects of chloroethane

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	NG	US National Toxicology Program (1989)
SA0, *Salmonella typhimurium* TA100, reverse mutation (gas exposure)	+	+	1% in air	Araki *et al.* (1994)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	NG	US National Toxicology Program (1989)
SA5, *Salmonella typhimurium* TA1535, reverse mutation (gas exposure)	+	+	3% in air	Araki *et al.* (1994)
SA7, *Salmonella typhimurium* TA1537, reverse mutation (gas exposure)	–	–	17% in air	Araki *et al.* (1994)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	US National Toxicology Program (1989)
SA9, *Salmonella typhimurium* TA98, reverse mutation (gas exposure)	–	–	17% in air	Araki *et al.* (1994)
ECW, *Escherichia coli* WP2 *uvrA*, reverse mutation (gas exposure)	+	+	1% in air	Araki *et al.* (1994)
GCO, Gene mutation, Chinese hamster ovary CHO cells *hprt* locus *in vitro*	+	(+)	940	Ebert *et al.* (1994)
TBM, Cell transformation, BALB/c 3T3 C11-13 mouse cells *in vitro*	–	NT	467	Tu *et al.* (1985)
UVM, Unscheduled DNA synthesis, B6C3F$_1$ mouse hepatocytes *in vivo*	–		25 500 inh. 6h/d × 3 d	Ebert *et al.* (1994)
CBA, Micronucleus test, B6C3F$_1$ mouse bone-marrow cells *in vivo*	–		25 500 inh. 6h/d × 3 d	Ebert *et al.* (1994)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; inh, inhalation

6. References

Aberer, W. & Zonzits, E. (1989) Allergy to ethyl chloride does occur, and might frequently be misdiagnosed. *Contact Derm.*, **21**, 352–353

Araki, A., Noguchi, T., Kato, F. & Matsushima, T. (1994) Improved method for mutagenicity testing of gaseous compounds by using a gas sampling bag. *Mutat. Res.*, **307**, 335–344

Bircher, A.J., Hampl, K., Hirsbrunner, P., Buechner, S.A. & Schneider, M. (1994) Allergic contact dermatitis from ethyl chloride and sensitization to dichlorodifluoromethane (CFC 12). *Contact Derm.*, **31**, 41–44

Bucher, J.R., Morgan, D.L., Adkins, B., Jr, Travlos, G.S., Davis, B.J., Morris, R. & Elwell, M.R. (1995) Early changes in sex hormones are not evident in mice exposed to the uterine carcinogens chloroethane or bromoethane. *Toxicol. appl. Pharmacol.*, **130**, 169–173

Ebert, R., Fedtke, N., Certa, H., Wiegand, H.J., Régnier, J.F., Marshall, R. & Dean, S.W. (1994) Genotoxicity studies with chloroethane. *Mutat. Res.*, **322**, 33–44

Fedtke, N., Certa, H., Ebert, R. & Wiegand, H.J. (1994a) Species differences in the biotransformation of ethyl chloride. I. Cytochrome P450-dependent metabolism. *Arch. Toxicol.*, **68**, 158–166

Fedtke, N., Certa, H., Ebert, R. & Wiegand, H.J. (1994b) Species differences in the biotransformation of ethyl chloride. II. GSH-dependent metabolism. *Arch. Toxicol.*, **68**, 217–223

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Soult, T.A. & Walker, J.S. (1993) Ethyl chloride intoxication [Letter to the Editor]. *Am. J. emerg. Med.*, **11**, 313–315

Tu, A.S., Murray, T.A., Hatch, K.M., Sivak, A. & Milman, H.A. (1985) In vitro transformation of BALB/c-3T3 cells by chlorinated ethanes and ethylenes. *Cancer Lett.*, **28**, 85–92

United States National Toxicology Program (1989) *Toxicology and Carcinogenesis Studies of Chloroethane (Ethyl chloride) (CAS No. 75-00-3) in F344/N Rats and B6C3F1 Mice (Gavage Studies)* (NTP Technical Report Series No. 346), Research Triangle Park, NC, United States Department of Health and Human Services

CHLOROFLUOROMETHANE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

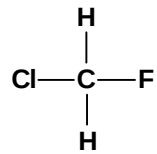
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Services Reg. No.: 593-70-4

Systematic name: Chlorofluoromethane

1.1.2 *Structural and molecular formulae and relative molecular mass*



CH_2ClF Relative molecular mass: 68.48

1.1.3 *Physical properties* (for details, see IARC, 1986)

(*a*) *Boiling-point*: –9.1°C

(*b*) *Melting-point*: –133°C

(*c*) *Conversion factor*: $mg/m^3 = 2.80 \times ppm$

1.2 Production and human exposure

Chlorofluoromethane has been reported as an impurity in dichlorofluoromethane, and thus limited human exposures may occur (IARC, 1986).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Chlorofluoromethane was tested for carcinogenicity in one study in rats by oral gavage at one dose level. High incidences of squamous-cell carcinomas and of fibrosarcomas of the forestomach and stomach were induced in rats of each sex (IARC, 1986).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Chlorofluoromethane is metabolized *in vitro* to carbon monoxide by rat hepatic microsomes and to formaldehyde by rat hepatic cytosolic preparations in the presence of glutathione (IARC, 1986).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Exposure of rats to chlorofluoromethane at 28 000 mg/m^3 by inhalation for 6 h per day on five days per week for two weeks caused moderate damage to kidneys, adrenal glands, testes, epididymides and haematopoietic tissues (IARC, 1986).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

The available data from experimental systems were not suitable for an evaluation (IARC, 1986).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

Chlorofluoromethane was mutagenic to *Salmonella typhimurium* and to mammalian cells in culture in both the presence and the absence of an exogenous metabolic activation system (IARC, 1986)

5. Evaluation

No epidemiological data relevant to the carcinogenicity of chlorofluoromethane were available.

There is *limited evidence* in experimental animals for the carcinogenicity of chlorofluoromethane.

Overall evaluation

Chlorofluoromethane is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

IARC (1986) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 41, *Some Halogenated Hydrocarbons and Pesticide Exposures*, Lyon, pp. 229–235

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 60

2-CHLORO-1,1,1-TRIFLUOROETHANE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 75-88-7

Chem. Abstr. Name: 2-Chloro-1,1,1-trifluoroethane

1.1.2 *Structural and molecular formulae and relative molecular mass*

$C_2H_2ClF_3$ Relative molecular mass: 118.49

1.1.3 *Physical properties* (for details, see IARC, 1986)

(*a*) *Boiling-point*: 6.9°C

(*b*) *Melting-point*: –105.5°C

(*c*) *Conversion factor*: $mg/m^3 = 4.85 \times ppm$

1.2 Use and human exposure

2-Chloro-1,1,1-trifluoroethane is used as a chemical intermediate in the production of the anaesthetic halothane. Human exposure occurs due to its presence as a low-level impurity in, and as a metabolite of, halothane (IARC, 1986).

2. Studies of Cancer in Humans

No data were available to the Working Group (IARC, 1986).

3. Studies of Cancer in Experimental Animals

2-Chloro-1,1,1-trifluoroethane was tested for carcinogenicity in one experiment in rats by gavage at one dose level. Increased incidences of uterine carcinomas and benign testicular tumours were observed (IARC, 1986).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Male Fischer 344 rats were exposed by inhalation to 1% 2-chloro-1,1,1-trifluoroethane for 2 h and then urine was collected for 24 h. Urinary metabolites identified by ^{19}F nuclear magnetic resonance and gas chromatography/mass spectrometry were 2,2,2-trifluoroethyl glucuronide (16%), trifluoroacetic acid (14%), trifluoroacetaldehyde hydrate (26%), trifluoroacetaldehyde-urea adduct (40%) and inorganic fluoride (3%). A minor, unidentified metabolite was also detected. No covalent binding of fluorine-containing metabolites was observed in the liver and kidney from the exposed rats (Yin *et al*., 1995). In-vitro incubation of 2-chloro-1,1,1-trifluoroethane with rat liver microsomes and an NADPH-generating system has been shown to involve a dechlorination reaction (Salmon *et al*., 1981) that produced trifluoroacetaldehyde hydrate as the only metabolite (Yin *et al*., 1995).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

The toxicity of 2-chloro-1,1,1-trifluoroethane was reviewed by a WHO task group which concluded that, in an inhalation experiment, the compound produced nasal and lung damage and atrophy of the thymus, spleen, testes and ovaries. In addition, thyroid weight was increased in male rats (WHO, 1992).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

2-Chloro-1,1,1-trifluoroethane was reviewed by a WHO task group, which concluded that it is embryotoxic at exposure concentrations that did not produce clear evidence of maternal toxicity and that there was evidence of teratogenicity (WHO, 1992).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

2-Chloro-1,1,1-trifluoroethane did not induce mutations in *Salmonella typhimurium* (IARC, 1986). 2-Chloro-1,1,1-trifluoroethane was reviewed by a WHO task group, which also concluded that it did not induce mutations in *S. typhimurium*, but additionally that it did not induce chromosomal aberrations in rat bone-marrow cells *in vivo*. Dominant lethal effects were observed in two of three studies in male mice (WHO, 1992).

5. Evaluation

No epidemiological data relevant to the carcinogenicity of 2-chloro-1,1,1-trifluoroethane were available.

There is *limited evidence* in experimental animals for the carcinogenicity of 2-chloro-1,1,1-trifluoroethane.

Overall evaluation

2-Chloro-1,1,1-trifluoroethane is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

IARC (1986) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 41, *Some Halogenated Hydrocarbons and Pesticide Exposures*, Lyon, pp. 253–259

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 60

Salmon, A.G., Jones, R.B. & Mackrodt, W.C. (1981) Microsomal dechlorination of chloroethanes: structure-reactivity relationships. *Xenobiotica*, **11,** 723–734

WHO (1992) *Partially Halogenated Chlorofluorocarbons (Ethane Derivatives)* (Environmental Health Criteria 139), Geneva, International Programme on Chemical Safety

Yin, H., Jones, J.P. & Anders, M.W. (1995) Metabolism of 1-fluoro-1,1,2-trichloroethane, 1,2-dichloro-1,1-difluoroethane, and 1,1,1-trifluoro-2-chloroethane. *Chem. Res. Toxicol.*, **8,** 262–268

CYCLOHEXANONE

Data were last evaluated in IARC (1989).

1. Exposure Data

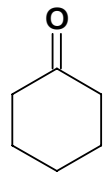
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 108-94-1

Chem. Abstr. Name: Cyclohexanone

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_6H_{10}O$ Relative molecular mass: 98.14

1.1.3 *Physical properties* (for details, see IARC, 1989)

(*a*) *Boiling point*: 155.6°C

(*b*) *Melting point*: –16.4°C

(*c*) *Conversion factor*: $mg/m^3 = 4.0 \times ppm$

1.2 Production, use and human exposure

Cyclohexanone is a synthetic organic liquid used primarily as an intermediate in the production of nylon. Other minor applications are as an intermediate, additive and solvent in a variety of products. Occupational exposure levels have been measured in some industries (IARC, 1989).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Cyclohexanone was tested for carcinogenicity by oral administration in the drinking-water in one strain of mice and one strain of rats. In mice, there was a slight increase in the incidence of tumours that occur commonly in this strain, but only in animals given the low dose. In rats, a slight increase in the incidence of adrenal cortical adenomas occurred in males treated with the low dose (IARC, 1989).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Cyclohexanone is metabolized to cyclohexanol, which is conjugated with glucuronic acid and excreted mainly in urine, where very little cyclohexanone or cyclohexanol is found (IARC, 1989).

The metabolism and kinetics of cyclohexanone were studied in a group of volunteers (four men and four women) during and after 8-h exposures to 101, 207 and 406 mg/m^3. After exposure to 207 mg/m^3, the metabolic yields of urinary cyclohexanol, 1,2- and 1,4-cyclohexanediol and their glucuronide conjugates were 1%, 39% and 18%, respectively. The elimination half-times ($t_{½}$) of the 1,2- and 1,4-diols, respectively, were 16 h and 18 h. Consequently, after repeated exposure over five days, there was no cumulation of urinary cyclohexanol, whereas there was cumulative excretion of the diols. The permeation rate of cyclohexanone liquid through the skin was 37–69 mg/cm^2 per hour, indicating that occupational exposure by this route is of minor importance (Mráz *et al*., 1994).

Monitoring of exhaled breath and urine of workers occupationally exposed to an average of 9 ppm [36 mg/m^3] cyclohexanone (range, 1–40 ppm [4–160 mg/m^3]) throughout an 8-h workshift showed a proportionality between environmental and exhaled breath concentrations; 9 ppm environmental cyclohexanone produced end-of-workshift breath concentrations of approximately 1 ppm cyclohexanone and urinary cyclohexanol of 9 mg/g creatinine (Ong *et al*., 1991).

Following the deliberate ingestion by a 61-kg man of 720 mL sake (ethanol, 10% w/v) and then about 100 mL of liquid cement containing cyclohexanone (39%), methyl ethyl ketone (28%), acetone (18%) and polyvinyl chloride (15%), cyclohexanone was not detectable in blood at the first sampling time (5 h after ingestion), when the plasma level of cyclohexanol was about 215 µg/mL. Urinary excretion of cyclohexanone was minimal, most excretion by this route being of cyclohexanol glucuronide and unconjugated cyclohexanol. For cyclohexanol, the plasma half-life was 4.75 h and the elimination constant (K_e) was 0.145 per hour (Sakata *et al*., 1989).

Isomers of cyclohexanediol were found in 101 of 584 urine samples from newborn babies in a special care unit. The most abundant was *trans*-1,2-cyclohexanediol. No glucuronide conjugates were detected. Cyclohexanone was found as a contaminant in dextrose infusion fluids. From the five samples analysed, at an average concentration of 0.89 mg, cyclohexanone would have been delivered in 150 mL dextrose over 24 h (Mills & Walker, 1990).

4.1.2 *Experimental systems*

Groups of six rabbits were given cyclohexanone (4.8 mmol/kg bw) and ethanol (4.8 mmol/kg bw) either together or separately by gavage. When cyclohexanone was given alone, maximum plasma concentrations of cyclohexanone and cyclohexanol were approximately 100 μg/mL at 15 min and 200 μg/mL at 120–180 min, respectively; after administration of the combined substances, maximum plasma concentrations of cyclohexanone and cyclohexanol were approximately 35 μg/mL at 15 min and 220 μg/mL at 120–180 min, respectively, indicating an interaction between cyclohexanone and ethanol (Sakata *et al*., 1993).

4.2 Toxic effects

4.2.1 *Humans*

No difference in nervous system function, blood and respiration was reported in workers exposed by inhalation and via skin contact, but there was some indication of liver disorders among a subgroup of workers (30–39 years old) with more than five years' exposure (IARC, 1989).

One report concerning five patients with contact dermatitis caused by a cyclohexanone resin made no attempt to identify the sensitizer (Bruze *et al*., 1988), while a report of one patient indicated that the patient was reacting to cyclohexanone itself (Sanmartín & De la Cuadra, 1992).

4.2.2 *Experimental systems*

No major effect of cyclohexanone on hepatic drug-metabolizing enzymes was observed in mice and beagle dogs. It is irritant to the eye and skin in rabbits and has been shown to cause central nervous system depression in rabbits and beagle dogs. Target organs for toxicity are kidney in beagle dogs and liver in beagle dogs and mice. No evidence for sensitizing potential has been shown in guinea-pigs (IARC, 1989).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Cyclohexanone did not impair the intrauterine development of mice. Variable results have been reported with regard to postnatal development, but the findings were not reproducible and some of the studies were inadequate (IARC, 1989).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Cyclohexanone did not induce mutations in bacteria, whereas chromosomal aberrations and aneuploidy were induced in cultured human lymphocytes and in the bone-marrow cells of rats treated *in vivo*.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of cyclohexanone were available.

There is *inadequate evidence* in experimental animals for the carcinogenicity of cyclohexanone.

Overall evaluation

Cyclohexanone is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

Bruze, M., Boman, A., Bergqvist-Karlsson, A., Björkner, B., Wahlberg, J.E. & Voog, E. (1988) Contact allergy to a cyclohexanone resin in humans and guinea pigs. *Contact Derm.,* **18,** 46–49

Collin, J.-P. (1971) Cytogenetic effect of sodium cyclamate, cyclohexanone and cyclohexanol. *Diabète*, **19**, 215–221 (in French)

de Hondt, H.A., Temtamy, S.A. & Abd-Aziz, K.B. (1983) Chromosomal studies on laboratory rats (*Rattus norvegicus*) exposed to an organic solvent (cyclohexanone). *Egypt. J. genet. Cytol*., **12**, 31–40

Dyshlovoi, V.D., Boiko, N.L., Shemetun, A.M. & Kharchenko, T.I. (1981) Cytogenetic action of cyclohexanone. *Gig. Sanit*., **5**, 76–77 (in Russian)

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W. & Zeiger, E. (1983) *Salmonella* mutagencity test results for 250 chemicals. *Environ. Mutag*., **Suppl. 1**, 3–142

IARC (1989) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 47, *Some Organic Solvents, Resin Monomers and Related Compounds, Pigments and Occupational Exposures in Paint Manufacture and Painting,* Lyon, pp. 157–169

Lederer, J., Collin, J.-P., Pottier-Armould, A.-M. & Gondry, E. (1971) Cytogenetic and teratogenetic effect of cyclamate and its metabolites. *Thérapeutique*, **47**, 357–363 (in French)

Mills, G.A. & Walker, V. (1990) Urinary excretion of cyclohexanediol, a metabolite of the solvent cyclohexanone, by infants in a special care unit. *Clin. Chem.,* **36,** 870–874

Table 1. Genetic and related effects of cyclohexanone

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	Haworth *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	Haworth *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Haworth *et al.* (1983)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	0.005	Dyshlovoi *et al.* (1981)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	10	Lederer *et al.* (1971)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	NG	Collin (1971)
AIH, Aneuploidy, human lymphocytes *in vitro*	+	NT	0.005	Dyshlovoi *et al.* (1981)
CBA, Chromosomal aberrations, rat bone-marrow cells *in vivo*	+		100 sc × 1	de Hondt *et al.* (1983)

[a] +, positive;–, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; sc, subcutaneous

Mráz, J., Gálová, E., Nohová, H. & Vítková, D. (1994) Uptake, metabolism and elimination of cyclohexanone in humans. *Int. Arch. occup. environ. Health,* **66,** 203–208

Ong, C.N., Chia, S.E., Phoon, W.H., Tan, K.T. & Kok, P.W. (1991) Monitoring of exposure to cyclohexanone through the analysis of breath and urine. *Scand. J. Work Environ. Health,* **17,** 430–435

Sakata, M., Kikuchi, J., Haga, M., Ishiyama, N., Maeda, T., Ise, T. & Hikita, N. (1989) Disposition of acetone, methyl ethyl ketone and cyclohexanone in acute poisoning. *Clin. Toxicol.,* **27,** 67–77

Sakata, M., Take, J., Watanabe, T., Sakata, K., Wada, K. & Haga, M. (1993) Metabolic interaction of ethanol and cyclohexanone in rabbits. *J. Toxicol. environ. Health,* **38,** 33–42

Sanmartín, O. & De la Cuadra, J. (1992) Occupational contact dermatitis from cyclohexanone as a PVC adhesive. *Contact Derm.,* **27,** 189–190

DECABROMODIPHENYL OXIDE

Data were last evaluated in IARC (1990).

1. Exposure Data

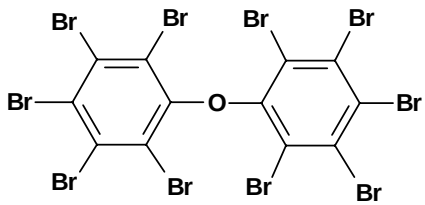
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 1163-19-5

Chem. Abstr. Name: Benzene, 1,1′-oxybis[2,3,4,5,6-pentabromo]-

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{12}OBr_{10}$ Relative molecular mass: 959.17

1.1.3 *Physical properties* (for details, see IARC, 1990)

(*a*) *Boiling point*: Decomposes at 425°C

(*b*) *Melting point*: 290–305°C

(*c*) *Conversion factor*: $mg/m^3 = 39.2 \times ppm$

1.2 Production, use and human exposure

Decabromodiphenyl oxide has been produced since the late 1970s as a flame retardant for use in plastics, especially high-impact polystyrene, and to treat textiles, such as automotive fabrics and tents. Occupational exposure to decabromodiphenyl oxide may occur during its production and use. It has also been detected in environmental samples collected near some production facilities (IARC, 1990).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Decabromodiphenyl oxide was tested for carcinogenicity by oral administration in one strain of mice and in two strains of rats. In one study in rats, it induced hepatocellular adenomas in animals of each sex and acinar-cell adenomas of the pancreas and mononuclear-cell leukaemia in males (IARC, 1990).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

When labelled decabromodiphenyl oxide was given by gavage to rats, over 99% of the label appeared in the faeces, approximately 0.5% of mainly unchanged compound was found in the liver, less than 1% of the label was detected in the urine over 16 days and trace amounts of label were found in the kidneys, spleen, lungs, brain, muscle, fat and skin. After an intravenous dose, the faeces and gut contents contained 75% of the dose, suggesting significant biliary excretion, and of the extracted faecal label, 63% was metabolites. No evidence of induction of cytochrome c reductase or cytochrome P450 activities was seen in male rats. In a two-year dietary study in rats, the bromine content of liver and adipose tissue was slightly increased (IARC, 1990).

4.2 Toxic effects

No data were available to the Working Group.

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

In single studies, decabromodiphenyl oxide did not induce gene mutations in either bacteria or mouse lymphoma L5178Y cells and neither did it induce sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary CHO cells.

Table 1. Genetic and related effects of decabromodiphenyl oxide

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	US National Toxicology Program (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	US National Toxicology Program (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	US National Toxicology Program (1986)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	US National Toxicology Program (1986)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	–	10	US National Toxicology Program (1986)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	500	US National Toxicology Program (1986)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	500	US National Toxicology Program (1986)

[a] –, negative
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL

5. Evaluation

No epidemiological data relevant to the carcinogenicity of decabromodiphenyl oxide were available.

There is *limited evidence* in experimental animals for the carcinogenicity of decabromodiphenyl oxide.

Overall evaluation

Decabromodiphenyl oxide is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

IARC (1990) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 48, *Some Flame Retardants and Textile Chemicals, and Exposures in the Textile Manufacturing Industry*, Lyon, pp. 73–84

United States National Toxicology Program (1986) *Toxicological and Carcinogenesis Studies of Decabromodiphenyl Oxide (CAS No. 1163-19-5) in F344/N Rats and B6C3F$_1$ Mice (Feeding Studies)* (Tech. Rep. Ser. No. 309), Research Triangle Park, NC, United States Department of Health and Human Services

DIBROMOACETONITRILE

Data were last evaluated in IARC (1991).

1. Exposure Data

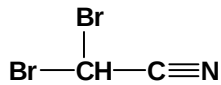
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 3252-43-5

Chem. Abstr. Name: Dibromoacetonitrile

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_2HNBr_2 Relative molecular mass: 198.84

1.1.3 *Physical properties* (for details, see IARC, 1991)

(*a*) *Boiling point*: 169–170°C

(*b*) *Conversion factor*: $mg/m^3 = 8.1 \times ppm$

1.2 Production and human exposure

Halogenated acetonitriles are not produced on an industrial scale. Several halogenated acetonitriles have been detected in chlorinated drinking-water in a number of countries as a consequence of the reaction of bromine with natural organic substances (and chlorine in the case of chlorinated acetonitriles) present in untreated water. The only known route of human exposure is through chlorinated drinking-water (IARC, 1991).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Dibromoacetonitrile was tested in a limited carcinogenicity study in female SEN mice by skin application, in an initiation/promotion study in female SEN mice by skin application and in a screening assay for lung tumours in female strain A mice by oral administration. No skin tumour was produced after skin application in mice. In the initiation/promotion study, in which dibromoacetonitrile was applied topically as six equal doses over a two-week period, followed by repeated doses of 12-*O*-tetradecanoylphorbol 13-acetate for 20 weeks, there was a dose-related increase in the number of mice with skin tumours except in the highest-dose group: control, 9/105; low dose (total dose 1200 mg/kg bw), 8/36 ($p < 0.05$); mid dose (total dose 2400 mg/kg bw), 33/70 ($p < 0.01$); high dose (total dose 4800 mg/kg bw), 10/74 (not significant). There was no increase in either the proportion of mice with lung tumours or the number of lung tumours per mouse (IARC, 1991).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Approximately 8% of a single oral dose of 149 mg/kg bw of dibromoacetonitrile to rats was excreted in urine within 24 h as thiocyanate, the product of released cyanide metabolized by rhodanese (IARC, 1991).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

A daily dose of 45 mg/kg bw given by gavage for 90 days to male and female CD rats did not produce any consistent adverse effects (IARC, 1991).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

There were slight decreases in early postnatal body weight of pups born to rats given dibromoacetonitrile orally at a dose of 50 mg/kg bw per day on gestation days 7–21. This dose was also toxic to the dams (IARC, 1991).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Dibromoacetonitrile induced DNA damage but not mutations in bacteria. It induced sister chromatid exchanges and DNA strand breaks in mammalian cell lines. Micronuclei were induced in the erythrocytes of newt (*Pleurodeles waltl*) larvae exposed for 12 days, but in mice dosed for five days, neither micronuclei in bone marrow nor abnormal sperm morphology were induced.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of dibromoacetonitrile were available.

There is *inadequate evidence* in experimental animals for the carcinogenicity of dibromoacetonitrile.

Overall evaluation

Dibromoacetonitrile is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

Bull, R.J., Meier, J.R., Robinson, M., Ringhand, H.P., Laurie, R.D. & Stober, J.A. (1985) Evaluation of mutagenic and carcinogenic properties of brominated and chlorinated acetonitriles: by-products of chlorination. *Fundam. appl. Toxicol.,* **5,** 1065–1074

Daniel, F.B., Schenck, K.M., Mattox, J.K., Lin, E.L., Haas, D.L. & Pereira, M.A. (1986) Genotoxic properties of haloacetonitriles: drinking water by-products of chlorine disinfection. *Fundam. appl. Toxicol.,* **6,** 447–453

IARC (1991) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 52, *Chlorinated Drinking-Water; Chlorination By-Products; Some Other Halogenated Compounds; Cobalt and Cobalt Compounds,* Lyon, pp. 269–296

Le Curieux, F., Giller, S., Gauthier, L., Erb, F. & Marzin, D. (1995) Study of the genotoxic activity of six halogenated acetonitriles, using the SOS chromotest, the Ames-fluctuation test and the newt micronucleus test. *Mutat. Res.*, **341**, 289–302

Table 1. Genetic and related effects of dibromoacetonitrile

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS chromotest, *Escherichia coli* PQ37	+	–	10	Le Curieux *et al.* (1995)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	58	Bull *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation (fluctuation test)	–	–	30	Le Curieux *et al.* (1995)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	58	Bull *et al.* (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Bull *et al.* (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	NG	Bull *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	58	Bull *et al.* (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		200 ppm inj.	Valencia *et al.* (1985)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	0.03	Bull *et al.* (1985)
DIH, DNA strand breaks, human lymphoblastic cell line *in vitro*	+	NT	NG	Daniel *et al.* (1986)
Micronucleus test, *Pleurodeles waltl* erythrocytes *in vivo*	+		0.12	Le Curieux *et al.* (1995)
MVM, Micronucleus test, CD-1 mouse bone-marrow cells *in vivo*	–		50 po × 5	Bull *et al.* (1985)
SPM, Sperm morphology, B6C3F$_1$ mice, *in vivo*	–		50 po × 5	Meier *et al.* (1985)

[a] +, positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; NG, not given; po, oral; inj., injection

Meier, J.R., Bull, R.J., Stober, J.A. & Cimino, M.C. (1985) Evaluation of chemicals used for drinking water disinfection for production of chromosomal damage and sperm-head abnormalities in mice. *Environ. Mutag.,* **7,** 201–211

Valencia, R., Mason, J.M., Woodruff, R.C. & Zimmering, S. (1985) Chemical mutagenesis testing in *Drosophila*. III. Results of 48 coded compounds tested for the National Toxicology Program. *Environ. Mutag.*, **7**, 325–348

DICHLOROACETONITRILE

Data were last evaluated in IARC (1991).

1. Exposure Data

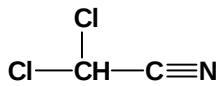
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 3018-12-0

Chem. Abstr. Name: Dichloroacetonitrile

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_2HNCl_2 Relative molecular mass: 109.94

1.1.3 *Physical properties* (for details, see IARC, 1991)

(*a*) *Boiling point*: 112–113°C

(*b*) *Conversion factor*: $mg/m^3 = 4.5 \times ppm$

1.2 Production and human exposure

Halogenated acetonitriles are not produced on an industrial scale. Several halogenated acetonitriles have been detected in chlorinated drinking-water in a number of countries as a consequence of the reaction of chlorine with natural organic substances present in untreated water. The only known route of human exposure is through chlorinated drinking-water (IARC, 1991).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Dichloroacetonitrile was tested in a limited carcinogenicity study in female SEN mice by skin application, in an initiation/promotion study in female SEN mice by skin application and in a screening assay for lung tumours in female strain A mice by oral administration. No skin tumour was produced after skin application in mice or in the initiation/promotion study, in which dichloroacetonitrile was applied topically as six equal doses over a two-week period, followed by repeated doses of 12-*O*-tetradecanoylphorbol 13-acetate for 20 weeks. There was no increase in either the proportion of mice with lung tumours or the number of lung tumours per mouse (IARC, 1991).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Studies with [1-^{14}C]dichloroacetonitrile in rats and mice and [2-^{14}C]dichloroacetonitrile in rats indicated that the substance is rapidly absorbed after oral administration in water. Excretion of radioactivity following dosing with [1-^{14}C]dichloroacetonitrile is more rapid in mice than in rats. In mice, approximately 84% of the dose was excreted in 24 h (67% in urine, 11% in faeces, 5% as CO_2), compared with 67% in rats in six days (44% in urine, 17% in faeces, 6% as CO_2). Excretion of [2-^{14}C]dichloroacetonitrile radioactivity in rats accounted for about 84% of the dose within 48 h (38% in urine, 12% in faeces, 34% as CO_2). The quantitative differences in the route of excretion of the two labels in rats indicate that dichloroacetonitrile is being cleaved *in vivo*. The 1-^{14}C-labelled compound behaved like cyanide (IARC, 1991).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Dichloroacetonitrile did not induce γ-glutamyltranspeptidase-positive foci in rat liver. An oral dose by gavage of 65 mg/kg bw per day for 90 days to male and female CD rats reduced body weights, spleen and gonad weights and serum cholesterol levels; other blood chemistry and haematological parameters were generally unchanged. Liver weights relative to body or brain weight were increased in female rats (IARC, 1991).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Dichloroacetonitrile given orally to rats at a dose of 45 mg/kg bw per day on gestation days 6–18 was associated in the full-term fetuses with an increased frequency of malformations of soft tissues, particularly of the cardiovascular and urogenital organs, and some skeletal malformations. This dose was also severely embryotoxic and toxic to the pregnant rats (IARC, 1991).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Dichloroacetonitrile induced DNA damage and mutation in bacteria. Sex-linked recessive lethal mutations were induced in *Drosophila melanogaster*. It weakly induced sister chromatid exchanges and DNA strand breaks in mammalian cell lines. Micronuclei were induced in the erythrocytes of newt (*Pleurodeles waltl*) larvae exposed for 12 days, but in mice dosed for five days, neither micronuclei in bone marrow nor abnormal sperm morphology were induced.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of dichloroacetonitrile were available.

There is *inadequate evidence* in experimental animals for the carcinogenicity of dichloroacetonitrile.

Overall evaluation

Dichloroacetonitrile is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

Bull, R.J., Meier, J.R., Robinson, M., Ringhand, H.P., Laurie, R.D. & Stober, J.A. (1985) Evaluation of mutagenic and carcinogenic properties of brominated and chlorinated acetonitriles: by-products of chlorination. *Fundam. appl. Toxicol.,* **5,** 1065–1074

Table 1. Genetic and related effects of dichloroacetonitrile

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS chromotest, *Escherichia coli* PQ37	–	(+)	50	Le Curieux *et al.* (1995)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	250	Simmon *et al.* (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	88	Bull *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation (fluctuation test)	+	+	10	Le Curieux *et al.* (1995)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	11	Bull *et al.* (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Bull *et al.* (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	NG	Bull *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	+	+	680	Bull *et al.* (1985)
SCH, *Saccharomyces cerevisiae*, mitotic recombination	–	NT	NG	Simmon *et al.* (1977)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		200 ppm feed	Valencia *et al.* (1985)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	(+)	+	2.3	Bull *et al.* (1985)
DIH, DNA strand breaks, human lymphoblastic cell line *in vitro*	(+)	NT	NG	Daniel *et al.* (1986)
Micronucleus test, *Pleurodeles waltl* erythrocytes *in vivo*	+		0.25	Le Curieux *et al.* (1995)
MVM, Micronucleus test, CD-1 mouse bone-marrow cells *in vivo*	–		50 po × 5	Bull *et al.* (1985)
SPM, Sperm morphology, B6C3F_1 mice *in vivo*	–		50 po × 5	Meier *et al.* (1985)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; NG, not given; po, oral

Daniel, F.B., Schenck, K.M., Mattox, J.K., Lin, E.L.C., Haas, D.L. & Pereira, M.A. (1986) Genotoxic properties of haloacetonitriles: drinking water by-products of chlorine disinfection. *Fundam. appl. Toxicol.,* **6,** 447–453

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Le Curieux, F., Giller, S., Gauthier, L., Erb, F. & Marzin, D. (1995) Study of the genotoxic activity of six halogenated acetonitriles, using the SOS chromotest, the Ames-fluctuation test and the newt micronucleus test. *Mutat. Res.*, **341**, 289–302

Meier, J.R., Bull, R.J., Stober, J.A. & Cimino, M.C. (1985) Evaluation of chemicals used for drinking water disinfection for production of chromosomal damage and sperm-head abnormalities in mice. *Environ. Mutag.,* **7,** 201–211

Simmon, V.F., Kauhanen, K. & Tardiff, R.G. (1977) Mutagenic chemicals identified in drinking water. In: Scott, D., Bridges, B.A. & Sobels, F.H., eds, *Progress in Genetic Toxicology*, Vol. 2, *Development in Toxicology and Environmental Sciences*, Amsterdam, Elsevier, pp. 249–258

Valencia, R., Mason, J.M., Woodruff, R.C. & Zimmering, S. (1985) Chemical mutagenesis testing in *Drosophila*. III. Results of 48 coded compounds tested for the National Toxicology Program. *Environ. Mutag.*, **7**, 325–348

DICHLOROACETYLENE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 7572-29-4

Chem. Abstr. Name: Dichloroethyne

IUPAC Systematic Name: Dichloroacetylene

1.1.2 *Structural and molecular formulae and relative molecular mass*

C_2Cl_2 Relative molecular mass: 94.93

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Volatile liquid with a disagreeable, sweetish odour (American Conference of Governmental Industrial Hygienists, 1991)

(*b*) *Boiling-point*: 33°C (explodes) (American Conference of Governmental Industrial Hygienists, 1991; Lide, 1997)

(*c*) *Melting-point*: –66°C (Lide, 1997)

(*d*) *Solubility*: Soluble in ethanol, diethyl ether and acetone (Lide, 1997)

(*e*) *Stability*: Explodes on heating strongly, ignites on contact with air; severe explosion hazard when shocked or exposed to heat or air; can react vigorously with oxidizing materials (American Conference of Governmental Industrial Hygienists, 1991)

(*f*) *Conversion factor*: $mg/m^3 = 3.9 \times ppm$

1.2 Production and use

Dichloroacetylene is not available in commercial quantities. It is reported to be a by-product in the synthesis of vinylidene chloride (Reichert *et al*., 1980). The compound may also be produced from the pyrolysis of various chlorohydrocarbons.

Dichloroacetylene is not known to be used commercially.

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), fewer than 100 workers in the United States were potentially exposed to dichloroacetylene (see General Remarks).

1.3.2 *Environmental occurrence*

No data were available to the Working Group.

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not recommended an 8-h time-weighted average threshold limit value but has recommended 0.39 mg/m^3 as the ceiling value for occupational exposures to dichloroacetylene in workplace air. Similar values have been used as standards or guidelines in other countries (International Labour Office, 1991).

No international guideline for dichloroacetylene in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Dichloroacetylene was tested for carcinogenicity in mice and rats by inhalation. A treatment-related increase was observed in the incidence of adenocarcinomas of the kidney in male mice. In rats, the occurrence of benign tumours of the liver and kidney and an increased incidence of lymphomas were reported (IARC, 1986).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

The metabolism of inhaled [^{14}C]dichloroacetylene has been studied in male Wistar rats exposed to 20 or 40 ppm [78 or 156 mg/m^3] atmospheres for 1 h. During the next

96 h, elimination of retained (approximately 17%) 20 and 40 ppm doses, respectively, was: urine, 68% and 60%; faeces, 28% and 27%. About 3.5% remained in the carcasses. Metabolites of dichloroacetylene that were identified were: *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine (62%), dichloroethanol (12%), dichloroacetic acid (9%), oxalic acid (8%) and chloroacetic acid (5%) in urine; and *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine in faeces. In bile, only *S*-(1,2-dichlorovinyl)glutathione was identified. Biliary cannulation did not influence the renal excretion of *N*-acetyl-*S*-(1,2-dichlorovinyl)-L-cysteine, a result that was interpreted to indicate that glutathione conjugation also occurs in the kidney. The identified metabolites are consistent with the existence of two metabolic pathways (Figure 1): the major pathway involves glutathione conjugation, while a minor pathway is cytochrome P450-dependent oxidation that accounts for the formation of 1,1-dichloro-compounds after chlorine migration (Kanhai *et al*., 1991).

In-vitro studies have demonstrated that glutathione conjugation of dichloroacetylene is predominantly enzymatic and the rate of reaction resulting in the formation of *S*-(1,2-dichlorovinyl)glutathione is similar for microsomes from rat kidney and liver (Kanhai *et al*., 1989). However, under different reaction conditions (20–500-fold higher protein concentrations), the rate was highest for microsomes from liver, followed by lung, brain and kidney (Patel *et al*., 1994). The further handling of this metabolite in kidney is not clearly defined, but it is known that γ-glutamyltranspeptidase and dipeptidases (e.g., in biliary epithelium) can transform *S*-(1,2-dichlorovinyl)glutathione to *S*-(1,2-dichlorovinyl)-L-cysteine, which can then be acetylated in tissues or by intestinal bacteria (Meister, 1988). An alternative to acetylation is β-lyase-mediated metabolism to form chlorothioketene as an intermediate (Dekant *et al*., 1988), which can react with tissue nucleophiles or with water, when it forms chloroacetic acid. Cysteine conjugate β-lyase activity has also been shown in rat cerebellar tissue (Patel *et al*., 1994).

4.2 Toxic effects

4.2.1 *Humans*

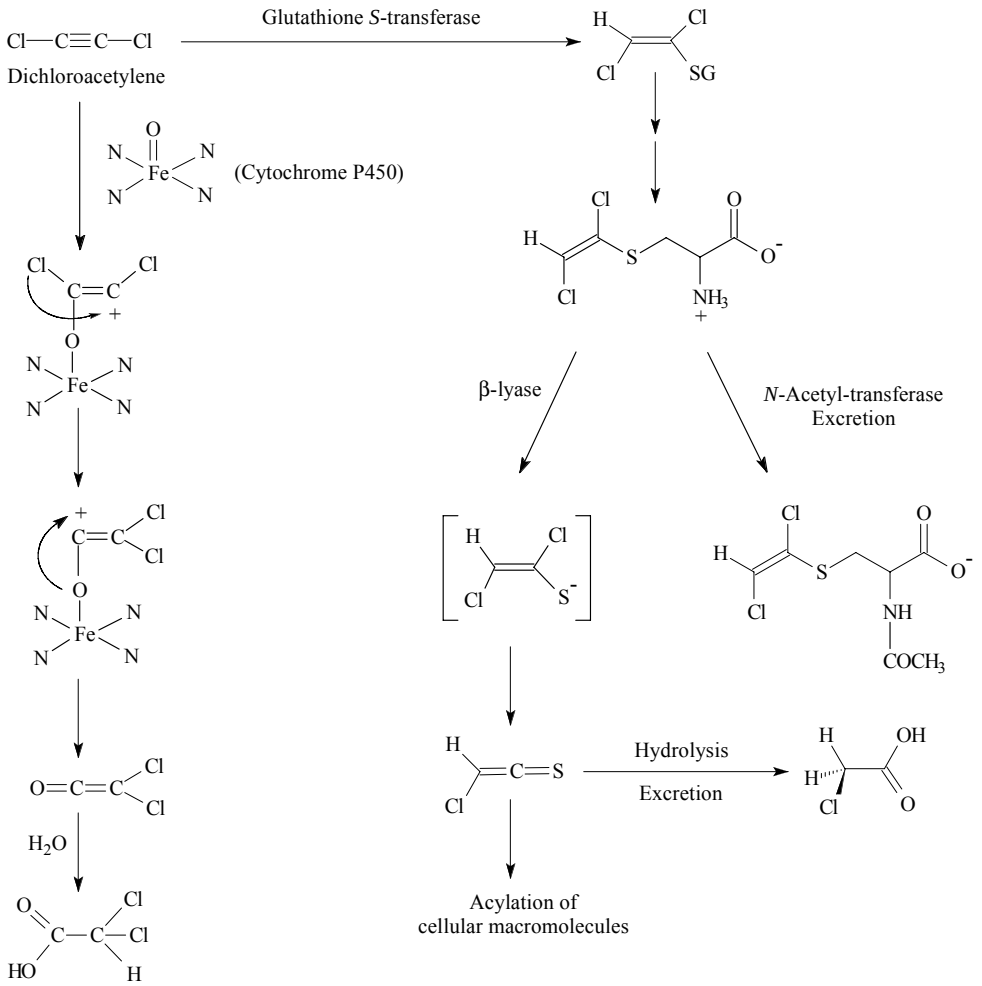
Toxic effects observed after accidental exposure to dichloroacetylene were mainly neurological disorders that persisted for periods ranging from several days to years (IARC, 1986).

4.2.2 *Experimental systems*

Dichloroacetylene inhalation induced nephrotoxic effects in rats and male rabbits including tubular and focal necrosis in the collecting tubules and increased mitotic activity in the renal epithelium. Hepatotoxic effects and neuropathological changes were also reported in male rabbits (IARC, 1986).

In-vitro studies on LLC-PK_1 cells with *S*-(1,2-dichlorovinyl)-L-cysteine have shown that Ca^{++} release from mitochondria is followed by DNA double-strand breaks and increased poly(ADP-ribosyl)ation of nuclear proteins. The DNA fragmentation is secondary to the activation of Ca^{++} and Mg^{++}-dependent endonucleases (Vamvakas *et al*., 1992). However, at low, nontoxic concentrations (1 or 5 μM), *S*-(1,2-dichlorovinyl)-L-cysteine exposure for

Figure 1. Proposed metabolic pathways of dichloroacetylene by glutathione conjugate formation and cytochrome P450 oxidation



Redrawn from Kanhai *et al.* (1991)
SG, glutathionyl

seven weeks resulted in the appearance of stable, dedifferentiated clones that had lost a number of the characteristics of the renal tubule cells from which they were derived. There was also increased poly(ADP-ribosyl)ation and enhanced *c-fos* expression (Vamvakas *et al.*, 1996). Increased poly(ADP-ribosyl)ation was also induced *in vivo*, following administration to male Wistar rats of *S*-(1,2-dichlorovinyl)-L-cysteine. A similar response was observed with certain renal carcinogens (potassium bromate and ferric nitriloacetate) but not others (trimethylpentane and *N*-nitrosodimethylamine) (McLaren *et al.*, 1994).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see also Table 1 for references)

Dichloroacetylene was mutagenic to *Salmonella typhimurium* in the presence or absence of an exogenous metabolic system (IARC, 1986).

5. Evaluation

No epidemiological data relevant to the carcinogenicity of dichloroacetylene were available.

There is *limited evidence* in experimental animals for the carcinogenicity of dichloroacetylene.

Overall evaluation

Dichloroacetylene is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

American Conference of Governmental Industrial Hygienists (1991) *Documentation of the Threshold Limit Values and Biological Exposure Indices*, 6th Ed., Vol. 1, Cincinnati, OH, pp. 403–405

American Conference of Governmental Industrial Hygienists (1997) *1997 Threshold Limit Values and Biological Exposure Indices*, Cincinnati, OH, p. 21

Dekant, W., Berthold, K., Vamavkas, S., Henschler, D. & Anders, M.W. (1988) Thioacylating intermediates as metabolites of *S*-(1,2-dichlorovinyl)-L-cysteine and *S*-(1,2,2-trichlorovinyl)-L-cysteine formed by cysteine conjugate β-lyase. *Chem. Res. Toxicol.*, **1**, 175–178

IARC (1986) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 39, *Some Chemicals Used in Plastics and Elastomers*, Lyon, pp. 369–378

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 62

International Labour Office (1991) *Occupational Exposure Limits for Airborne Toxic Substances*, 3rd Ed. (Occupational Safety and Health Series No. 37), Geneva, p. 138

Kanhai, W., Dekant, W. & Henschler, D. (1989) Metabolism of the nephrotoxin dichloroacetylene by glutathione conjugation. *Chem. Res. Toxicol.*, **2,** 51–56

Table 1. Genetic and related effects of dichloroacetylene

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	5000 ppm	Reichert *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000 ppm	Reichert *et al.* (1983)

[a] +, positive; –, negative
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL

Kanhai, W., Koob, M., Dekant, W. & Henschler, D. (1991) Metabolism of ^{14}C-dichloroethyne in rats. *Xenobiotica*, **21,** 905–916

Lide, D.R., ed. (1997) *CRC Handbook of Chemistry and Physics*, 78th Ed., Boca Raton, FL, CRC Press, p. 3–164

McLaren, J., Boulikas, T. & Vamvakas, S. (1994) Induction of poly(ADP-ribosyl)ation in the kidney after in vivo application of renal carcinogens. *Toxicology*, **8**, 101–102

Meister, A. (1988) Glutathione metabolism and its selective modification. *J. biol. Chem.*, **263**, 17205–17208

NOES (1997) *National Occupational Exposure Survey 1981–83*, Unpublished data as of November 1997, Cincinnati, OH, United States Department of Health and Human Services, Public Health Service, National Institute for Occupational Safety and Health

Patel, N., Birner, G., Dekant, W. & Anders, M.W. (1994) Glutathione-dependent biosynthesis and bioactivation of *S*-(1,2-dichlorovinyl)glutathione and *S*-(1,2-dichlorovinyl)-L-cysteine, the glutathione and cysteine *S*-conjugates of dichloroacetylene, in rat tissues and subcellular fractions. *Drug Metab. Dispos.,* **22,** 143–147

Reichert, D., Metzler, M. & Henschler, D. (1980) Decomposition of the neuro- and nephrotoxic compound dichloroacetylene in the presence of oxygen: separation and identification of novel products. *J. environ. Pathol. Toxicol.*, **4**, 525–532

Reichert, D., Neudecker, T., Spenger, U. & Henschler, D. (1983) Mutagenicity of dichloroacetylene and its degradation products trichloroacetyl chloride, trichloroacryloyl chloride and hexachlorobutadiene. *Mutat. Res.*, **117**, 21–29

Vamvakas, S., Bittner, D., Dekant, W. & Anders, M.W. (1992) Events that precede and that follow *S*-(1,2-dichlorovinyl)-L-cysteine-induced release of mitochondrial Ca^{2+} and their association with cytotoxicity to renal cells. *Biochem. Pharmacol.*, **44**, 1131–1138

Vamvakas, S., Richter, H. & Bittner, D. (1996) Induction of dedifferentiated clones of LLC-PK_1 cells upon long-term exposure to dichlorovinylcysteine. *Toxicology,* **106,** 65–74

WHO (1993) *Guidelines for Drinking Water Quality*, 2nd Ed., Vol. 1, *Recommendations*, Geneva

trans-1,4-DICHLOROBUTENE

Data were last reviewed in IARC (1977) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

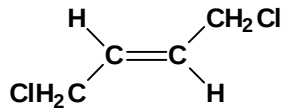
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 110-57-6

Chem. Abstr. Name: *trans*-1,4-Dichloro-2-butene

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_4H_6Cl_2$ Relative molecular mass: 125

1.1.3 *Physical properties* (for details, see IARC, 1977)

(*a*) *Boiling-point*: 155.5°C at 101 kPa

(*b*) *Melting-point*: 1–3°C

(*c*) *Conversion factor*: $mg/m^3 = 5.1 \times ppm$

1.2 Production and use

trans-1,4-Dichlorobutene has been produced commercially since about 1963 in several countries by the chlorination of 1,3-butadiene. By far its major use is as an intermediate in the manufacture of hexamethylenediamine and chloroprene (IARC, 1977).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

trans-1,4-Dichlorobutene was tested for carcinogenicity in mice by skin application and by subcutaneous and intraperitoneal administration. It produced a low incidence of local sarcomas when injected subcutaneously or intraperitoneally (IARC, 1977).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Toxic effects

No data were available to the Working Group.

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

trans-1,4-Dichlorobutene is mutagenic to bacteria (IARC, 1977).

5. Evaluation

No epidemiological data relevant to the carcinogenicity of *trans*-1,4-dichlorobutene were available.

There is *inadequate evidence* in experimental animals for the carcinogenicity of *trans*-1,4-dichlorobutene.

Overall evaluation

trans-1,4-Dichlorobutene is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

IARC (1977) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*, Vol. 15, *Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals*, Lyon, pp. 149–154

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 62

1,2-DICHLOROPROPANE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

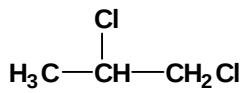
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 78-87-5

Chem. Abstr. Name: 1,2-Dichloropropane

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_3H_6Cl_2$ Relative molecular mass: 113.0

1.1.3 *Physical properties* (for details, see IARC, 1986)

(*a*) *Boiling-point*: 96.4°C (Lide, 1995)

(*b*) *Melting-point*: –100.4°C (Lide, 1995)

(*c*) *Conversion factor*: $mg/m^3 = 4.6 \times ppm$

1.2 Production, use and human exposure

1,2-Dichloropropane has been used as an industrial solvent, as a chemical intermediate and in soil fumigants. Human exposure may occur during its production and industrial and domestic use, and due to the presence of low levels in ambient air and in water (IARC, 1986).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

1,2-Dichloropropane was tested for carcinogenicity by gavage in one experiment in mice and one experiment in rats. A dose-related increase in the incidence of hepatocellular tumours was observed in male and female mice. Inconclusive results were obtained in female rats, and no effect was seen in male rats (IARC, 1986).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

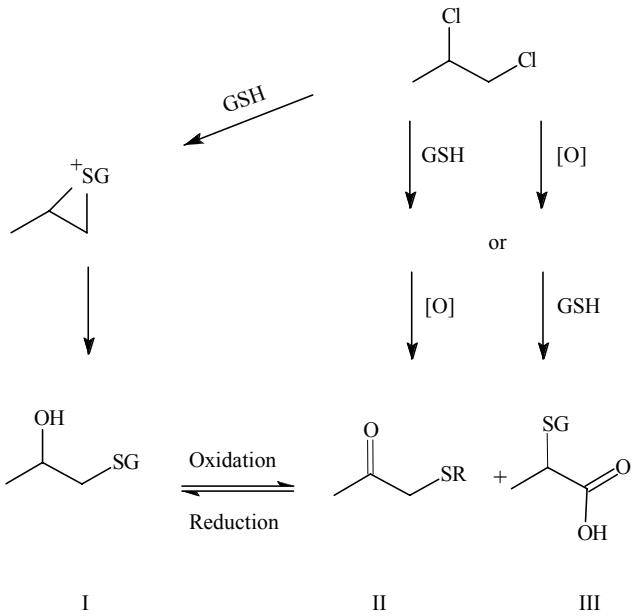
No data were available to the Working Group.

4.1.2 *Experimental systems*

Rats receiving single oral doses of 1,2-dichloro[1-^{14}C]propane excreted about 50% in the urine and 5% in faeces in 24 h. There was little further excretion over the next 72 h. A total of 19% of the dose was excreted as $^{14}CO_2$ and 23% as other volatile substances. At 96 h, 5% remained in the carcass. The major urinary metabolite found after oral dosing was *N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine. Identified minor metabolites were β-chlorolactate and *N*-acetyl-*S*-(2,3-dihydroxypropyl)-L-cysteine (IARC, 1986).

In later studies, male and female Fischer 344 rats were either exposed to atmospheres of 5, 50 or 100 ppm [23, 230 or 460 mg/m^3] of singly labelled 1,2-dichloro[^{14}C]propane for a 6-h period or dosed orally once with 1 or 100 mg/kg bw or on seven consecutive days with 1 mg/kg bw. During inhalation exposure, maximum blood concentrations were reached after 2 h, the values being approximately 0.06, 0.9 and 4.0 μg/g blood, respectively. Once exposure stopped, 1,2-dichloropropane was rapidly eliminated from blood. Analysis of expired air provided evidence for saturation of metabolism, the proportion of expired 1,2-dichloro[^{14}C]propane increasing with dose. For both gavage and inhalation administration, the principal routes of elimination were urine (37–65%) and expired air (18–40%), most of the radioactivity being eliminated within 24 h, irrespective of the route or sex. Tissues, faeces and the cage wash accounted for < 11%, about 10% and about 4% of the dose, respectively. The major urinary metabolites were *N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine (I), *N*-acetyl-*S*-(2-oxopropyl)-L-cysteine (II) and *N*-acetyl-*S*-(1-carboxyethyl)-L-cysteine (III) (Timchalk *et al*., 1991). It has been proposed that these metabolites could arise as follows (Figure 1): oxidation of C-1 and subsequent conjugation on C-2 gives III; conjugation on C-1 and oxidation of C-2 gives II; reduction of II gives I. This mechanism is supported by studies with D_6-labelled compounds (Bartels & Timchalk, 1990) as well as studies that strongly suggest that 1,2-

Figure 1. Proposed metabolic scheme for the formation of mercapturic acid metabolites of 1,2-dichloropropane in the rat



SG, glutathionyl; I, *N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine; II, *N*-acetyl-*S*-(2-oxopropyl)-L-cysteine; III, *N*-acetyl-*S*-(1-carboxyethyl)-L-cysteine
From Bartels and Timchalk (1990)

dichloropropane is activated by human CYP2E1, by oxidation, to a product trapped as a glutathione conjugate (Guengerich *et al*., 1991).

4.2 Toxic effects

4.2.1 *Humans*

Human exposures resulting in toxicity indicate that the main target organs are liver and kidney (IARC, 1986). Sublethal exposure also causes central nervous system depression (Imberti *et al*., 1987; Lucantoni *et al*., 1992). In a case series of 10 painters or engineers with contact allergic dermatitis, all patients demonstrated a positive response to 1,2-dichloropropane (Baruffini *et al*., 1989).

4.2.2 *Experimental systems*

Liver damage follows short-term exposure of rats to 1,2-dichloropropane by inhalation (IARC, 1986).

Adult male Sprague-Dawley rats were dosed by gavage with 0, 100, 250, 500 or 1000 (750 in the 13-week study) mg/kg bw 1,2-dichloropropane per day for one day, for up to 10 days or for 13 weeks. In the single-dose study, the main effects were a reduction in body weight gain and central nervous system depression; morphological changes were

restricted to centrilobular hepatocytes in rats of the 500 and 1000 mg/kg bw dose groups. Non-protein sulfhydryl (thiols) were decreased in the liver and increased in the kidney. Over the 10-day period, resistance to hepatotoxicity developed, but there was clear evidence of haemolytic anaemia and haemosiderosis. In the 13-week study, many deaths occurred in the groups given 500 and 750 mg/kg bw, but none occurred in the lower-dose groups. There was limited hepatotoxicity and no apparent nephrotoxicity, while splenic haemosiderosis was evident in most rats of all dose groups (Bruckner *et al*., 1989).

Exposure of rats by inhalation to 1,2-dichloropropane concentrations of 100 mg/m^3 for 4 h produced blood concentrations of 0.2 μg/mL and resulted in a reduction of hepatic non-protein thiols immediately following the treatment, while there was no evidence of hepatic lipid peroxidation or change in total protein content (Di Nucci *et al*., 1990). A dose-dependent decrease in hepatic reduced glutathione content was also found after single intraperitoneal injections of rats with 1,2-dichloropropane. Daily dosing for four weeks resulted in a dose-dependent increase in reduced glutathione and glutathione-*S*-transferase activity and a decrease in cytochrome P450 content. Areas of focal necrosis observed after five days dosing tended to disappear after the longer dosing period. Steatosis was evident after five days' dosing at 100 mg/kg bw, while hyperplasia of the liver was seen in 5/5 rats examined at 10 mg/kg bw (Trevisan *et al*., 1989). Treatment of rats with buthionine sulfoximine, a glutathione-depleting agent, increased the lethal toxicity of 1,2-dichloropropane (2 mL/kg bw), while administration of *N*-acetylcysteine, a glutathione precursor, decreased the toxicity (Imberti *et al*., 1990).

In contrast to the increased concentration of non-protein thiols in the kidney, mentioned above, there has been report of a dose-dependent decrease in angiotensin-converting enzyme activity of the proximal tubule brush border, fraying of the microvilli and epithelial coagulative necrosis of the brush border after intraperitoneal treatment of rats with 250 and 500 mg/kg bw 1,2-dichloropropane. The earliest renal changes are alterations of the glomeruli, but the most sensitive parameter is angiotensin-converting enzyme activity. The biochemical changes are reversible (Trevisan *et al*., 1988, 1991). In-vitro studies in which rat renal cortical slices were exposed to 1,2-dichloropropane show that a depletion in glutathione content occurs which can be prevented by carbon monoxide and the loss of organic anion accumulation (lactate and 4-aminohippurate) can be partially inhibited by acivicin and aminooxyacetic acid, which are inhibitors of γ-glutamyltranspeptidase and β-lyase activities, respectively (Trevisan *et al*., 1993).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Pregnant Sprague-Dawley rats and New Zealand White rabbits were dosed orally (gavage) with 1,2-dichloropropane on gestation days 6–15 and 7–19, respectively. Maternal toxicity in both rats and rabbits was observed at doses of 125 mg/kg bw and

150 mg/kg bw, respectively. At these maternally toxic doses only, there were increases in the incidence of delayed ossification of the skull of the fetuses. No teratogenic effects were observed in either rats or rabbits (Kirk *et al*., 1995).

D-D, a commercial mixture of chlorinated hydrocarbons that contained 25.6% 1,2-dichloropropane (other major components being *cis(Z)*-1,3-dichloropropene, 28.1%, and *trans(E)*-1,3-dichloropropene, 25.6%) was tested for effects on reproduction in male and female rats exposed by inhalation to D-D concentrations up to 90 ppm (v/v) for 6 h per day on five days per week for 10 weeks before they were mated. There were decreases in body weight gain and slight increases in the weights of liver and kidney in 90-ppm rats of both sexes, but there were no effects upon reproductive performance (Linnett *et al*., 1988).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

1,2-Dichloropropane was mutagenic to *Salmonella typhimurium* but not to *Streptomyces coelicolor*. It induced mutations weakly but not chromosomal effects in *Aspergillus nidulans*. It did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster*.

In Chinese hamster ovary CHO cells in culture, it induced sister chromatid exchanges and chromosomal aberrations.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of 1,2-dichloropropane were available.

There is *limited evidence* in experimental animals for the carcinogenicity of 1,2-dichloropropane.

Overall evaluation

1,2-Dichloropropane is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

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Table 1. Genetic and related effects of 1,2-dichloropropane

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	5000	De Lorenzo *et al*. (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	565	Stolzenberg & Hine (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	2900	Principe *et al*. (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	–	5000	Haworth *et al*. (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	5000	De Lorenzo *et al*. (1977)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	2900	Principe *et al*. (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	(+)	–	5000	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5800	Principe *et al*. (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1666	Haworth *et al*. (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	5800	Principe *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5800	Principe *et al*. (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Haworth *et al*. (1983)
SAS, *Salmonella typhimurium* TA1978, reverse mutation	–	–	25 000	De Lorenzo *et al*. (1977)
STF, *Streptomyces coelicolor*, forward mutation	–	NT	58 000	Principe *et al*. (1981)
ANG, *Aspergillus nidulans*, genetic crossing-over	–	NT	17 400	Crebelli *et al*. (1984)
ANF, *Aspergillus nidulans*, forward mutation	(+)	NT	58 000	Principe *et al*. (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		5040 feed	Woodruff *et al*. (1985)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	113	Galloway *et al*. (1987)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	370	Von der Hude *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	(+)	(+)	660	Galloway *et al*. (1987)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day

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1,2-DIETHYLHYDRAZINE

Data were last reviewed in IARC (1974) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

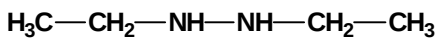
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 1615-80-1

Chem. Abstr. Name: *N*,*N*′-Diethylhydrazine

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_4H_{12}N_2$ Relative molecular mass: 88.2

1.1.3 *Physical properties* (for details, see IARC, 1974)

(*a*) *Boiling point*: 85–86°C

(*b*) *Conversion factor*: $mg/m^3 = 3.61 \times ppm$

1.2 Production and use

It is unlikely that 1,2-diethylhydrazine is produced and has uses outside chemical laboratories (IARC, 1974).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

1,2-Diethylhydrazine was tested for carcinogenicity in rats by subcutaneous administration, producing tumours of the brain, olfactory bulbs, mammary glands and liver, and by transplacental exposure, producing tumours of the brain, spinal cord and peripheral nervous system (IARC, 1974).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Toxic effects

No data were available to the Working Group.

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

1,2-Diethylhydrazine is weakly mutagenic to *Salmonella typhimurium* TA100 and particularly TA102, but only in the absence of an exogenous metabolic activation system. The activity in strain TA102 rapidly disappears with time of incubation, so that after 7 h it is halved and after 11 h, there is no activity.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of 1,2-diethylhydrazine were available.

There is *sufficient evidence* for the carcinogenicity of 1,2-diethylhydrazine in experimental animals.

1,2-Diethylhydrazine *is possibly carcinogenic to humans (Group 2B)*.

6. References

IARC (1974) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*, Volume 4, *Some Aromatic Amines, Hydrazine and Related Substances, N-Nitroso Compounds and Miscellaneous Alkylating Agents*, Lyon, pp. 153–157

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 62

Table 1. Genetic and related effects of 1,2-diethylhydrazine salts

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Shimizu *et al*. (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	–	220	Matsushita *et al*. (1993)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Shimizu *et al*. (1978)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Shimizu *et al*. (1978)
SAS, *Salmonella typhimurium* TA102, reverse mutation	+	–	NG	Matsushita *et al*. (1993)

[a] +, positive; (+), weak positive; –, negative
[b] LED, lowest effective dose; HID, highest ineffective dose; NG, not given; in-vitro tests, µg/mL

Matsushita, H., Jr, Endo, O., Matsushita, H., Yamamoto, M. & Mochizuki, M. (1993) Mutagenicity of alkylhydrazine oxalates in *Salmonella typhimurium* TA100 and TA102 demonstrated by modifying the growth conditions of the bacteria. *Mutation Res.*, **301**, 213–222

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DIETHYL SULFATE

Data were last evaluated in IARC (1992a).

1. Exposure Data

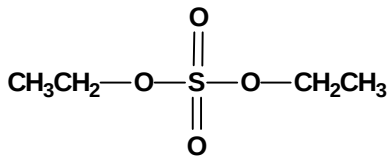
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 98503-29-8

Chem. Abstr. Name: Sulfuric acid, diethyl ester

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_4H_{10}O_4S$ Relative molecular mass: 154.19

1.1.3 *Physical properties* (for details, see IARC, 1992a)

(*a*) *Boiling point*: 208–209.5°C

(*b*) *Melting point*: –25°C

(*c*) *Conversion factor*: $mg/m^3 = 6.31 \times ppm$

1.2 Production, use and human exposure

Diethyl sulfate is manufactured from ethylene and sulfuric acid. It is used principally as an intermediate (ethylating agent) in the manufacture of dyes, pigments and textile chemicals, and as a finishing agent in textile production. It is an intermediate in the indirect hydration (strong acid) process for the preparation of synthetic ethanol from ethylene. No data were available on levels of occupational exposure to diethyl sulfate (IARC, 1992a).

2. Studies of Cancer in Humans

2.1 Cohort studies

Exposure to diethyl sulfate occurs in ethanol production. One cohort study at an isopropanol (see this volume) and ethanol manufacturing plant in the United States revealed

a significantly increased risk for laryngeal cancer (standardized mortality ratio [SMR], 5.0 (95% CI, 1.4–12.9), based on four cases; after including some additional groups of workers, the SMR was 3.2 (95% CI, 1.3–6.6) based on seven cases (IARC, 1992a).

A cohort study at two plants producing ethanol and isopropanol in the United States showed nonsignificant excess risks based on two cancers of the larynx and three buccal cavity and pharynx cancers in strong-acid workers (IARC, 1992a).

2.2 Case–control studies

A subsequent case–control study nested in an expanded cohort at the aforementioned isopropanol and ethanol manufacturing plant in the United States indicated that the increased risk of laryngeal cancer was related to exposure to sulfuric acid; the risk persisted even after exclusion of workers in the ethanol and isopropanol units (IARC, 1992a).

An association between estimated exposure to diethyl sulfate and risk for brain tumours was suggested in a case-control study of workers at a petrochemical plant in the United States. Seventeen glioma cases and six times as many controls were included and an odds ratio of 2.1 (90% confidence interval [CI], 0.6–7.7) was obtained; a parallel study of 21 cases (including the 17 of this other study) and with another set of controls showed no clear increase in risk, however (IARC, 1992a).

[No measurement of exposure to diethyl sulfate was available for the industrial processes investigated in the epidemiological studies. It is therefore difficult to assess the contribution of diethyl sulfate to the increased cancer risks. Furthermore, exposure to mists and vapours from strong inorganic acids, primarily sulfuric acid (see IARC, 1992b), may play a role in increasing these risks.]

3. Studies of Cancer in Experimental Animals

Diethyl sulfate was tested for carcinogenicity by oral and subcutaneous administration in one strain of rats. After subcutaneous administration, a high incidence of malignant tumours at the injection site was observed. Following oral gavage of diethyl sulfate, tumours of the forestomach were observed. A low incidence of malignant tumours of the nervous system was observed in the same strain of rats after prenatal exposure (IARC, 1992a).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

In male rats administered diethyl sulfate solution by gavage or by intraperitoneal or subcutaneous injection, ethylmercapturic acid and a sulfoxide were identified as metabolites (IARC, 1992a).

4.2 Toxic effects

Diethyl sulfate is a strong skin irritant in animals. No other data were available to the Working Group (IARC, 1992a)

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Groups of adult female (C3H/R1 × 101/R1)F_1 mice were treated with diethyl sulfate by a single intraperitoneal injection of 150 mg/kg bw within four days before mating or at 1, 6, 9 or 25 h after mating with untreated males. Control groups were treated with vehicle only (0.1 mL dimethyl sulfoxide) four days before mating or 6 or 25 h after mating. Control and treated females were killed and their uterine contents examined 17–18 days after mating. Resorptions were significantly increased ($p < 0.01$) following treatment 1, 6 or 9 h after mating (30%, 24% and 14%, respectively) in comparison with available control group frequencies of 4.1%, 10% and 3.9%. Treatment had no effect if given before mating or 25 h after mating. Midgestational and late deaths were significantly increased at 1 h (15% and 14%, respectively) and at 6 h (16% and 21%, respectively), in comparison with available control frequencies of 0.9% and 1.3%. No effect was observed at other times. The incidences of live fetuses with malformations were (numbers of fetuses examined in parentheses): before mating control, 0.6% (338), treated, 0.2% (441); 1 h after mating control, 0.3% (371), treated, 15% (113); 6 h treated, 25% (157); 9 h treated, 3% (213); 25 h treated, 2% (314). In contrast to other alkylating agents with similar DNA-binding properties but different effects upon exposed zygotes, there appeared to be no site-specific alkylation product identifiable as the critical target. The authors speculated that the lethal effects were due to an epigenetic disruption of gene expression during early embryogenesis (Generoso *et al.*, 1991).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

As previously summarized, diethyl sulfate induced mutation and DNA damage in bacteria and induced reverse mutation and mitotic recombination in yeast. In plant cells, diethyl sulfate induced chromosomal aberrations. In a single study, diethyl sulfate did not induce heritable translocation in *Drosophila melanogaster* but did induce autosomal recessive lethal mutations, sex-linked recessive lethal mutations and genetic crossing-over. In cultured mammalian cells, diethyl sulfate induced chromosomal aberrations, micronucleus formation, sister chromatid exchanges, forward mutation and DNA single-strand breaks; it also induced unscheduled DNA synthesis in primary cultures of rat

Table 1. Genetic and related effects of diethyl sulfate

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage, induction, SOS repair test, DNA strand breaks, cross-links or related damage	+	NT	30	De Oliveira *et al*. (1986)
PRB, Prophage, induction, SOS repair test, DNA strand breaks, cross-links or related damage	NT	+	0.2	Nakamura *et al*. (1987)
PRB, Prophage, induction, SOS repair test, DNA strand breaks, cross-links or related damage	+	NT	40	Barbé *et al*. (1983)
PRB, *Salmonella typhimurium* TA1535/pSK1002, *umu* test,	+	NT	1170	Vericat *et al*. (1986)
SAF, *Salmonella typhimurium* SV50, forward mutation, arabinose resistance test (Ara test)	+	NT	75	Xu *et al*. (1984)
SAF, *Salmonella typhimurium* TM677, forward mutation	+	NT	65	Skopek & Thilly (1983)
SAF, *Salmonella typhimurium* BA13 and BAL13, forward mutation, arabinose resistance test (Ara test)	+	NT	154	Roldán-Arjona *et al*. (1990)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	NG	McCann *et al*. (1975)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	2500	Waskell (1978)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	NG	Probst *et al*. (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	NG	McCann *et al*. (1975)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	+	NT	2500	Levin *et al*. (1982)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	2500	Levin *et al*. (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	Waskell (1978)
SAS, *Salmonella typhimurium* TA97 (*hisO1242 hisD6610* pKM101), reverse mutation	+	NT	2500	Levin *et al*. (1982)
SAS, *Salmonella typhimurium* TA90 (*hisO1242 hisD6610*), reverse mutation	+	NT	2500	Levin *et al*. (1982)
SAS, *Salmonella typhimurium* TA88 (*hisO1242 hisD6610*), reverse mutation	+	NT	5000	Levin *et al*. (1982)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SAS, *Salmonella typhimurium* TA2637 (*hisC3076* pKM101), reverse mutation	+	NT	2500	Levin *et al*. (1982)
SAS, *Salmonella typhimurium* TR3243 (*hisD6610*), reverse mutation	+	NT	5000	Levin *et al*. (1982)
SAS, *Salmonella typhimurium hisC3076*, reverse mutation	+	NT	5000	Levin *et al*. (1982)
SAS, *Salmonella typhimurium hisD3052*, reverse mutation	–	NT	5000	Levin *et al*. (1982)
SAS, *Salmonella typhimurium* TS1121 (*aroC321 hisG46*), reverse mutation	+	NT	600	Hoffmann *et al*. (1988)
SAS, *Salmonella typhimurium* TS1157 (*aroC321 hisG46* pKM101), reverse mutation	+	NT	600	Hoffmann *et al*. (1988)
ECK, *Escherichia coli* K12, forward or reverse mutation	+	NT	308	Mohn & Van Zeeland (1985)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	+	NT	NG	Probst *et al*. (1981)
EC2, *Escherichia coli* WP2, reverse mutation	+	NT	NG	Probst *et al*. (1981)
SCH, *Saccharomyces cerevisiae*, homozygosis by mitotic recombination or gene conversion	+	NT	4500	Zimmermann *et al*. (1966)
SCR, *Saccharomyces cerevisiae*, reverse mutation	+	NT	4500	Zimmermann *et al*. (1966)
ACC, *Allium cepa*, chromosomal aberrations	–	NT	4600	Gohil & Kaul (1983)
PLC, Plant cells, chromosomal aberrations	+	NT	1200	Floria & Ghiorghita (1980)
PLC, Plant cells, chromosomal aberrations	+	NT	3850	Gohil & Kaul (1983)
DMG, *Drosophila melanogaster*, genetic crossing-over or recombination	+		6000 feed	Pelecanos (1966)
DMM, *Drosophila melanogaster*, autosomal recessive lethal mutations	+		6000 feed	Pelecanos (1966)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		NG	Abraham *et al*. (1979)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		1500 inj	Vogel (1989)
DMC, *Drosophila melanogaster*, heritable translocations	–		6000 feed	Pelecanos (1966)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DIA, DNA strand breaks, cross-links or related damage, Chinese hamster ovary CHO cells *in vitro*	+	NT	154	Abbondandolo *et al*. (1982)
DIA, DNA strand breaks, cross-links or related damage, Chinese hamster ovary CHO cells *in vitro*	+	NT	385	Dogliotti *et al*. (1984)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	NT	15.4	Probst *et al*. (1981)
GCO, Gene mutation, Chinese hamster ovary CHO cells *in vitro*	+	NT	46	Couch *et al*. (1978)
GCO, Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	+	NT	154	Bignami *et al*. (1988)
GCO, Gene mutation, Chinese hamster ovary CHO cells, Na/K ATPase *in vitro*	+	NT	154	Bignami *et al*. (1988)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	+	NT	308	Mohn & Van Zeeland (1985)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	+	NT	100	Nishi *et al*. (1984)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	NT	100	Nishi *et al*. (1984)
MIA, Micronucleus test, Chinese hamster lung V79 cells *in vitro*	+	NT	154	Bonatti *et al*. (1986)
MIA, Micronucleus test, Chinese hamster lung V79 cells *in vitro*	+	NT	40	De Ferrari *et al*. (1988)
MIA, Micronucleus test, Chinese hamster lung V79 cells *in vitro*	+	NT	460	Nüsse *et al*. (1989)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	NT	100	Asita (1989)
DIH, DNA strand breaks, cross-links or related damage, human leukocytes *in vitro*	+	NT	154	Schutte *et al*. (1988)
MIH, Micronucleus test, human lymphocytes *in vitro*	+	NT	154	De Ferrari *et al*. (1988)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	154	De Ferrari *et al*. (1988)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
AIH, Aneuploidy, human lymphocytes *in vitro*	+	NT	15	De Ferrari *et al*. (1988)
DVA, DNA strand breaks, cross-links or related damage, SD rat brain cells *in vivo*	+		40 iv × 1	Robbiano & Brambilla (1987)
MST, Mouse spot test, C57BL/6 Jena XT mice *in vivo*	?		225 ip × 1	Braun *et al*. (1984)
SLP, Mouse specific locus test, (101/E1 × C3H/E1)F_1 mice, post-spermatogonia *in vivo*	(+)		200 ip × 1	Ehling & Neuhäuser-Klaus (1988)
MVM, Micronucleus test, ddY mice *in vivo*	+		400 ip × 1	Asita *et al*. (1992)
COE, Chromosomal aberrations, NMRI mice embryos *in vivo*	+		150 ip × 1	Braun *et al*. (1986)
DLM, Dominant lethal test, (101/E1 × C3H/E1)F_1 mice *in vivo*	+		100 ip × 1	Ehling & Neuhäuser-Klaus (1988)
Micronucleus test, *Pleurodeles waltl*, larvae erythrocytes	+		6 (water)	Jaylet *et al*. (1986)
BVD, Binding (covalent) to DNA, (102/E1 × C3H/E1)F_1 mouse germ/testis/bone-marrow/liver *in vivo*	+		48 ip × 1	Van Zeeland *et al*. (1990)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; inj., injection; ip, intraperitoneally; iv, intravenous

hepatocytes. Diethyl sulfate induced chromosomal aberrations, micronucleus formation and aneuploidy in cultured human lymphocytes. It induced alkali-labile sites in cultured human leukocytes in one study. It was clastogenic in mice and newts (*Pleurodeles waltl*), induced DNA damage in mice and rats and ethylated DNA in mice. Diethyl sulfate induced specific locus mutations in mouse germ-line cells. In mice, diethyl sulfate alkylated DNA to produce mainly *N*7-ethylguanine in germ cells, testis tubules, bone marrow and liver (IARC, 1992a).

DNA base sequence changes were analysed in 31 transmissible *vermilion* mutants recovered from *Drosophila melanogaster*, the male germ cells of which had been treated with diethyl sulfate. There were 93% base-pair substitutions and 7% deletions. The most frequent base-pair changes were GC→AT transitions (73%) and AT→TA transversions (10%) (Sierra *et al.*, 1993).

5. Evaluation

There is *inadequate evidence* for the carcinogenicity in humans of diethyl sulfate.

There is *sufficient evidence* for the carcinogenicity of diethyl sulfate in experimental animals.

Overall evaluation

Diethyl sulfate is *probably carcinogenic to humans (Group 2A)*.

In making the overall evaluation, the Working Group took into account that diethyl sulfate is a strong direct-acting alkylating agent which ethylates DNA and that, as a result, it is genotoxic in virtually all test systems examined, including induction of potent effects in somatic and germ cells of mammals exposed *in vivo*.

6. References

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DIGLYDICYL RESORCINOL ETHER

Data were last reviewed in IARC (1985) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

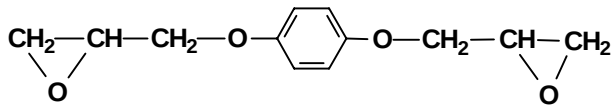
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 101-90-6

Chem. Abstr. Name: Oxirane, 2,2′-[phenylenebis(oxymethylene)]bis-

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{12}H_{14}O_4$ Relative molecular mass: 222.2

1.1.3 *Physical properties* (for details, see IARC, 1985)

(*a*) *Boiling point*: 172°C at 106 Pa

(*b*) *Conversion factor*: $mg/m^3 = 9.09 \times ppm$

1.2 Production and use

Diglycidyl resorcinol ether has been produced since at least 1974. It has only limited application, principally in the aerospace industry (IARC, 1985).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Diglycidyl resorcinol ether (technical grade) was tested for carcinogenicity by gavage in mice of one strain and in rats of one strain. It induced squamous-cell carcinomas and

papillomas of the forestomach in animals of both species. In female mice, an increased incidence of hepatocellular tumours was observed. In one experiment in mice, no skin tumour was observed after skin application (IARC, 1985).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Toxic effects

4.2.1 *Humans*

Diglycidyl resorcinol ether causes burns and skin sensitization (IARC, 1985).

4.2.2 *Experimental systems*

Application of diglycidyl resorcinol ether caused irritation to the eyes and skin of rabbits. Once-monthly intravenous injection of the compound at doses of 100–200 mg/kg bw produced a progressive lowering of the leukocyte count in monkeys. Hyperkeratosis and basal-cell hyperplasia in the forestomach were observed in rats and mice exposed daily to intragastric doses of 12.5 mg/kg bw and higher for 13 weeks. In a two-year study in rats, dose-related bronchopneumonia occurred, which was not consistent with chemical pneumonitis, but was characterized by polymorphonuclear leukocytes in the alveoli. The compound also inhibited the growth of Walker carcinoma in rats (IARC, 1985). The occurrence of forestomach hyperkeratosis and epithelial cell proliferation was confirmed in a two-week study in rats with doses of 25 mg/kg bw, but not with 12 mg/kg bw (Ghanayem *et al*., 1986).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Diglycidyl resorcinol ether (technical grade) was mutagenic to *Salmonella typhimurium* and at the *tk* locus but not the *hprt* locus of cultured mouse lymphoma cells. It induced chromosomal aberrations in Chinese hamster ovary CHO cells, but did not increase the proportion of micronucleated cells in the bone marrow of treated mice.

Table 1. Genetic and related effects of diglycidyl resorcinol ether (technical grade)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	25	Seiler (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	16.5	US National Toxicology Program (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	5	US National Toxicology Program (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	167	US National Toxicology Program (1986)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	167	US National Toxicology Program (1986)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	0.125	McGregor *et al*. (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	0.1	McGregor *et al*. (1996)
G51, Gene mutation, mouse lymphoma L5178Y cells, *hprt* locus *in vitro*	–	NT	0.4	McGregor *et al*. (1996)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	NT	2.5	Seiler (1984)
MVM, Micronucleus test, ICR mouse bone-marrow cells *in vivo*	–		600 po × 1	Seiler (1984)

[a] +, positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; po, oral

5. Evaluation

No epidemiological data relevant to the carcinogenicity of diglycidyl resorcinol ether were available.

There is *sufficient evidence* for the carcinogenicity of a technical grade of diglycidyl resorcinol ether in experimental animals.

Overall evaluation

Diglycidyl resorcinol ether (technical grade) is *possibly carcinogenic to humans (Group 2B)*.

6. References

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DIISOPROPYL SULFATE

Data were last evaluated in IARC (1992).

1. Exposure Data

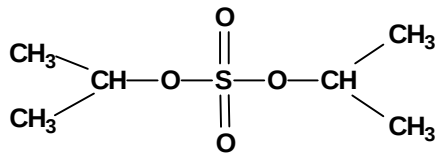
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 2973-10-6

Chem. Abstr. Name: Sulfuric acid, bis(1-methylethyl)ester

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_6H_{14}O_4S$ Relative molecular mass: 182.24

1.1.3 *Physical properties* (for details, see IARC, 1992)

(*a*) *Boiling point*: 94°C at 931 Pa; 106°C at 2394 Pa (decomposes)

(*b*) *Melting point*: –19°C

(*c*) *Conversion factor*: $mg/m^3 = 7.45 \times ppm$

1.2 Production and use

Diisopropyl sulfate is an intermediate in the indirect hydration (strong- or weak-acid) process for the preparation of isopropanol from propylene. It has no other known industrial use. No data were available on levels of occupational exposure to diisopropyl sulfate (IARC, 1992).

2. Studies of Cancer in Humans

2.1 Cohort studies

Exposure to diisopropyl sulfate occurs in production of isopropanol (see this volume). An early cohort study of isopropanol manufacture using the strong-acid process at a

petrochemical plant in the United States showed a significant excess risk for nasal sinus cancer. An increased risk for cancer of the buccal cavity and pharynx was suggested in a cohort of workers at an isopropanol unit in the United States. A cohort study at an isopropanol plant in the United Kingdom indicated an increased risk for nasal cancer (based on one case only) and for brain tumours (IARC, 1992).

As described in the monograph on diethyl sulfate (see this volume), a cohort study at an isopropanol and ethanol manufacturing plant in the United States revealed an increased risk for laryngeal cancer. As also mentioned in the same monograph, a cohort study at a plant producing ethanol and isopropanol in the United States suggested an increased risk for cancers of the larynx, buccal cavity and pharynx in strong-acid workers (IARC, 1992).

2.2 Case–control studies

A subsequent case–control study nested in an expanded cohort at the aforementioned isopropanol and ethanol manufacturing plant in the United States indicated that the increased risk of laryngeal cancer was related to exposure to sulfuric acid; the risk persisted even after exclusion of workers in the ethanol and isopropanol units (IARC, 1992).

[No measurement of exposure to diisopropyl sulfate was available for the industrial processes investigated in the epidemiological studies. It is therefore difficult to assess the contribution of diisopropyl sulfate to the increased cancer risks. Furthermore, exposure to mists and vapours from strong inorganic acids, primarily sulfuric acid, probably plays a role in increasing these risks.]

3. Studies of Cancer in Experimental Animals

Diisopropyl sulfate was tested for carcinogenicity by subcutaneous injection in one strain of rats and by skin application in one strain of mice. It produced local sarcomas in rats and skin papillomas and carcinomas in mice. In a screening study in two strains of mice, an increased incidence of lung adenomas was observed following subcutaneous injection (IARC, 1992).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

No data were available to the Working Group.

5. Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of diisopropyl sulfate.

There is *sufficient evidence* in experimental animals for the carcinogenicity of diisopropyl sulfate.

Overall evaluation

Diisopropyl sulfate is *possibly carcinogenic to humans (Group 2B)*.

6. Reference

IARC (1992) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 54, *Occupational Exposures to Mists and Vapours from Strong Inorganic Acids; and Other Industrial Chemicals*, Lyon, pp. 41–130, 229–235

1,1-DIMETHYLHYDRAZINE

Data were last reviewed in IARC (1974) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

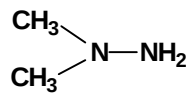
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 57-14-7
Chem. Abstr. Name: 1,1-Dimethylhydrazine
IUPAC Systematic Name: 1,1-Dimethylhydrazine
Synonyms: Dimazine; dimazin; UDMH

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2H_8N_2$ Relative molecular mass: 60.10

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Flammable, hygroscopic liquid. Fumes in air and gradually turns yellow. Characteristic ammonia-like fishy odour of aliphatic hydrazines (Budavari, 1996)
(*b*) *Boiling-point*: 63.9°C (Lide, 1995)
(*c*) *Melting-point*: –58°C (Lide, 1995)
(*d*) *Solubility*: Miscible with water with evolution of heat. Also miscible with ethanol, diethyl ether, dimethylformamide and hydrocarbons (Budavari, 1996)
(*e*) *Vapour pressure*: 17 kPa at 25°C; relative vapour density (air = 1), 2.07 (Verschueren, 1996)
(*f*) *Flash point*: –15°C, closed cup (Lewis, 1993)
(*g*) *Explosive limits*: upper limits, 95%; lower, 2% by volume in air (American Conference of Governmental Industrial Hygienists, 1991)
(*h*) *Conversion factor*: $mg/m^3 = 2.46 \times ppm$

1.2 Production and use

1,1-Dimethylhydrazine is used as a component of jet and rocket fuels, for chemical synthesis, as a stabilizer for organic peroxide fuel additives, as an absorbent for acid gases, in photography and as a plant growth control agent (Lewis, 1993).

1.3 Occurrence

1.3.1 *Occupational exposure*

No data were available to the Working Group.

1.3.2 *Environmental occurrence*

Production and use of 1,1-dimethylhydrazine may result in its release to the environment (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 0.025 mg/m^3 as the threshold limit value for occupational exposures to 1,1-dimethylhydrazine in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for 1,1-dimethylhydrazine in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

1,1-Dimethylhydrazine was tested for carcinogenicity in mice after oral administration, producing tumours at various sites including a high incidence of vascular tumours. The observation of a few liver tumours after high oral doses of 1,1-dimethylhydrazine occurring in rats after a long latent period was inadequate for evaluation of the carcinogenic effect in this species (IARC, 1974).

3.1 Subcutaneous injection

Hamster: Groups of 15 male and 15 female European hamsters (*Cricetus cricetus*) [age unspecified] were given weekly subcutaneous injections of 1/10 of the LD_{50} (LD_{50}: 373 mg/kg bw for males and 325 mg/kg bw for females) of 1,1-dimethylhydrazine in saline for life. A group of eight males and eight females served as controls. Hamsters were observed until spontaneous death. Six males and six females treated with 1,1-dimethylhydrazine developed peripheral nerve sheath tumours (neurofibrosarcoma,

melanotic and unpigmented schwannoma). No tumour of this type was observed in the untreated controls (Ernst *et al*., 1987).

Groups of 12 male and 12 female Syrian golden hamsters (*Mesocricetus auratus*) were given subcutaneous injections of 8, 17 or 35 (1/10 of the LD_{50}) mg/kg bw 1,1-dimethylhydrazine weekly for life. A group of seven males and seven females given saline served as controls. Nonsignificant increases in the incidence of malignant lymphomas in females given the intermediate dose (4/12 versus 1/7) and that of benign phaeochromocytoma in males given the lowest dose (4/12 versus 1/7) were observed (Jeong & Kamino, 1993).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Godoy *et al*. (1984) have studied the metabolic fate of 1,1-dimethylhydrazine in the context of its role as a reductive metabolite of the carcinogen *N*-nitrosodimethylamine. In rat liver slices, [^{14}C]dimethylhydrazine is activated to metabolites which bind to nucleic acids. In rat liver microsomes and 9000 × *g* supernatants (microsomes plus cytosol), it is converted to formaldehyde. In microsomes, this process has the characteristics of a cytochrome P450-mediated reaction, requiring NADPH and oxygen, but in the 9000 × *g* supernatant, this cofactor dependence was not seen, suggesting that the reaction was non-enzymatic. Metabolism in these systems also resulted in covalent binding of radioactivity that showed comparable enzymatic and non-enzymatic components. These data show that 1,1-dimethylhydrazine might not be a detoxication product of *N*-nitrosodimethylamine but contributes to its covalent binding to nucleic acids and proteins.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

In female Sprague-Dawley rats, daily intraperitoneal injections of 10, 30, 50 or 70 mg/kg bw 1,1-dimethylhydrazine resulted in the death of 0, 5, 6 and 9 out of 10 animals per group (Cornish & Hartung, 1969). Surviving animals showed diuresis, increased serum transaminase levels and histopathological signs of mild kidney damage.

Daily intraperitoneal injection of BALB/c and C57BL/6 mice with 5, 10, 25, 50 or 75 mg/kg bw 1,1-dimethylhydrazine for seven days resulted in a significant increase in one-way mixed lymphocyte response (MLR) (Tarr *et al*., 1988). When only the responder

mice (C57BL/6) were treated, the response was also increased. The authors suggested that B cells and/or macrophages may represent a target cell subpopulation for the immunoenhancing effect of 1,1-dimethylhydrazine. Since prostaglandin E_2 production by adherent splenocytes (enriched for macrophages) *in vitro* was significantly reduced at 10 μg/mL of 1,1-dimethylhydrazine, the authors suggested that inhibition of prostaglandin E_2 synthesis, a suppressor of the MLR, might explain the immunoenhancement by 1,1-dimethylhydrazine.

The 48-h concanavalin A-induced lymphoblastogenic responses in splenocytes isolated from BALB/c mice treated with *Corynebacterium parvum* and 1,1-dimethylhydrazine mice were significantly increased in comparison with *C. parvum* treatment alone (Frazier *et al*., 1992), indicating that 1,1-dimethylhydrazine can overcome certain types of immunosuppression.

In murine splenocytes in culture, however, 10–50 μg/mL 1,1-dimethylhydrazine inhibited concanavalin A-stimulated DNA synthesis (Bauer *et al*., 1990). Similar suppression was observed in interleukin 2-dependent CTLL-20 cells when DNA synthesis was stimulated with interleukin 2.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Keller *et al*. (1984) investigated the embryotoxicity and teratogenicity of 1,1-dimethylhydrazine in pregnant Fischer 344 rats. In the high-dose group treated intraperitoneally with 60 mg/kg bw 1,1-dimethylhydrazine per day on days 6–15 of gestation, maternal weight gains and mean fetal weights were significantly reduced. The numbers of implants and of viable fetuses per litter were also less than in controls, although not reduced significantly, and the number of malformations (unfused ossification centres of vertebrae, anophthalmia or severe microphthalmia, hydronephrosis, agenesis of kidney, hydrocephalic fetus, unossified sternebrae) was moderately increased. At a daily dose of 30 mg/kg bw, these effects were not observed.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

In mammalian cells treated *in vitro*, 1,1-dimethylhydrazine induced gene mutations in Chinese hamster lung V79 cells and in mouse lymphoma L5178Y cells, chromosomal aberrations in Chinese hamster ovary cells and unscheduled DNA synthesis in mouse hepatocytes but not in rat hepatocytes. In a single study, it induced somatic mutations in *Drosophila melanogaster*. There is conflicting evidence as to its mutagenicity to bacteria.

Table 1. Genetic and related effects of 1,1-dimethylhydrazine

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage	NT	–	17000	Ho & Ho (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2000	Brusick & Matheson (1976)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	+	250	Bruce & Heddle (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1500	Von Wright & Tikkanen (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	4800	De Flora (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	4015	Parodi *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	De Flora *et al*. (1984)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	–	NG	Matsushita *et al.* (1993)
SA2, *Salmonella typhimurium* TA102, reverse mutation	+	–	NG	Matsushita *et al.* (1993)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	(+)	NT	5000	Tosk *et al.* (1979)
SA3, *Salmonella typhimurium* TA1530, reverse mutation	–	–	15000	Bartsch *et al*. (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2000	Brusick & Matheson (1976)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	250	Bruce & Heddle (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	4800	De Flora (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	4015	Parodi *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Rogan *et al*. (1982)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	NG	De Flora *et al*. (1984)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2000	Brusick & Matheson (1976)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	250	Bruce & Heddle (1979)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	4800	De Flora (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	4015	Parodi *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Rogan *et al*. (1982)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	De Flora *et al*. (1984)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2000	Brusick & Matheson (1976)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	4800	De Flora (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	4015	Parodi *et al.* (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	NG	De Flora *et al*. (1984)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	4800	Brusick & Matheson (1976)
SA9, *Salmonella typhimurium* TA98, reverse mutation	(+)	(+)	NG	De Flora (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	(+)	(+)	1262	Parodi *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	(+)	(+)	NG	De Flora *et al*. (1984)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	+	250	Bruce & Heddle (1979)
SAS, *Salmonella typhimurium* TAG46, reverse mutation	–	–	15000	Bartsch *et al*. (1980)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	–	2000	Brusick & Matheson (1976)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	NT	120	Von Wright & Tikkanen (1980)
SCG, *Saccharomyces cerevisiae*, gene conversion	–	–	2000	Brusick & Matheson (1976)
ANF, *Aspergillus nidulans*, forward mutation	+	NT	100	Bignami *et al*. (1981)
DMM, *Drosophila melanogaster*, somatic mutation (*white/white+)*	+		150 feed	Vogel & Nivard (1993)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		1200 inj	Zijlstra & Vogel (1988)
DIA, DNA strand breaks, rat hepatocytes *in vitro*	+	NT	2	Sina *et al*. (1983)
URP, Unscheduled DNA synthesis, ACI/N rat primary hepatocytes *in vitro*	–	NT	60	Mori *et al.* (1988)
UIA, Unscheduled DNA synthesis, C3HeN mouse primary hepatocytes *in vitro*	+	NT	60	Mori *et al.* (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	80	Brusick & Matheson (1976)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	6	Rogers & Back (1981)
G51, Gene mutation, mouse lymphoma L5178Y cells, ouabain resistance and cytosine arabinoside resistance *in vitro*	–	NT	300	Rogers & Back (1981)
G9H Gene mutation, Chinese hamster lung V79 cells, *hprt* locus (metabolic activation with rat liver perfusate) *in vitro*	–	+	300	Beije *et al.* (1984)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	(+)	20	JETOC (1997)
HMM, Host-mediated assay, *Salmonella typhimurium* TA1950 in NMRI mouse host	–		140 po × 1	Von Wright & Tikkanen (1980)
DVA, DNA fragmentation, Swiss albino mouse lung *in vivo*	+		42 ip × 5	Parodi *et al.* (1981)
DVA, DNA fragmentation, Swiss albino mouse liver *in vivo*	+		42 ip × 5	Parodi *et al.* (1981)
UVR, Unscheduled DNA synthesis, Fischer 344 rat kidney cells *in vivo*	–		50 ip × 1	Tyson & Mirsalis (1985)
MVM, Micronucleus test, CD1 mouse splenocytes *in vivo*	+		13.8 ip × 1	Benning *et al.* (1994)
MVM, Micronucleus test, CD1/CR mouse bone-marrow cells *in vivo*	–		83 ip × 1	Cliet *et al.* (1993)
MVM, Micronucleus test, CD1/CR mouse spermatids *in vivo*	+		83 ip × 1	Cliet *et al.* (1993)
MVM, Micronucleus test, CD1/CR mouse hepatocytes *in vivo*	+		14 ip × 2	Cliet *et al.* (1989)
MVM, Micronucleus test, (C57BL/6 × C3H/He) F_1 mouse bone-marrow *in vivo*	–		500 ip × 5	Bruce & Heddle (1979)
MVM, Micronucleus test, mouse bone marrow (BALB/c AnNCrj) *in vivo*	–		20 ip × 1	Suzuki *et al.* (1994)
DLM, Dominant lethal test, ICR/Ha Swiss mice *in vivo*	–		63 ip × 1	Epstein *et al.* (1972)
DLM, Dominant lethal test, mice *in vivo*	–		12.5 ip × 5	Brusick & Matheson (1976)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BVD, Binding (covalent) to DNA, formation of *N*7-methylguanine in Sprague-Dawley rat liver DNA *in vivo*	+		19 po × 1	Sagelsdorff *et al.* (1988)
SPM, Sperm abnormality test, (C57BL/6 × C3H/He) F_1 mouse *in vivo*	–		500 ip × 5	Bruce & Heddle (1979)
SPM, Sperm morphology, (C57BL/6 × C3H/He) F_1 mice *in vivo*	–		100 ip × 5	Wyrobek & Bruce (1975)
Colonic nuclear aberration assay in C57BL/6J mice, *in vivo*	–		100 po × 1	Wargovich *et al.* (1983)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; NG, not given; inj, injection; ip, intraperitoneal; po, oral

In a single study, 1,1-dimethylhydrazine formed *N*7-methylguanine with DNA in the liver of rats treated *in vivo*. Given to mice *in vivo*, it did not induce sperm abnormalities, nuclear aberrations in the colon or micronucleus formation in the bone marrow, but, in single studies, it did induce micronucleus formation in spermatids, splenocytes and hepatocytes. In one study, 1,1-dimethylhydrazine induced DNA fragmentation in lung and in liver of mice *in vivo.* It failed to induce unscheduled DNA synthesis in kidney cells of rats in a single study conducted *in vivo*. It produced negative results in a host-mediated assay using mice.

5. Evaluation

No epidemiological data on the carcinogenicity of 1,1-dimethylhydrazine were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,1-dimethylhydrazine.

Overall evaluation

1,1-Dimethylhydrazine is *possibly carcinogenic to humans (Group 2B).*

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DIMETHYL HYDROGEN PHOSPHITE

Data were last evaluated in IARC (1990).

1. Exposure Data

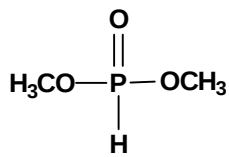
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 868-85-9

Chem. Abstr. Name: Dimethyl phosphonate

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2H_7O_3P$ Relative molecular mass: 110.05

1.1.3 *Physical properties* (for details, see IARC, 1990)

(*a*) *Boiling point:* 170–17°C at 2.6 kPa

(*b*) *Conversion factor*: $mg/m^3 = 4.52 \times ppm$

1.2 Production, use and human exposure

Dimethyl hydrogen phosphite is used as a flame retardant on nylon 6 fibres, as a chemical intermediate in the production of pesticides and in lubricant additives and adhesives. No data on occupational exposure levels were available. A potential source of exposure to this chemical is from its occurrence as a degradation product of the chemical intermediate trimethyl phosphite and of pesticides such as trichlorphon and malathion (IARC, 1990).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Dimethyl hydrogen phosphite was tested for carcinogenicity by oral administration in one strain of mice and in one strain of rats. In rats, it caused an increase in the incidence of alveolar/bronchiolar carcinomas in animals of each sex and of squamous-cell carcinomas of the lung and of papillomas and carcinomas of the forestomach in males (IARC, 1990).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

As previously summarized, rats treated with 200 mg/kg bw dimethyl hydrogen phosphite by gavage developed chemical pneumonia, and lung adenomatous and alveolar epithelial hyperplasia. They also showed hyperkeratosis, submucosal oedema and hyperplasia of the forestomach and urinary bladder calculi. Serum angiotensin-converting enzyme level and soluble forestomach nonprotein sulfhydryls were increased, whereas renal and hepatic microsomal cytochrome P450 activity and some phase II enzyme activities in liver, kidney, lung and stomach remained unchanged.

In mice, hepatocellular vacuolization, cardiac mineralization, lung congestion, and testicular calcification and atrophy were reported (IARC, 1990).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental data* (see Table 1 for references)

In single studies, dimethyl hydrogen phosphite induced sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells in culture and mutations in mouse cells in culture but did not induce sex-linked recessive lethal mutations in

Table 1. Genetic and related effects of dimethyl hydrogen phosphite

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	US National Toxicology Program (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	US National Toxicology Program (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000	US National Toxicology Program (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	US National Toxicology Program (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		1500 ppm inj.	Woodruff *et al*. (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		650 ppm feed	Woodruff *et al*. (1985)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–[c]	NT	1	Shaddock *et al*. (1990)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	+	2100	McGregor *et al*. (1988)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	250	Tennant *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	+	1600	Tennant *et al*. (1987)

[a] +, positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; inj., injection
[c] UDS induced in Aroclor-induced rat hepatocytes

Drosophila melanogaster. It was not mutagenic to bacteria in the presence or absence of an exogenous metabolic system.

Dimethyl hydrogen phosphite has been reported to induce unscheduled DNA synthesis in hepatocytes of rats that had been treated with Aroclor or 3-methylcholanthrene.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of dimethyl hydrogen phosphite were available.

There is *limited evidence* for the carcinogenicity of dimethyl hydrogen phosphite in experimental animals.

Overall evaluation

Dimethyl hydrogen phosphite is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

IARC (1990) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Volume 48, *Some Flame Retardants and Textile Chemicals, and Exposures in the Textile Manufacturing Industry*, Lyon, pp. 85–93

McGregor, D.B., Brown, A., Cattanach, P., Edwards, I., McBride, D. & Caspary, W.J. (1988) Responses of the L5178T tk^+/tk^- mouse lymphoma cell forward mutation assay. II: 18 coded chemicals. *Environ. mol. Mutag.*, **11**, 91–118

Shaddock, J.G., Robinson, B.Y. & Casciano, D.A. (1990) Effect of pretreatment with hepatic mixed-function oxidase inducers on the genotoxicity of four rat carcinogens in the hepatocyte/DNA repair assay. *Mutagenesis*, **5**, 387–391

Tennant, R.W., Margolin, B.H., Shelby, M.D., Zeiger, E., Haseman, J.K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B. & Minor, R. (1987) Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. *Science*, **236**, 933–941

United States National Toxicology Program (1985) *Toxicology and Carcinogenesis Studies of Dimethyl Hydrogen Phosphite (CAS No. 868-85-9) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)* (Technical Report No. 287; HIN Publ. No. 86-2543), Research Triangle Park, NC, United States Department of Health and Human Services

Woodruff, R.C., Mason, J.M., Valencia, R. & Zimmering, S. (1985) Chemical mutagenesis testing in *Drosophila*. V. Results of 53 coded compounds tested for the National Toxicology Program. *Environ. Mutag.*, **7**, 677–702

3,4-EPOXY-6-METHYLCYCLOHEXYLMETHYL 3,4-EPOXY-6-METHYLCYCLOHEXANE CARBOXYLATE

Data were last reviewed in IARC (1976) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

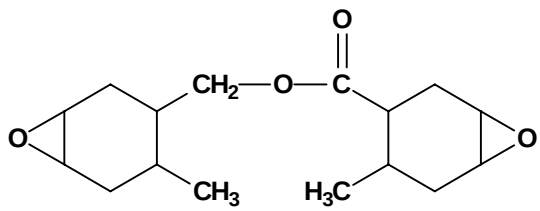
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abst. Serv. Reg. No.: 141-37-7

Systematic name: 4-Methyl-7-oxabicyclo[4.1.0]heptane-3-carboxylic acid, 4-methyl-7-oxabicyclo[4.1.0]hept-3-yl methyl ester

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{16}H_{24}O_4$ Relative molecular mass: 280.4

1.1.3 *Physical properties* (for details, see IARC, 1976)

(*a*) *Boiling-point*: 215°C at 665 Pa

(*b*) *Conversion factor*: $mg/m^3 = 11.47 \times ppm$

1.2 Production and use

This chemical was produced by one company in the United States until 1965 for use in the manufacture of cured resins (IARC, 1976).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3,4-Epoxy-6-methylcyclohexylmethyl 3,4-epoxy-6-methylcyclohexane carboxylate was tested for carcinogenicity in mice by skin application: it produced skin carcinomas (IARC, 1976).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

No data were available to the Working Group.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of 3,4-epoxy-6-methylcyclohexylmethyl 3,4-epoxy-6-methylcyclohexane carboxylate were available.

There is *limited evidence* in experimental animals for the carcinogenicity of 3,4-epoxy-6-methylcyclohexylmethyl 3,4-epoxy-6-methylcyclohexane carboxylate.

Overall evaluation

3,4-Epoxy-6-methylcyclohexylmethyl 3,4-epoxy-6-methylcyclohexane carboxylate is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

IARC (1976) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 11, *Cadmium, Nickel, Some Epoxides, Miscellaneous Industrial Chemicals and General Considerations on Volatile Anaesthetics*, Lyon, pp. 147–151

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 63

cis-9,10-EPOXYSTEARIC ACID

Data were last reviewed in IARC (1976) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

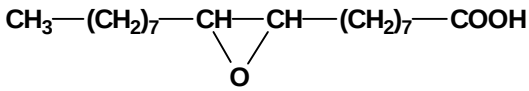
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abst. Serv. Reg. No.: 2443-39-2

Systematic name: *cis*-3-Octyl-oxiraneoctanoic acid

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{18}H_{34}O_3$ Relative molecular mass: 298.5

1.1.3 *Physical properties* (for details, see IARC, 1976)

(*a*) *Melting-point*: 59.5–59.8°C

(*b*) *Conversion factor*: $mg/m^3 = 12.29 \times ppm$

1.2 Production and use

cis-9,10-Epoxystearic acid is produced only in small quantities for research purposes, but it can occur in seed oils (e.g., sunflower) upon prolonged storage (IARC, 1976).

2. Studies of Cancer in Humans

No data were available to the working group.

3. Studies of Cancer in Experimental Animals

cis-9,10-Epoxystearic acid was tested for carcinogenicity in mice by skin application and subcutaneous injection, and in rats by subcutaneous injection. No significant increase in the incidence of tumours was associated with treatment (IARC, 1976).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

9,10-Epoxystearic acid has been detected in human urine at concentrations of about 2 nmol/L (Ulsaker & Teien, 1995), probably occurring as a result of the oxidation of unsaturated fatty acid by cytochrome P450 (Laniado-Schwartzman *et al.*, 1988).

4.1.2 *Experimental systems*

No data were available to the Working Group.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Daily oral doses of *cis*-9,10-epoxystearic acid of up to 250 mg/kg bw administered by gavage to male and female Sprague-Dawley rats did not affect growth rate, food consumption, organ weights, haematology, blood chemistry or histology. Deaths which occurred during the experiment were not substance-related, but the result of mechanical injury during dosing (Chu *et al.*, 1980).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental data* (see Table 1 for references)

cis-9,10-Epoxystearic acid was not mutagenic in *Salmonella typhimurium* in the presence or in the absence of exogenous metabolic activation.

Table 1. Genetic and related effects of *cis*-9,10-epoxystearic acid

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	2500	Chu *et al*. (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	2500	Chu *et al*. (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	125	Chu *et al*. (1980)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	2500	Chu *et al*. (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	2500	Chu *et al*. (1980)

[a] –, negative

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL

5. Evaluation

No epidemiological data relevant to the carcinogenicity of *cis*-9,10-epoxystearic acid were available.

There is *inadequate evidence* in experimental animals for the carcinogenicity of *cis*-9,10-epoxystearic acid.

Overall evaluation

cis-9,10-Epoxystearic acid is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

Chu, I., Villeneuve, D.C., Nestmann, E.R., Douglas, G., Becking, G.C., Lough, R. & Matula, T.I. (1980) Subacute toxicity and mutagenicity of cis-9,10-epoxystearic acid. *Bull. environ. Contam. Toxicol*., **25**, 400–403

IARC (1976) *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 11, *Cadmium, Nickel, Some Epoxides, Miscellaneous Industrial Chemicals and General Considerations on Volatile Anaesthetics*, Lyon, pp. 153–156

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 63

Laniado-Schwartzman, M., Davis, K.L., McGiff, J.C., Levere, R.D. & Abraham, N.G. (1988) Purification and characterization of cytochrome P-450-dependent arachidonic acid epoxygenase from human liver. *J. biol. Chem*., **263**, 2536–2542

Ulsaker, G.A. & Teien, G. (1995) Identification of 9,10-epoxyoctadecanoic acid in human urine using gas chromatography-mass spectrometry. *Biomed. Chromatogr*., **9**, 183–187

ETHYL ACRYLATE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

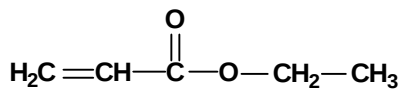
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 140-88-5
Chem. Abstr. Name: 2-Propenoic acid, ethyl ester
IUPAC Systematic Name: Acrylic acid, ethyl ester
Synonym: Ethyl propenoate

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_5H_8O_2$ Relative molecular mass: 100.12

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Liquid with an acrid, penetrating odour (Budavari, 1996)
(*b*) *Boiling-point*: 99.4°C (Lide, 1995)
(*c*) *Melting-point:* –71.2°C (Lide, 1995)
(*d*) *Solubility*: Slightly soluble in water; soluble in chloroform; miscible with diethyl ether and ethanol (Lide, 1995)
(*e*) *Vapour pressure*: 3.9 kPa at 20°C; relative vapour density (air = 1), 3.5 (Verschueren, 1996)
(*f*) *Flash-point*: 15°C, open cup (Budavari, 1996)
(*g*) *Explosive limits*: Lower explosive limit, 1.8% by volume in air (American Conference of Governmental Industrial Hygienists, 1991)
(*h*) *Conversion factor*: $mg/m^3 = 4.09 \times ppm$

1.2 Production and use

Production of ethyl acrylate in the United States in 1993 was reported to be 160 345 tonnes (United States International Trade Commission, 1994).

Ethyl acrylate is used as a monomer in acrylic resins (American Conference of Governmental Industrial Hygienists, 1991).

1.3 Occurrence

1.3.1 *Occupational exposure*

The 1981–83 National Occupational Exposure Survey (NOES) estimated that 34 000 workers in the United States were potentially exposed to ethyl acrylate (NOES, 1997).

National estimates of exposure were not available from other countries.

1.3.2 *Environmental occurrence*

Ethyl acrylate may be released into the environment in escape or stack emissions or in wastewater during its production and use. It is also a volatile component of pineapple and Beaufort cheese (a type manufactured in a small area of the French Alps). It has been detected at low levels in wastewater samples (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 20 mg/m^3 as the threshold limit value for occupational exposures to ethyl acrylate in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for ethyl acrylate in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Ethyl acrylate was tested for carcinogenicity by oral gavage in mice and rats. Dose-related increases in the incidence of squamous-cell papillomas and carcinomas of the forestomach were observed in both species. Ethyl acrylate was tested by inhalation in the same strains of mice and rats; no treatment-related neoplastic lesion was observed. No treatment-related tumour was observed following skin application of ethyl acrylate for lifespan to male mice (IARC, 1986).

3.1 Oral administration

Rat: Three groups of 25 male Fischer 344 rats, two months of age, were treated with 200 mg/kg bw ethyl acrylate (purity, 99%) by gavage in corn oil on five days per week

for six or 12 months. Control rats received 5 mL corn oil/kg bw per day on five days per week for 12 months. Five rats from each treatment group were killed 24 h after the last dose. The remaining rats were killed at 24 months of age. All animals were examined for gross lesions and the stomachs were collected and fixed in formalin. Microscopic examination was restricted to three or four sections of the stomach. No treatment-related neoplastic lesions were observed in the forestomach of rats exposed to ethyl acrylate for six months and autopsied at 24 months of age. After 12 months of ethyl acrylate administration, all rats showed hyperplastic lesions but no neoplastic lesions were detected. However, when rats received ethyl acrylate for 12 months and were killed after nine months of recovery, they developed squamous-cell carcinomas (3/13) and papillomas (1/13) (Ghanayem *et al*., 1993). [The Working Group noted that histopathological evaluation was limited to the stomach.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

De Bethizy *et al*. (1987) administered ethyl [2,3-^{14}C]acrylate to rats orally by gavage at doses of 2, 20 and 200 mg/kg bw. The total recovery in specific tissues and excreta fell with increasing dose from 108% at 2 mg/kg bw to 73% at 200 mg/kg bw. The major metabolite was $^{14}CO_2$, with 52–61% exhaled within 24 h. The proportion of radioactivity excreted in the urine fell with increasing dose, from 28% at 2 mg/kg bw to 8% at 200 mg/kg bw. Three metabolites were identified: 3-hydroxypropionic acid and two mercapturic acids. *N*-Acetyl-*S*-(2-carboxyethyl)cysteine arises by glutathione conjugation of acrylic acid, while *N*-acetyl-*S*-(2-carboxyethyl)cysteine ethyl ester derives from the conjugation of intact ethyl acrylate. The percentage of the dose excreted as these mercapturic acids falls with increasing dose, consistent with depletion of glutathione. Although ethyl acrylate does not reduce non-protein sulfhydryls in the liver, marked and dose-dependent depletion occurs in the forestomach and glandular stomach, which is enhanced by pretreatment of rats with the esterase inhibitor tri(*ortho*-cresyl)phosphate. These data are consistent with the hydrolysis of ethyl acrylate being a systemic detoxication reaction, since acrylic acid has no effect on non-protein sulfhydryl levels.

Linhart *et al*. (1994) reported increases in urinary levels of 3-hydroxypropanoic, lactic and acetic acids after administration of ethyl acrylate to rats.

Potter and Tran (1992) showed that ethyl acrylate reacts spontaneously with glutathione and protein sulfhydryl groups in many tissues: in liver alone, conjugation with glutathione was catalysed by cytosolic glutathione *S*-transferase. Miller *et al*. (1981)

showed a major role for the liver in the hydrolysis of ethyl acrylate, the order of activities among tissues being liver >> blood >> lung > kidney. The hydrolysis of ethyl acrylate in various regions of the nose and respiratory tract was region-dependent (Frederick *et al*., 1994): high activity was found in homogenates of the dorsal meatus and olfactory septum, with much lower activity in respiratory epithelium. This distribution of activity does not correlate well with the distribution of cytotoxicity of ethyl acrylate after inhalation exposure. Stott and McKenna (1985) found that ethyl acrylate was hydrolysed in homogenates of mouse nasal epithelium.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Frederick *et al*. (1990) treated male Fischer 344/N rats with 0, 2, 20, 50, 100 and 200 mg/kg bw ethyl acrylate by daily gavage for two weeks. Another group of animals received 200, 1000, 2000 and 4000 ppm (mg/L) in the drinking-water for two weeks. In the 20–200 mg/kg bw dose range, dose-dependent irritation of the forestomach, but not of the glandular stomach, was observed. In the animals dosed with ethyl acrylate in the drinking-water, much lower effects were observed at corresponding dose levels. Dosage of 200 mg/kg bw led to a reduction of about 90% in non-protein sulfhydryl content in the forestomach, but not in the glandular stomach or the liver. Interestingly, Ghanayem *et al*. (1991a) found that sulfhydryl-containing agents (cysteine and cysteamine) enhanced ethyl acrylate-induced oedema of the forestomach, whereas depletion of the sulfhydryl content by fasting or pretreatment with diethyl maleate was protective.

In Fischer 344 rats of both sexes receiving a single dose of 100, 200 or 400 mg/kg bw ethyl acrylate, dose- and time-dependent occurrence of mucosal and submucosal oedema, vacuolization of the tunica muscularis of the forestomach and mild submucosal oedema in the glandular stomach were observed (Ghanayem *et al*., 1985a). Equivalent subcutaneous or intraperitoneal dosing did not produce similar gastric lesions. Profound gastric toxicity was also obtained with methyl or ethyl acrylate, while acrylic acid, *n*-butyl acrylate, methyl and ethyl propionate and methacrylic acid esters were inactive (Ghanayem *et al*., 1985b). Depending on dose and time, forestomachs of rats either returned to normal or showed (reversible) mucosal hyperplasia (Ghanayem *et al*., 1991b; Gillette & Frederick, 1993), while submucosal fibrosis became more prevalent in high-dose animals with time (Ghanayem *et al*., 1986a). Another study (Ghanayem *et al*., 1993) provided evidence that a certain time of sustained hyperplasia of the forestomach is required for effective tumorigenesis of ethyl acrylate in the forestomach of rats.

Daily gavage doses of 100 and 200 mg/kg bw ethyl acrylate on five days per week for two weeks resulted in a dramatic increase in forestomach epithelial cell proliferation in male Fischer 344 rats (Ghanayem *et al*., 1986b).

Exposure of male and female Fischer 344 rats and $B6C3F_1$ mice to 0, 0.1 or 0.31 mg/L ethyl acrylate vapour for 6 h per day on five days per week for 27 months resulted in dose-dependent occurrence of basal-cell hyperplasia, an increase in intra-epithelial glands, respiratory metaplasia and diffuse atrophy of the olfactory epithelium in rats, and in hyperplasia of submucosal glands and respiratory metaplasia of olfactory epithelium in mice (Miller *et al*., 1985). Inhalation exposure of male Wistar rats to 1000 mg/m^3 ethyl acrylate for 6 h led to a significant increase in urinary thioether excretion (Vodička *et al*., 1990). The average concentrations of ethyl acrylate in inhaled air that caused 50% depletion of non-protein sulfhydryl groups were estimated at 41.7 mmol/m^3 for blood, 50.4 mmol/m^3 for liver, 63.8 mmol/m^3 for lung and 81.5 mmol/m^3 for brain.

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

In single studies, ethyl acrylate did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster* but did induce mitotic recombination in *Saccharomyces cerevisiae.* It was not mutagenic to bacteria.

In mammalian cells treated *in vitro*, it induced mutation at the *tk* locus in mouse L5178Y lymphoma cells, in the absence of exogenous metabolic activation, but not at the *hprt* locus in Chinese hamster ovary CHO cells. It induced chromosomal aberrations in mouse L5178Y lymphoma cells, Chinese hamster ovary CHO and Chinese hamster lung CHL cells *in vitro.*

In a single study, ethyl acrylate failed to induce DNA binding in forestomach or liver of rats when given by gavage at doses up to 400 mg/kg (Ghanayem *et al*., 1987) [The Working Group noted the inadequate method for determining DNA binding.] It induced micronucleus formation in mouse bone marrow and weakly in mouse splenocytes; another study performed under the same conditions was negative. In single studies, ethyl acrylate failed to induce sister chromatid exchanges or chromosomal aberrations in mouse splenocytes *in vivo*. It did not induce DNA damage in peripheral white blood cells of mice or in the forestomach of rats treated *in vivo*.

4.4.3 *Mechanistic considerations*

Ethyl acrylate appears to be clastogenic to mammalian cells *in vitro*. The preferential induction of small colonies rather than large ones in the mouse lymphoma L5178Y *tk* mutagenicity assay is thought to indicate that mutations arise from chromosomal damage rather than by point mutation. The clastogenic activity of ethyl acrylate seen *in vitro* is

Table 1. Genetic and related effects of ethyl acrylate

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1666	Haworth *et al.* (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	670	Waegemaekers & Bensink (1984)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1666	Haworth *et al.* (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	670	Waegemaekers & Bensink (1984)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1666	Haworth *et al.* (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	670	Waegemaekers & Bensink (1984)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	670	Waegemaekers & Bensink (1984)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1666	Haworth *et al.* (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	670	Waegemaekers & Bensink (1984)
SCH, *Saccharomyces cerevisiae* D61.M, homozygosis by mitotic recombination or gene conversion	+	NT	733	Zimmermann & Mohr (1992)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		40 000 ppm feed	Valencia *et al.* (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	20	Moore *et al.* (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	(+)	NT	20	McGregor *et al.* (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	20	Moore *et al.* (1989)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	(+)	20	Dearfield *et al.* (1991)
GCO Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	–	NT	23	Moore *et al.* (1989)
GCO Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	–	NT	80	Moore *et al.* (1991)
SIM, Sister chromatid exchange, mouse splenocytes *in vitro*	–	NT	25	Kligerman *et al.* (1991)
CIM, Chromosomal aberrations, mouse splenocytes *in vitro*	(+)	NT	2	Kligerman *et al.* (1991)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic activation	With exogenous metabolic activation		
CIM, Chromosomal aberrations, mouse lymphoma L5178Y cells *in vitro*	+	NT	20	Moore *et al.* (1988)
CIM, Chromosomal aberrations, mouse lymphoma L5178Y cells *in vitro*	+	NT	20	Moore *et al.* (1989)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	+	NT	21	Moore *et al.* (1989)
CIC, Chromosomal aberrations, Chinese hamster lung cells *in vitro*	+	NT	9.8	Ishidate *et al.* (1981)
DVA, DNA strand breaks, Fischer 344 rat forestomach *in vivo*	–	NT	4% po × 1	Morimoto *et al.* (1990)
DVA, DNA strand breaks, Tg.AC mouse peripheral blood leukocytes *in vivo*	–		12 µg/mouse skin × 3/wk 20 wk	Tice *et al.* (1997)
SVA, Sister chromatid exchange, C57BL/6 mouse splenocytes *in vivo*	–		1000 ip × 1	Kligerman *et al.* (1991)
MVM, Micronucleus test, C57BL/6 mouse splenocytes *in vivo*	(+)		1000 ip × 1	Kligerman *et al.* (1991)
MVM, Micronucleus test, BALB/c mouse bone marrow *in vivo*	+		225 ip × 2	Przybojewska *et al.* (1984)
MVM, Micronucleus test, BALB/c and C57BL/6J mouse bone marrow *in vivo*	–		812 ip × 2	Ashby *et al.* (1989)
MVM, Micronucleus test, C57BL/6J mouse bone marrow *in vivo*	–		738 ip × 2	Ashby *et al.* (1989)
MVM, Micronucleus test, Tg.AC mouse peripheral blood cells *in vivo*	–		12 µg/mouse skin × 3/wk 20 wk	Tice *et al.* (1997)
CVA, Chromosomal aberrations, C57BL/6 mouse splenocytes *in vivo*	–		1000 ip × 1	Kligerman *et al.* (1991)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; ip, intraperitoneal; po, oral; wk, week

not readily expressed *in vivo*. Ethyl acrylate did not bind to deoxyribonucleosides *in vitro* (McCarthy *et al.*, 1994).

5. Evaluation

No epidemiological data relevant to the carcinogenicity of ethyl acrylate were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of ethyl acrylate.

Overall evaluation

Ethyl acrylate is *possibly carcinogenic to humans (Group 2B)*.

6. References

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GLYCIDALDEHYDE

Data were last reviewed in IARC (1976) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

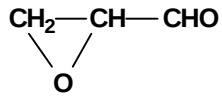
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 765-34-4

Systematic name: Oxirane-carboxaldehyde

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_3H_4O_2$ Relative molecular mass: 72.1

1.1.3 *Physical properties* (for details, see IARC, 1976)

(*a*) *Boiling-point*: 112–113°C

(*b*) *Conversion factor*: $mg/m^3 = 2.95 \times ppm$

1.2 Production and use

Glycidaldehyde has been used as a cross-linking agent for the finishing of wool, for the oil tanning and fat liquoring of leather and surgical sutures (IARC, 1976).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Glycidaldehyde was tested for carcinogenicity in mice by skin application and by subcutaneous injection and in rats by subcutaneous injection. It produced malignant tumours at the site of application in both species (IARC, 1976).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Toxic effects

4.2.1 *Humans*

Glycidaldehyde produces skin irritation and sensitization (IARC, 1976).

4.2.2 *Experimental systems*

Repeated inhalation of glycidaldehyde by rats resulted in a reduction in nucleated marrow cells and focal necrosis of liver and kidney. Repeated intravenous injections into rabbits lowered the leukocyte count and the proportion of polymorphonuclear cells. It is also irritating to the skin and mucous membranes (IARC, 1976). The carcinogenicity and genotoxicity of glycidaldehyde have been reviewed (Feron *et al.*, 1991).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

The genotoxicity of glycidaldehyde has been reviewed (Feron *et al*., 1991).

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Glycidaldehyde induces mutations in bacteriophage T4, bacteria, yeast and sex-linked recessive lethal mutations in *Drosophila melanogaster*, but not *hprt* locus mutations in mouse lymphoma L5178Y. DNA adducts can be found *in vitro* using calf thymus DNA and *in vivo* in skin cells of mice treated topically with glycidaldehyde. The main adduct, in this case, has been identified as a cyclic adduct of deoxyadenosine, namely, 3-β-D-deoxyribofuranosyl-7-(hydroxymethyl)-3*H*-imidazo[2,1-*i*]purine-3′-monophosphate. The

Table 1. Genetic and related effects of glycidaldehyde

Test system	Result[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	5	Wade *et al*. (1979)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	100	Wade *et al*. (1979)
ECF, Bacteriophage T4-infected *Escherichia coli*, forward mutation	+	NT	3400	Corbet *et al*. (1970)
KPF, *Klebsiella pneumoniae*, forward mutation	+	NT	1.4	Knaap *et al*. (1982)
SCR, *Saccharomyces cerevisiae* S211, reverse mutation	–	NT	11000	Izard (1973)
SCR, *Saccharomyces cerevisiae* S138, reverse mutation	–	NT	11000	Izard (1973)
DMX, *Drosophila melanogaster* germ cell, sex-linked recessive lethal mutations	+		1800 inj	Knaap *et al*. (1982)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *hprt* locus *in vitro*	–	NT	7.2	Knaap *et al*. (1982)
TCS, Cell transformation, Syrian hamster embryo cells *in vitro*	(+)	NT	1	Pienta *et al*. (1977)
BID, Binding (covalent) to calf thymus DNA *in vitro*	+		600	Van Duuren & Loewengart (1977)
BID, Binding (covalent) to calf thymus DNA *in vitro*	+	NT	685	Steiner *et al*. (1992)
BVD, Binding (covalent) to DNA, male C3H mice *in vivo*	+		2 mg/mouse skin-painting	Steiner *et al*. (1992)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; inj, injection

frequency of this adduct was 1 adduct per 6.4×10^4 nucleotides after a 2-mg application and 1 adduct per 7.6×10^3 nucleotides after a 10-mg skin-painting (Steiner *et al.*, 1992). Glycidaldehyde gave a marginally positive result of uncertain significance for the morphological transformation of Syrian hamster embryo cells *in vitro*.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of glycidaldehyde were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of glycidaldehyde.

Overall evaluation

Glycidaldehyde is *possibly carcinogenic to humans (Group 2B)*.

6. References

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HEXAMETHYLPHOSPHORAMIDE

Data were last reviewed in IARC (1977) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

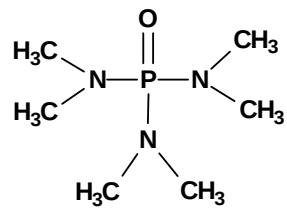
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 680-31-9

Systematic name: Hexamethylphosphoric triamide

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_6H_{18}N_3OP$ Relative molecular mass: 179.2

1.1.3 *Physical properties* (for details, see IARC, 1977)

(*a*) *Melting-point*: 7°C

(*b*) *Boiling-point*: 233°C

(*c*) *Conversion factor*: $mg/m^3 = 7.33 \times ppm$

1.2 Production and use

Hexamethylphosphoramide has been produced commercially in relatively small quantities in several countries of Europe, in Japan and in the United States. It is used as a solvent for polymers, a selective solvent for gases and as a thermal and ultraviolet radiation degradation stabilizer in various polymers (IARC, 1977).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Hexamethylphosphoramide was tested for carcinogenicity in rats, the only species tested, by inhalation; in this study, which was reported as a preliminary note, it produced squamous-cell carcinomas of the nasal cavity. It has also been inadequately tested in rats by oral administration (IARC, 1977).

3.1 Inhalation exposure

Rat: Four groups of 120 male and 120 female Sprague-Dawley rats were exposed to 0 (control), 50, 400 and 4000 ppb [0, 0.37, 2.9 and 29 mg/m^3] hexamethylphosphoramide vapour for 6 h per day on five days per week for periods ranging from nine months to two years. In an additional study, four groups of 100 male and 100 female rats were similarly exposed to 0, 10, 50 and 100 ppb [0, 73, 370 and 730 μg/m^3] atmospheres. Nasal tumours were first found after approximately seven months of exposure at 400 and 4000 ppb, after nine months at 100 ppb and after 12 months at 50 ppb. No exposure-related tumours were found at 10 ppb. Tumour incidences at 24 months were: 50 ppb, 15% (12 months of exposure) and 25% (24 months of exposure); 100 ppb, 19% (six months of exposure) and 56% (13 months of exposure); 400 ppb, 82% (10 months of exposure); 4000 ppb, 83% (nine months of exposure). Most tumours developed in the squamous or respiratory epithelium and nasal glands, all of which showed squamous metaplasia or dysplasia in the anterior nasal cavity. Exposure concentrations correlated with tumour incidence and latency, but not with tumour type. The total of 473 nasal tumours included 72% epidermoid carcinomas, 15% adenoid squamous carcinomas and 8% papillomas. Most tumours (59%) developed in the anterior nasal cavity and then progressed to the posterior nasal cavity (41%) (Lee & Trochimowicz, 1982a).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

When labelled hexamethylphosphoramide was given intraperitoneally to rats and mice, 70% of the label was excreted within 20 h in the urine. The parent compound undergoes a sequence of *N*-demethylations to yield pentamethylphosphoramide, *N*′,*N*′,*N*″,*N*‴-tetramethylphosphoramide and *N*′,*N*″,*N*‴-trimethylphosphoramide. In-vitro studies with rat liver slices indicated oxidative demethylation with the simultaneous formation of

formaldehyde. Hexamethylphosphoramide is also excreted in cows' milk after oral administration (IARC, 1977).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Repeated inhalation of hexamethylphosphoramide by rats resulted in severe degenerative changes in renal convoluted tubules. Rats given this compound in the diet showed severe bronchiectasis and bronchopneumonia with areas of squamous metaplasia (IARC, 1977).

Inhalation by rats of 351 ppm [*sic*] for 15 min did not cause any decrease in respiratory rate (Gardner *et al*., 1985). In the carcinogenicity experiment described above (Lee & Trochimowicz, 1982a), rhinitis, nasal epithelium degeneration, squamous metaplasia and dysplasia were observed in rats exposed to hexamethylphosphoramide by inhalation at concentrations of 10, 50, 100, 400 or 4000 ppb for 6–24 months. No pathological lesions were found in the 10-ppb group after 24 months. Incidence of tracheitis, degeneration of the tracheobronchial epithelium and murine pneumonia was dose-related in the 100-, 400- and 4000-ppb groups. The ciliated cells were the most susceptible to hexamethylphosphoramide. Keratinized squamous metaplasia developed at 4000 ppb (Lee & Trochimowicz, 1982b,c).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

When rats were given daily doses of 200 mg/kg bw hexamethylphosphoramide on days 7–20 of gestation, no abnormalities were found in the offspring. The fertility of rats was not impaired by 10 mg/kg bw per day administered by gavage for 169 days (IARC, 1977).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Hexamethylphosphoramide gave negative results in several conventional assays for bacterial mutagenicity which employed commonly used *Salmonella typhimurium* strains in the presence or absence of exogenous metabolic activation systems. In one study, it gave positive results in two strains of *Escherichia coli* WP2, in the presence of an

Table 1. Genetic and related effects of hexamethylphosphoramide

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SAF, *Salmonella typhimurium*, forward mutation, 8-azaguanine	NT	–	1000	Skopek *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Baker & Bonin (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Garner *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation (fluctuation test)	–	–	500	Hubbard *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	MacDonald (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Martire *et al.* (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Nagao & Takahashi (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Richold & Jones (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1000	Rowland & Severn (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Simmon & Shepherd (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	NG	Venitt & Crofton-Sleigh (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000	Zeiger & Haworth (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation (liquid suspension test)	–	+	2000	Sarrif *et al.* (1997)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	NG	Baker & Bonin (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	NG	Martire *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	NG	Garner *et al.* (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Richold & Jones (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1000	Rowland & Severn (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	NG	Simmon & Shepherd (1981)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000	Zeiger & Haworth (1985)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA5, *Salmonella typhimurium* TA1535, reverse mutation (liquid suspension test)	–	–	40000	Sarrif *et al.* (1997)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Baker & Bonin (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Martire *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Garner *et al.* (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	2500	MacDonald (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Nagao & Takahashi (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Richold & Jones (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1000	Rowland & Severn (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Simmon & Shepherd (1981)
SA7, *Salmonella typhimurium* TA1537, reverse mutation (liquid suspension test)	–	+	10000	Sarrif *et al.* (1997)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	NG	Baker & Bonin (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	NG	Martire *et al.* (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	500	Richold & Jones (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1000	Rowland & Severn (1981)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	NG	Simmon & Shepherd (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Baker & Bonin (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	Brooks & Dean (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Martire *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Garner *et al.* (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation (fluctuation test)	–	–	500	Hubbard *et al.* (1981)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	MacDonald (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Nagao & Takahashi (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Richold & Jones (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1000	Rowland & Severn (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Simmon & Shepherd (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	NG	Venitt & Crofton-Sleigh (1981)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000	Zeiger & Haworth (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation (liquid suspension test)	–	+	10000	Sarrif *et al.* (1997)
SAS, *Salmonella typhimurium* TA92, reverse mutation	–	–	1000	Brooks & Dean (1981)
SAS, *Salmonella typhimurium* TA97, reverse mutation	–	–	5000	Zeiger & Haworth (1985)
SAS, *Salmonella typhimurium* TA97, reverse mutation (liquid suspension test)	–	+	5000	Sarrif *et al.* (1997)
ECK, *Escherichia coli* K-12/343/113, forward or reverse mutation	–	–	4000	Mohn *et al.* (1981)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	–	+	NG	Venitt & Crofton-Sleigh (1981)
EC2, *Escherichia coli* WP2, reverse mutation	–	+	NG	Venitt & Crofton-Sleigh (1981)
ECW, *Escherichia coli* WP2*uvrA*, reverse mutation (fluctuation test)	–	–	1000	Gatehouse (1981)
EC2, *Escherichia coli* WP2, reverse mutation	–	–	NG	Matsushima *et al.* (1981)
ECW, *Escherichia coli* WP2*uvr*A, reverse mutation	–	–	NG	Matsushima *et al.* (1981)
ECR, *Escherichia coli* WP2*uvr*ApKM101, reverse mutation	–	–	NG	Matsushima *et al.* (1981)
SCH, *Saccharomyces cerevisiae* JD1, homozygosis by mitotic gene conversion	–	+	50	Sharp & Parry (1981)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SCH, *Saccharomyces cerevisiae D7*, homozygosis by mitotic gene conversion	–	+	2000	Zimmermann & Scheel (1981)
SCH, *Saccharomyces cerevisiae* DEL, homozygosis by mitotic gene conversion	(+)	(+)	50000	Carls & Schiestl (1994)
SCR, *Saccharomyces cerevisiae* XV-185-14C, reverse mutation	–	(+)	100	Mehta & von Borstel (1981)
SZF, *Schizosaccharomyces pombe*, forward mutation, five loci	–	–	30	Loprieno (1981)
DMM, *Drosophila melanogaster*, *white/white*$^+$ eye mosaic test, somatic mutation and mitotic recombination	+		18 feed	Vogel & Nivard (1993)
DMM, *Drosophila melanogaster*, *white/white*$^+$ eye mosaic test, somatic mutation and mitotic recombination host (SMART)	+		18 feed	Aguirrezabalaga *et al.* (1994)
DMM, *Drosophila melanogaster*, *white-ivory* eye test, somatic mutation and mitotic recombination	+		9 feed	Ferreiro *et al.* (1995)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		250 ppm feed	Valencia & Houtchens (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		100 feed	Vogel *et al.* (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		100 feed	Wurgler & Graf (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		25 feed	Vogel *et al.* (1985)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		100 ppm feed	Foureman *et al.* (1994)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	+		45 feed	Aguirrezabalaga *et al.* (1995)
DMH, *Drosophila melanogaster*, heritable translocation test	+		25 feed	Vogel *et al.* (1985)
DMH, *Drosophila melanogaster*, heritable translocation test	+		100 ppm feed	Foureman *et al.* (1994)
Drosophila melanogaster, survival of DNA repair-deficient mus homozygotes relative to their repair-proficient heterozygous siblings	+		896 feed	Henderson & Grigliatti (1992)
Micronucleus test, *Pleurodeles waltl in vivo*	(+)		30	Fernandez *et al.* (1989)
DIA, DNA–protein cross-links, rat nasal epithelial cells *in vitro*	+	NT	179	Kuykendall *et al.* (1995)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
GCO, Gene mutation, Chinese hamster ovary CHO cells *in vitro*, five loci	–	–	31000	Carver *et al.* (1981)
DMN, *Drosophila melanogaster*, chromosome loss (ring-X)	+		11 feed	Vogel *et al.* (1985)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	?	200	Knaap *et al.* (1981)
GML, Gene mutation, mouse lymphoma P388F cells *tk* locus *in vitro*	NT	+	8.28	Anderson & Cross (1985)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	–	+	1500	Jotz & Mitchell (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	1000	Evans & Mitchell (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	339	Natarajan & van Kesteren-van Leeuwen (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	+	10	Perry & Thomson (1981)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	+[c]	5.4	Darroudi & Natarajan (1993)
MIA, Micronucleus test, Chinese hamster ovary CHO cells *in vitro*	–	+[c]	2.7	Darroudi & Natarajan (1993)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	339	Natarajan & van Kesteren-van Leeuwen (1981)
CIR, Chromosomal aberrations, rat liver RL_1 cells *in vitro*	–	NT	100	Dean (1981)
SIH, Sister chromatid exchange, human hepatoma Hep G2 cells *in vitro*	+	NT	1.6	Natarajan & Darroudi (1991)
MIH, Micronucleus test, human hepatoma Hep G2 cells *in vitro*	+	NT	1.6	Natarajan & Darroudi (1991)
MIH, Micronucleus test, human hepatoma Hep G2 cells *in vitro*	+[d]	NT	0.5	Darroudi *et al.* (1996)
MIH, Micronucleus test, human lymphocytes *in vitro*	–	NT	1.8	Darroudi *et al.* (1996)
CHL, Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	900	Chang & Klassen (1968)
SVA, Sister chromatid exchange, CBA/J mouse bone marrow *in vivo*	+		15.4 ip × 1	Paika *et al.* (1981)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SVA, Sister chromatid exchange, CBA/J mouse liver *in vivo*	–		1304 ip × 1	Paika *et al.* (1981)
MVM, Micronucleus test, B6C3F_1 mouse bone marrow *in vivo*	+		1232 ip × 2	Salamone *et al.* (1981)
MVM, Micronucleus test, ICR mouse bone marrow *in vivo*	+		205 ip × 2	Kirkhart (1981)
MVM, Micronucleus test, CDI mouse bone marrow *in vivo*	+		205 ip × 2	Tsuchimoto & Matter (1981)
MVM, Micronucleus test, C57BL/6J mouse bone marrow *in vivo*	+		1850 ip × 2	Richardson *et al.* (1983)
MVM, Micronucleus test, C57BL/6J mouse bone marrow *in vivo*	+		1315 ip × 2	Styles *et al.* (1983)
MVM, Micronucleus test, C3H/C57 mouse bone marrow *in vivo*	+		1315 ip × 2	Styles *et al.* (1983)
MVM, Micronucleus test, BALB/c/CBA mouse bone marrow *in vivo*	+		1315 ip × 2	Styles *et al.* (1983)
MVR, Micronucleus test, Alderley Park rat bone-marrow cells *in vivo*	+		1850 ip × 1	Albanese (1987)
CBA, Chromosomal aberrations, mouse bone-marrow cells *in vivo*	–		15 ip × 1	Manna & Das (1973)
CBA, Chromosomal aberrations, Alderley Park rat bone-marrow cells *in vivo*	+		1850 ip × 1	Albanese (1987)
DLM, Dominant lethal test, A/L and C57BL/6J mice	+		50 ip × 2	Srám *et al.* (1970)
DLM, Dominant lethal test, ICR/Ha Swiss mice	–		2000 ip × 1	Epstein *et al.* (1972)
SPM, Sperm morphology, B6C3F_1/CRL mice *in vivo*	–		2630 ip × 5	Wyrobek *et al.* (1981)
SPM, Sperm morphology (CBA×BALB/c)F_1 mice *in vivo*	?		1030 ip × 5	Topham (1981)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; ip, intraperitoneal

[c] Activation system using human hepatoma (Hep62) S-9; rat liver S-9 was negative

[d] The fluorescent in situ hybridization assay shows ~80% of the micronuclei are centromere-positive compared to ~50% in controls.

exogenous metabolic activation system. In a recent study, it was claimed to be mutagenic to several strains of *S. typhimurium*, in the presence of an exogenous metabolic activation system, when tested in a liquid suspension assay.

In *Drosophila melanogaster*, hexamethylphosphoramide gave positive results in tests for sex-linked recessive lethal mutations, heritable translocations, somatic mutation and differential survival in DNA repair-proficient and -deficient strains. In yeast, it induced mitotic gene conversion. Hexamethylphosphoramide induced micronuclei in newts treated *in vivo*.

In mammalian cells, it induced sister chromatid exchanges, micronuclei and gene mutations *in vitro*. In one study, it induced DNA–protein cross-links in rat nasal epithelial cells treated *in vitro*. In human cells *in vitro*, hexamethylphosphoramide induced micronuclei and sister chromatid exchanges.

In a single study *in vivo*, hexamethylphosphoramide induced sister chromatid exchanges in mouse bone marrow but not in mouse liver. In single studies *in vivo*, hexamethylphosphoramide did not induce chromosomal aberrations in mouse bone marrow, but did so in rat bone marrow. In several independent studies, it induced micronuclei in bone marrow of mice treated *in vivo*. Of two studies in which hexamethylphosphoramide was tested for induction of dominant lethal mutations in mice, one was positive and one was negative. It gave inconclusive or negative results in tests for abnormal sperm morphology in mice.

4.4.3 *Mechanistic considerations*

Studies of the pattern of mutagenicity of hexamethylphosphoramide in *D. melanogaster* strongly suggest that this compound is a DNA cross-linking agent (Vogel & Natarajan, 1995). The cross-linking activity of hexamethylphosphoramide is supported by the detection of DNA–protein cross-links in rat nasal epithelial cells treated *in vitro* (Kuykendall *et al.*, 1995). High-performance liquid chromatographic analysis of DNA extracted from flies injected with [^{14}C]hexamethylphosphoramide revealed no methylation at O^6 or *N*7 of guanine (Vogel *et al.*, 1985). This finding suggests that the formation of DNA adducts by hexamethylphosphoramide may not be the result of simple methylation reactions.

The metabolism of hexamethylphosphoramide in nasal tissues of rats leads to the production of formaldehyde via cytochrome P450-mediated *N*-demethylation (Ashby & Lefevre, 1982; Dahl & Hadley, 1983). Formaldehyde, like hexamethylphosphoramide, is carcinogenic to rat nasal epithelium when given by inhalation and, like hexamethylphosphoramide, induces DNA–protein cross-links in target tissues (IARC, 1995). It is possible, therefore, that metabolism of hexamethylphosphoramide at the target tissue leads to the local production of formaldehyde, which then forms DNA–protein cross-links (and possibly other DNA modifications) which in turn initiate carcinogenesis. However, formaldehyde appears to be significantly more potent (about 60-fold) in forming DNA–protein cross-links than is hexamethylphosphoramide at equimolar concentrations, although the latter is substantially more carcinogenic (by nearly 100-fold) to the rat nasal

epithelium than is formaldehyde (Bogdanffy *et al.*, 1997). This suggests that DNA–protein cross-links alone may not be critical to the mechanism of the carcinogenicity of hexamethylphosphoramide. Based on their studies of the mitogenic and tissue-damaging effects on the rat nasal epithelium of inhaled hexamethylphosphoramide (single exposures or five daily 1-h exposures at 3 ppm), Harman *et al.* (1997) postulated that its high carcinogenic potency could be explained by its ability to liberate formaldehyde intracellularly and to stimulate mitogenesis in the absence of cytoxicity. This is in contrast to formaldehyde, which appears to be carcinogenic only at doses that cause substantial tissue damage and which does not appear to be mitogenic at lower doses that do not damage the nasal epithelium (IARC, 1995). It is argued, therefore (Bogdanffy *et al.*, 1997; Harman *et al.*, 1997), that the stimulus for formaldehyde-induced cell proliferation is cytotoxicity, whereas for hexamethylphosphoramide it is mitogenesis. The efficiency with which promutagenic lesions induced by formaldehyde are converted to mutations would be low, since the death rate of epithelial cells (cytotoxicity) would counteract the birth rate (cell proliferation). In contrast, metabolites of hexamethylphosphoramide that accumulate in the tissue induce a mitogenic response such that the low levels of promutagenic lesions produced from formaldehyde would be more likely to be converted into mutations.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of hexamethylphosphoramide were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of hexamethylphosphoramide.

Overall evaluation

Hexamethylphosphoramide is *possibly carcinogenic to humans (Group 2B)*.

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ISOPROPYL OILS

Data were last reviewed in IARC (1977) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

Isopropyl oils are produced as by-products in the reaction of propylene with sulfuric acid during the manufacture of isopropanol and consist of the residue after distillation. They do not appear to have been used commercially (IARC, 1977).

No biological data additional to those described for isopropanol were available to the Working Group (IARC, 1977, 1987).

2. Studies of Cancer in Humans

An increased incidence of cancer of the paranasal sinuses was observed in workers at factories where isopropanol was manufactured by the strong-acid process. The risk for laryngeal cancer may also have been elevated in these workers. It is unclear whether the cancer risk was due to the presence of diisopropyl sulfate, which is an intermediate in the process, to isopropyl oils, which are formed as by-products, or to other factors, such as sulfuric acid. Epidemiological data concerning the manufacture of isopropanol by the weak-acid process are insufficient for an evaluation of carcinogenicity (IARC, 1987).

3. Studies of Cancer in Experimental Animals

Isopropyl oils, formed during the manufacture of isopropanol by both the strong-acid and weak-acid processes, were tested inadequately in mice by inhalation, skin application and subcutaneous administration. Isopropyl oils formed during the strong-acid process were also tested inadequately in dogs by inhalation and instillation into the sinuses (IARC, 1977, 1987).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

No data were available to the Working Group.

5. Evaluation

There is *inadequate evidence* for the carcinogenicity of isopropyl oils in humans.

There is *inadequate evidence* for the carcinogenicity of isopropyl oils in experimental animals.

Overall evaluation

Isopropyl oils are *not classifiable as to their carcinogenicity to humans (Group 3).*

6. References

IARC (1977) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*, Vol. 15, *Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals*, Lyon, pp. 223–243

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 229

LAUROYL PEROXIDE

Data were last reviewed in IARC (1985) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

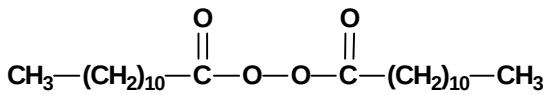
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 105-74-8

Systematic name: Peroxide, bis(1-oxododecyl)

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{24}H_{46}O_4$ Relative molecular mass: 398.6

1.1.3 *Physical properties* (for details, see IARC, 1985)

Melting-point: 54.7–55°C

1.2 Production and use

Lauroyl peroxide was first produced commercially in about 1941. It is used principally in the production of polymers; small amounts are employed in food packaging (IARC, 1985).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Lauroyl peroxide was tested for carcinogenicity by subcutaneous administration in mice and rats and by skin application in mice. In one study in mice by subcutaneous

administration, the evidence concerning a carcinogenic effect was inconclusive; in two other studies, no increase in tumour incidence was observed. Two studies in mice by skin application were inadequate for an evaluation of complete carcinogenicity; one study indicated that lauroyl peroxide has promoting activity in mouse skin (IARC, 1985).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

A single application of lauroyl peroxide to mouse skin induced mild hyperplasia and a temporary increase in dark basal keratinocytes. No major inflammatory or vascular change was noted (IARC, 1985).

4.3 Reproductive and developmental effects

No adequate data were available to the Working Group.

4.4 Genetic and related effects

No adequate data were available to the Working Group.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of lauroyl peroxide were available.

There is *inadequate evidence* in experimental animals for the carcinogenicity of lauroyl peroxide.

Overall evaluation

Lauroyl peroxide is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

IARC (1985) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 36, *Allyl Compounds, Aldehydes, Epoxides and Peroxides*, Lyon, pp. 315–321

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 65

METHYL ACRYLATE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

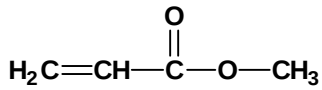
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 96-33-3
Chem. Abstr. Name: 2-Propenoic acid, methyl ester
IUPAC Systematic Name: Acrylic acid, methyl ester
Synonym: Methyl propenoate

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_4H_6O_2$ Relative molecular mass: 86.09

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Liquid with an acrid odour (Budavari, 1996)
(*b*) *Boiling-point*: 80.6°C (American Conference of Governmental Industrial Hygienists, 1992)
(*c*) *Melting-point*: –76.5°C (Budavari, 1996)
(*d*) *Solubility*: Slightly soluble in water (6 g/100 mL at 20°C, 5 g/100 mL at 40°C); soluble in ethanol, diethyl ether, acetone and benzene (American Conference of Governmental Industrial Hygienists, 1992)
(*e*) *Vapour pressure*: 9.3 kPa at 20°C; relative vapour density (air = 1), 3.0 (Verschueren, 1996)
(*f*) *Flash point*: –2.8°C, closed cup; 6.7°C, open cup (American Conference of Governmental Industrial Hygienists, 1992)
(*g*) *Explosive limits:* upper, 25%; lower, 2.8% by volume in air (American Conference of Governmental Industrial Hygienists, 1992)
(*h*) *Conversion factor*: $mg/m^3 = 3.52 \times ppm$

1.2 Production and use

Production of methyl acrylate in the United States was reported to be 14 100 tonnes in 1983 (United States National Library of Medicine, 1997).

Methyl acrylate is used in manufacture of acrylic and modacrylic fibres, amphoteric surfactants, leather finish resins, textile and paper coatings and plastic films (United States National Library of Medicine, 1997).

1.3 Occurrence

1.3.1 *Occupational exposure*

National estimates of exposure were not available.

1.3.2 *Environmental occurrence*

Methyl acrylate is a volatile component of pineapples. It may be released into the environment in fugitive and stack emissions or in wastewater during its production and use in the manufacture of acrylic fibres and resins. Methyl acrylate has been detected at low levels in wastewater and ambient air samples (United States National Library of Medicine, 1997).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 7 mg/m^3 as the threshold limit value for occupational exposures to methyl acrylate in workplace air. Similar values have been used as standards or guidelines in many countries (International Labour Office, 1991).

No international guideline for methyl acrylate in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

In one study, reported as an abstract, in which rats were exposed to methyl acrylate by inhalation for two years, no neoplastic effect was reported (IARC, 1986).

3.1 Inhalation exposure

Rat: In the full report of the study cited previously in IARC (1986), four groups of 86 male and 86 female Sprague-Dawley rats, 35 days of age, were exposed (whole body) to 0, 15, 45 and 135 ppm [0, 53, 158 and 475 mg/m^3] methyl acrylate (purity, > 99.8%; main impurities methyl propionate and ethyl acrylate) in air for 6 h per day on five days

per week for 24 months. Interim kills were performed after 12 (10 males and 10 females) and 18 months (15 males and 15 females) of exposure. No significant difference in mortality was observed between the groups. The incidence of soft-tissue sarcomas varied considerably among the groups but there was no dose-dependence. No increased frequency of any tumour type in any organ could be related to a carcinogenic effect of the test substance (Reininghaus *et al*., 1991).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Autoradiography of guinea-pigs exposed dermally to methyl [2,3-^{14}C]acrylate showed that the radioactivity was seen in the subcutaneous tissues and throughout the body. Following oral administration of methyl [2,3-^{14}C]acrylate to guinea-pigs, the radioactivity was distributed in internal organs, especially the liver and bladder, and brain within 2 h; at 16 h it was seen only in mucous linings of the stomach, intestine and mouth epithelium. One hour after intraperitoneal injection of the same dose (no vehicle), radioactivity was concentrated in the peritoneum and liver and present in most other organs. Radioactivity quickly decreased in most organs, except the liver and bladder. After 24 and 48 h most organs had lost the radioactive material, but some was retained in mucous linings. After an intraperitoneal dose of methyl [2,3-^{14}C]acrylate to young male guinea-pigs, 35% of the radioactivity was excreted as $^{14}CO_2$ in expired air within 8 h and 40% after 72 h (22.6% was excreted in the urine over 72 h) (IARC, 1986).

Conjugation with sulfhydryl groups appears to be an important detoxification process for methyl acrylate in the guinea-pig. The thioethers were identified as *N*-acetyl-*S*-(2-carboxyethyl)-L-cysteine and the corresponding monomethyl ester, with a ratio between the two metabolites of 20:1. In male rats exposed to methyl acrylate by inhalation, depletion of nonprotein sulfhydryl compounds was most pronounced in the lung, compared to liver, kidney and blood. After administration of methyl acrylate to Wistar rats, no hydroxymercapturic acid that might be derived from the epoxide of methyl acrylate was detected. It seems unlikely, therefore, that epoxidation of the acrylic esters occurs *in vivo* (IARC, 1986).

Two further metabolic studies of methyl [2,3-^{14}C]acrylate have confirmed these results (Sapota, 1988, 1993).

4.2 Toxic effects

4.2.1 *Humans*

Methyl acrylate in nail lacquer has been shown to be a possible cause of contact dermatitis (Kanerva *et al*., 1995, 1996).

4.2.2 *Experimental systems*

No exposure-related clinical signs or lesions of systemic toxicity were observed in male and female Sprague-Dawley rats exposed by inhalation to methyl acrylate for 6 h per day on five days per week, at concentrations of 0, 15, 45 and 135 ppm [0, 53, 158 and 475 mg/m^3] over 24 months (Reininghaus *et al*., 1991). Atrophy of the neurogenic epithelial cells and hyperplasia of reserve cells were observed in the nasal mucosa of all methyl acrylate-treated animals. These changes were dose-related and mainly affected the anterior part of the olfactory epithelium. Opacity and neovascularization of the cornea were seen in all methyl acrylate-exposed groups.

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Methyl acrylate did not induce mutations in bacteria.

In mammalian cells treated *in vitro*, methyl acrylate induced mutations at the *tk* locus in mouse cells, in the absence of exogenous metabolic activation, but not at the *hprt* locus in Chinese ovary hamster cells. It induced chromosomal aberrations in mouse and Chinese hamster cells *in vitro*.

Of three micronucleus assays of methyl acrylate using mouse bone marrow *in vivo*, two were negative.

4.4.3 *Mechanistic considerations*

Methyl acrylate appears to be clastogenic to mammalian cells *in vitro*. The preferential induction of small colonies rather than large ones in the mouse lymphoma L5178Y *tk* mutagenicity assay is thought to indicate that mutations arise from chromosomal damage rather than by point mutation. The clastogenic activity of methyl acrylate seen *in vitro* is not readily detected *in vivo*.

Table 1. Genetic and related effects of methyl acrylate

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation (spot test)	–	–	258	Florin *et al.* (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	590	Hachiya *et al.* (1982)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	1250	Waegemaekers & Bensink (1984)
SA5, *Salmonella typhimurium* TA1535, reverse mutation (spot test)	–	–	258	Florin *et al.* (1980)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	590	Hachiya *et al.* (1982)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	1250	Waegemaekers & Bensink (1984)
SA7, *Salmonella typhimurium* TA1537, reverse mutation (spot test)	–	–	258	Florin *et al.* (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	590	Hachiya *et al.* (1982)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	1250	Waegemaekers & Bensink (1984)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	590	Hachiya *et al.* (1982)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	1250	Waegemaekers & Bensink (1984)
SA9, *Salmonella typhimurium* TA98, reverse mutation (spot test)	–	–	258	Florin *et al.* (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	590	Hachiya *et al.* (1982)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	1250	Waegemaekers & Bensink (1984)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	14	Moore *et al.* (1988)
GCO Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	–	NT	18	Moore *et al.* (1989)
GCO Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	–	NT	60	Moore *et al.* (1991)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
GCO, Gene mutation, Chinese hamster ovary A552 cells, *xprt* locus *in vitro*	–	NT	25	Oberly *et al.* (1993)
CIM, Chromosomal aberrations, mouse lymphoma L5178Y cells *in vitro*	+	NT	16	Moore *et al.* (1988)
CIC, Chromosomal aberrations, Chinese hamster lung cells *in vitro*	+	NT	6.5	Ishidate *et al.* (1981)
CIC, Chromosomal aberrations, Chinese hamster lung cells *in vitro*	+	NT	75	Sofuni *et al.* (1984a)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	NT	14	Moore *et al.* (1989)
MVM, Micronucleus test, ddY mouse bone-marrow cells *in vivo*	–		250 po × 1	Hachiya *et al.* (1981)
MVM, Micronucleus test, BALB/c mouse bone-marrow cells *in vivo*	+		37.5 ip × 2	Przybojewska *et al.* (1984)
MVM, Micronucleus test, ddY mouse bone-marrow cells *in vivo*	–		2100 ppm inh 3 h	Sofuni *et al.* (1984b)

[a] +, positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; po, orally; ip, intraperitoneally; inh, inhalation

5. Evaluation

No epidemiological data relevant to the carcinogenicity of methyl acrylate were available.

There is *inadequate evidence* in experimental animals for the carcinogenicity of methyl acrylate.

Overall evaluation

Methyl acrylate is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

American Conference of Governmental Industrial Hygienists (1992) *Documentation of the Threshold Limit Values and Biological Exposure Indices*, 6th Ed., Vol. 2, Cincinnati, OH, pp. 931–934

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2-METHYLAZIRIDINE (PROPYLENEIMINE)

Data were last reviewed in IARC (1975) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

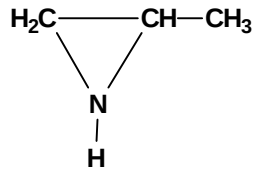
1.1.1 *Nomenclature*

Chem. Abst. Serv. Reg. No.: 75-55-8

Systematic name: 2-Methylaziridine

Synonym: Propylene-1,2-imine

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_3H_7N Relative molecular mass: 57.1

1.1.3 *Physical properties* (for details, see IARC, 1975)

(*a*) *Melting-point*: –65°C

(*b*) *Boiling-point*: 66–67°C

(*c*) *Conversion factor*: $mg/m^3 = 2.34 \times ppm$

1.2 Production and use

2-Methylaziridine is a reactive alkylating agent that is used as an intermediate in the production of polymers, coatings, adhesives, textiles and paper finishes (IARC, 1975).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

2-Methylaziridine was administered to male and female rats by gavage at doses of 0, 10 and 20 mg/kg bw. Treatment-related toxicity was found at both doses and increased mortality was seen at the high dose, which was discontinued after 28 weeks. Animals were killed at week 60. The treatment produced mammary adenocarcinomas in females at both doses, gliomas in both sexes at both doses, squamous-cell carcinomas of the ear duct in both sexes, leukaemia in males and a small number of intestinal tumours in males (IARC, 1975; Weisburger *et al.*, 1981).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

In a nephrotoxicity study, six male Sprague-Dawley rats were given a single intraperitoneal injection of 2-methylaziridine and their urine was collected over the following 16 days. At a dose level of 20 μL/kg bw, urine volume was increased and *N*-acetyl-β-D-glucosaminidase activity in the urine increased sharply on day 2, reached a maximum on day 3 and remained elevated until day 12, after which it decreased to near normal levels. β-D-Glucosidase and β-D-galactosidase activities increased nine days after the administration of 2-methylaziridine. In contrast to these markers of renal papillary damage, brush border marker enzymes were not consistently affected. A dose level of 30 μL/kg bw induced a sharp decrease in urinary volume until day 7, when the rats became anuric and died. Histology revealed that the 20 μL/kg bw dose induced coagulative necrosis at the tip of the renal papilla (Halman *et al.*, 1986). Renal papillary damage has also been observed in Fischer 344 and Sprague-Dawley rats at the same dose, whereas the multimammate desert mouse, *Mastomys natalensis*, was more resistant, even to a dose of 30 μL/kg bw (Gartland *et al.*, 1989; Holmes *et al.*, 1997).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

2-Methylaziridine is mutagenic to bacteria and induces mitotic recombination in *Saccharomyces cerevisiae*. In *Drosophila melanogaster*, it induced somatic mutations of several different types in feeding experiments and sex-linked recessive lethal mutations in an inhalation experiment using repair-deficient genotype of *D. melanogaster*. While transformation was not induced in mouse C3H 10T½ cells when the standard assay was used, transformed colonies did arise if the treated cells were replated.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of 2-methylaziridine were available.

There is *sufficient evidence* for the carcinogenicity in experimental animals of 2-methylaziridine.

Overall evaluation

2-Methylaziridine is *possibly carcinogenic to humans (Group 2B).*

6. References

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Table 1. Genetic and related effects of 2-methylaziridine

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	2.5	Simmon (1979a)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	+	5.0	Dunkel *et al.* (1984)[c]
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	75	McCann *et al.* (1975)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	NT	2.5	Simmon (1979a)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	1.6	Dunkel *et al.* (1984)[c]
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	NT	NG	Simmon (1979a)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	167	Dunkel *et al.* (1984)[c]
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	NG	Simmon (1979a)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	167	Dunkel *et al.* (1984)[c]
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	NG	Simmon (1979a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	167	Dunkel *et al.* (1984)[c]
SAS, *Salmonella typhimurium* TA1536, reverse mutation	–	NT	NG	Simmon (1979a)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	+	+	167	Dunkel *et al.* (1984)[c]
SCH, *Saccharomyces cerevisiae* D3, homozygosis by mitotic recombination	+	+	100	Simmon (1979b)
DMM, *Drosophila melanogaster*, somatic mutation (unstable *zeste-white* eye)	+		57 feed	Batiste-Alentorn *et al.* (1991)
DMM, *Drosophila melanogaster*, somatic mutation (*white-ivory* eye)	+		57 feed	Batiste-Alentorn *et al.* (1994)
DMM, *Drosophila melanogaster*, somatic mutation (wing spot)	+		114 feed	Batiste-Alentorn *et al.* (1995)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–[d]		2000 ppm inh 24 h	Vogel & Nivard (1997)
TCM, Cell transformation, C3H/10T½ mouse cells *in vitro*	–[e]	NT	4	Schechtman *et al.* (1987)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
HMM, Host-mediated assay, *Salmonella typhimurium* TA1535, Swiss-Webster mouse peritoneal cavity	+		355 po	Simmon *et al.* (1979)
HMM, Host-mediated assay, *Saccharomyces cerevisiae* D3, Swiss-Webster mouse peritoneal cavity	–		NG	Simmon *et al.* (1979)

[a] +, positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; inh, inhalation; po, oral
[c] Data from four laboratories
[d] Positive in excision repair deficient genotype
[e] Positive if treated cells are replated

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METHYL IODIDE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

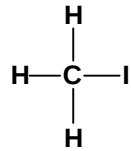
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 74-88-4
Chem. Abstr. Name: Iodomethane
IUPAC Systematic Name: Iodomethane

1.1.2 *Structural and molecular formulae and relative molecular mass*



CH_3I Relative molecular mass: 141.94

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless transparent liquid, with a sweet ethereal odour (American Conference of Governmental Industrial Hygienists, 1992; Budavari, 1996)

(*b*) *Boiling-point*: 42.5°C (Lide, 1997)

(*c*) *Melting-point*: –66.4°C (Lide, 1997)

(*d*) *Solubility*: Slightly soluble in water (14 g/L at 20°C); soluble in acetone; miscible with diethyl ether and ethanol (Budavari, 1996; Verschueren, 1996; Lide, 1997)

(*e*) *Vapour pressure*: 53 kPa at 25.3°C; relative vapour density (air = 1), 4.9 (Verschueren, 1996)

(*f*) *Octanol/water partition coefficient (P)*: log *P*, 1.51 (Hansch *et al*., 1995)

(*g*) *Conversion factor*: $mg/m^3 = 5.81 \times ppm$

1.2 Production and use

No information on the global production of methyl iodide was available to the Working Group. Production in the United States in 1983 was about 50 tonnes (IARC, 1986).

Because of its high refractive index, methyl iodide is used in microscopy. It is also used as an embedding material for examining diatoms, in testing for pyridine, as a methylating agent in pharmaceutical (e.g., quaternary ammonium compounds) and chemical synthesis, as a light-sensitive etching agent for electronic circuits, and as a component in fire extinguishers (IARC, 1986; American Conference of Governmental Industrial Hygienists, 1992; Budavari, 1996).

1.3 Occurrence

1.3.1 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1997), approximately 2000 workers in the United States were potentially exposed to methyl iodide (see General Remarks). Occupational exposures to methyl iodide may occur in its production and use in organic synthesis and as a laboratory reagent.

1.3.2 *Environmental occurrence*

Methyl iodide is produced by many marine photosynthetic organisms and therefore the ocean is thought to be a major natural source of methyl iodide. Some of this is released to the atmosphere and some reacts with seawater to form methyl chloride. Industrial emissions of methyl iodide may occur in conjunction with its use as a methylating agent and in organic synthesis. Humans are exposed to methyl iodide from the ambient air and from ingesting seafood (United States National Library of Medicine, 1998).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has recommended 12 mg/m^3 as the 8-h time-weighted average threshold limit value for occupational exposures to methyl iodide in workplace air. Values of 1–28 mg/m^3 have been used as standards or guidelines in other countries (International Labour Office, 1991). Methyl iodide is considered to be a human carcinogen in Germany (Deutsches Forschungsgemeinschaft, 1998).

No international guideline for methyl iodide in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Methyl iodide was tested for carcinogenicity in one experiment in rats by subcutaneous administration and in a screening test for lung adenomas in strain A mice by intraperitoneal injection. It induced local sarcomas in rats after single or repeated subcutaneous injections; a marginally increased incidence of lung tumours was observed in mice (IARC, 1986).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

The inhalation of trace amounts of [^{132}I]methyl iodide was followed by a decrease in plasma radioactivity, a thyroid uptake pattern and urinary excretion that were similar to those observed after oral administration of inorganic iodide (IARC, 1986).

Methyl iodide incubated with human erythrocytes conjugates nonenzymatically with glutathione. In addition, an enzymatic conjugation was apparent with erythrocytes from 10/17 donors, but even among these people, the nonenzymatic process was dominant (Hallier *et al*., 1990).

4.1.2 *Experimental animals*

Data from several experiments with rats suggest that absorbed methyl iodide is excreted mainly in bile. Approximately 25% of an oral dose of 50 mg/kg bw was excreted in bile as *S*-methylglutathione, while 2% of the same subcutaneous dose was recovered from urine and 1% of a 76 mg/kg bw oral dose was present, unchanged, in expired air within 30 min. The urinary metabolites detected in rats after subcutaneous injection, presumed to originate from *S*-methylglutathione, were *S*-methylcysteine, *N*-acetyl-*S*-methylcysteine, *S*-methylthioacetic acid and *N*-(methylthioacetyl)glycine (IARC, 1986).

4.2 Toxic effects

4.2.1 *Humans*

Workers affected by non-fatal poisoning showed many neurological symptoms such as visual and psychological disturbances, vertigo and weakness (IARC, 1986).

4.2.2 *Experimental animals*

Toxic effects observed in rodents after exposure to methyl iodide included narcosis, lung congestion and liver and kidney damage. Oral administration to rats reduced non-protein thiol concentrations in liver and kidney (IARC, 1986).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Methyl iodide induces DNA damage and is mutagenic to bacteria in the presence or absence of an exogenous metabolic system. It induces mitotic recombination in yeast and mutations in cultured mammalian cells. It induces transformation in Syrian hamster embryo cells but not in C3H 10T½ cells.

DNA adducts were detected in the stomach, forestomach, liver and lung of male and female Fischer 344 rats exposed to [^{14}C]methyl iodide orally or by inhalation in a closed exposure system. [^{14}C]3-Methyladenine, [^{14}C]7-methylguanine and [^{14}C]*O*^{6}-methylguanine were identified by a combination of three different methods of hydrolysing DNA and subsequent high-performance liquid chromatography or gas chromatography–mass spectrometry analysis. The higher levels of methylated guanines were found in the stomach and forestomach following both oral and inhalation exposure (Gansewendt *et al*., 1991).

5. Evaluation

No epidemiological data relevant to the carcinogenicity of methyl iodide were available.

There is *limited evidence* in experimental animals for the carcinogenicity of methyl iodide.

Overall evaluation

Methyl iodide is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

Amacher, D.E. & Dunn, E.M. (1985) Mutagenesis at the ouabain-resistance locus of 3.7.2C L5178Y cells by chromosomal mutagens. *Environ. Mutag*., **7,** 523–533

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Table 1. Genetic and related effects of methyl iodide

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ECD, *Escherichia coli pol A*/W3110-P3478, differential toxicity (spot test)	(+)	NT	23000	Rosenkranz & Poirier (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	(+)	NT	NG	McCann *et al*. (1975)
SA0, *Salmonella typhimurium* TA100, reverse mutation	+	NT	7.6	Simmon *et al*. (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	250	Simmon (1979a)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	+	+	4600	Rosenkranz & Poirier (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	250	Simmon (1979a)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	250	Simmon (1979a)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	23000	Rosenkranz & Poirier (1979)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	250	Simmon (1979a)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	250	Simmon (1979a)
SAS, *Salmonella typhimurium* TA1536, reverse mutation	–	–	250	Simmon (1979a)
ECW, *Escherichia coli* WP2 *uvr*A, reverse mutation	+	NT	NG	Hemminki *et al*. (1980)
EC2, *Escherichia coli* WP2, reverse mutation	+	NT	2850	Takahashi & Kawazoe (1987)
SCH, *Saccharomyces cerevisiae* D3, mitotic recombination	+	+	2300	Simmon (1979b)
ANR, *Aspergillus nidulans*, reverse mutation	–	NT	NG	Moura Duarte (1972)
VFC, *Vicia faba*, chromosomal aberrations	–	NT	140	Rieger *et al*. (1988)
GCO, Gene mutation, Chinese hamster ovary CHO cells, *hprt* locus *in vitro*	+	NT	0.75	Amacher & Zelljadt (1984)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	+	50	Clive *et al*. (1979)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	7.5	Moore & Clive (1982)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	7.5	Moore *et al*. (1985)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
G51, Gene mutation, mouse lymphoma L5178Y cells, *hprt* locus *in vitro*	NT	–	50	Clive *et al*. (1979)
G51, Gene mutation, mouse lymphoma L5178Y cells, *hprt* locus *in vitro*	(+)	NT	10	Moore & Clive (1982)
G51, Gene mutation, mouse lymphoma L5178Y cells, ouabain resistance *in vitro*	+	NT	3.6	Amacher & Dunn (1985)
TCM, Cell transformation, C3H 10T½ mouse cells	–	NT	250	Oshiro *et al*. (1981)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	1	Pienta *et al*. (1977)
BVD, Binding (covalent) to DNA, male and female Fischer 344 rat stomach, forestomach, liver and lungs *in vivo*	+		3.1 po × 1	Gansewendt *et al.* (1991)
BVD, Binding (covalent) to DNA, male and female Fischer 344 rat stomach, forestomach, liver and lungs *in vivo*	+		744 mg/m^3 inh 6 h × 1	Gansewendt *et al.* (1991)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; po, oral; inh, inhalation

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Moore, M.M. & Clive, D. (1982) The quantitation of TK$^{-/-}$ and HGPRT^{-} mutants of L5178Y/TK$^{+/-}$ mouse lymphoma cells at varying times post-treatment. *Environ. Mutag*., **4**, 499–519

Moore, M.M., Clive, D., Howard, B.E., Batson, A.G. & Turner, N.T. (1985) In situ analysis of trifluorothymidine-resistant (TFT^{r}) mutants of L5178Y/TK$^{+/-}$ mouse lymphoma cells. *Mutat. Res*., **151**, 147–159

Moura Duarte, F.A. (1972) Mutagenic effects of some inorganic acid esters in *Aspergillus nidulans* (Eidam) winter. *Cienc. Cult*., **24**, 42–52 (in Spanish)

NOES (1997) *National Occupational Exposure Survey 1981–83*, Unpublished data as of November 1997, Cincinnati, OH, United States Department of Health and Human Services, Public Health Service, National Institute for Occupational Safety and Health

Oshiro, Y., Balwierz, P.S. & Molinary, S.V. (1981) Morphological transformation of C3H/10T½ CL8 cells by alkylating agents. *Toxicol. Lett.*, **9**, 301–306

Pienta, R.J., Poiley, J.A. & Lebherz, W.B., III (1977) Morphological transformation of early passage golden Syrian hamster embryo cells derived from cryopreserved primary cultures as a reliable *in vitro* bioassay for identifying diverse carcinogens. *Int. J. Cancer*, **19**, 642–655

Rieger, R., Michaelis, A., Schubert, I., Veleminsky, J., Gichner, T. & Angelis, K.J. (1988) Induction of chromatid aberrations by TEM and maleic hydrazide is differently affected by pretreatment of *Vicia faba* root-tip meristems with methyl iodide. *Mutat. Res*., **208**, 101–104

Rosenkranz, H.S. & Poirier, L.A. (1979) Evaluation of the mutagenicity and DNA-modifying activity of carcinogens and noncarcinogens in microbial systems. *J. natl Cancer Inst*., **62**, 873–892

Simmon, V.F. (1979a) In vitro mutagenicity assays of chemical carcinogens and related compounds with *Salmonella typhimurium*. *J. natl Cancer Inst.*, **62**, 893–899

Simmon, V.F. (1979b) In vitro assays for recombinogenic activity of chemical carcinogens and related compounds with *Saccharomyces cerevisiae* D3. *J. natl Cancer Inst.*, **62**, 901–909

Simmon, V.F., Kauhanen, K. & Tardiff, R.G. (1977) Mutagenic activity of chemicals identified in drinking water. In: Scott, D. Bridges, B.A. & Sobels, F.H., eds, *Progress in Genetic Toxicology*, Vol. 2, *Development in Toxicology and Environmental Sciences*, Amsterdam, Elsevier/North-Holland Biomedical Press, pp. 249–258

Takahashi, K. & Kawazoe, Y. (1987) Potent induction of the adaptive response by a weak mutagen, methyl iodide, in *Escherichia coli*. *Mutat. Res*., **180**, 163–169

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Verschueren, K. (1996) *Handbook of Environmental Data on Organic Chemicals*, 3rd Ed., New York, Van Nostrand Reinhold, pp. 1289–1290

WHO (1993) *Guidelines for Drinking Water Quality*, 2nd Ed., Vol. 1, *Recommendations*, Geneva

MORPHOLINE

Data were last evaluated in IARC (1989).

1. Exposure Data

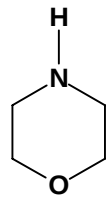
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 110-91-8

Chem. Abstr. Name: Morpholine

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_4H_9NO Relative molecular mass: 87.12

1.1.3 *Physical properties* (for details, see IARC, 1989)

(*a*) *Boiling-point*: 128.3°C; 24.8°C at 1.3 kPa

(*b*) *Melting-point*: –4.7°C; –4.9°C

(*c*) *Conversion factor*: $mg/m^3 = 3.56 \times ppm$

1.2 Production, use and human exposure

Morpholine is a synthetic organic liquid used mainly as an intermediate in the production of rubber chemicals and optical brighteners, as a corrosion inhibitor in steam condensate systems, as an ingredient in waxes and polishes and as a component of protective coatings on fresh fruits and vegetables. Occupational exposure may occur during the production of morpholine and in its various uses, but data on exposure levels are sparse. It has been detected in samples of foodstuffs and beverages (IARC, 1989).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Morpholine was tested for carcinogenicity by oral administration in two strains of mice, one strain of rats and one strain of hamsters. The studies in one of the strains of mice and in hamsters were considered inadequate for evaluation. In the other strain of mice, no significant increase in the incidence of tumours was seen in treated animals. In the study in rats, a few tumours of the liver and lung occurred in treated animals. Morpholine was also tested by inhalation exposure in rats; it did not increase the incidence of tumours over that found in controls (IARC, 1989).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Morpholine is absorbed after oral, dermal and inhalation administration. In rats it was distributed to all organs and eliminated rapidly. In rabbits, mice, rats and hamsters, almost all morpholine was excreted unchanged in the urine following its administration by any route, whereas guinea-pigs excreted 20% of the dose as *N*-methylmorpholine-*N*-oxide. *N*-Nitrosomorpholine was formed in rodents following concomitant administration with nitrite or nitrous oxide and *in vitro* when added to human saliva (IARC, 1989).

4.2 Toxic effects

4.2.1 *Humans*

Rhinitis, lower airway irritation and corneal oedema have been reported in workers exposed to morpholine (IARC, 1989).

4.2.2 *Experimental systems*

Diluted morpholine is a skin and eye irritant in rabbits and guinea-pigs. Its inhalation is irritant in rats and damages the airways in rabbits. Skin application in rabbits, oral administration and skin application in guinea-pigs or inhalation in rats caused necrosis of kidney tubules and liver. Oral administration induced stomach and small intestine haemorrhages in guinea-pigs and rats, liver degeneration in rats and renal insufficiency in mice (IARC, 1989).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

Peripheral blood lymphocytes were analysed for chromosomal aberrations in 24 workers (16 men and 8 women) occupationally exposed to morpholine for 3–10 years. The average atmospheric concentration of morpholine was 0.53–0.93 mg/m^3, reaching maxima of 0.74–2.14 mg/m^3. The percentages of cells with any aberrations were: controls, 1.61 ± 0.2%; exposed, 2.08 ± 0.2%; and the percentages of cells with deletions were: controls, 0.69 ± 0.2%; exposed, 0.86 ± 0.2%. According to the database of the Institute of Medical Genetics (Moscow, Russia), the expected percentage of cells with aberrations is 1.19 (437 individuals analysed) (Katosova *et al.*, 1991). [The Working Group noted the lack of information regarding other exposures, the characteristics of the control group and the types of aberration recorded.]

4.4.2 *Experimental systems*

Morpholine did not induce mutations in bacteria, unscheduled DNA synthesis in primary cultures of rat hepatocytes or chromosomal aberrations in Chinese hamster lung fibroblasts. According to an abstract, it induced a small increase in *tk* locus mutations of mouse lymphoma cells and increased the frequency of morphologically transformed BALB/c 3T3 cells. In a transplacental exposure study with Syrian hamsters, it did not induce micronuclei, chromosomal aberrations or mutations in embryo cells (IARC, 1989).

5. Evaluation

No epidemiological data relevant to the carcinogenicity of morpholine were available.

There is *inadequate evidence* in experimental animals for the carcinogenicity of morpholine.

Overall evaluation

Morpholine is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

IARC (1989) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 47, *Some Organic Solvents, Resin Monomers and Related Compounds, Pigments and Occupational Exposures in Paint Manufacture and Painting*, Lyon, pp. 199–213

Katosova, L.D., Fomenko V.N. & Davydenko, L.N. (1991) Results of cytogenetic examination of workers exposed to morpholine. *Gig. Tr. prof. Zabol.*, **6**, 35–36 (in Russian)

1,5-NAPHTHALENE DIISOCYANATE

Data were last reviewed in IARC (1979) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

1.1 Chemical and physical data

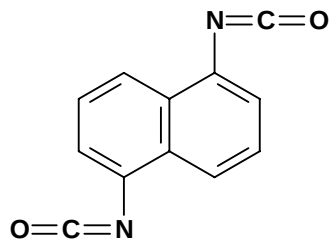
1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 3173-72-6

Chem. Abstr. Name: 1,5-Diisocyanatonaphthalene

IUPAC Systematic Name: Isocyanic acid, 1,5-naphthylene ester

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{12}H_6N_2O_2$ Relative molecular mass: 210.20

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: White to light yellow, crystalline solid (Lewis, 1993)

(*b*) *Boiling-point*: 183°C (Ulrich, 1989)

(*c*) *Melting-point*: 130–132°C (Ulrich, 1989)

(*d*) *Conversion factor*: $mg/m^3 = 8.60 \times ppm$

1.2 Production and use

1,5-Naphthalene diisocyanate is reported to be produced by two companies in Japan and one in Germany (Chemical Information Services, 1995).

1.3 Occurrence

No data were available to the Working Group.

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not proposed any occupational exposure limit for 1,5-naphthalene diisocyanate in workplace air. Values ranging from 0.02 to 0.095 mg/m^3 for a time-weighted average and from 0.01 to 0.07 mg/m^3 for short-term exposure limits have been used as standards or guidelines in several countries (International Labour Office, 1991; United States National Library of Medicine, 1997).

No international guideline for 1,5-naphthalene diisocyanate in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

No data were available to the Working Group.

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Toxic effects

4.2.1 *Humans*

Acute damage to the respiratory tract as well as conjunctivitis were observed in workers employed in the production of polyurethane rubber, in which 1,5-naphthalene diisocyanate was the basic chemical. An association has been reported between bronchitis and exposure to 1,5-naphthalene diisocyanate (IARC, 1979).

The irritative effects of 1,5-naphthalene diisocyanate were confirmed in a further study of rubber production workers, in which exposure over the range of 0.002–0.007 mg/m^3 was associated with increased frequency of eye irritation, cough and exertion dyspnoea. Also, an increase in the closing volume, especially among the older workers, was observed, suggesting airway irritation (Alexandersson *et al.*, 1986)

4.2.2 *Experimental systems*

No data were available to the Working Group.

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

No data were available to the Working Group.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of 1,5-naphthalene diisocyanate were available.

No experimental data relevant to the carcinogenicity of 1,5-naphthalene diisocyanate were available.

Overall evaluation

1,5-Naphthalene diisocyanate is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

Alexandersson, R., Gustafsson, P., Hedenstierna, G. & Rosen, G. (1986) Exposure to naphthalene-diisocyanate in a rubber plant: symptoms and lung function. *Arch. environ. Health*, **41**, 85–89

American Conference of Governmental Industrial Hygienists (1997) *1997 TLVs® and BEIs®*, Cincinnati, OH, pp. 1013–1014

Chemical Information Services (1995) *Directory of World Chemical Producers—1995–1996, Standard Edition*, Dallas, TX, p. 278

IARC (1979) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 19, *Some Monomers, Plastics, Synthetic Elastomers, and Acrolein*, Lyon, pp. 303–340

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 67

International Labour Office (1991) *Occupational Exposure Limits for Airborne Toxic Substances*, 3rd Ed. (Occupational Safety and Health Series No. 37), Geneva, pp. 286–287

Lewis, R.J., Jr (1993) *Hawley's Condensed Chemical Dictionary*, 12th Ed., New York, Van Nostrand Reinhold, pp. 805–806

Ulrich, H., (1989) Acrylic acid and derivatives. In: Gerhartz, W. & Yamamoto, Y.S., eds., *Ullmann's Encyclopedia of Industrial Chemistry*, 5th rev. Ed., Vol. A 14, VCH Publishers, pp. 611–625

United States National Library of Medicine (1997*) Registry of Toxic Effects of Chemical Substances (RTECS) Database*, Bethesda, MD [Record No. 95536]

WHO (1993) *Guidelines for Drinking Water Quality*, 2nd Ed., Vol. 1, *Recommendations*, Geneva

PENTACHLOROETHANE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

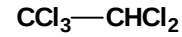
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 76-01-7

Systematic name: Pentachloroethane

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_2HCl_5 Relative molecular mass: 202.29

1.1.3 *Physical properties* (for details, see IARC, 1986)

(*a*) *Boiling-point*: 162°C

(*b*) *Melting-point*: –29°C

(*c*) *Conversion factor*: $mg/m^3 = 8.27 \times ppm$

1.2 Production, use and human exposure

Pentachloroethane was produced commercially as a chemical intermediate, and occupational exposure may have occurred. Trace levels have been reported in ambient air and water (IARC, 1986).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Technical-grade pentachloroethane (containing 4.2% hexachloroethane) was tested for carcinogenicity by oral administration by gavage in one experiment in mice and one

experiment in rats. Hepatocellular carcinomas were induced in mice of each sex and hepatocellular adenomas in female mice; a marginally increased incidence of kidney tubule-cell adenomas was observed in male rats but not in female rats (IARC, 1986).

No data were available on the carcinogenicity of pure pentachloroethane (IARC, 1986).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Following subcutaneous administration of 1.1–1.8 mg/kg bw pentachloroethane to female mice, 12–51% of the dose was expired as the parent compound, 2–16% as trichloroethylene and 3–9% as tetrachloroethylene; the major urinary metabolites were trichloroethanol (16–32%) and trichloroacetic acid (9–18%) (IARC, 1986).

Pentachloroethane is dechlorinated in the presence of a rabbit liver reconstituted cytochrome P450 system or by rat liver microsomes, NADPH and oxygen (without oxygen, it is metabolized to 96% trichloroethylene and 4% 1,1,2,2-tetrachloroethane) (IARC, 1986).

Kinetic constants of pentachloroethane metabolism have been determined in male Fischer 344 rats exposed to the vapour phase at a concentration of 2895 mg/m^3 for 6 h and then placed in exhaled breath chambers. The maximum metabolic rate V_{max} was 9.2 mg/kg per hour (45.5 μmol/kg per hour) and the Michaelis constant K_m was 0.9 mg/L (4.45 μM) (Gargas & Andersen, 1989).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Pentachloroethane given to rats by gavage during a two-year study caused chronic, diffuse kidney inflammation and renal papilla mineralization. A single dose also reduced hepatic cytochrome P450 content and microsomal epoxide hydrolase activities. Inhalation exposure of rabbits to pentachloroethane decreased their total antibody titres (IARC, 1986).

Male and female Fischer 344 rats were dosed with pentachloroethane (purity, 96%) by gavage for 10 days at a dose level of 150 mg/kg bw. The rat strain, dose and dose route were the same as used in the carcinogenicity study (see Section 3). In male rats, there were increases in the renal α_{2u}-globulin protein droplet concentration from 9.1 ± 2.3 mg/kg wet

kidney weight in controls to 25.9 mg/kg in the treated group. Specifically, in the P_2 renal tubule segment, the [^{3}H]thymidine cell-labelling index increased from 11.5 ± 0.7% in controls to 38.8 ± 3.9% in the treated group. The corresponding values for the P_1 and P_3 segments, respectively, were 8.7 ± 0.6% and 8.5 ± 1.5% in controls and 9.2 ± 1.6% for both segments in the treated group. The labelling index in the P_2 segment in female rats was 1.8 ± 0.7% in controls and 0.8 ± 0.2% in the treated group (Goldsworthy *et al.*, 1988).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Pentachloroethane is not mutagenic to *Salmonella typhimurium*. In the presence of an exogenous metabolic activation system, there was weak induction of mutation and gene conversion in yeast. In Chinese hamster ovary CHO cells, there was induction of sister chromatid exchanges, but not of chromosomal aberrations. In Chinese hamster lung CHL cells, chromosomal aberrations and aneuploidy were induced. Mutations were induced at the *tk* locus of mouse lymphoma L5178Y cells.

[U-^{14}C]Pentachloroethane injected into the peritoneal cavity of male Wistar rats and BALB/c mice was found to bind to DNA (as well as RNA and proteins) in liver, stomach, lung and kidney. Adducts were not identified (Turina *et al.*, 1989).

5. Evaluation

No epidemiological data relevant to the carcinogenicity of pentachloroethane were available.

There is *limited evidence* in experimental animals for the carcinogenicity of pentachloroethane.

Overall evaluation

Pentachloroethane is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

Bronzetti, G., Morichetti, E., Del Carratore, R., Rosellini, D., Paolini, M., Cantelli-Forti, G., Grilli, S. & Vellosi, R. (1989) Tetrachloroethane, pentachloroethane, and hexachloroethane: genetic and biochemical studies. *Teratog. Carcinog. Mutag.*, **9**, 349–357

Table 1. Genetic and related effects of pentachloroethane

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	38	Haworth *et al*. (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	38	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	38	Haworth *et al*. (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	38	Haworth *et al*. (1983)
SCG, *Saccharomyces cerevisiae* D7, gene conversion *trp* locus	–	(+)	506	Bronzetti *et al*. (1989)
ANG, *Aspergillus nidulans* P1, mitotic segregation	–	NT	336	Crebelli *et al*. (1988)
SCR, *Saccharomyces cerevisiae* D7, forward mutation *ilv* locus	–	(+)	1517	Bronzetti *et al*. (1989)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–[c]		800 ppm inj	Foureman *et al*. (1994)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	–	100	Galloway *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	206	Galloway *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster lung CHL cells *in vitro*	+	+	80	Matsuoka *et al*. (1996)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	70	McGregor *et al*. (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus (microwell method) *in vitro*	+[d]	–	80	Sofuni *et al*. (1996)
AIA, Aneuploidy, Chinese hamster lung CHL cells *in vitro*	+	+	80	Matsuoka *et al*. (1996)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; inj, injection
[c] Also negative with 300 ppm in feed
[d] Two laboratories

Crebelli, R., Benigni, R., Franekic, J., Conti, G., Conti, L. & Carere, A. (1988) Induction of chromosome malsegregation by halogenated organic solvents in *Aspergillus nidulans*: unspecific or specific mechanism? *Mutat. Res.*, **201**, 401–411

Foureman, P., Mason, J.M., Valencia, R. & Zimmering, S. (1994) Chemical mutagenesis testing in *Drosophila*. X. Results of 70 coded chemicals tested for the National Toxicology Program. *Environ. mol. Mutag.*, **23**, 208–227

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B. & Zeiger, E. (1987) Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. *Environ. mol. Mutag.*, **10** (Suppl. 10), 1–175

Gargas, M.L. & Andersen, M.E. (1989) Determining kinetic constants of chlorinated ethane metabolism in the rat from rates of exhalation. *Toxicol. appl. Pharmacol.*, **99**, 344–353

Goldsworthy, T.L., Lyght, O., Burnett, V.L. & Popp, J.A. (1988) Potential role of α-2μ-globulin, protein droplet accumulation, and cell replication in the renal carcinogenicity of rats exposed to trichloroethylene, perchloroethylene, and pentachloroethane. *Toxicol. appl. Pharmacol.*, **96**, 367–379

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W. & Zeiger, E. (1983) *Salmonella* mutagenicity test results for 250 chemicals. *Environ. Mutag.*, **5** (Suppl. 1), 3–142

IARC (1986) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 41, *Some Halogenated Hydrocarbons and Pesticide Exposures*, Lyon, pp. 99–111

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 69

Matsuoka, A., Yamakage, K., Kusakabe, H., Wakuri, S., Asakura, M., Noguchi, T., Sugiyama, T., Shimada, H., Nakayama, S., Kasahara, Y., Takahashi, Y., Mima, K.F., Hatanaka, M., Ishidate, M., Morita, T., Watanabe, D., Hara, M., Odawara, K., Tanaka, N., Hayashi, M. & Sofuni, T. (1996) Re-evaluation of chromosomal aberration induction on nine mouse lymphoma assay 'unique positive' NTP carcinogens. *Mutat. Res.*, **369**, 243–252

McGregor, D.B., Brown, A., Cattanach, P., Edwards, I., McBride, D., Riach, C. & Caspary, W.J. (1988) Responses of the L5178Y tk^+/tk^- mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. *Environ. mol. Mutag.*, **12**, 85–154

Sofuni, T., Honma, M., Hayashi, M., Shimada, H., Tanaka, N., Wakuri, S., Awogi, T., Yamamoto, K.I., Nishi, Y. & Nakadate, M. (1996) Detection of in vitro clastogens and spindle poisons by the mouse lymphoma assay using the microwell method: interim report of an international collaborative study. *Mutagenesis*, **11**, 349–355

Turina, M.P., Colacci, A., Grilli, S., Mazzullo, M., Prodi, G., Lattanzi, G., Bonora, B., Bartoli, S. & Guidotti, L. (1989) Metabolic activation and covalent binding to nucleic acids of pentachloroethane as short-term test of genotoxicity. *Res. Commun. chem. Pathol. Pharmacol.*, **63**, 81–91

PHENYL GLYCIDYL ETHER

Data were last evaluated in IARC (1989).

1. Exposure Data

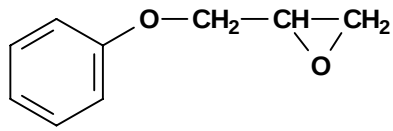
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 122-60-1

Systematic name: (Phenoxymethyl)oxirane

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_9H_{10}O_2$ Relative molecular mass: 150.18

1.1.3 *Physical properties* (for details, see IARC, 1989)

(*a*) *Boiling-point*: 245°C

(*b*) *Melting-point*: 3.5°C

(*c*) *Conversion factor*: $mg/m^3 = 6.14 \times ppm$

1.2 Production, use and human exposure

Glycidyl ethers are basic components of epoxy resins which have been commercially available since the late 1940s. Bisphenol A diglycidyl ether and its oligomers are major components of epoxy resins. Other glycidyl ethers, including phenyl glycidyl ether, are frequently incorporated into epoxy resin systems as reactive modifiers. Epoxy resins based on bisphenol A diglycidyl ether are widely used in protective coatings, including paints, in reinforced plastic laminates and composites, in tooling, casting and moulding resins, in bonding materials and adhesives, and in floorings and aggregates. Occupational exposure to bisphenol A diglycidyl ether and phenyl glycidyl ether may occur during their production, during the production of epoxy products and during various uses of epoxy products, but data on exposure levels are sparse (IARC, 1989).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Pure phenyl glycidyl ether was tested for carcinogenicity by inhalation exposure in male and female rats of one strain, producing carcinomas of the nasal cavity in animals of each sex (IARC, 1989).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Percutaneous absorption of phenyl glycidyl ether is high in rats and rabbits. It binds to glutathione in the presence of liver microsomes from various avian species (IARC, 1989).

4.2 Toxic effects

4.2.1 *Humans*

Phenyl glycidyl ether has been recognized as a contact allergen using patch tests on exposed workers from various factories who developed dermatitis and dermatosis (IARC, 1989).

4.2.2 *Experimental systems*

Blood, urine and histopathological analysis of rats and beagle dogs exposed by inhalation to 6–73.5 mg/m^3 phenyl glycidyl ether for three months did not reveal any treatment-related effect. This compound caused skin irritation and corneal injury in rabbits and hair loss in rats. Its sensitizing potential in guinea-pigs is low (IARC, 1989).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Prenatal toxicity was not induced in rats exposed by inhalation to phenyl glycidyl ether (IARC, 1989).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

Phenyl glycidyl ether induced mutations in bacteria and transformation in mammalian cells *in vitro* (in a Syrian hamster embryo cell clonal assay and in an assay for the enhancement of viral transformation), but did not induce chromosomal aberrations in animal cells *in vitro* or either micronuclei or chromosomal aberrations *in vivo*. It did not induce dominant lethal effects in rats (IARC, 1989).

Thymidine and 2′-deoxyadenosine react with phenyl glycidyl ether *in vitro*. The main adducts are *N*-3-(2-hydroxy-3-phenoxypropyl)thymidine and *N*-1-(2-hydroxy-3-phenoxypropyl)-2′-deoxyadenosine, respectively. With longer reaction times, a small amount of dialkylated 2′-deoxyadenosine was also formed (Van den Eeckhout *et al.*, 1990).

5. Evaluation

No epidemiological data relevant to the carcinogenicity of phenyl glycidyl ether were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of phenyl glycidyl ether.

Overall evaluation

Phenyl glycidyl ether is *possibly carcinogenic to humans (Group 2B)*.

6. References

IARC (1989) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 47, *Some Organic Solvents, Resin Monomers and Related Compounds, Pigments and Occupational Exposures in Paint Manufacture and Painting*, Lyon, pp. 237–261

Van den Eeckhout, E., De Bruyn, A., Pepermans, H., Esmans, E.L., Vryens, I., Claereboudt, J., Claeys, M. & Sinsheimer, J.E. (1990) Adduct formation identification between phenyl glycidyl ether and 2′-deoxyadenosine and thymidine by chromatography, mass spectrometry and nuclear magnetic resonance spectroscopy. *J. Chromatogr.*, **504**, 113–128

TETRAKIS(HYDROXYMETHYL)PHOSPHONIUM SALTS

Data were last evaluated in IARC (1990).

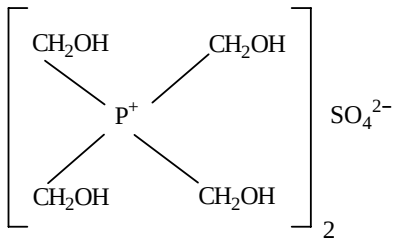
1. Exposure Data

1.1 Chemical and physical data

Tetrakis(hydroxymethyl)phosphonium sulfate

Chem. Abstr. Serv. Reg. No.: 55566-30-8

Chem. Abstr. Name: Phosphonium, tetrakis(hydroxymethyl)-, sulfate (2:1) (salt)

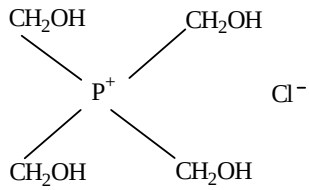


$C_8H_{24}O_{12}P_2S$ Relative molecular mass: 406.28

Tetrakis(hydroxymethyl)phosphonium chloride

Chem. Abstr. Serv. Reg. No.: 124-64-1

Chem. Abstr. Name: Phosphonium, tetrakis(hydroxymethyl)-, chloride

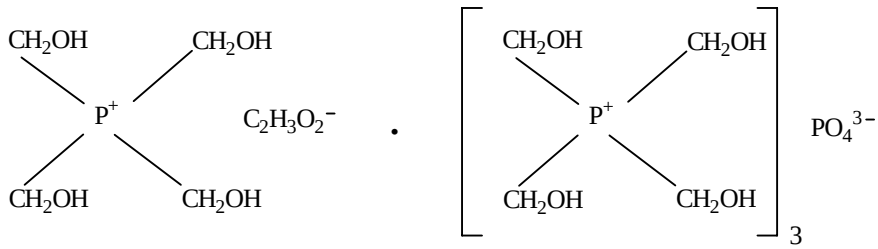


$C_4H_{12}ClO_4P$ Relative molecular mass: 190.56

Tetrakis(hydroxymethyl)phosphonium acetate/phosphate

Chem. Abstr. Serv. Reg. No.: 55818-96-7

Chem. Abstr. Name: Phosphonium, tetrakis(hydroxymethyl)-, acetate (salt), mixture with tetrakis(hydroxymethyl)phosphonium phosphate (3:1) (salt)



$C_4H_{12}O_4P$. $C_2H_3O_2/(C_4H_{12}O_4P)_3$. PO_4 Relative molecular mass: 214.16/560.30

1.2 Production and use

Tetrakis(hydroxymethyl)phosphonium salts are used to produce crease-resistant and flame-retardant finishes on textile fabrics, including children's nightwear. No data on occupational exposure levels were available (IARC, 1990).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Tetrakis(hydroxymethyl)phosphonium sulfate was tested for carcinogenicity by oral administration in one strain of mice and one strain of rats. No dose-related increase in the incidence of any tumour was observed, but in males receiving the low dose there was an increased incidence of malignant lymphomas in mice and of mononuclear-cell leukaemia in rats.

Tetrakis(hydroxymethyl)phosphonium chloride was tested for carcinogenicity by oral administration in one strain of mice and one strain of rats. No dose-related increase in the incidence of any tumour was observed; however, in male rats receiving the low dose, there was an increased incidence of mononuclear-cell leukaemia. Tetrakis(hydroxymethyl)-phosphonium chloride did not show significant promoting activity in a two-stage skin carcinogenicity test in mice.

A mixed acetate/phosphate salt of tetrakis(hydroxymethyl)phosphonium base showed weak promoting activity in a two-stage skin carcinogenicity study (IARC, 1990).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Tetrakis(hydroxymethyl)phosphonium chloride can be absorbed through the skin (Ulsamer *et al.*, 1980).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

During 90-day gavage studies, hepatocyte vacuolar degeneration was seen in rats and mice receiving tetrakis(hydroxymethyl)phosphonium sulfate or chloride. Neurotoxicity was also caused by the latter salt (IARC, 1990).

In two-year studies, lesions attributed to administration of the sulfate salt in rats included liver cystic degeneration in males and hepatocyte cytoplasmic vacuolization in animals of each sex. No lesion was reported in treated mice. When treated with the chloride salt, mice and rats showed hepatocyte cytoplasmic vacuolization. Moreover, male rats showed liver cystic degeneration, female mice displayed thyroid follicular-cell hyperplasia and female rats spleen haematopoeisis (IARC, 1990).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

Tetrakis(hydroxymethyl)phosphonium chloride and sulfate salts were not mutagenic to bacteria in either the presence or absence of an exogenous metabolic system. In single studies, the sulfate salt induced mutations in mouse lymphoma L5178Y cells *in vitro* at the *tk* locus and, in mouse bone marrow *in vivo*, it caused a marginal increase in the frequency of chromosomal aberrations, but did not induce micronuclei (IARC, 1990).

Tetrakis(hydroxymethyl)phosphonium chloride was not mutagenic to bacteria in either the presence or absence of an exogenous metabolic system. It induced sister chro-

matid exchanges and chromosomal aberrations in Chinese hamster ovary cells *in vitro* and, in a single study, it induced mutations in mouse lymphoma L5178Y cells *in vitro* at the *tk* locus (IARC, 1990).

5. Evaluation

No epidemiological relevant to the carcinogenicity of tetrakis(hydroxymethyl)-phosphonium salts were available.

There is *inadequate evidence* in experimental animals for the carcinogenicity of tetrakis(hydroxymethyl)phosphonium salts.

Overall evaluation

Tetrakis(hydroxymethyl)phosphonium salts are *not classifiable as to their carcinogenicity to humans (Group 3)*.

6. References

Grasselli, J.G. & Ritchey, W.M., eds (1975) *CRC Atlas of Spectral Data and Physical Constants for Organic Compounds*, Vol. 4, Cleveland, OH, CRC Press, p. 120

IARC (1990) *IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans*, Volume 48, *Some Flame Retardants and Textile Chemicals, and Exposures in the Textile Manufacturing Industry*, Lyon, pp. 95–107

Ulsamer, A.G., Osterberg, R.E. & McLaughlin, J., Jr (1980) Flame-retardant chemicals in textiles. *Clin. Toxicol.*, **17**, 101–131

TRICHLOROACETONITRILE

Data were last evaluated in IARC (1991).

1. Exposure Data

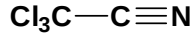
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 545-06-2

Systematic name: Trichloroacetonitrile

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_2NCl_3 Relative molecular mass: 144.39

1.1.3 *Physical properties* (for details, see IARC 1991)

(*a*) *Boiling point*: 85.7°C

(*b*) *Melting-point*: –42°C

(*c*) *Conversion factor*: $mg/m^3 = 5.91 \times ppm$

1.2 Production, use and human exposure

Halogenated acetonitriles are not produced on an industrial scale. Trichloroacetonitrile has been used on a limited basis in the past as a pesticide. Several halogenated acetonitriles have been detected in chlorinated drinking-water in a number of countries as a consequence of the reaction of chlorine with natural organic substances. The only known route of human exposure is through chlorinated drinking-water (IARC, 1991).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Trichloroacetonitrile was tested in a limited carcinogenicity study in female SEN mice by skin application, in an initiation/promotion study in female SEN mice by skin application and in a screening assay for lung tumours in female strain A mice by oral administration. No skin tumour was produced after skin application in mice or in the initiation/promotion study, in which trichloroacetonitrile was applied topically as six equal doses over a two-week period, followed by repeated doses of 12-*O*-tetradecanoyl-phorbol 13-acetate for 20 weeks. A small, significant increase in the proportion of mice with lung tumours and in the number of tumours per mouse was observed: control, 3/31 and 0.1; treated group (10 mg/kg bw, three times per week, eight weeks), 9/32 and 0.38 ($p < 0.05$) (IARC, 1991).

4. Other Data Relevant to an Evaluation Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

About 2% of a single oral dose of 108 mg/kg bw trichloroacetonitrile to rats was excreted in urine within 24 h as thiocyanate, the product of released cyanide metabolized by rhodanese (IARC, 1991).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Trichloroacetonitrile did not induce γ-glutamyltranspeptidase-positive foci in rat liver (IARC, 1991).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Trichloroacetonitrile given orally to rats at doses of 15–55 mg/kg bw per day on gestation days 6–18 was associated in the full-term fetuses with an increased frequency of

malformations, particularly of the cardiovascular and urogenital organs. Embryolethality occurred at dose levels below those which caused maternal toxicity and malformations (IARC, 1991).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Trichloroacetonitrile did not induce DNA damage in bacteria. Conflicting results were obtained in bacterial mutation studies. It induced sister chromatid exchanges and DNA strand breaks in mammalian cell lines. Micronuclei were induced in the erythrocytes of newt (*Pleurodeles waltl*) larvae exposed for 12 days, but in mice dosed for five days, neither micronuclei in bone marrow nor abnormal sperm morphology were induced.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of trichloroacetonitrile were available.

There is *inadequate evidence* for the carcinogenicity of trichloroacetonitrile in experimental animals.

Overall evaluation

Trichloroacetonitrile is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

Bull, R.J., Meier, J.R., Robinson, M., Ringhand, H.P., Laurie, R.D. & Stober, J.A. (1985) Evaluation of mutagenic and carcinogenic properties of brominated and chlorinated acetonitriles: by-products of chlorination. *Fundam. appl. Toxicol.*, **5**, 1065–1074

Daniel, F.B., Schenck, K.M., Mattox, J.K., Lin, E.L., Haas, D.L. & Pereira, M.A. (1986) Genotoxic properties of haloacetonitriles: drinking water by-products of chlorine disinfection. *Fundam. appl. Toxicol.*, **6**, 447–453

IARC (1991) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 52, *Chlorinated Drinking-water; Chlorination By-products; Some Other Halogenated Compounds; Cobalt and Cobalt Compounds*, Lyon, pp. 269–296

Le Curieux, F., Giller, S., Gauthier, L., Erb, F. & Marzin, D. (1995) Study of the genotoxic activity of six halogenated autonitriles, using the SOS chromotest, the Ames-fluctuation test and the newt micronucleus test. *Mutat. Res.*, **341**, 289–302

Table 1. Genetic and related effects of trichloroacetonitrile

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS chromotest, *Escherichia coli* PQ37	–	–	100	Le Curieux *et al.* (1995)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	844	Bull *et al.* (1985)
SA0, *Salmonella typhimurium* TA100, reverse mutation (fluctuation test)	+	–	30	Le Curieux *et al.* (1995)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	844	Bull *et al.* (1985)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	NG	Bull *et al.* (1985)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	NG	Bull *et al.* (1985)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	844	Bull *et al.* (1985)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	2	Bull *et al.* (1985)
DIH, DNA strand breaks, human lymphoblastic line (of T-cell origin) *in vitro*	+	NT	NG	Daniel *et al.* (1986)
MVM, Micronucleus test, CD-1 mouse bone-marrow cells *in vivo*	–		50 po × 5	Bull *et al.* (1985)
Micronucleus test, *Pleurodeles waltl* erythrocytes *in vivo*	+		0.1 µg/mL	Le Curieux *et al.* (1995)
SPM, Sperm morphology, B6C3F_1 mice, *in vivo*	–		50 po × 5	Meier *et al.* (1985)

[a] +, positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NG, not given; po, oral

Meier, J.R., Bull, R.J., Stober, J.A. & Cimino, M.C. (1985) Evaluation of chemicals used for drinking water disinfection for production of chromosomal damage and sperm-head abnormalities in mice. *Environ. Mutag.*, **7**, 201–211

TRIETHYLENE GLYCOL DIGLYCIDYL ETHER

Data were last reviewed in IARC (1976) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

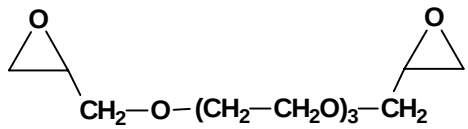
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.:1954-28-5

Systematic name: 2,2′-(2,5,8,11-Tetraoxadodecane-1,12-diyl)bisoxirane

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{12}H_{22}O_6$ Relative molecular mass: 262.3

1.1.3 *Physical properties* (for details, see IARC, 1976)

(*a*) *Boiling point*: 133–149°C at 13.3 Pa; 195–197°C at 266 Pa

(*b*) *Melting-point*: –15 to –11°C

(*c*) *Conversion factor*: $mg/m^3 = 10.73 \times ppm$

1.2 Production and use

Triethylene glycol diglycidyl ether was first prepared in 1962, but has been produced commercially only on a small scale in the United Kingdom for use as an antineoplastic drug given by intravenous or intraarterial injection (IARC, 1976).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Triethylene glycol diglycidyl ether was tested by intraperitoneal injection in a single study for lung adenoma induction in strain A mice. It increased the incidence of lung tumours at the high dose (IARC, 1976).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

In rats treated intravenously or subcutaneously with triethylene glycol diglycidyl ether, 75% of the dose was excreted in the urine as triethylene glycol-bis-2,3-dihydroxypropyl ether, together with a hydroxymercapturic acid and an olefinic mercapturate derived from that hydroxymercapturate. A glutathione conjugate of triethylene glycol diglycidyl ether and the corresponding cysteinylglycine and cysteine conjugates were excreted into the bile.

When this compound was incubated with rat-liver homogenates, only the cysteinylglycine conjugate was found (IARC, 1976).

4.2 Toxic effects

4.2.1 *Humans*

The severe kidney damage seen in rats and dogs after intravenous administration of triethylene glycol diglycidyl ether has not been observed in human patients; however, haematological depression (leukopenia for instance) and temporary dysuria were observed (IARC, 1976).

4.2.2 *Experimental systems*

Rats and dogs given intravenous doses of triethylene glycol diglycidyl ether showed necrosis of the renal tubule epithelium, of the adrenal cortex and of the intestinal epithelium. In dogs, blood neutrophils disappeared and lymphocyte counts fell to 50% of normal. Though the erythrocyte and platelet counts remained constant, the brief appearance of polychromatic and nucleated red cells indicated that erythropoiesis was also affected.

Testicular atrophy and decreased spermatogenesis were observed in mice after intraperitoneal injection (IARC, 1976).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

No data were available to the Working Group.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of triethylene glycol diglycidyl ether were available.

There is *inadequate evidence* for the carcinogenicity of triethylene glycol diglycidyl ether in experimental animals.

Overall evaluation

Triethylene glycol diglycidyl ether is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

IARC (1976) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*, Volume 11, *Cadmium, Nickel, Some Epoxides, Miscellaneous Industrial Chemicals and General Considerations on Volatiles Anaesthetics*, Lyon, pp. 209–214

IARC (1987) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Supplement 7, *Overall Evaluations of Carcinogenicity: An Updating of* IARC Monographs *Volumes 1 to 42*, Lyon, p. 73

TRIS(2-CHLOROETHYL) PHOSPHATE

Data were last evaluated in IARC (1990).

1. Exposure Data

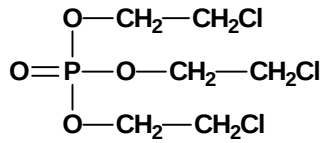
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 115-96-8

Systematic name: Tris(2-chloroethyl) phosphate

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_6H_{12}Cl_3O_4P$ Relative molecular mass: 285.49

1.1.3 *Physical properties* (for details, see IARC, 1990)

(*a*) *Boiling-point*: 330°C

(*b*) *Melting-point*: –55°C

1.2 Production, use and human exposure

Tris(2-chloroethyl) phosphate is used as a flame retardant in plastics, especially in flexible foams used in automobiles and furniture, and in rigid foams used for building insulation. No data on occupational exposure levels were available. Tris(2-chloroethyl) phosphate has been detected in drinking-water, river water, sea water and sediments in various parts of the world (IARC, 1990).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Tris(2-chloroethyl) phosphate was tested for initiating and promoting activity and for complete carcinogenicity in one strain of mice by skin application. No initiating activity was found; promoting activity and complete carcinogenicity could not be evaluated (IARC, 1990).

3.1 Oral administration

3.1.1 *Mouse*

Groups of 60 male and 60 female B6C3F_1 mice, eight to nine weeks of age, were administered 0, 175 or 350 mg/kg bw tris(2-chloroethyl) phosphate (purity, ≈ 98%) by gavage on five days per week for up to 104 weeks. Eight to ten mice of each sex from each group were evaluated at 66 weeks for organ weights and clinical pathology. There was no significant difference in survival between treated and control groups of either sex, and final mean body weights of mice were similar among all groups. The principal chemical-related effects occurred in the kidney, in which nuclear enlargement (karyomegaly) of tubule epithelial cells was present in approximately 80% of high-dose mice. In the original diagnosis, renal tubule adenomas were seen in one control male, one high-dose male and one low-dose female. A carcinoma was also seen in one high-dose male. In a subsequent examination of step sections of all the mouse kidneys, adenomas were found in one low-dose male and two high-dose males. The incidences of renal tubule neoplasms in the original and step sections combined were (control, low-dose, high-dose) 1/50, 1/50, and 4/50 for males. Treated female mice demonstrated a marginally increased incidence of neoplasms (primarily adenomas) of the Harderian gland (3/50, 8/50, 7/50); in addition, three Harderian gland neoplasms occurred in high-dose female mice evaluated after 66 weeks (United States National Toxicology Program, 1991).

3.1.2 *Rat*

Groups of 60 male and 60 female Fischer 344/N rats, eight to ten weeks of age, were administered 0, 44 or 88 mg/kg bw tris(2-chloroethyl) phosphate (purity, ≈ 98%) by gavage on five days per week for up to 104 weeks. Nine or ten rats of each sex from each group were evaluated at 66 weeks for organ weights and clinical pathology. There were no clinical signs attributable to the treatment. Survival in the high-dose groups of male and female rats was significantly reduced, so that, at two years, the survivors in the control, low- and high-dose groups, respectively, were: males—36/50, 33/50, 25/50; females—32/50, 33/50, 17/50. Female rats dying early or that were killed while moribund frequently had brain lesions, whereas male rats did not. Final mean body weights of surviving rats were similar to those of controls. All rats were examined microscopically. The principal treatment-related effects occurred in the kidney and brain. The incidences of focal hyperplasia of the renal tubule epithelium and renal tubule adenomas were markedly increased in male rats receiving 88 mg/kg tris(2-chloroethyl) phosphate and, to a lesser

extent, in female rats (control, low-dose, high-dose; renal tubule hyperplasia, male rats: 0/50, 2/50, 24/50; female rats: 0/50, 3/50,16/50; renal tubule adenoma, male rats: 1/50, 5/50, 24/50 ($p < 0.001$); female rats: 0/50, 2/50, 5/50) ($p = 0.003$)). Renal tubule carcinomas occurred in one control and one high-dose male rat. Degenerative lesions consisting of gliosis, mineralization, haemorrhage and/or haemosiderin accumulation occurred in the cerebrum and brain stem of more than 50% of female rats receiving 44 or 88 mg/kg bw tris(2-chloroethyl) phosphate; similar lesions were seen in only a few treated males (United States National Toxicology Program, 1991).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Neurotoxic effects of tris(2-chloroethyl) phosphate have been reported in rats and hens (IARC, 1990).

Neurotoxic effects were observed in 16-week dose-range finding studies preliminary to the carcinogenicity studies described in Section 3.1 (United States National Toxicology Program, 1991). Mild inhibition of serum cholinesterase activity was seen in female rats receiving 175 and 350 mg/kg bw for either 16 days or 16 weeks, but not in male rats or in male or female mice. Clinical signs of toxicity in female rats included ataxia, excessive salivation, gasping and convulsions, which may have been related to the cholinesterase inhibition. Alternatively, some of the clinical signs may be attributed to the observed neuronal necrosis in the hippocampus and thalamus. Female rats were also more seriously affected by neurotoxicity in the subsequent carcinogenicity study.

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

Tris(2-chloroethyl) phosphate was not mutagenic to bacteria in the absence of an exogenous metabolic system but gave equivocal results in the presence of an exogenous metabolic system (IARC, 1990). It caused cell transformation and, in single studies, sister chromatid exchanges but not chromosomal aberrations or mutations in rodent cells *in vitro*. In single studies, it gave equivocal results in a micronucleus test in Chinese hamsters and caused dominant lethal mutations in rats *in vivo*.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of tris(2-chloroethyl) phosphate were available.

There is *limited evidence* for the carcinogenicity of tris(2-chloroethyl) phosphate in experimental animals.

Overall evaluation

Tris(2-chloroethyl) phosphate is *not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

Galloway, S.W., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J, Margolin, B.H., Resnick, M.A., Anderson, B. & Zeiger, E. (1987) Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. *Environ. Mutag.*, **10** (Suppl. 10), 1–175

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Table 1. Genetic and related effects of tris(2-chloroethyl) phosphate

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	6950	Prival *et al*. (1977)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	(+)	1427	Nakamura *et al*. (1979)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	500	Haworth *et al*. (1983)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	6950	Prival *et al*. (1977)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	+	143	Nakamura *et al*. (1979)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	500	Haworth *et al*. (1983)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	500	Haworth *et al*. (1983)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	–	6950	Prival *et al*. (1977)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	500	Haworth *et al*. (1983)
G9H, Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	–	2000	Sala *et al*. (1982)
SIC, Sister chromatid exchange, Chinese hamster lung V79 cells *in vitro*	+	+	700	Sala *et al*. (1982)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	?	500	Galloway *et al*. (1987)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	1600	Galloway *et al*. (1987)
TCM, Cell transformation, C3H 10T½ mouse cells	–	(+)	900	Sala *et al*. (1982)
TCS, Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	400	Sala *et al*. (1982)
MVM, Micronucleus test, Chinese hamster lung V79 cells *in vivo*	?		250 ip × 1	Sala *et al*. (1982)
DLR, Dominant lethal test, rats *in vivo*	+		0.5 mg/m^3 inh	Shepel'skaia & Dyshginevich (1981)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; ip, intraperitoneal; inh, inhalation

United States National Toxicology Program (1991) *Toxicology and Carcinogenesis Studies of Tris(2-chloroethyl)phosphate (CAS No.115-96-8) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies)* (Technical Report Series No. 391; NIH Publication No. 91-2846), Research Triangle Park, NC, United States Department of Health and Human Services

1,2,3-TRIS(CHLOROMETHOXY)PROPANE

Data were last reviewed in IARC (1977) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

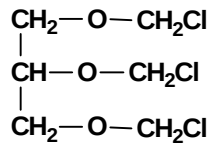
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 38571-73-2

Systematic name: 1,2,3-Tris(chloromethoxy)propane

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_6H_{11}Cl_3O_3$ Relative molecular mass: 237.5

1.1.3 *Physical properties* (for details, see IARC, 1977)

(*a*) *Boiling-point*: 155°C at 2.5 kPa

1.2 Production and use

1,2,3-Tris(chloromethoxy)propane appears not to have been produced commercially, although its use as a resin-hardening agent was investigated (IARC, 1977).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

1,2,3-Tris(chloromethoxy)propane was tested for carcinogenicity in mice by subcutaneous and intraperitoneal administration; it produced malignant tumours at the sites

of administration. Skin papillomas and a low incidence of skin carcinomas were observed in skin-painting studies in mice (IARC, 1977).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

No data were available to the Working Group.

5. Evaluation

No epidemiological data relevant to the carcinogenicity of 1,2,3-tris(chloromethoxy)-propane were available.

There is *limited evidence* for the carcinogenicity of 1,2,3-tris(chloromethoxy)-propane in experimental animals.

Overall evaluation

1,2,3-Tris(chloromethoxy)propane is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

IARC (1977) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man*, Vol. 15, *Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals*, Lyon, pp. 301–305

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VINYLIDENE FLUORIDE

Data were last reviewed in IARC (1986) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

1. Exposure Data

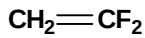
1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Reg. Serv. No.: 75-38-7

Systematic name: 1,1-Difluoroethene

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2H_2F_2$ Relative molecular mass: 64.04

1.1.3 *Physical properties* (for details, see IARC, 1986)

(*a*) *Boiling-point*: –83°C

(*b*) *Melting-point*: –144°C

(*c*) *Conversion factor*: $mg/m^3 = 2.62 \times ppm$

1.2 Production, use and human exposure

Vinylidene fluoride has been produced commercially since the 1940s. It is used in the manufacture of polyvinylidene fluoride and elastomeric copolymers. Human exposure can occur in the manufacture of the monomer and its use in polymer production (IARC, 1986).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

In a limited study in one strain of rats by oral administration, a small number of liposarcomas was observed in treated animals (IARC, 1986).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Vinylidene fluoride is taken up rapidly via the pulmonary route in rats, but at equilibrium the mean concentration (by volume) in rats was only 23% of that in the gaseous phase. Metabolism proceeded very slowly and was saturable at exposure concentrations of about 260 mg/m^3. Its maximum rate was 1% that of vinyl chloride and less than 20% that of vinyl fluoride; there has been a report of an increase in the urinary excretion of fluoride in exposed rats. No alkylating intermediate was demonstrated after passage through a mouse-liver microsomal system. However, vinylidene fluoride inhibits mixed-function oxidase activity *in vitro* and, like similar halogenated compounds that are transformed to reactive metabolites, it alters rat intermediary metabolism, leading to acetone exhalation (IARC, 1986).

Male Fischer 344/N rats were exposed via the nose only for 6 h to concentrations of vinylidene fluoride ranging from 27 to 16 000 ppm [71–42 000 mg/m^3]. Tidal volume (mean, 1.51 mL/breath) and respiratory frequency (mean, 132 breaths/min) were not influenced by exposure concentration. Steady-state blood levels of vinylidene fluoride increased linearly with increasing exposure concentration up to 16 000 ppm. Vinylidene fluoride tissue/air partition coefficients were determined experimentally to be 0.07, 0.18, 0.8, 1.0, and 0.29 for water, blood, liver, fat and muscle, respectively. Previously published determinations (Filser & Bolt, 1979) for the maximum velocity of metabolism (V_{max} in mg/h/kg) and Michaelis–Menten constant (K_m in mg/L) are 0.07 and 0.13, respectively. Time to reach steady-state blood levels of vinylidene fluoride was less than 15 min for all concentrations. After cessation of exposure, blood levels of vinylidene fluoride decreased to 10% of steady-state levels within 1 h. Simulation of the metabolism of vinylidene fluoride indicated that although blood levels of vinylidene fluoride increased linearly with increasing exposure concentration, the amount of vinylidene fluoride metabolized per 6-h exposure period approached a maximum at about 2000 ppm [5240 mg/m^3] vinylidene fluoride (Medinsky *et al*., 1988).

Concentrations of vinylidene fluoride were measured in blood of B6C3F$_1$ mice during 6-h exposures to nominal concentrations of 250, 3750 or 15 000 ppm [650, 9800 or 39 000 mg/m^3] vinylidene fluoride. Measured steady-state levels of vinylidene fluoride in blood of mice increased with increasing exposure concentration. At the two lower exposure concentrations, vinylidene fluoride was not detected in blood taken 15 min or longer after cessation of exposure, suggesting that the post-exposure levels were at or below the detection limit (4 ng vinylidene fluoride/mL blood). For the 15 000 ppm

exposure, vinylidene fluoride was detected in blood up to 15 min after exposure (Medinsky *et al*., 1990).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Exposure of rats to 215 000 mg/m^3 vinylidene fluoride for 3.5 h produced no sign of hepatoxicity. However, treatment of rats with Aroclor 1254 on three consecutive days followed by exposure for 6 h to 65 500 mg/m^3 did produce some hepatotoxicity. No excess ATPase-deficient foci were observed in the livers of Wistar rats that had been exposed from birth to 5200 mg/m^3 for 8 h per day on five days per week for 10–14 weeks (IARC, 1986).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

Vinylidene fluoride (50% v/v in air for 24 h) gave equivocal results for mutagenicity to *Salmonella typhimurium* when tested in the presence of an exogenous metabolic system (IARC, 1986).

5. Evaluation

No epidemiological data relevant to the carcinogenicity of vinylidene fluoride were available.

There is *inadequate evidence* for the carcinogenicity of vinylidene fluoride in experimental animals.

Overall evaluation

Vinylidene fluoride is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

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The volume, page and year of publication are given. References to corrigenda are given in parentheses.

B

C

D

E

F

H

I

J

K

L

M

O

Q

R

Zineb	*12*, 245 (1976); *Suppl. 7*, 74 (1987)
Ziram	*12*, 259 (1976); *Suppl. 7*, 74 (1987); *53*, *423* (1991)

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