

**WORLD HEALTH ORGANIZATION
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER**



**IARC MONOGRAPHS
ON THE EVALUATION
OF CARCINOGENIC
RISKS TO HUMANS**

**VOLUME 60
SOME INDUSTRIAL CHEMICALS**

1994
I A R C
L Y O N
F R A N C E



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IARC MONOGRAPHS
ON THE
EVALUATION OF CARCINOGENIC
RISKS TO HUMANS

Some Industrial Chemicals

VOLUME 60

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,

15-22 February 1994

1994

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. In 1980 and 1986, the programme was expanded to include evaluations of carcinogenic risks associated with exposures to complex mixtures and other agents.

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.

This project is supported by PHS Grant No. 5-UO1 CA33193-12 awarded by the US National Cancer Institute, Department of Health and Human Services. Additional support has been provided since 1986 by the European Commission.

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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.

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SOME INDUSTRIAL CHEMICALS**

Lyon, 15–22 February 1994

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PREAMBLE

IARC MONOGRAPHS PROGRAMME ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS¹

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

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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. 22) 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 different 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. 28-30).

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 may 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 users' survey, made in 1988, indicated that the *Monographs* are consulted by various agencies in 57 countries. Each volume is generally printed in 4000 copies for distribution to governments, regulatory bodies and interested scientists. The *Monographs* are also available *via* the Distribution and Sales Service of the World Health Organization.

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 surveys of chemicals being tested for carcinogenicity (IARC, 1973-1992) and directories of on-going research in cancer epidemiology (IARC, 1976-1994) often indicate those exposures that may be scheduled for future meetings. Ad-hoc working groups convened by IARC in 1984, 1989, 1991 and 1993 gave recommendations as

to which agents should be evaluated in the *IARC Monographs* series (IARC, 1984, 1989, 1991b, 1993).

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 *et seq.*). 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 IARC from recognized sources of information on carcinogenesis, including data storage and retrieval systems such as BIOSIS, Chemical Abstracts, CANCERLIT, MEDLINE and TOXLINE—including EMIC and ETIC for data on genetic and related effects and reproductive and developmental effects, respectively.

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 US 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, prior to 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 publishes 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 USA. 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 individual exposures under study to the occurrence of cancer in individuals and provide an estimate of relative risk (ratio of incidence/mortality in those exposed to incidence/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 to appear 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 quality of individual epidemiological studies of cancer has been summarized and 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 their judgement, the Working Group considers several criteria for causality. A strong association (i.e., 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 from 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 (i.e., 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 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, BCME and CMME (technical grade), chlorambucil, chlornaphazine, ciclosporin, coal-tar pitches, coal-tars, combined oral contraceptives, cyclophosphamide, diethylstilboestrol, melphalan, 8-methoxypsoralen plus UVA, 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 the carcinogenicity in humans (Vainio *et al.*, 1994). 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. 27) 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. 28) 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 genetic activity profiles, DNA adduct profiles, 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 that is being evaluated 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. 15), 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 both sexes 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 preneoplastic 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 undergo transition to malignancy, it should nevertheless be suspected of being a carcinogen and it 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 p. 28), 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 and involving cultural habits (e.g., tobacco smoking), the other data considered to be relevant are divided into those on absorption, distribution, metabolism and excretion; those on 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 from humans and 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. 21. 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; bar graphs (activity profiles) and corresponding summary tables with detailed information on the preparation of the profiles (Waters *et al.*, 1987) are given in appendices.

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. 15 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) *Exposures*

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 (i.e., 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 (i.e., 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 (i.e., 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 dimension, 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 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, or no data on cancer in humans are available.

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, or no data on cancer in experimental animals are available.

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, 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 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 and *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 ON THE AGENTS CONSIDERED

This sixtieth volume of *IARC Monographs* covers a number of industrial chemicals, several of which have been reviewed by previously convened working groups. Their conclusions are listed in Table 1. Some of these chemicals have considerable commercial importance, as the building blocks of widely used polymers and copolymers. Justification for their re-evaluation is that a substantial body of new data has become available, and the Preamble has been modified (IARC, 1991, 1992a) to permit more explicit inclusion of data on aspects other than cancer in the evaluation process.

Table 1. Previous evaluations of agents considered in this volume

Agent	Degree of evidence of carcinogenicity		Overall evaluation of carcinogenicity to humans	Volume (year of publication)
	Human	Animal		
Ethylene	ND	ND	3	19 (1979)
Ethylene oxide	L	S	2A	11 (1976); 36 (1985); Suppl. 7 (1987)
Propylene	ND	ND	3	19 (1979)
Propylene oxide	I	S	2A	11 (1976); 36 (1985); Suppl. 7 (1987)
Styrene	I	L	2B ^a	19 (1979); Suppl. 7 (1987)
Styrene oxide	ND	S	2A ^a	11 (1976); 19 (1979); 36 (1985)
4-Vinylcyclohexene	ND	L	3	11 (1976); 39 (1986)
4-Vinylcyclohexene diepoxide	ND	L	3	11 (1976)
Acrylamide	ND	S	2B	39 (1986)
Methyl methacrylate	ND	I	3	19 (1979)

S, sufficient evidence; L, limited evidence; I, inadequate evidence; ND, no data available
For definitions of categories, see preamble, pp. 27-30.

^aOther relevant data taken into account in making the overall evaluation

Several of these chemicals are characterized by unsaturated chemical bonds, which are necessary for polymerization. These bonds may also be targets for metabolic reactions in which epoxides are formed, some of which are also industrially important chemicals in their own right and are also reviewed and evaluated in this volume.

The Working Group noted the scarcity of data on occupational exposure to such industrially important chemicals as ethylene and propylene. Even though this is explainable

by the absence of regulations on occupational exposure levels in most countries, the gathering and publication of detailed exposure data are to be encouraged.

Parent compounds and electrophilic metabolites

When an alkene and its epoxide are both tested for toxicity, the epoxide is often mutagenic and carcinogenic, while the data are much less clear for the parent alkene, even when it is administered at higher levels than the epoxide. How can an epoxide be carcinogenic and its alkene not?

Pharmacokinetic differences in the way in which the alkene and its epoxide are handled appear to be important. For instance, activation of the parent compound to the epoxide can be saturated and rate-limiting, so that the tissue concentrations reached after administration of the epoxide cannot be attained after administration of the parent compound. Alternatively, rapid detoxification of the epoxide in the cell in which it is formed can result in a lower systemic burden of the epoxide than would be expected if all of it left the site of formation. Furthermore, the reactivity of the epoxide with DNA may be so weak that DNA damage severe enough to give rise to a detectable increase in tumour incidence can be attained only at the site of administration of the epoxide. Finally, detoxification processes may be saturated after administration of high doses of the reactive metabolite, whereas epoxide generated intracellularly from the parent compound may be inactivated.

The carcinogenicity of an epoxide is dependent on the rate of its reaction with critical as opposed to non-critical molecules, on the biological consequences of the adducts formed and on repair of the adducted molecule. The last may be cell type- and adduct-specific. At high levels of the epoxide, repair mechanisms can be saturated, especially at the site of administration by injection or gavage.

In view of these differences, it is not surprising that the cancer risk from exposure to a parent compound may be much lower than that from exposure to its preformed electrophilic metabolite.

Background DNA damage

Some of the adducts formed by the chemicals evaluated in this volume are the same as those found in apparently unexposed individuals, which are probably derived from endogenous and/or environmental agents and processes. How should the additional adducts formed be evaluated in terms of cancer risk?

Ethylene oxide, for instance, reacts with DNA in experimental animals to form predominantly 7-hydroxyethylguanine. This is one of a number of adducts that have recently been identified in humans and animals without specific exposure to a known DNA-reactive carcinogen. This (and other) background DNA damage might contribute to what is called 'spontaneous' mutagenesis and carcinogenesis. Such background damage can be evaluated in appropriately designed studies that take into account host and confounding factors.

Repair of many types of DNA adducts and lesions is well known. It is now becoming evident that efficient repair systems have evolved to handle many of the adducts formed by endogenous and ubiquitous DNA damaging agents. This appears to be true also for the repair of 7-hydroxyethylguanine formed by ethylene oxide. As in other biological processes,

individuals differ with respect to their repair capacities. In any population of animals and humans, DNA damage induced by a specific exposure is detectable only if it is significantly higher than that in the control population. Thus, the limit of detection of 'background' DNA adducts that are formed endogenously is no longer different from that for other genetic end-points which make a background contribution and which vary among and within individuals.

Estimates of dose

Pharmacokinetic models allow description and prediction of the concentrations of a parent alkene and its epoxide metabolite in different tissues and as a function of time. Exposure conditions can be taken into account, and species-specific susceptibility derived from quantitative differences in the rates of metabolic processes can be explained.

Data on haemoglobin adducts are also available for a number of chemicals evaluated in this volume, in both humans and experimental animals. The concentration of haemoglobin adducts formed by a given compound/metabolite may be proportional to the concentration of that agent integrated over the lifetime of erythrocytes during the period preceding blood sampling and may be proportional to the formation of DNA adducts in various tissues. Chemicals with different reactivities bind to different sites in haemoglobin. Haemoglobin binding may take place via another route than DNA binding. While both acrylamide itself and glycidamide, its metabolite, bind to haemoglobin, the main binding to DNA is through glycidamide. Thus, comparison of protein binding, as indicative of binding potency, by different compounds should be carried out with caution.

Combination of data on haemoglobin binding with pharmacokinetic models and historical information on exposure may allow estimation of levels of past human exposures to a few chemicals. Such quantitative information may improve epidemiological analyses, in particular with respect to exposure assessment.

Covalent binding indices

The efficiency of DNA binding can be exemplified by indices such as the 'covalent binding index' (Lutz, 1979). This is the concentration of the adduct in DNA (e.g. adducts per milligram) per dose and body weight. The interpretation of covalent binding indices is complex, and they should therefore be used with caution. The data are typically calculated from a single dose and single time-point. The rate of adduct formation (and often even the identity of the adduct) and its disappearance have not been addressed in many of the studies from which indices were calculated. Single time-point experiments fail to take into consideration differential repair of adducts at various sites of DNA. It is well known that the main adduct seen at the beginning of an experiment may not be the main one seen later (Swenberg *et al.*, 1990). Ideally, a DNA binding indicator should reflect steady-state levels, mimicking chronic human exposure.

Design of studies of genetic effects in humans

Many of the studies of human populations that involve biological markers of exposure or chromosomal or genetic damage need to be considered not only in terms of the biological

mechanisms involved but also for their epidemiological and statistical features, including how and which subjects are selected and their number and personal characteristics. Potential confounding factors, such as cigarette smoking, diet, alcohol and caffeine use, medications and medical history, should be taken into account in subject selection and data analysis. The more powerful the effect of a confounding factor on the end-point, the greater the need to measure it accurately.

Many of the cytogenetic studies reviewed in this volume are smaller than most of the epidemiological studies. Having too few subjects reduces the statistical power to detect an effect of a given magnitude and can make the results of a study appear to be negative when the study is really uninformative.

In studies to compare groups of people for biological markers of effect, such as cytogenetic outcomes, it may be necessary to consider whether the unit of statistical analysis is the person or cells. Comparisons of groups of people allow assessment of confounding and effect-modifying factors, which may not be possible in analyses based only on cells. The fact that changes in an individual's cells are not independent of each other must be taken into account in the statistical analysis. In particular, when small groups of people are studied, the variability within an individual can overshadow variability between people, which is generally the object of studying human populations.

One factor that may strongly affect the risk of an effect is genetic susceptibility. When only a small proportion of a study population is susceptible to a genotoxic agent, differences between groups can be masked. This factor may be of particular importance when it involves a genetically conditioned host response, such as metabolic activation, or the capacity to repair genetic lesions.

Cancer clusters

For several of the chemicals evaluated in this volume, the earliest epidemiological studies were carried out because an apparently high incidence of cancer had been noted in a work force (a case cluster). If subsequent studies included the index cases which prompted the investigation in their analyses, minimal weight should be attached to any risk estimate for cancer found, because case clusters frequently occur by chance, and analyses that include clusters can be expected to show excess cancer. This comment does not detract, however, from the value of case clusters as an initial clue to cancer hazard.

Exposure assessment in studies of humans

Detailed attention must be paid to exposure assessment. Too often, assessment is purely dichotomous and qualitative. In studies in which exposure-response relationships are evaluated, the method of exposure characterization should take into account whether exposures are continuous or intermittent, constant at the same level or fluctuate. The exposure measurement must also correspond to the biological attributes of the outcome and is thus relevant to the time-frame of the biological events being measured. Subjects in epidemiological studies are often misclassified with regard to exposure, so that any effect of exposure will tend to be obscured. When important exposure misclassification occurs and is nondifferential (i.e. does not differ systematically between subjects with different biological outcomes), not much weight can be given to findings that indicate no effect.

Several of the epidemiological studies summarized in the monographs were designed to assess the risk for cancer in relation to quantitative indices of exposure. Various indices are examined, including cumulative exposure (expressed as parts per million-years or -days), duration of exposure (expressed in years), average intensity of exposure (expressed in parts per million and calculated as cumulative exposure divided by duration of exposure) and maximal exposure (expressed in parts per million). The results obtained are not always consistent. For example, in a European study of the glass-reinforced plastics industry (Kogevinas *et al.*, 1994), a positive relationship was found for mortality from lymphatic and haematopoietic cancer with estimated average intensity of exposure to styrene, but not with cumulative exposure.

It is not clear which index of exposure is most relevant for the chemicals under review. The answer to this question will depend on the pharmacokinetics and postulated mechanisms of action of the substances and also on the nature of any relevant repair mechanisms. On the one hand, for example, if a carcinogenic effect depends on metabolic activation and that activation becomes saturated above a certain exposure level, it is possible that no increase in risk occurs once the saturation threshold has been exceeded. The risk from long-term, lower intensity exposure might then be higher than that from the same cumulative exposure experienced over a very short time. On the other hand, if repair mechanisms become saturated above a critical exposure level, risk may be elevated only when exposures exceed the relevant threshold.

In the absence of clear indications about the most appropriate exposure index, the Working Group set most store by findings for cumulative exposure, especially when allowance was made for a latent interval between exposure and the development of risk. It is recognized, however, that with better information about pharmacokinetics and biological mechanisms, it may be necessary to revise this view in the future.

Genetic changes in carcinogenesis

The development in our understanding of the importance of mutational change, at the gene and chromosome level, in the genesis of human cancers has reinforced the conclusion that cancers emerge as a consequence of alterations in the structure and/or expression of a number of genes that are normally involved in controlling the processes of cellular proliferation, differentiation and programmed cell death (apoptosis). Both gene mutations and cancers arise 'spontaneously', but their prevalence is increased in humans exposed to mutagenic and carcinogenic agents.

Many of the agents that are known to induce cancers in humans also induce cancers in animals and DNA damage and mutations in somatic cells of exposed humans and in various mammalian and nonmammalian cells *in vitro*. For example, human populations exposed to ionizing and certain types of nonionizing radiation have increased levels of DNA damage and chromosomal and gene mutations in their somatic cells and increased frequencies of cancers over those in controls. In the case of skin cancers resulting from exposure to solar radiation, the nature of the changes resulting in mutation of specific oncogenes in the emerging tumours reflects the specific nature of the DNA lesions (pyrimidine dimers) induced by ultraviolet light (IARC, 1992b). Similarly, oncogenic mutations in lung carcinomas of miners exposed to radon (Vähäkangas *et al.*, 1992) reflect changes (deletions

of nucleotide sequences) associated with ionizing radiation-induced lesions in DNA. Liver tumours in humans exposed to aflatoxins also reflect specific mutations in oncogenes induced by this agent, and similar associations are found in liver and skin tumours of experimental animals exposed to other chemical mutagens (IARC, 1993). Many of the mutations induced in exposed individuals by radiation or chemicals may be irrelevant in an oncogenic sense, but what is relevant is that a *proportion* of any induced *additional* mutations will have important oncogenic consequences (Bos & van Kreijl, 1992).

The association between mutation and human cancer has been especially highlighted by:

(i) the much increased cancer incidence in individuals who inherit single gene mutations that result in increased genomic instability and/or inefficient DNA repair. Such inherited defects predispose these individuals to various cancers, e.g. of skin (xeroderma pigmentosum), of lymphocytes (ataxia telangiectasia) or of colon (hereditary nonpolyposis coli).

(ii) studies on other familial cancer predispositions, in which the inheritance of a specific mutation (usually deletion of a chromosomal segment containing a tumour suppressor gene or a mutation that inactivates that gene) results in a considerable elevation in the frequencies of specific cancers, e.g. of the breast, ovary and colon; and

(iii) the identification of specific genes—oncogenes, tumour suppressor and apoptotic genes—whose activation, inactivation and loss by gene mutation, chromosomal deletion or translocation (fusion genes) are essential steps in the development of cancers, whether they arise as a consequence of an evident inherent predisposition, are sporadic or are associated with exposure to a known environmental carcinogen.

Much of the initial information on genes involved in human cancers came from studies on experimental animals and on mammalian and other cells *in vitro*; moreover, the chromosomal mutations and the genes involved are common (if not identical) across a wide range of species. Information on agents that produce these changes in experimental animals and other organisms *in vitro* and *in vivo* clearly must be of relevance to the carcinogenic potential of these agents to induce cancers in humans.

Those agents that induce mutations in the germinal cells of rodents are considered to be especially relevant in terms of their carcinogenic potential. Few agents—about 30—many of which are known carcinogens, have thus far been documented as germ-cell mutagens in animals, perhaps because germinal cells represent a distal target with a high capacity for exclusion of genetic damage. Those agents capable of inducing heritable genetic effects in germinal cells may also be expected to do so in somatic cells, thereby increasing the burden of viable genetic lesions and predisposing to carcinogenesis.

The importance of epidemiological evidence for cancer in humans cannot be overstated. Nevertheless, epidemiological evidence requires relatively large populations, unless the risks are very high, whereas small groups may be exposed to potential carcinogens. The advances in our understanding of the processes involved in carcinogenesis indicate the importance of attaching increased relevance to data obtained from studies on genetic and related effects of exposure to an agent. Data from such studies were therefore considered by the Working Group in arriving at their evaluations; the instances in which such data influenced the final evaluation of an agent are described in the text relating to that agent.

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THE MONOGRAPHS

ETHYLENE

This substance was considered by a previous Working Group, in February 1978 (IARC, 1979). 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.: 74-85-1

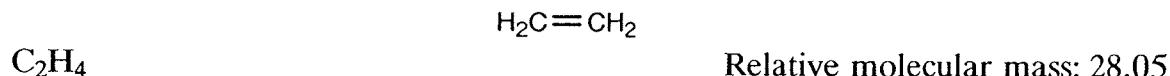
Replaced CAS Reg. No.: 33060-30-9; 87701-64-2; 87701-65-3

Chem. Abstr. Name: Ethene

IUPAC Systematic Name: Ethylene

Synonyms: Acetene; bicarburetted hydrogen; elayl; olefiant gas

1.1.2 Structural and molecular formulae and relative molecular mass



1.1.3 Chemical and physical properties of the pure substance

- (a) *Description:* Colourless gas (Lide, 1991)
- (b) *Boiling-point:* -103.7°C (Lide, 1991)
- (c) *Melting-point:* -169°C (Lide, 1991)
- (d) *Spectroscopy data:* Infrared [prism, 1131], ultraviolet and mass spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991).
- (e) *Solubility:* Very slightly soluble in water (0.26% vol/vol); slightly soluble in acetone, benzene and ethanol; soluble in diethyl ether (American Conference of Governmental Industrial Hygienists, 1991; Lide, 1991)
- (f) *Volatility:* Vapour pressure, 4270 kPa at 0°C ; relative vapour density (air = 1), 0.9686 (Grantom & Royer, 1987)
- (g) *Stability:* Lower explosive limit (in air), 2.75 vol% or 34.6 g/m^3 at 100 kPa and 20°C (Grantom & Royer, 1987)
- (h) *Octanol-water partition coefficient (P):* log P, 1.13 (Hansch & Leo, 1979)

(i) Conversion factor: $\text{mg/m}^3 = 1.15 \times \text{ppm}^a$

1.1.4 Technical products and impurities

The purity of ethylene is normally greater than 99.9 wt%; quality is adjusted to meet specific requirements. Sulfur, oxygen and acetylene are the most troublesome but carefully controlled impurities, especially when ethylene from multiple sources is mixed for transportation. Specification ranges (mg/kg) for maximal levels of key contaminants in ethylene are: methane + ethane, 50–2000; propylene and heavier, 7–200; acetylene, 1.4–10; hydrogen, 0.1–10; carbon monoxide, 0.15–10; carbon dioxide, 2.2–50; oxygen, 0.6–10; sulfur, 1–10; and water, 0.6–20 (Grantom & Royer, 1987 [results of a survey of 10 US producers]; Dow Chemical Co., 1989; Amoco Chemical Co., 1993). Specifications for the quality of ethylene in Europe, Japan and the USA are similar (Grantom & Royer, 1987).

1.1.5 Analysis

Atmospheric hydrocarbons, including ethylene, can be determined by capillary column gas chromatography with flame ionization detection (Locke *et al.*, 1989; Khalil & Rasmussen, 1992). The lower limit of detection with this method is 10 ppb (10 $\mu\text{L/L}$) by volume (Locke *et al.*, 1989). A variation on this method consists of preconcentration with a two-stage cryotrap system and an aluminium oxide-coated column; the limit of detection is 2.5 ppt (Schmidbauer & Oehme, 1985) or 2 pg (Matuška *et al.*, 1986). A similar method is based on sample enrichment with a solid sorbent, a zeolite, at room temperature, followed by heat desorption for gas chromatographic separation and flame ionization detection (Persson & Berg, 1989). Use of solid sorbent tubes in series (Tenax TA + Carbosphere S) has been suggested, with analysis by gas chromatography and an electron capture detection system parallel to a tandem photoionization and flame ionization system; the limit of detection for ethylene was 24 ppt (Reineke & Bächmann, 1985).

Methods have been developed for the biological monitoring of occupational exposure to ethylene, which are based on determination of a haemoglobin adduct [*N*-(2-hydroxyethyl)valine] of the metabolite, ethylene oxide, using gas chromatography/mass spectrometry (Törnqvist *et al.*, 1986a) and gas chromatography/electron capture detection (Kautiainen & Törnqvist, 1991).

1.2 Production and use

1.2.1 Production

Ethylene is the petrochemical produced in largest quantities worldwide. Recovered from coke-oven gas and other sources in Europe since 1930, ethylene emerged as a large-volume intermediate in the 1940s when US oil and chemical companies began separating it from refinery waste gas and producing it from ethane obtained from refinery by-product streams and from natural gas. After that time, the industry rapidly switched its raw material base from coal to hydrocarbons (Grantom & Royer, 1987).

^aCalculated from: $\text{mg/m}^3 = (\text{relative molecular mass}/24.45) \times \text{ppm}$, assuming normal temperature (25 °C) and pressure (101.3 kPa)

Over 95% of the worldwide annual commercial production of ethylene is currently based on steam cracking of petroleum hydrocarbons. Various feedstocks, including ethane, propane, butanes, naphthas and gas oils, are used to produce ethylene. Naphthas are the principal raw material used in western Europe and Japan, accounting for over 80% of the ethylene produced. Ethane is the primary feedstock in the USA, followed by propane, naphthas, gas oils and butane. Small amounts of ethylene are recovered from other feedstocks, such as retrograde-field condensates and refinery waste gases. Dehydration of ethanol is the third commercial route to ethylene (Grantom & Royer, 1987). Production of ethylene in 19 countries and regions is presented in Table 1. Total European Union production in 1990 was 12 820 thousand tonnes (European Commission, 1993).

Table 1. Worldwide production of ethylene (thousand tonnes)

Country or region	1982	1984	1986	1988	1990	1992
Argentina	NR	255	258	NR	NR	NR
Belarus ^a	-	-	-	-	145	NA
Canada	1 013	1 464	1 909	2 346	2 434	2 521
China	565	648	642	1 231	1 572	1 982 ^b
Former Czechoslovakia	NR	NR	NR	683	619	NA
France	1 865	2 078	2 259	2 432	2 244	2 650
Germany ^c	2 634	3 217	2 662	3 125	3 072	3 393
Hungary	NR	265	269	264	234	281
Italy	872	1 136	NR	NR	NR	NR
Japan	3 590	4 384	4 291	5 057	5 810	6 104
Mexico	396	643	767	916	NR	NR
Poland	175	256	279	328	308	NA
Republic of Korea	374	526	534	609	1 054	2 769
Romania	NR	317	312	335	243	132 ^d
Russia ^a	2 000	2 543	2 799	3 175	2 318	NA
Taiwan	452	660	868	852	776	734
Ukraine ^a	-	-	-	-	446	NA
United Kingdom	1 113	1 153	1 736	2 025	1 495	1 934
USA	11 113	14 235	14 905	16 875	16 541	18 327 ^b

From Scientific & Technical Information Research Institute of the Ministry of Chemical Industry of China (1984); Anon. (1985, 1987, 1988, 1989, 1990); Giménez *et al.* (1990); Anon. (1991a, 1992, 1993); NA, not available; NR, not reported

^aReported as part of USSR from 1981 through 1988

^bPreliminary

^cWestern

^dEstimate

Information available in 1991 indicated that ethylene was produced by 17 companies in the USA, 13 in Japan, nine in Germany, five in France, four each in Brazil and the United Kingdom, three each in Canada and the Netherlands, two each in Argentina, Australia, Belgium, China, the Republic of Korea, Saudi Arabia and the former Yugoslavia, and one each in Austria, the former Czechoslovakia, Finland, India, Italy, Mexico, Norway,

Singapore, South Africa, Spain, Thailand, Turkey and Venezuela (Chemical Information Services Ltd, 1991).

1.2.2 Use

About 80% of the ethylene used in western Europe, Japan and the USA is for producing polyethylene (high density, low density and linear low density), ethylene oxide/ethylene glycols and ethylene dichloride/vinyl chloride. Significant amounts are also used to make ethylbenzene/styrene, oligomer products (e.g. alcohols and α -olefins), acetaldehyde/acetic acid and vinyl acetate (Grantom & Royer, 1987). Typical patterns for use of ethylene in western Europe, Japan and the USA are presented in Table 2.

Table 2. Use patterns (%) for ethylene in western Europe, Japan and the USA

Use	Western Europe ^a (1983)	Japan		USA	
		1983	1991	1983	1991
LD-LLD polyethylene ^b	35	30	29	28.5	27
HD polyethylene ^c	15	20	19	20	24
Ethylene oxide	12	11	11	17	14
Ethylene dichloride	19	18	14	14	13
Ethylbenzene	8	9	10	7	7
Ethanol + acetaldehyde	6	5	4	4	2
Vinyl acetate monomer	- ^d	3	-	2.5	3
Miscellaneous	5	4	13	7	10

From Grantom and Royer (1987), Anon. (1991b) and Japan Petrochemical Industry Association (1993)

^aBelgium, Germany, France, Italy, Luxembourg, the Netherlands and the United Kingdom

^bLD, low density; LLD, linear low density

^cHD, high density

^dIncluded in 'miscellaneous'

While most commercially produced ethylene is used as a feedstock in the production of polymers and industrial chemicals, a relatively small amount is used for the controlled ripening of citrus fruits, tomatoes, bananas and many other fruits, vegetables and flowers. Endogenous production of ethylene in plant tissue generally increases rapidly during ripening. Application of ethylene to plants before the time of this natural increase not only initiates the ripening process but also increases endogenous ethylene production. Ethylene has commonly been used in this way since the early part of this century (Nickell, 1982; Kader & Kasmire, 1984; Bridgen, 1985; Reid, 1985; Kader, 1986; Watada, 1986).

1.3 Occurrence

Ethylene is ubiquitous in the environment, arising from both natural and man-made sources. Major sources are as a natural product from vegetation of all types (Sawada &

Totsuka, 1986; Rudolph *et al.*, 1989); as a product of burning vegetation, agricultural wastes and refuse, and the incomplete combustion of fossil fuels; and releases during the production and use of ethylene (Sawada & Totsuka, 1986).

Total annual emission of ethylene from the global surface has been estimated to be 18–45 million tonnes per year, of which approximately 74% is released from natural sources and 26% from anthropogenic sources. Releases from terrestrial ecosystems comprise about 89% of the natural sources and aquatic ecosystems, about 11%. Burning of biomass to clear land for agriculture or other uses is believed to be the largest anthropogenic source of ethylene emissions (77%); the combustion of various fossil fuels also accounts for a significant fraction (21%) of anthropogenic emissions (Sawada & Totsuka, 1986).

1.3.1 *Natural occurrence*

Ethylene is a natural product emitted by fruits, flowers, leaves, roots and tubers (Altshuller, 1983). The rate of release varies during the life cycle of the plant. Plants that normally produce 0.6–6 µg/kg fresh weight per hour may produce up to 120 µg/kg per hour during ripening of fruits and during senescence and loss of leaves (Dörffling, 1982; Tille *et al.*, 1985). Ethylene is also produced endogenously by humans and other mammals (see section 4.1).

Other natural sources of ethylene include volcanic emissions and natural gas. Volcanos emit only trace concentrations of ethylene, and leaked natural gas contains mainly saturated hydrocarbons (Sawada & Totsuka, 1986).

1.3.2 *Occupational exposure*

The National Occupational Exposure Survey conducted by the US National Institute for Occupational Safety and Health between 1981 and 1983 indicated that 12 280 US employees were potentially exposed occupationally to ethylene (US National Institute for Occupational Safety and Health, 1993). The estimate is based on a survey of US companies and did not involve measurements of actual exposures.

There is thought to be little opportunity for occupational exposure to ethylene during its manufacture in a closed system. Exposure may occur as a result of leaks, spills and other accidents and from work in tanks that contained ethylene (Dooley, 1983). No data on measured levels of exposure to ethylene during its manufacture or processing were available to the Working Group. Hogstedt *et al.* (1979) estimated that during the period 1941–47, ethylene levels in an ethylene oxide production plant in Sweden would have been approximately 600 mg/m³.

Personal and stationary monitoring of ethylene in a company where this gas was used for controlling the ripening of bananas showed air concentrations to be in the range of 0.02–3.35 ppm [0.02–3.85 mg/m³], with an estimated average concentration of 0.3 ppm [0.35 mg/m³] (Törnqvist *et al.*, 1989a). In a study on exposure of firefighters, samples taken during the 'knockdown' phase of a fire showed a concentration of 46 ppm [53 mg/m³] ethylene; none was detected during the 'overhaul' phase (Jankovic *et al.*, 1991).

1.3.3 Air

Ethylene concentrations in ambient air at rural and remote sites worldwide are generally in the range of $< 1\text{--}5\ \mu\text{g}/\text{m}^3$ (Altshuler, 1983; Anlauf *et al.*, 1985; Colbeck & Harrison, 1985; Davidson *et al.*, 1986; Van Valin & Luria, 1988; Kanakidou *et al.*, 1989; Lightman *et al.*, 1990; Hov *et al.*, 1991; Mowrer & Lindskog, 1991; Satsumabayashi *et al.*, 1992).

In urban and indoor air contaminated with combustion products, ethylene concentrations typically range from a few to over $1000\ \mu\text{g}/\text{m}^3$. For example, a median concentration of 21.4 ppb as carbon (ppbC) [$12.3\ \mu\text{g}/\text{m}^3$] ethylene, with a maximum of 1001 ppbC [$573\ \mu\text{g}/\text{m}^3$], was measured in over 800 ambient air samples obtained from 39 US cities during 1984–86 (Seinfeld, 1989). In 1985, geometric mean atmospheric concentrations of ethylene ranging from 3.2 to 45.8 ppb [$3.7\text{--}52.7\ \mu\text{g}/\text{m}^3$] were determined in an industrial suburb of Bombay, India (Rao & Pandit, 1988). In northwest England, geometric mean ambient air concentrations of ethylene during the summer of 1983 were 41.2 ppbC [$23.6\ \mu\text{g}/\text{m}^3$] in urban air samples and 1.5 ppbC [$0.86\ \mu\text{g}/\text{m}^3$] in rural air samples (Colbeck & Harrison, 1985). Ethylene concentrations averaged 4.0 ppb [$4.6\ \mu\text{g}/\text{m}^3$] in 1980 and 2.2 ppb [$2.5\ \mu\text{g}/\text{m}^3$] in 1981 in 258 air samples taken over Tokyo, Japan (Uno *et al.*, 1985).

One of the major sources of atmospheric ethylene globally—the burning of biomass (Sawada & Totsuka, 1986)—can also be a source of locally high concentrations. A mean ethylene concentration of 490 ppbC [$281\ \mu\text{g}/\text{m}^3$] was measured in the indoor air of rural Nepali houses where biomass combustion is the main source of energy; the mean concentration in outdoor air at Katmandu was 2.1 ppbC [$1.2\ \mu\text{g}/\text{m}^3$] (Davidson *et al.*, 1986).

Vehicle exhaust emissions make an important contribution to urban air concentrations of ethylene. Estimates in the mid-1980s for countries of the European Union (Table 3) show that emissions from gasoline- and diesel-fuelled vehicles make a significant contribution in that region (Bouscaren *et al.*, 1987).

Although ethylene is not a fuel component, it is present in motor vehicle exhaust as a result of fuel-rich combustion of hydrocarbon fuels (Stump *et al.*, 1989). Mean ethylene emissions from 25 vehicles in the United Kingdom were 211.94 mg/km in urban road tests, 123.20 mg/km in suburban road tests and 93.9 mg/km in rural road tests (Bailey *et al.*, 1990a,b). The following levels of ethylene were determined in air samples representative of various traffic emissions in Sweden: 68 and 64 $\mu\text{g}/\text{m}^3$ (two sites, urban intersection); 13 and 9.8 $\mu\text{g}/\text{m}^3$ (two sites, fast suburban traffic); and 56 $\mu\text{g}/\text{m}^3$ (cold starts at a garage exit) (Löfgren & Petersson, 1992). Ethylene concentrations of 51–405 $\mu\text{g}/\text{m}^3$ were measured in the Tingstad Tunnel in Göteborg, Sweden (Barrefors & Petersson, 1992).

Industrial emissions of ethylene to the air in the USA in 1991 were reported to amount to 17 400 tonnes (US National Library of Medicine, 1993); industrial emissions in the countries of the European Union are shown in Table 3.

Cigarette smoke is also a significant source of exposure to ethylene, as 1–2 mg ethylene are released per cigarette. The exposure of the average cigarette smoker to ethylene is roughly 10 times that from urban air pollution (Persson *et al.*, 1988; Shaikh *et al.*, 1988). In two studies of smokey tavern air, the ethylene levels were 56 and 110 $\mu\text{g}/\text{m}^3$; the corresponding outdoor air concentrations at the time were 16 and 12 $\mu\text{g}/\text{m}^3$ ethylene (Löfroth *et al.*, 1989).

Table 3. Estimated ethylene emissions in member states of the European Union (thousand tonnes/year)

Country	Source			
	Road traffic		Chemical industry ^a	Other sources
	Gasoline	Diesel		
Belgium	4.7	1.3	1	0.9
Denmark	2	0.7	NR	NR
Germany	27	9.5	2.2	11
France	28	8.8	2.5	1
Greece	2.8	1.4	NR	0.01
Ireland	1.3	0.2	NR	NR
Italy	28	8.1	2	1
Luxemburg	0.23	0.05	NR	NR
Netherlands	5.1	1.6	2.6	3.1
Portugal	1.1	1.3	0.3	0.1
Spain	8.9	4.4	1	0.8
United Kingdom	33	5.5	1	1.5
Total (approximate)	145	45	13	20

From Bouscaren *et al.* (1987); NR, not reported

^aProduction of ethylene and ethylene polymers and copolymers

In laboratory studies, ethylene has been detected as a thermal degradation product of polyethylene and polypropylene (Hoff *et al.*, 1982; Frostling *et al.*, 1984).

Ethylene is degraded in the troposphere mainly by reactions with OH radical and ozone. Its average atmospheric lifetime is estimated at two to four days (Sawada & Totsuka, 1986; Rudolph *et al.*, 1989).

1.3.4 Water

Although ethylene is only slightly soluble in water, low concentrations have been measured in various surface waters. Pacific and Atlantic Ocean surface waters (77° N to 75° S) contained 0.7–12.1 nl/L, fresh water lakes and rivers in the USA from 4.8 to 13.0 nl/L, and more polluted waters in the Mississippi River delta and near the shore in Miami, FL, from 26 to 35 nl/L (Sawada & Totsuka, 1986).

1.4 Regulations and guidelines

Ethylene has been classified in several countries as an asphyxiant because its presence at high concentrations in air lowers the available oxygen concentration. Countries in which it is classified as an asphyxiant include Australia, Belgium, Canada, Finland, Hungary, the Netherlands, the United Kingdom and the USA. Nevertheless, the major hazard is due to its inflammable and explosive character. No exposure limits have been recommended in most

countries, but Switzerland established a time-weighted average occupational exposure limit of 11 500 mg/m³ (about one-half the lower exposure limit) in 1987 (ILO, 1991; American Conference of Governmental Industrial Hygienists, 1993; UNEP, 1993). In Germany, no exposure limit is given for ethylene because it is 'justifiably suspected of having carcinogenic potential' (Deutsche Forschungsgemeinschaft, 1993).

In the USA, ethylene is exempted from the requirement of a tolerance for residues when it is used as a plant regulator on fruit and vegetable crops and when it is injected into the soil to cause premature germination of witchweed for a variety of crops (US Environmental Protection Agency, 1992). The US Food and Drug Administration (1993) permits use of ethylene-containing polymers in products in contact with food.

2. Studies of Cancer in Humans

Some cohorts involved in the manufacture of ethylene oxide are likely to have been exposed to ethylene; however, in the only study in which exposure to ethylene was assessed (Hogstedt *et al.*, 1979), described in detail on pp. 89–90, the risk for cancer in relation to ethylene was not assessed separately.

3. Studies of Cancer in Experimental Animals

3.1 Inhalation exposure

Rat: Groups of 120 male and 120 female Fischer 344 rats, six to seven weeks of age, were exposed by inhalation to 0, 300, 1000 or 3000 ppm (0, 345, 1150 or 3450 mg/m³) ethylene (> 99.9% pure) for 6 h per day on five days per week for up to 24 months, at which time the experiment was terminated. The high dose was chosen to avoid the hazard of explosion. Necropsies were performed at six (5 rats/dose and per sex), 12 (5 rats/dose and per sex), 18 (19–20 rats/dose and per sex) and 24 (all survivors) months. All rats that died spontaneously were also necropsied. There was no significant difference in survival between control and treated groups. The high-dose and control animals were examined histologically. The authors reported that there was no evidence of treatment-related toxicity and no increase in tumour incidence (Hamm *et al.*, 1984).

3.2 Induction of enzyme-altered foci in a two-stage liver system

Rat: Groups of male and female Sprague-Dawley rats, three to five days of age, were exposed by inhalation to 0 (5 male and 9 female rats) or 10 000 ppm (11 500 mg/m³, 2 males and 10 females) ethylene [purity unspecified] for 8 h per day on five days per week for three weeks. One week later, the rats received oral administrations of 10 mg/kg bw Clophen A 50 (a mixture of polychlorinated biphenyls [not otherwise specified]) by gavage twice a week for up to eight additional weeks (promotion), at which time the experiment was terminated and the livers were examined for ATPase-deficient foci. The number of ATPase-deficient foci in

the rats exposed to ethylene did not exceed the control values. In the same experiment, ethylene oxide, administered as a positive control, produced a significant increase in the incidence of ATPase-deficient foci in females (Denk *et al.*, 1988).

3.3 Carcinogenicity of metabolites

See the monograph on ethylene oxide.

4. Other Data Relevant for an Evaluation of Carcinogenicity and Its Mechanisms

The toxicology of ethylene has been reviewed (National Research Council Canada, 1985; Gibson *et al.*, 1987; Angerer *et al.*, 1988; Greim, 1993).

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

The inhalation pharmacokinetics of ethylene have been investigated in human volunteers at atmospheric concentrations of up to 50 ppm [57.5 mg/m³] by gas uptake in a closed spirometer system (Shen *et al.*, 1989; Filser *et al.*, 1992). The uptake, exhalation and metabolism of ethylene can be described by first-order kinetics.

Uptake of ethylene into the body is low. Clearance due to uptake, which reflects the transfer rate of ethylene from the atmosphere into the body, was 25 L/h for a man of 70 kg. This value represents only 5.6% of the experimentally obtained alveolar ventilation rate of 450 L/h. The majority (94.4%) of ethylene inhaled into the lungs is exhaled again without becoming systemically available via the blood stream. Maximal accumulation of ethylene in the same man, determined as the thermodynamic partition coefficient whole body:air ($K_{eq} = \text{Conc}_{\text{animal}}/\text{Conc}_{\text{air}}$), was 0.53. The concentration ratio at steady state was even smaller (0.33), owing to metabolic elimination. Clearance due to metabolism, in relation to the concentration in the atmosphere, was calculated to be 9.3 L/h for a man of 70 kg. This indicates that at steady state about 36% of systemically available ethylene is eliminated metabolically and 64% is eliminated by exhalation as the unchanged substance, as can be calculated from the values of clearance of uptake and of clearance of metabolism. The biological half-life of ethylene was 0.65 h. The alveolar retention of ethylene at steady state was calculated to be 2% (Filser *et al.*, 1992). From theoretical considerations of the lung uptake of gases and vapours (Johanson & Filser, 1992), it can be deduced that the low uptake rate of ethylene is due to its low solubility in blood: Ostwald's solubility coefficient for human blood at 37 °C, 0.15 (Steward *et al.*, 1973).

(a) Endogenous formation

Endogenous production of ethylene can be deduced from its exhalation by unexposed subjects (Ram Chandra & Spencer, 1963; Shen *et al.*, 1989; Filser *et al.*, 1992). For a man of

70 kg, a mean production rate of 32 nmol/h [0.9 µg/h] and a corresponding mean body burden of 0.011 nl/ml tissue [equivalent to 0.44 nmol/kg bw or 0.012 µg/kg bw] was calculated for ethylene gas (Filser *et al.*, 1992). The amount of ethylene in the breath of women is increased significantly at the time of ovulation; no difference was observed in the basal ethylene outputs of non-pregnant and pregnant women and of men (Harrison, 1981). The biochemical sources of ethylene are unknown; however, several mechanisms by which it might be produced in mammals are discussed below.

(b) *Haemoglobin adducts*

The ethylene metabolite, ethylene oxide, reacts with nucleophilic centres in cellular macromolecules (see monograph on ethylene oxide). In several studies, the haemoglobin (Hb) adducts *N*-(2-hydroxyethyl)histidine (HOEtHis) and *N*-(2-hydroxyethyl)valine (HOEtVal) have been used as internal dose monitors of the formation of ethylene oxide from ethylene. In nonsmokers, the background levels of HOEtVal range between 11 and 188 pmol/g Hb (Törnqvist *et al.*, 1986b; Bailey *et al.*, 1988; Törnqvist *et al.*, 1989a; Sarto *et al.*, 1991; Filser *et al.*, 1992; Törnqvist *et al.*, 1992; van Sittert *et al.*, 1993; van Sittert & van Vliet, 1994). Farmer *et al.* (1986) reported background levels of 30–930 pmol/g Hb in three subjects, without considering smoking habits. In Hb of subjects presumed not to be exposed to ethylene, the levels of 2-hydroxyethyl adducts were 1500–4300 pmol/g Hb *N*-(2-hydroxyethyl)cysteine (HOEtCys) in three subjects, 30–530 pmol/g Hb HOEtVal in five subjects; 60 and 300 pmol/g Hb N^T -HOEtHis in two subjects and 110–290 pmol/g Hb N^T -HOEtHis in five. Tobacco smoke, urban air and endogenous production were included as possible sources of ethylene (Calleman, 1986). HOEtHis levels in 31 control subjects ranged from < 20 to 4700 pmol/g Hb. Smoking did not contribute to these background alkylations (van Sittert *et al.*, 1985).

Exposure to environmental ethylene concentrations of 10–20 ppb [11.5–23 µg/m³] was associated with an HOEtVal increment of 4–8 pmol/g globin at steady state (Törnqvist & Ehrenberg, 1990). Background levels of HOEtVal were predicted on the basis of pharmacokinetic parameters of ethylene and ethylene oxide, together with the rate constant of the reaction of ethylene oxide with the *N*-terminal valine in Hb and confirmed by measured data. HOEtVal levels resulting from endogenous ethylene only were calculated to be about 12 pmol/g Hb. Those resulting from both endogenous and environmental ethylene (15 ppb [17.25 µg/m³]) in the area of Munich (Germany) were computed to be about 18 pmol/g Hb; the measured level was about 20 pmol/g Hb and, hence, in close agreement with that predicted (Filser *et al.*, 1992). No difference in HOEtVal adduct levels was seen in nonsmoking workers in an ethylene plant and in nonsmoking controls not occupationally exposed (van Sittert & van Vliet, 1994).

Significantly higher levels of HOEtVal (129–690 pmol/g Hb) were found in cigarette smokers (10–30 cigarettes/day) than in nonsmokers (Törnqvist *et al.*, 1986b; Passingham *et al.*, 1988; Persson *et al.*, 1988; Törnqvist *et al.*, 1989a; Sarto *et al.*, 1991). Ethylene (0.25 mg/cigarette; Elmenhorst & Schultz, 1968) and ethylene oxide (0.005 mg/cigarette; Binder & Lindner, 1972) present in tobacco smoke were considered to be major causes of the elevated adduct levels (Törnqvist *et al.*, 1986b; Persson *et al.*, 1988; Törnqvist *et al.*, 1989a). Smoking

10 cigarettes per day was associated with an additional 60–114 pmol/g Hb HOEtVal (Törnqvist *et al.*, 1986b; Bailey *et al.*, 1988; Passingham *et al.*, 1988; van Sittert *et al.*, 1993).

Nonsmoking fruit store workers exposed occupationally to atmospheric ethylene (0.02–3.35 ppm [0.023–3.85 mg/m³]) used for the ripening of bananas had levels of 22–65 pmol/g Hb HOEtVal, whereas nonsmoking controls had 12–27 pmol/g Hb. On the basis of a mean exposure concentration of 0.3 ppm [0.345 mg/m³], it was estimated that about 3% (range, 1–10%) of inhaled ethylene was metabolized to ethylene oxide (Törnqvist *et al.*, 1989a). This percentage is equal to the alveolar retention at steady state calculated from inhalation pharmacokinetics (see above). The two values are in agreement. An increment of 100–120 pmol/g Hb HOEtVal was estimated for a time-weighted average exposure (40 h/week) to 1 ppm ethylene [1.15 mg/m³] (Kautiainen & Törnqvist, 1991; Ehrenberg & Törnqvist, 1992).

On the basis of the relationship between HOEtVal levels and exposure levels of ethylene or ethylene oxide, an 'uptake' (i.e. amount metabolized) of 1 mg ethylene/kg bw was calculated to be equivalent to a tissue dose of ethylene oxide of 0.7×10^{-6} mol \times h/L [0.03 mg \times h/kg bw] (Törnqvist *et al.*, 1988). This value is in agreement with the value of 0.5×10^{-6} mol \times h/L that can be calculated from the pharmacokinetic data for ethylene and ethylene oxide published by Filser *et al.* (1992).

4.1.2 Experimental systems

Four male CBA mice (average body weight, 31 g) were exposed together for 1 h in a closed glass chamber (5.6 L) to ¹⁴C-ethylene (22 mCi/mmol) in air at 17 ppm \times h [22.3 (mg/m³) \times h, equivalent to about 1 mg/kg bw]. Blood and organs from two mice were pooled 4 h after the end of exposure. Radioactivity was about the same in kidney (0.16 μ Ci/g wet weight) and liver (0.14 μ Ci/g) but lower in testis (0.035 μ Ci/g), brain (0.02 μ Ci/g) and Hb (0.0094 μ Ci/g Hb). Urine was collected from the two other mice during 48 h, and blood was collected after 21 days. *S*-(2-Hydroxyethyl)cysteine was identified as a metabolite of ethylene in urine (3% of ¹⁴C in urine) by thin-layer chromatography. The radioactivity in Hb was 0.011 μ Ci/g Hb. These data, together with those on specific hydroxyethyl derivatives at amino acid residues of Hb (see below), indicated that ethylene was metabolized to ethylene oxide (Ehrenberg *et al.*, 1977).

In several experiments, disposition of ¹⁴C-ethylene (free of ¹⁴C-acetylene or $\geq 97\%$ pure) in male Fischer 344 rats (170–220 g) was determined over 36 h following 5-h exposures in a closed chamber (35 L) to 10 000 ppm [11 500 mg/m³]. In each experiment, up to four rats were exposed together in a single chamber. Within about 1 min after the end of exposure, animals were transferred to individual all-glass metabolism cages. Most of the eliminated ¹⁴C was exhaled as ethylene (18 μ mol [504 μ g] per rat exposed to acetylene-containing ethylene); smaller amounts were excreted in urine (2.7 μ mol ethylene equivalents/rat) and faeces (0.4 μ mol) and exhaled as CO₂ (0.16 μ mol). Radioactivity was also found in blood (0.022 μ mol ethylene equivalents/ml), liver (0.047 μ mol ethylene equivalents/liver), gut (0.034 μ mol ethylene equivalents/gut) and kidney (0.006 μ mol ethylene equivalents/kidney). Pre-treatment of animals with a mixture of polychlorinated biphenyls (Aroclor 1254; 500 mg/kg bw; single intraperitoneal injection five days before exposure) had no measurable influence on ethylene exhalation but resulted in a significant ($p < 0.05$) increase in exhaled ¹⁴CO₂ (2.04 μ mol ethylene equivalents/rat) and of ¹⁴C in urine (11.1 μ mol ethylene equivalents/rat)

and in blood (0.044 μmol ethylene equivalents/ml). The organ burden of ^{14}C was one to two orders of magnitude greater in Aroclor 1254-treated than in untreated animals. Radioactivity also became detectable in lungs, brain, fat, spleen, heart and skeletal muscle. The data were interpreted as indicating that the metabolism of ethylene can be stimulated by an inducer of the mixed-function oxidase system (Guest *et al.*, 1981).

The pharmacokinetics of inhaled ethylene have been investigated in male Sprague-Dawley rats using closed exposure chambers in which the atmospheric concentration-time course was measured after injection of a single dose into the chamber atmosphere (Bolt *et al.*, 1984; Bolt & Filser, 1987; Shen *et al.*, 1989; Filser, 1992). Uptake of ethylene into the body was low. Clearance due to uptake (as described above) was 20 ml/min for one rat of 250 g, which represents only 17% of the alveolar ventilation (117 ml/min; Arms & Travis, 1988). Most (83%) inhaled ethylene that reaches the lungs is exhaled again without becoming systemically available via the blood stream. Maximal accumulation of ethylene in the organism, determined as the thermodynamic partition coefficient, whole body:air ($K_{\text{eq}} = \text{Conc}_{\text{animal}}/\text{Conc}_{\text{air}}$), was 0.7. The concentration ratio at steady-state whole body:air was somewhat lower owing to metabolic elimination, and it decreased from 0.7 to 0.54 at exposure concentrations below 80 ppm [92 mg/m^3]; however, at very low atmospheric concentrations, the concentration ratio at steady-state whole body:air increased again, owing to endogenous production of ethylene: For instance, it was almost twice the value of the thermodynamic partition coefficient whole body:air at an exposure concentration of 0.05 ppm [0.06 mg/m^3] (calculated using the pharmacokinetic parameters and equation 18 of Filser, 1992). At concentrations between 80 and 0.1 ppm [92 and 0.12 mg/m^3], clearance was seen, due to metabolism related to the concentration in the atmosphere of about 4.7 ml/min for a 250-g rat. In that concentration range at steady state, therefore, about 24% of systemically available ethylene is eliminated by metabolism and 76% by exhalation of the unchanged substance (taking into account values of clearance of uptake and clearance of metabolism). The alveolar retention of ethylene at steady state was 3.5%, and the biological half-life was 4.7 min (Filser *et al.*, 1992). At atmospheric concentrations greater than 80 ppm [92 mg/m^3], metabolism of ethylene became increasingly saturated, reaching a maximal rate of metabolism (V_{max}) of 0.035 $\mu\text{mol}/(\text{min} \times 250 \text{ g bw})$ [0.24 $\text{mg}/(\text{h} \times \text{kg bw})$] at about 1000 ppm [1150 mg/m^3]. The apparent Michaelis constant (K_{m}) related to the average concentration of ethylene gas within the organism was 130 nl/ml tissue, which corresponds to an atmospheric concentration of 208 ppm [239 mg/m^3] at $V_{\text{max}/2}$, calculated by means of the kinetic parameters given by Filser (1992).

Gas uptake studies with male Fischer 344 rats gave values for V_{max} of 0.24 $\text{mg}/(\text{h} \times \text{kg bw})$ and an 'inhalational K_{m} ' (related to the atmospheric concentration) of 218 ppm [251 mg/m^3] (Andersen *et al.*, 1980).

Involvement of cytochrome P450-dependent monooxygenases in the metabolism of ethylene in male Sprague-Dawley rats was suggested by the complete inhibition of metabolic elimination after intraperitoneal treatment with 200 mg/kg diethyldithiocarbamate 15 min before exposure and by an increase in the rate of its metabolism with a V_{max} of about 14 $\mu\text{mol}/(\text{h} \times \text{kg bw})$ [0.33 $\text{mg}/(\text{h} \times \text{kg bw})$], after treatment with a single dose of Aroclor 1254 (500 mg/kg bw) six days before the experiment (Bolt *et al.*, 1984).

The metabolism of ^{14}C -ethylene in 15 male CBA mice kept for 7 h in a closed exposure chamber (11 L), in which the atmospheric concentration-time course was measured after generation of an initial atmospheric concentration of 10 ppb [$11.5 \mu\text{g}/\text{m}^3$], was reduced by co-exposure to propylene at 1260 ppm [$1267 \text{mg}/\text{m}^3$], suggesting inhibition of ethylene metabolism by propylene (Svensson & Osterman-Golkar, 1984).

In liver microsomes prepared from male Sprague-Dawley rats, ethylene at concentrations of up to 10% [$115 \text{g}/\text{m}^3$] in the gas phase was metabolized to ethylene oxide in the presence of an NADPH regenerating system (1 h, pH 7.5, 37°C). The rate of formation of ethylene oxide was saturable (V_{max} , $0.67 \text{nmol}/\text{h}$ per mg protein) and could be reduced by the addition of diethyldithiocarbamate or β -naphthoflavone to the microsomal suspension. Treatment of the rats with phenobarbital (single intraperitoneal injection of $80 \text{mg}/\text{kg}$ bw followed by three days of 0.1% in drinking-water) before preparation of liver microsomes did not change the V_{max} (Schmiedel *et al.*, 1983).

Male Sprague-Dawley rats exposed to ethylene exhaled ethylene oxide. In these experiments, two animals were kept together up to 21 h in a closed exposure chamber (6.4 L). The concentration of ethylene in the atmosphere of the chamber was maintained at greater than 1000 ppm [$1150 \text{mg}/\text{m}^3$] by repeated additions, in order to maintain V_{max} conditions for ethylene. One hour after the beginning of exposure, the atmospheric concentration of exhaled ethylene oxide reached a peak value of 0.6 ppm [$0.69 \text{mg}/\text{m}^3$]. After about 2.5 h, the concentration had decreased to about 0.3 ppm [$0.345 \text{mg}/\text{m}^3$] and then remained constant. On the basis of the concentration-time courses of atmospheric ethylene, it was speculated that this decrease was due to rapid induction of ethylene oxide metabolizing enzymes, whereas the rate of ethylene metabolism remained unaffected (Filser & Bolt, 1984). In male Sprague-Dawley rats exposed to concentrations greater than 1000 ppm, the amount of ethylene taken up per unit time from the atmosphere of a closed chamber remained constant over exposure times of up to 30 h (Bolt *et al.*, 1984). Pharmacokinetic data for ethylene and ethylene oxide indicated that under steady-state conditions only 29% of metabolized ethylene is available systemically as ethylene oxide. Therefore, assuming that the liver is the principal organ in which ethylene is metabolized, an intrahepatic first-pass effect for the intermediate ethylene oxide was suggested (Filser & Bolt, 1984).

In view of the saturability of ethylene metabolism, the maximal possible average body concentration of its metabolite, ethylene oxide, was calculated to be $0.34 \text{nmol}/\text{ml}$ tissue [$15 \mu\text{g}/\text{kg}$ bw] in an open exposure system (infinitely large atmospheric volume). The same value was computed to result from exposure to ethylene oxide at an atmospheric concentration of 5.6 ppm [$10.2 \text{mg}/\text{m}^3$] at steady state (Bolt & Filser, 1987).

Ethylene oxide was found in the blood of male Fischer 344/N rats during exposure to an atmospheric ethylene concentration of 600 ppm [$690 \text{mg}/\text{m}^3$]. A maximal value of about $3 \mu\text{g}/\text{g}$ blood of ethylene oxide was seen 8 min after the start of exposure to ethylene; this value was followed 4 min later by an immediate decrease to about $0.6 \mu\text{g}/\text{g}$, and the level remained constant for the following 46 min. During exposure, the cytochrome P450 content in the liver was reduced to 94% after 20 min and to 68% after 360 min. It was speculated that an ethylene-specific cytochrome P450 isozyme was rapidly deactivated during exposure to ethylene, resulting in reduced formation of ethylene oxide (Maples & Dahl, 1993). This speculation is based on results obtained by an unspecific method for the determination of

cytochrome P450 which is not suitable for the determination of cytochrome P450 isozymes; however, under certain conditions, suicide metabolism of ethylene in rat liver does seem to occur, as indicated from experiments of induction of cytochrome P450-dependent monooxygenases. In male Sprague-Dawley rats treated with phenobarbital (80 mg/kg bw, intraperitoneal injection daily for four days, exposure to ethylene on day 5) and then exposed for 3 h to a mixture of commercial ethylene (contaminated with about 10 ppm acetylene) and air (1:1 v/v), a green pigment was found in the liver 4 h after exposure. The same pigment was formed *in vitro* during incubation of acetylene-free ethylene with the 9000 × g supernatant of a rat liver homogenate (from phenobarbital-pretreated animals) in the presence of NADPH. No controls were used (Ortiz de Montellano & Mico, 1980). The pigment was identified as a *N*-(2-hydroxyethyl)protoporphyrin IX, an alkylation product of the prosthetic haem of cytochrome P450-dependent monooxygenases. It was concluded that the phenobarbital-inducible form of cytochrome P450 was destroyed during oxidative metabolism of ethylene (Ortiz de Montellano *et al.*, 1980, 1981).

The further metabolic fate of ethylene oxide is described in the monograph on that chemical.

(a) *Endogenous formation*

Four possible sources of endogenous ethylene have been suggested: lipid peroxidation (Lieberman & Mapson, 1964; Lieberman & Hochstein, 1966; Frank *et al.*, 1980; Sagai & Ichinose, 1980; Törnqvist *et al.*, 1989b; Kautiainen *et al.*, 1991); enzyme- (Fu *et al.*, 1979), copper- (Lieberman *et al.*, 1965) or iron- (Kessler & Remmer, 1990) catalysed oxidative destruction of methionine; oxidation of haemoglobin (Clemens *et al.*, 1983); and the metabolism of intestinal bacteria (Törnqvist *et al.*, 1989b).

Ethylene is also exhaled by untreated rats (Frank *et al.*, 1980; Sagai & Ichinose, 1980; Shen *et al.*, 1989). The endogenous production rate in a Sprague-Dawley rat (250 g bw) was determined to be 2.8 nmol/h [0.31 µg/(h × kg bw)], resulting in a body burden of ethylene gas of 0.032 nl/ml tissue [0.036 µg/kg bw] (Filser, 1992). The corresponding exhalation rate may be calculated from the pharmacokinetic parameters of Filser (1992) as 0.24 µg/(h × kg bw).

(b) *Haemoglobin adducts*

Hydroxyethyl adducts at cysteine, histidine and the N-terminal valine of Hb were identified in several animal species exposed to ethylene and have been ascribed to the formation of ethylene oxide (Ehrenberg *et al.*, 1977; Osterman-Golkar *et al.*, 1983; Segerbäck, 1983; Törnqvist *et al.*, 1986a, 1988, 1989b; Kautiainen *et al.*, 1991). Background levels of Hb adducts, partially due to exposure to endogenous and environmental ethylene, are listed in Table 4.

In male CBA mice exposed for 70 h to an atmospheric concentration of 9100 ppm ethylene [10 465 mg/m³], the level of the Hb adduct HOEtCys was 7200 pmol/g Hb (Ehrenberg *et al.*, 1977).

Further support for the proposal that ethylene oxide is the reactive metabolite of ethylene arose from the finding of similar relative patterns of the Hb adducts HOEtCys, HOEtHis and HOEtVal in male CBA mice either exposed in a closed chamber to atmospheric ¹⁴C-ethylene at initial concentrations of 0.25, 1.1 or 11 ppm [0.29, 1.27, 12.7 mg/m³]

(exposure dose 1, 6.5 or 50 ppm \times h [1.15, 7.48, 57.5 mg \times h/m³]) or treated intraperitoneally with ¹⁴C-ethylene oxide (44 μ mol/kg bw [1.9 mg/kg bw]) (Segerbäck, 1983).

Table 4. Hydroxyethyl (HOEt) haemoglobin adducts measured in animals after endogenous and environmental exposure to ethylene and related metabolites

Species and strain	Sex	Haemoglobin adducts measured (pmol/g Hb)			Reference
		HOEtCys	HOEtHis	HOEtVal	
CBA mouse	Male	1400			Ehrenberg <i>et al.</i> (1977)
Mouse	NR			20–120	Törnqvist <i>et al.</i> (1986a)
B6C3F ₁ mouse	Male			58	Walker <i>et al.</i> (1992a)
F344 rat	Male		1300, 2800		Osterman-Golkar <i>et al.</i> (1983)
Rat	NR			~100	Törnqvist <i>et al.</i> (1986a)
F344 rat	Male, female			75, 60	Törnqvist <i>et al.</i> (1988)
F344 rat	Male			42	Walker <i>et al.</i> (1992a)
Syrian hamster	NR			~100	Törnqvist <i>et al.</i> (1986a)
Syrian hamster	Male, female			120, 105	Törnqvist <i>et al.</i> (1988)

NR, not reported

It was calculated from the value of 2–2.4 pmol HOEtCys/g Hb per (ppm h) of ethylene and the value of 30 pmol HOEtCys/g Hb per (ppm \times h) of ethylene oxide that 7–8% of inhaled ethylene is metabolized in male CBA mice to ethylene oxide (Ehrenberg *et al.*, 1977; Segerbäck, 1983). These mice had been exposed to ethylene at concentrations below 20 ppm [23 mg/m³], at which first-order kinetics of metabolism can be assumed. The value is equal to the alveolar retention of ethylene at steady state and is similar to the values calculated for rats and humans (see above).

HOEtVal was determined in Hb of male and female Fischer 344 rats and male and female Syrian hamsters exposed for six months to gasoline and diesel exhausts (mean atmospheric concentrations of ethylene, < 0.1–2.28 ppm [$<$ 0.115–2.62 mg/m³]). In hamsters, the levels of HOEtVal increased almost linearly with dose. The increments at the highest dose were similar in female rats (505 pmol/g Hb) and hamsters (615 pmol/g Hb) and in male rats (450 pmol/g Hb) and hamsters (420 pmol/g Hb). These values were about 50–90% of those predicted from the data on mice, indicating that ethylene behaves similarly in these species. It was estimated from the results of studies on animals that an uptake (i.e. amount metabolized) of 1 mg ethylene/kg bw is associated with a tissue dose of ethylene oxide of 0.7×10^{-6} mol \times h/L [0.03 mg \times h/kg bw], similar to the value obtained for humans (Törnqvist *et al.*, 1988).

4.1.3 Comparison between humans and experimental animals

Formation of ethylene oxide was determined directly in rats exposed to ethylene, but no such data are available for humans. Assuming that the metabolism of ethylene in humans proceeds quantitatively via ethylene oxide, however, the average body burden of ethylene

oxide resulting from exposure to ethylene can be calculated from pharmacokinetic parameters obtained for the two compounds. A value for ethylene oxide of 0.17 pmol/ml tissue [7.5 ng/kg bw] would result from ethylene produced endogenously; taking into account additional exposure to 15 ppb [17.3 $\mu\text{g}/\text{m}^3$] atmospheric ethylene, as measured in Munich, Germany, the body burden of ethylene oxide can be computed as 0.25 pmol/ml tissue [11 ng/kg bw] (Filser *et al.*, 1992).

For exposure concentrations below 50 ppm [57.5 mg/m^3], the pharmacokinetic parameters of inhaled ethylene obtained in rats were extrapolated to humans by means of an allometric method based on a surface factor equal to two-thirds of body weight (Filser, 1992). The deviation between the values predicted from rats and the measured values did not exceed a factor of 2.3.

4.2 Toxic effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

Exposure to atmospheric ethylene alone did not lead to toxic effects, whether after single exposures of male Holtzman rats (4 h up to 57 000 ppm [65 550 mg/m^3]) or male Fischer 344 rats (5 h to 10 000 ppm [11 500 mg/m^3]) (Conolly & Jaeger, 1977; Conolly *et al.*, 1978; Conolly & Jaeger, 1979; Guest *et al.*, 1981), after 90-day exposures of male and female Sprague-Dawley rats (6 h/day, 5 days/week, up to 10 000 ppm [11 500 mg/m^3]) (Rhudy *et al.*, 1978) or after two-year exposures of male and female Fischer 344 rats (6 h/day, 5 days/week, up to 3000 ppm [3450 mg/m^3]) (Hamm *et al.*, 1984). This lack of toxicity, which might be predicted from results obtained for ethylene oxide, is due to saturation of the metabolic activation of ethylene (see section 4.1.2).

Single exposures of male Holtzman rats to atmospheric ethylene (4 h; 10 000, 23 000–30 000, 50 000–57 000 ppm [11 500, 26 450–34 500, 57 500–65 550 mg/m^3]) one day after treatment with Aroclor 1254 (100 mg/kg bw, equivalent to 300 $\mu\text{mol}/\text{kg}$ bw, once daily by gavage for three days induced dose-dependent acute hepatotoxicity. Hepatic effects were indicated 24 h after beginning of exposure by elevated serum concentrations of sorbitol dehydrogenase and of alanine- α -ketoglutarate transaminase and by histological findings such as cell ballooning and haemorrhagic necrosis in centrilobular zones (Conolly & Jaeger, 1977; Conolly *et al.*, 1978; Conolly & Jaeger, 1979). Treatment 0.5 h before start of the exposure with diethylmaleate (0.5 ml/kg bw), in order to deplete reduced glutathione, or with trichloropropene oxide (0.1 ml/kg bw), in order to inhibit epoxide hydrolase, had no effect on the hepatotoxicity of ethylene in Aroclor 1254-pretreated rats (Conolly & Jaeger, 1979).

In two male Fischer 344 rats treated with Aroclor 1254 (500 mg/kg ; single intraperitoneal injection five days before exposure), a 5-h exposure to 10 000 ppm [11 500 mg/m^3] ^{14}C -ethylene (free of ^{14}C -acetylene) in a closed recirculating system (35 L) caused uniform hepatic centrilobular necrosis, which was seen 36 h after exposure. Treatment with Aroclor 1254 without subsequent exposure to ethylene resulted in slight

hypertrophy of centrilobular liver cells without hepatocellular necrosis. The authors suggested that Aroclor 1254 affects the metabolism of ethylene in such a way that a toxic metabolite is produced in sufficient quantities to elicit hepatotoxicity (Guest *et al.*, 1981).

4.3 Reproductive and prenatal effects

No data were available to the Working Group.

4.4 Genetic and related effects (see also Table 5 and Appendices 1 and 2)

4.4.1 Humans

In the DNA of peripheral lymphocytes of eight people not occupationally exposed to ethylene or ethylene oxide, 7-(2-hydroxyethyl)guanine (7-HOEtGua) was detected at a background level of 8.5 ± 5.7 nmol/g DNA. Possible sources for this DNA adduct were not discussed (Föst *et al.*, 1989).

No other data were available to the Working Group.

4.4.2 Experimental systems

(a) DNA adducts

The ratio between 7-HOEtGua in DNA in various organs and HOEtVal in Hb of rats exposed to ethylene oxide was over 100 times higher in unexposed than in animals exposed for four weeks (Walker *et al.*, 1992a,b). [This suggests that factors other than ethylene oxide are involved in the formation of 7-HOEtGua.]

7-HOEtGua was found by gas chromatography-mass spectrometry at background levels of 2-6 nmol/g DNA in DNA of lymphocytes from blood of untreated male Sprague-Dawley rats (Föst *et al.*, 1989) and in DNA of various tissues from male Fischer 344 rats and B6C3F1 mice (Walker *et al.*, 1992b). Alkylation of 7-guanine was measured in DNA from liver, spleen and testis of mice 14 h after exposure by inhalation to ^{14}C -ethylene at an initial concentration of 11 ppm [12.9 mg/m^3] (exposure dose, $50 \text{ ppm} \times \text{h}$ [$58.5 \text{ mg} \times \text{h/m}^3$]) for 8 h. The values of degree of alkylation were 0.17 for liver, 0.098 for spleen and 0.068 nmol/g DNA for testis, representing < 10% of the background levels. The ratios of 7-guanine in DNA to N^T -His in Hb were approximately the same as those obtained after intraperitoneal injection of ethylene oxide (Segerbäck, 1983).

(b) Mutation and allied effects

Gene mutations were not induced in *Salmonella typhimurium* TA100 exposed for 7 h to 20% ethylene in air, either with or without an exogenous metabolic system. Ethylene did not induce micronuclei in bone-marrow cells of rats or of mice exposed to up to 3000 ppm (3500 mg/m^3) for 6 h/day, five days/week for four weeks.

Table 5. Genetic and related effects of ethylene

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	225.0000 ^c	Victorin & Ståhlberg (1988)
MVM, Micronucleus test, mouse bone-marrow cells <i>in vivo</i>	-		1200, inhal. 6 h 5 d/wk 4 wks	Vergnes & Pritts (1994)
MVR, Micronucleus test, rat bone-marrow cells <i>in vivo</i>	-		725, inhal. 6 h 5 d/wk 4 wks	Vergnes & Pritts (1994)
BVD, Binding (covalent) to mouse DNA <i>in vivo</i>	+		5.9000, inhal. 8 h	Segerbäck (1983)
Protein binding				
BHP, Binding (covalent) to human haemoglobin <i>in vivo</i>	+		0.075, inhal. 8 h	Törnqvist <i>et al.</i> (1989a)
BHP, Binding (covalent) to human haemoglobin <i>in vivo</i>	+		0.0000	Filser <i>et al.</i> (1992)

^a+, positive; -, negative

^bIn-vitro tests, µg/ml; in-vivo tests, mg/kg bw

^cAtmospheric concentration in exposure chamber

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Ethylene, the petrochemical manufactured in largest volume worldwide, is produced primarily by the steam-cracking of hydrocarbons. It is used mainly as a chemical intermediate in the production of polymers and other industrial chemicals; small amounts are used to promote the ripening of fruits and vegetables. Ethylene is introduced into the environment from both natural and man-made sources, including emissions from vegetation, as a product of burning of organic material (such as cigarettes) and of incomplete combustion of fossil fuels, and in its production and use. Few data are available on levels of occupational exposure.

5.2 Human carcinogenicity data

The available data did not allow the Working Group to evaluate the carcinogenicity of ethylene to humans.

5.3 Animal carcinogenicity data

Ethylene was tested for carcinogenicity in one experiment in rats exposed by inhalation. No increase in tumour incidence was reported.

5.4 Other relevant data

Endogenous but unidentified sources of ethylene exist in man and experimental animals. Steady-state alveolar retention of ethylene is less than 10% in both man and rat. The biological half-time of ethylene in humans is about 0.65 h. In rats and man, the processes of uptake, exhalation and metabolism are described by first-order kinetics, at least up to 50 ppm; in rats, ethylene metabolism follows first-order kinetics up to about 80 ppm. The maximal rate of metabolism in rats is reached at about 1000 ppm, the initial metabolite being ethylene oxide; hydroxyethyl cysteine is a urinary metabolite in mice. Because ethylene metabolism can be saturated, the maximal possible concentration of ethylene oxide in rat tissues is about 0.34 nmol/ml (15 ng/g bw).

Exposure to ethylene results in the formation of adducts with proteins. In nonsmokers, the background concentrations of the hydroxyethyl valine adduct of haemoglobin were 12–188 pmol/g haemoglobin. Environmental ethylene contributes to these concentrations; the endogenous contribution was calculated to be about 12 pmol/g haemoglobin in nonsmoking control subjects. The increment of N-terminal hydroxyethyl valine formed during a 40-h work week has been estimated as 100–120 pmol/g haemoglobin per part per million of ethylene. Tobacco smoke contributes to formation of this adduct: smoking 10–30 cigarettes/day was reported to result in 600–690 pmol/g haemoglobin.

Background concentrations of 7-hydroxyethyl guanine were 8.5 nmol/g DNA in one study of human peripheral lymphocytes and ranged from 2 to 6 nmol/g DNA in various

tissues of rats and mice. A single exposure of mice to 50 ppm ethylene for 1 h resulted in 0.1–0.2 nmol/g DNA.

No data were available on the genetic and related effects of ethylene in exposed humans. In a single study, no micronuclei were induced in bone-marrow cells of mice and rats exposed *in vivo*. Gene mutation was not induced in *Salmonella typhimurium*. Although the genetic effects of ethylene have not been well studied, its metabolite, ethylene oxide, is genotoxic in a broad range of assays.

5.5 Evaluation¹

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

There is *inadequate evidence* in experimental animals for the carcinogenicity of ethylene.

Overall evaluation

Ethylene is *not classifiable as to its carcinogenicity to humans (Group 3)*.

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¹For definition of the italicized terms, see Preamble, pp. 27–30.

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ETHYLENE OXIDE

This substance was considered by previous Working Groups, in February 1976 (IARC, 1976), June 1984 (IARC, 1985) and March 1987 (IARC, 1987). 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.: 75-21-8

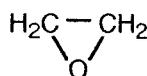
Replaced CAS Reg. No.: 19034-08-3; 99932-75-9

Chem. Abstr. Name: Oxirane

IUPAC Systematic Name: Oxirane

Synonyms: Dihydrooxirene; dimethylene oxide; 1,2-epoxyethane; epoxyethane; ethene oxide; EtO; ETO; oxacyclopropane; oxane; oxidoethane

1.1.2 Structural and molecular formulae and relative molecular mass



$\text{C}_2\text{H}_4\text{O}$

Relative molecular mass: 44.06

1.1.3 Chemical and physical properties of the pure substance

- (a) *Description:* Colourless gas (Rebsdats & Mayer, 1987)
- (b) *Boiling-point:* 13.2 °C at 746 mm Hg [99.4 kPa] (Lide, 1991); 10.8 °C at 760 mm Hg [101.3 kPa] (Rebsdats & Mayer, 1987)
- (c) *Melting-point:* -111 °C (Lide, 1991)
- (d) *Density (liquid):* 0.8824 at 10 °C/10 °C (Lide, 1991)
- (e) *Spectroscopy data:* Infrared [prism, 1109] and mass spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991).
- (f) *Solubility:* Soluble in water, acetone, benzene, ethanol and diethyl ether (Lide, 1991)

- (g) *Volatility*: Vapour pressure, 145.6 kPa at 20 °C (Rebsdats & Mayer, 1987; Hoechst Celanese Corp., 1992); relative vapour density (air = 1), 1.5 at 20 °C (Hoechst Celanese Corp., 1992)
- (h) *Stability*: Reacts readily with acids (Cawse *et al.*, 1980); reactions proceed mainly via ring opening and are highly exothermic; explosive decomposition of vapour may occur at higher temperatures if heat dissipation is inadequate (Rebsdats & Mayer, 1987). Lower explosive limit, 2.6–3.0% by volume in air (Rebsdats & Mayer, 1987; Dever *et al.*, 1994)
- (i) *Octanol–water partition coefficient (P)*: log P, -0.30 (Sangster, 1989)
- (j) *Conversion factor*: $\text{mg/m}^3 = 1.80 \times \text{ppm}^a$

1.1.4 Technical products and impurities

Ethylene oxide of high purity (99.5–99.95%) is available from several sources with the following typical specifications: acidity (as acetic acid), 0.002% max.; aldehydes (as acetaldehyde), 0.001–0.01% max.; chlorides (as Cl), 0.005%; water, 0.02–0.03% max.; acetylene, 0.0005%; carbon dioxide, 0.001–0.002%; and residue, 0.005–0.01 g/100 ml max. (Rebsdats & Mayer, 1987; Hoechst Celanese Corp., 1988; Dow Chemical Co., 1989; Union Carbide, 1993).

Ethylene oxide for use as a fumigant and sterilizing agent is available in mixtures with nitrogen, carbon dioxide or dichlorodifluoromethane. Mixtures of 8.5–80% ethylene oxide/91.5–20% carbon dioxide (Allied Signal Chemicals, 1993) and 12% ethylene oxide in dichlorodifluoromethane are commonly used (Cawse *et al.*, 1980). As a result of concern about the role of chlorofluorocarbons in causing depletion of stratospheric ozone, they are being replaced in such mixtures by nitrogen and other flame retardant diluent gases (Dever *et al.*, 1994).

1.1.5 Analysis

Ethylene oxide in workplace air can be determined by packed column gas chromatography (GC) with an electron capture detector (ECD). The sample is adsorbed on hydrobromic acid-coated charcoal and desorbed with dimethylformamide. The sample is derivatized to 2-bromoethylheptafluorobutyrate for analysis. This method (NIOSH Method 1614) has an estimated limit of detection of 1 µg ethylene oxide per sample (Eller, 1987a). A similar method is reported by the US Occupational Safety and Health Administration, in which the sample is adsorbed on charcoal, desorbed with a benzene:carbon disulfide solution, converted to 2-bromoethanol and analysed by GC/ECD (Tucker & Arnold, 1984; Cummins *et al.*, 1987; European Commission, 1989). In another method (NIOSH Method 3702), a portable gas chromatograph is used with a photoionization detector. The sample is either drawn directly into a syringe or collected as a bag sample; it is then injected directly into the gas chromatograph for analysis. The estimated limit of detection is 2.5 pg/ml injection (0.001 ppm [0.002 mg/m³]) (Eller, 1987b).

^aCalculated from: $\text{mg/m}^3 = (\text{relative molecular mass}/24.45) \times \text{ppm}$, assuming normal temperature (25 °C) and pressure (101.3 kPa)

Methods for the analysis and quantification of ethylene oxide in emissions from production plants and commercial sterilizers by GC with flame ionization detection have been reviewed (Gray *et al.*, 1985; Steger, 1989; Margeson *et al.*, 1990). Passive methods for personal sampling of ethylene oxide in air have also been reported (Kring *et al.*, 1984; Puskar & Hecker, 1989; Puskar *et al.*, 1990, 1991; Szopinski *et al.*, 1991).

Biological monitoring of occupational exposure to ethylene oxide has been conducted by analysis of alveolar air and blood (Brugnone *et al.*, 1986). Several methods have been reported for the determination of *N*-(2-hydroxyethyl) adducts in haemoglobin, with cysteine, valine and histidine: a radioimmunological technique, a modified Edman degradation procedure with GC/mass spectrometry; a GC method with selective ion monitoring mass spectrometry and a GC/ECD method (Gray *et al.*, 1985; Farmer *et al.*, 1986; Bailey *et al.*, 1987; Bolt *et al.*, 1988; Föst *et al.*, 1991; Kautiainen & Törnqvist, 1991; Sarto *et al.*, 1991; van Sittert *et al.*, 1993).

Methods have been reported for the detection of residues of ethylene oxide used as a sterilant: headspace GC (Marlowe *et al.*, 1987) and GC (Wojcik-O'Neill & Ello, 1991) for analysis of medical devices, capillary GC for analysis of drugs and plastics (Danielson *et al.*, 1990) and headspace GC for analysis of packaging materials and for ethylene oxide in ethoxylated surfactants and demulsifiers (Dahlgran & Shingleton, 1987). Methods have also been developed for the determination of ethylene oxide residues in processed food products. In one such method, ethylene oxide is converted to ethylene iodohydrin and analysed by GC/ECD (Jensen, 1988).

1.2 Production and use

1.2.1 Production

Ethylene oxide was produced from 1914 by the chlorohydrin process, in which ethylene chlorohydrin is prepared by reacting ethylene with hypochlorous acid (chlorine in water) and is converted to ethylene oxide by reaction with calcium oxide (Cawse *et al.*, 1980). This method is no longer used on an industrial scale, at least in the USA. The process was inefficient, as most of the chlorine that was used was lost as calcium chloride and unwanted organochlorine by-products were generated. Since 1931, that process has been gradually replaced by the direct vapour phase oxidation process, in which ethylene is oxidized to ethylene oxide with air or oxygen and a silver catalyst at 10–30 atm (1–3 MPa) at 200–300 °C (Rebsdatt & Mayer, 1987; Berglund *et al.*, 1990).

Table 1 gives production volumes in Germany, Japan and the USA. It has been estimated that worldwide production of ethylene oxide exceeds 5500 thousand tonnes per year (WHO, 1985). Information available in 1991 indicated that ethylene oxide was produced by eight companies in the USA, seven in Germany, five in Japan, four each in China and the United Kingdom, two each in Belgium, Brazil, Canada and Spain and one each in Australia, Bulgaria, the former Czechoslovakia, France, India, Italy, Mexico, the Netherlands, the Republic of Korea, Singapore, Sweden and Venezuela (Chemical Information Services Ltd, 1991).

Table 1. Production of ethylene oxide in selected countries, 1982–92 (thousand tonnes)

Country	Year					
	1982	1984	1986	1988	1990	1992
Germany ^a	393	474	498	626	629	630
Japan	471	533	489	510	674	721
USA	2262	2585	2463	2700	2429	2522 ^b

From Anon. (1985, 1989, 1993a); Japan Petrochemical Industry Association (1993)

^aWestern

^bPreliminary

1.2.2 Use

Ethylene oxide is an important raw material for making major consumer goods in virtually all industrialized countries. Table 2 presents the pattern of use of ethylene oxide as a chemical intermediate in the USA, which is typical of that elsewhere in the world. It is used directly in the gaseous form as a disinfectant, sterilizing agent, fumigant and insecticide (see Table 3), either alone or in nonexplosive mixtures with nitrogen, carbon dioxide or dichloro-fluoromethane. It is used as a fumigant to remove pests and microorganisms from spices and seasonings, furs, furniture, nut meats, tobacco, books, drugs, leather, motor oil, paper, soil, animal bedding, clothing and transport vehicles; and as a sterilant for foodstuffs, cocoa, flour, dried egg powder, coconut, fruits, dehydrated vegetables, cosmetics and dental, medical and scientific supplies (Popp *et al.*, 1986; US Environmental Protection Agency, 1986; Rebsdats & Mayer, 1987).

Table 2. Use patterns (%) for ethylene oxide in the USA

Use	Year				
	1981	1984	1987	1990	1993
Ethylene glycol	62	62	59	59	61
Non-ionic surfactants	12	12	14	13	16
Ethanolamines	5	7	8	8	8.5
Glycol ethers	6	7	6	6	5
Diethylene glycol	NR	NR	6	6	5
Triethylene glycol	NR	NR	2	2	2
Miscellaneous ^a	15	12	5	6	2.5

From Anon. (1981, 1984, 1987, 1990, 1993b); NR, not reported

^aIncludes higher glycols (polyethylene glycol), urethane polyols and exports

Table 3. Use of ethylene oxide as a fumigant and sterilant in the USA, 1983

Site and use	Amount used (tonnes)
Manufacturing facilities (production of sterile disposable items for medical use)	1500-2600
Medical facilities	500-550
Hospitals	400-450 ^a
Medical clinics	50
Dental clinics	29.7
Doctors' surgeries (private)	16.8
Dentists' surgeries (private)	3.3
Veterinarians (private and clinical)	0.045
Museums	0.3
Libraries, archives	0.86
Research laboratories	277-446
Animal breeding	22.7
Drugs and medical devices	250-410
Microbiological, cancer	2.3-11.4
USDA high-containment laboratories	2.0
Railroad cars	1.0
Beehives	0.68-0.9
USDA quarantine port of entry	0.3
Spices	340
Black walnuts	1.5
Cosmetics	11
Dairy packaging	14.5
Total	2600-3900

From US Environmental Protection Agency (1986); USDA, US Department of Agriculture

^a1976 value

Most ethylene oxide is converted into other products, including ethylene glycol; glycol ethers; ethanolamine; ethoxylation products of long-chain alcohols and amines, alkyl phenols, cellulose, starch and poly(propylene glycol); and ethylene carbonate. Ethylene glycol is used principally as an intermediate in the production of terephthalate polyester resins for fibres, films and bottles and in automotive antifreeze. Ethoxylation products of long-chain alcohols and alkylphenols are used as nonionic surfactants in household and industrial detergents. Glycol ethers, made by the addition of ethylene oxide to short-chain alcohols (including ethylene glycol to give di-, tri- and polyethylene glycols), are used as solvents, intermediates and in many other applications (Cawse *et al.*, 1980).

1.3 Occurrence

1.3.1 *Natural occurrence*

Ethylene oxide occurs as a metabolite of ethylene (see the monograph on ethylene, section 4.1). It is reactive in the environment. Its estimated atmospheric residence time, the time required for a given quantity to be reduced to 37% of its original level, is 5.8 days. In water, ethylene oxide reacts with anions such as chloride and carbonate; its half-life in fresh water (pH 7, 25 °C) is two weeks, and that in salt water is four days (US Environmental Protection Agency, 1986).

1.3.2 *Occupational exposure*

The National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health in the USA between 1981 and 1983 indicated that 270 000 US employees were potentially exposed at work to ethylene oxide (US National Institute for Occupational Safety and Health, 1993). Of this number, 22% were estimated to be exposed to ethylene oxide and 78% to materials containing ethylene oxide. Workers in hospitals and in the chemicals and allied products industry (plastics, synthetic materials and drugs manufacture) accounted for half of the number. The estimate is based on a survey of US companies and did not involve actual measurements of exposure. People with relatively high exposure include approximately 96 000 exposed in hospitals and 21 000 exposed during commercial sterilization of medical supplies, pharmaceutical products and spices (Steenland *et al.*, 1991). Most of the data on occupational exposure is related to the production of ethylene oxide and its use in industrial and hospital sterilization.

(a) *Production of ethylene oxide and its derivatives*

Rough estimates of exposure to ethylene oxide have been made for a Swedish company where ethylene oxide and derivatives were produced by the chlorohydrin process. Average exposure was estimated to be to less than 25 mg/m³ during the period 1941–47 and 10–50 mg/m³ during the 1950s and early 1960s, with occasional peaks above the odour threshold of 1300 mg/m³. After manufacture of ethylene oxide was stopped in 1963, exposure to 1–10 mg/m³ (with occasional higher values) continued to occur because of its use in the manufacture of other compounds (Hogstedt *et al.*, 1979a).

At another Swedish plant, where ethylene oxide was produced by oxygenation of ethylene, the 8-h time-weighted average (TWA) exposure to ethylene oxide was 9–15 mg/m³ in 1963–76 and 2–4 mg/m³ in 1977–82 during production of ethylene oxide and ethylene glycol, 6 mg/m³ in 1963–76 and 2 mg/m³ in 1977–82 in processing of ethylene oxide and 2–6 mg/m³ in 1963–76 and 1–3 mg/m³ in 1977–82 in maintenance and technical service work. Certain workers in each category are reported to have had higher exposures, up to 600–1800 mg/m³, during periods of minutes (Hogstedt *et al.*, 1986).

Area samples taken in the 1960s throughout a US plant where ethylene oxide was produced by direct oxidation of ethylene contained 0–55 ppm [0–100 mg/m³]; the majority contained 3–20 ppm [5–36 mg/m³]. On the basis of these results, the general long-term exposure of operators was estimated to be 5–10 ppm [9–18 mg/m³] (Joyner, 1964).

The following exposures were estimated for production workers in two US plants where manufacture and use of ethylene oxide started in 1925: 1925–39, > 14 ppm [25 mg/m³]; 1940–56, 14 ppm [25 mg/m³]; 1957–73, 5–10 ppm [9–18 mg/m³]; and 1974–88, < 1 ppm [1.8 mg/m³], with frequent peaks of several hundred parts per million in the earliest period and some peaks of similar intensity in the 1940s to mid-1950s. The chlorohydrin process was used from 1925 to 1957 (Teta *et al.*, 1993). Although the results of environmental monitoring in these plants since 1976 indicated that the 8-h TWA was less than 1 ppm [1.8 mg/m³], it was generally between 1 and 5 ppm [1.8 and 9 mg/m³] for maintenance employees and could go up to 66 ppm [119 mg/m³] (Greenberg *et al.*, 1990).

Area and personal samples were taken in five US plants where ethylene oxide and its derivatives were produced, including the two described above, by the US National Institute for Occupational Safety and Health during 1977 and 1978. In most of the 95 personal samples taken, representative of a shift, the concentration of ethylene oxide was below the detection limits (which varied from 0.1 to 8 mg/m³), although a few contained between 1 and 148 mg/m³ (82 ppm). Similarly, in most area samples, the concentration was below the detection limits or was in the range < 1–1.5 ppm [2–3 mg/m³], apart from exceptional situations such as leaks (Lovegren & Koketsu, 1977a,b,c; Oser *et al.*, 1978a,b, 1979). The fact that full-shift concentrations in these plants were usually well below the standards of the time (50 ppm, or 90 mg/m³) has been attributed to three main factors: use of completely closed systems for the storage, transfer and production of ethylene oxide; implementation of measures to prevent fire; and operation out of doors, resulting in dilution by natural air (Morgan *et al.*, 1981).

In one US chemical manufacturing complex, two groups of employees may have been exposed to ethylene oxide: during its production and during production of ethylene glycol, glycol ethers and ethanolamines. Yearly TWA exposures (1977–80) were reported to have been to less than 1 ppm [1.8 mg/m³] in all jobs except loading, where technicians were exposed to up to 1.7 ppm [3 mg/m³] yearly and 5.7 ppm [10 mg/m³] individually. Peak exposures were usually to less than 20 ppm [36 mg/m³], except in loading where exposure was to up to 235 ppm [420 mg/m³] (Currier *et al.*, 1984).

The typical average daily exposures of workers in a 1979 survey of US plants where ethylene oxide was manufactured and used were 0.3–4 ppm [0.5–7.3 mg/m³]; worst-case peak exposures of maintenance workers were up to 9600 ppm [17 300 mg/m³] (Flores, 1983).

Under the sponsorship of the Chemical Manufacturers Association, company data were collected on current exposures of workers to ethylene oxide in 11 ethylene oxide production units and 24 ethoxylation units in the USA in 1987 (Table 4). Respirators were reported to be used in specific operations, such as rail car loading and unloading, maintenance and product sampling, where engineering controls are not feasible (Heiden Associates, 1988a).

In a German plant where ethylene oxide is manufactured, 2-h area samples taken in 1978–79 contained less than 5 ppm [9 mg/m³] under normal working conditions. Concentrations rose to as much as 1900 ppm [3400 mg/m³] for several minutes in exceptional cases during plant breakdown (Thiess *et al.*, 1981a).

In a Dutch ethylene oxide manufacturing plant, geometric mean concentrations in 8-h personal samples were calculated to be < 0.01 ppm [< 0.02 mg/m³] for 1974, 1978 and 1980

and 0.12 ppm [0.2 mg/m³] for 1981, with individual values ranging overall from not detected (< 0.05 ppm [$< 0.1 \text{ mg/m}^3$]) to 8 ppm [14 mg/m³] (van Sittert *et al.*, 1985).

Table 4. Worker exposure to ethylene oxide by type of unit and job category in the US chemical manufacturing industry, 1987

Unit and job category	No. of samples	8-h TWA (mg/m ³)		No. of samples	Short-term (10–150 min) exposure (mg/m ³)	
		Mean ^a	Range		Mean ^a	Range
Ethylene oxide production						
Production workers	402	0.7	0.11–3.2	171	7.7	1.62–19.8
Maintenance workers	439	1.3	0.14–5.6	59	19.6	0.20–35.3
Supervisors	123	0.2	0.04–0.18	3	1.3	1.3–1.4
Distribution workers	218	2.9	0.36–6.8	111	11.7	3.6–17.6
Laboratory workers	189	0.7	0.12–4.3	39	1.4	0.4–2.2
Other workers	97	0.2	0.05–0.72			
Ethoxylation						
Production workers	640	0.4	0.12–1.26	172	2.0	0.02–9.9
Maintenance workers	191	1.1	0.02–4.7	56	13.3	0.11–54.9
Supervisors	54	0.4	0.05–0.72	5	8.6	0.9–23.8
Distribution workers	105	0.7	0.20–2.7	100	3.4	0.9–21.6
Laboratory workers	52	0.4	0.02–0.9	19	5.0	0.4–11.0
Other workers	24	0.4	0.18–0.54			

Adapted from Heiden Associates (1988a); TWA, time-weighted average

^aWeighted by number of workers exposed

In the former Czechoslovakia, the 8-h TWA concentrations of ethylene oxide measured in 1982–84 in the working environment of an ethylene oxide production plant were 0–8.25 mg/m³ (Karelová *et al.*, 1987).

Gardner *et al.* (1989) reported that monitoring since 1977 in four British plants where ethylene oxide and derivatives were produced indicated average exposures to less than 5 ppm [9 mg/m³] in almost all jobs and to < 1 ppm [1.8 mg/m³] in many jobs; occasional peaks up to several hundred parts per million occurred as a result of operating difficulties. In earlier years, peak exposures above the odour threshold of 700 ppm [1260 mg/m³] were reported.

In industries where ethylene oxide and its derivatives are manufactured, exposure may occur to a large variety of chemicals other than ethylene oxide, depending on the types of processes and jobs. They include unsaturated aliphatic hydrocarbons (e.g. ethylene, propylene), other epoxides (e.g. propylene oxide), chlorohydrins (e.g. epichlorohydrin, ethylene chlorohydrin), chlorinated aliphatic hydrocarbons (e.g. dichloromethane, dichloroethane), glycols and ethers (e.g. ethylene glycol, glycol ethers, bis(2-chloroethyl)ether), aldehydes (e.g. formaldehyde), amines (e.g. aniline), aromatic hydrocarbons (e.g. benzene, styrene), alkyl sulfates and other compounds (Shore *et al.*, 1993).

(b) *Use of ethylene oxide for industrial sterilization*

Industrial workers may be exposed to ethylene oxide during sterilization of a variety of products, such as medical equipment and products (e.g. surgical products, single-use medical devices), disposable health care products, pharmaceutical and veterinary products, spices and animal feed.

In an extensive survey of the industry in the USA, conducted by the National Institute for Occupational Safety and Health, exposure to ethylene oxide was estimated on the basis of data collected in 1976–85 by 21 out of 36 companies, most of which were involved in sterilization of medical supplies and spices. Individual 8-h TWA concentrations in workers' personal breathing zones, collected by active sampling on charcoal tubes, were included in a model in which regression analysis was used to link exposure concentration to seven significant variables: year of operation, volume of sterilizer or treatment vessel, period since product was sterilized, product type, aeration procedure, presence of a rear exhaust valve in the sterilizer, and exposure category (sterilizer, chamber area, maintenance, production, warehouse, clean room, quarantine and laboratory; Stayner *et al.*, 1993) (Greife *et al.*, 1988). When the model was applied in a cohort study to the job histories of exposed workers in 13 of the companies, the estimated historical average exposure concentrations ranged from 0.05 to 77.2 ppm [0.1–139 mg/m³], with a mean of 5.5 ppm [9.9 mg/m³] and a median of 3.2 ppm [5.8 mg/m³] (Stayner *et al.*, 1993). Wong and Trent (1993) used the industrial hygiene data from the same companies and estimated that sterilizer operators were exposed to an 8-h TWA concentration of 16 ppm [29 mg/m³] before 1978 and 4–5 ppm [7–9 mg/m³] after 1978, while production workers were exposed to about 5 ppm [9 mg/m³] before 1978 and 2 ppm [3.6 mg/m³] after that year.

Engineering controls and new work practices designed to lower workers' exposure were generally adopted by ethylene oxide users in the USA in 1978 and 1979 (Steenland *et al.*, 1991). Stolley *et al.* (1984) estimated that the 8-h TWA concentrations of sterilizer operators in three US facilities before 1980 had been 0.5, 5–10 and 5–20 ppm [1, 9–18 and 9–36 mg/m³], while data collected in the two plants that were still operating in 1980–82 indicated concentrations of less than 1 ppm [2 mg/m³].

Under the sponsorship of the Health Industry Manufacturers Association, company data were collected on current exposures of workers to ethylene oxide in 71 facilities in the USA in 1987 where medical devices and diagnostic products were sterilized. The workers included sterilizer operators, maintenance workers, supervisors, warehouse workers, laboratory workers and quality control personnel. Respirators were reported to be used in specific operations, such as unloading the sterilizer, maintenance, quality control sampling, emergencies, loading aeration, and changing ethylene oxide bottles, cylinders and tanks. Concentrations were measured outside the respirators. The routine 8-h TWA concentration, occurring two or more days per week, was > 1 ppm (> 1.8 mg/m³) for 12.6% of workers, 0.5–1 ppm (0.9–1.8 mg/m³) for 13.9%, 0.3–0.5 ppm (0.5–0.9 mg/m³) for 26.7% and < 0.3 ppm (< 0.5 mg/m³) for 46.8%. Short-term sampling (for 5–120 min; average, 28 min; except in one factory where sampling was for 210 min for workers in other jobs) showed routine short-term exposures of > 10 ppm (> 18 mg/m³) for 10.7% of workers, 5–10 ppm (9–18 mg/m³) for 17.1% and < 5 ppm (< 9 mg/m³) for 72.2%. Non-routine short-term

exposure, occurring one day per week or near areas where there was exposure was > 10 ppm (> 18 mg/m³) for 5.1% of workers, 5–10 ppm (9–18 mg/m³) for 2.6% and < 5 ppm (< 9 mg/m³) for 92.3% (Heiden Associates, 1988b).

In a Swedish factory where hospital equipment was sterilized, area samples taken in 1977 in the storage area showed concentrations of ethylene oxide ranging from 2 to 70 ppm [3.6–126 mg/m³]; the 8-h TWA concentration in the breathing zone of workers in the same area was 20 ppm [36 mg/m³] (Hogstedt *et al.*, 1979b). In a Swedish factory evaluated in 1978, full-shift personal sampling indicated that sterilizing room operators had an exposure concentration of 2.4 ppm [4.3 mg/m³]; area sampling indicated an exposure of 1.3 ppm [2.3 mg/m³]. Personal sampling showed a concentration of 0.1 ppm [0.2 mg/m³] in the packing room, and area sampling showed a concentration of 0.8 ppm [1.4 mg/m³] in the stockroom (Högstedt *et al.*, 1983). In another Swedish study, sterilizers and a laboratory technician in the production of disposable medical equipment were reported to have been exposed to bursts of ethylene oxide at concentrations of 5–10 ppm [9–18 mg/m³] for a total of 1 h per working day, while packers were exposed at an average of 0.5–1 ppm [1–2 mg/m³] for the entire week (Pero *et al.*, 1981). Sterilizers, packers and truck drivers at another Swedish factory, where single-use medical equipment was produced, were reported to be exposed to an 8-h TWA concentration of 0.5–1 ppm [1–2 mg/m³] (Pero *et al.*, 1982). In two Swedish disposable medical equipment plants, sterilizers and packers were the most heavily exposed, but levels decreased steadily from 35–40 ppm [about 70 mg/m³] in 1970 to < 0.2 – 0.75 ppm [< 1.5 mg/m³] in 1985; the average exposures of store workers and development engineers decreased from 5–20 ppm [9–36 mg/m³] to < 0.2 ppm [< 0.4 mg/m³] in the same period, while those of people in other job categories (repairmen, laboratory technicians, controllers and foremen) decreased from 1–4 ppm [2–7 mg/m³] to < 0.2 ppm [< 0.4 mg/m³] (Hagmar *et al.*, 1991).

In a plant in eastern Germany where disposable medical equipment was sterilized, workers were found to have been exposed to an average concentration of about 60 mg/m³ in 1985 and about 30 mg/m³ from 1989 onwards (Tates *et al.*, 1991a).

In Belgium, 12 workers involved in industrial sterilization in three plants were exposed to 8-h TWA concentrations of 0.1–9.3 ppm [0.2–16.7 mg/m³], with averages per plant of 1.7 ppm [3.1 mg/m³], 3.7 [6.7] and 4.5 [8.1] (Wolfs *et al.*, 1983).

Other substances to which workers involved in sterilizing medical products may be exposed include gases present with ethylene oxide in the sterilizing mixture, such as chlorofluorocarbons and carbon dioxide (Heiden Associates, 1988b) and methyl formate in Sweden (Hagmar *et al.*, 1991).

(c) *Use of ethylene oxide in hospitals*

Ethylene oxide is used widely in hospitals as a gaseous sterilant for heat-sensitive medical items, surgical instruments and other objects and fluids that come into contact with biological tissues (Babich, 1985). The US National Institute for Occupational Safety and Health estimated that there were more than 10 000 sterilizers in use in US health care facilities. Large sterilizers are found in central supply areas of most hospitals, and smaller sterilizers are found in clinics, operating rooms, tissue banks and research facilities (Glaser, 1979).

Exposure to ethylene oxide may result during any of the following operations and conditions: changing pressurized ethylene oxide gas cylinders; leaking valves, fittings and piping; leaking sterilizer door gaskets; opening of the sterilizer door at the end of a cycle; improper ventilation at the sterilizer door; improperly or unventilated air gap between the discharge line and the sewer drain; removal of items from the sterilizer and transfer of the sterilized load to an aerator; improper ventilation of aerators and aeration areas; incomplete aeration of items; inadequate general room ventilation; passing near sterilizers and aerators during operation (Mortimer & Kercher, 1989).

The US National Institute for Occupational Safety and Health conducted a series of studies over 10 years to document the exposure of US hospital sterilization staff to ethylene oxide. The main results are summarized in Table 5. Levels found in other studies in the USA and in other countries are presented in Table 6.

In a unit in Argentina equipped with old gas sterilizers with no mechanical ventilation, the 8-h TWA concentration was 60–69 ppm [108–124 mg/m³] (Lerda & Rizzi, 1992).

In most studies, exposure appears to result mostly from peak emissions during such operations as opening the door of the sterilizer and unloading and transferring sterilized material. Proper engineering controls and work practices are reported to result in full-shift exposure levels of less than 0.1 ppm [0.18 mg/m³] and short-term exposure levels of less than 2 ppm [3.6 mg/m³] (Mortimer & Kercher, 1989). In a survey of 125 US hospitals, however, use of personal protective equipment was found to be limited to the wearing of various types of gloves while transferring sterilized items. No respirators were used (Elliott *et al.*, 1988).

Other substances to which sterilizer operators in hospitals may be exposed include other gases, such as chlorofluorocarbons (e.g. dichlorodifluoromethane) and carbon dioxide present in the sterilizing mixture (Wolfs *et al.*, 1983; Deschamps *et al.*, 1989). Some operating room personnel exposed to ethylene oxide may also be exposed to anaesthetic gases and X-rays (Sarto *et al.*, 1984a), and some may have occasional exposure to low concentrations of formaldehyde (Gardner *et al.*, 1989).

(d) Other uses

In a US waste-water treatment plant in the starch processing area, where ethylene oxide is used as a reaction chemical to modify starch, full-shift personal breathing zone concentrations ranged from undetectable to 0.43 mg/m³ for operators and from undetectable to [2.5 mg/m³] for mechanics (McCammon *et al.*, 1990).

1.3.3 Air

Estimated ethylene oxide emissions in member states of the European Union in the mid-1980s are presented in Table 7 (Bouscaren *et al.*, 1987). In 1985, US emissions of ethylene oxide were estimated to have been approximately 5000 tonnes per year. Sterilization and fumigation sites accounted for 57% of total emissions, production and captive use for 31%, medical facilities for 8% and ethoxylation for 4%. Most emissions from producer and ethoxylator sites are due to equipment leaks. Less than 0.1% of the ethylene oxide produced is used in sterilizer and fumigator processes, but nearly all of the ethylene oxide used for this purpose is released into the atmosphere or mixed with water and routed to a sewer system (Markwordt, 1985).

Table 5. Exposure of hospital sterilizer operators to ethylene oxide (personal samples) in studies conducted by the US National Institute for Occupational Safety and Health, 1977-90

No. of hospitals	Operation or conditions	Duration of sampling	No. of samples	Concentration (mg/m ³)	Period of measurements	Reference
12	Good engineering controls and good work practice	8-h TWA	4	ND	[1984-85]	Elliott <i>et al.</i> (1988)
		Short-term (2-30 min)	3	ND		
	Good engineering controls and poor work practices	8-h TWA	15	[ND-0.29]		
		Short-term (2-30 min)	19	[ND-5.4]		
	No engineering controls and good work practices	8-h TWA	14	[ND-0.83]		
		Short-term (2-30 min)	4	[0.43-7.2]		
No engineering controls and poor work practices	8-h TWA	24	[ND-8.3]			
	Short-term (2-30 min)	8	[0.43-186]			
8		Full-shift TWA (6-8 h)	50	[ND-0.5]	1984-86	Mortimer & Kercher (1989)
		Short-term (1-30 min)	59	[ND-10.4]		
1	Decontamination room Sterile room	8-h TWA	2	[0.58-0.77]	1987	Boeniger (1988a)
		8-h TWA	6	[0.02-1.37]		
1		8-h TWA	8	[< 0.02]	1988	Newman & Freund (1989)
1	Before installation of controls (1984)	Full-shift TWA	NR	[0.43] (average)	1987	Kercher & Mortimer (1987)
		Short-term (15-20 min)	NR	[3.4] (average)		
		Short-term (1-2 min)	NR	[4.3] (average)		
	After installation of controls (1985)	Full-shift TWA	NR	[< 0.1] (average)		
		Short-term (15-20 min)	NR	[< 0.4] (average)		
		Short-term (1-2 min)	NR	[1]		
1	Full shift Cracking sterilizer door open Transferring load to aerator	4-7-h TWA	8	[0.04-0.40]	1987	Boeniger (1988b)
		30 sec	6	[< 0.05-7.7]		
		30 sec	15	[0.23-18.9]		
1		6-8-h TWA	3	[< 0.02]	1991	Shults & Seitz (1992)

ND, not detected; NR, not reported

Table 6. Ethylene oxide concentrations observed in hospitals in various countries

Country	No. of hospitals	Year of sampling	Job or operation	Duration of sampling	No. of samples	Concentration (mg/m ³)		Reference
						Range	Mean	
Belgium	3		Sterilizer operators	8-h TWA	28	0.4-4.5	0.5-2.9	Wolfs <i>et al.</i> (1983)
	1		Sterilizer operators; leaking equipment	8-h TWA	16	0.5-32.9	14.0	
	1		Sterilizer operators; box sterilizer with capsules	8-h TWA	5	16.2-95.2	27.0	
Former Czechoslovakia		1984	Sterilization workers; area sampling	8-h TWA	NR	0-4.8		Karelová <i>et al.</i> (1987)
Finland	24	1981	Sterilizer operators	8-h TWA	NR	0.2-0.9		Hemminki <i>et al.</i> (1982)
	24	1981	Sterilizing chamber open	Peaks 20 min	NR	≤ 450 9-18		Hemminki <i>et al.</i> (1982)
France	4 ^a	1983-86	Loading, sterilizing, unloading, aerating; area sampling	Few min 6-8-h TWA	270 14	0.9-414 0.1-9		Mouilleseaux <i>et al.</i> (1983)
	5		Opening sterilizer and handling sterilized material; personal sampling	2.5-102 min	10	0.4-70		Deschamps <i>et al.</i> (1989)
Italy	1		Sterilization workers	8-h TWA	10 subjects	1.90-4.71		Brugnone <i>et al.</i> (1985)
	1		Sterilizer operators	7-8-h TWA	4 subjects	11.5-16.7	14.3	Sarto <i>et al.</i> (1987)
			Helpers	7-8-h TWA	4 subjects	6.8-9.0	7.7	
	6		Old sterilizers					Sarto <i>et al.</i> (1984a)
			Opening sterilizer; area sampling	5 min	NR	23-288	113	
			One sterilization cycle; personal sampling	Variable	NR	6.7-63.9	28.4	
			Standard working day; personal sampling	8-h TWA	19 subjects	6.7-36	19.3	
	2		Second-generation sterilizers					
		Opening sterilizer, area sampling	5 min	NR	9-47	15.5		
		One sterilization cycle; personal sampling	Variable	NR	0.5-4.7	2.0		
		Standard working day; personal sampling	8-h TWA	NR	0.4-0.9	0.63		

Table 6 (contd)

Country	No. of hospitals	Year of sampling	Job or operation	Duration of sampling	No. of samples	Concentration (mg/m ³)		Reference
						Range	Mean	
Italy (contd)	1		Sterilization workers	6.5-h TWA	5 subjects	0.68 ^b		Sarto <i>et al.</i> (1991)
			Preparation workers	6.5-h TWA	5 subjects	0.045		
Mexico	1		Sterilizer operators	8-h TWA	22 subjects	0-2.4		Schulte <i>et al.</i> (1992)
USA	1		Sterilizer workers	8-h TWA	14	< 0.13-7.7		Hansen <i>et al.</i> (1984)
			Sterilizer unloading; personal sampling	15 min	17	< 4.3-81		
	1	1985-86 1987 1988	Sterilizer unloading; maximum	Instantaneous	13	4-1430		Mayer <i>et al.</i> (1991)
			Sterilizer operators; personal sampling	8-h TWA	34 subjects NR 31	≤ 4.3 < 1.8 < 0.18]		
	9		Sterilizer operators	8-h TWA	51 subjects	0-0.54		Schulte <i>et al.</i> (1992)

^aOne was a municipal sterilization and disinfection facility

^bEach has the same concentration.

Table 7. Estimated ethylene oxide emissions in member states of the European Union

Country	Emissions (thousand tonnes/year)	
	From chemical industry	Other sources
Belgium	0.41	NR
France	0.40	NR
Germany	0.8	0.45
Italy	0.5	0.28
Netherlands	0.2	0.23
Spain	0.12	NR
United Kingdom	0.41	NR
Total	2.8	

From Bouscaren *et al.* (1987); NR, not reported

Emissions of ethylene oxide reported to the US Environmental Protection Agency by industrial facilities in the USA declined from approximately 2900 tonnes in 1987 to 835 tonnes in 1991 (US National Library of Medicine, 1993).

1.3.4 Other occurrence

Of 204 food products from Danish retail shops in 1985 examined for ethylene oxide residues, 96 samples were found to have concentrations of ethylene oxide ranging from 0.05 to 1800 mg/kg. The food products surveyed included herbs and spices (14–580 mg/kg); dairy (0.06–4.2 mg/kg), pickled fish (0.08–2.0 mg/kg), meat (0.05–20 mg/kg) and cocoa (0.06–0.98 mg/kg) products; and black and herb teas (3–5 mg/kg; one sample contained 1800 mg/kg). In a follow-up survey of 59 honey samples, no ethylene oxide residue was detected (Jensen, 1988).

Ethylene oxide has also been reported to be a product of incomplete combustion and has been identified in automobile and diesel exhaust and in tobacco smoke (Gray *et al.*, 1985).

Patients on dialysis units sterilized with ethylene oxide showed allergic symptoms due to sensitization to residual ethylene oxide (see section 4.2.1).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for ethylene oxide in a number of countries are presented in Table 8.

A tolerance of 50 ppm (mg/kg) has been established in the USA for residues of ethylene oxide when used as a postharvest fumigant in or on raw black walnut meats, copra and whole spices (US Environmental Protection Agency, 1992a).

Ethylene oxide, either alone or admixed with carbon dioxide or dichlorodifluoromethane, is permitted in the USA as a fumigant for the control of microorganisms and insect

infestation in ground spices and other processed natural seasoning materials, except mixtures to which salt has been added. Residues of ethylene oxide in ground spices must not exceed the established tolerance of 50 ppm (mg/kg) in whole spices (US Environmental Protection Agency, 1992b).

The US Food and Drug Administration (1993) permits the use of ethylene oxide in various products that may come into contact with food.

Table 8. Occupational exposure limits and guidelines for ethylene oxide

Country or region	Year	Concentration (mg/m ³)	Interpretation
Argentina	1991	2	TWA; suspected of having carcinogenic potential
Australia	1983	2	TWA; suspected human carcinogen
Austria	1982	18	TWA
Belgium	1984	1.8	TWA; probable human carcinogen
Brazil	1978	70	TWA
Canada	1986	2	TWA; suspected human carcinogen
Chile	1983	16	TWA
Denmark	1988	1.8	TWA; suspected carcinogen
Finland	1993	1.8	TWA; suspected of having carcinogenic potential
France	1993	2 10	TWA; suspected carcinogen STEL
Germany	1993	None	Carcinogenic in animals; skin
Hungary	1978	1	Ceiling (30-min); probable human carcinogen; irritant; sensitizer
Indonesia	1978	90	TWA
Italy	1978	60	TWA; sensitizer
Japan	1991	1.8	TWA; suspected of having carcinogenic potential (tentative)
Mexico	1989	2	TWA
Netherlands	1986	90	TWA
Poland	1982	1	TWA
Romania	1975	30 60	Average Maximum
Sweden	1991	2 9	TWA; probable human carcinogen; skin STEL
Switzerland	After 1987	2	TWA; suspected carcinogen; skin
Taiwan	1981	90	TWA
United Kingdom	1992	10	TWA; maximum exposure limit
USA			
ACGIH (TLV)	1994	1.8	TWA; suspected human carcinogen ^a
OSHA (PEL)	1992	1.8 9	TWA STEL
NIOSH (REL)	1992	0.18 9	TWA Ceiling

Table 8 (contd)

Country or region	Year	Concentration (mg/m ³)	Interpretation
Venezuela	1978	90 135	TWA Ceiling

From Arbeidsinspectie (1986); Cook (1987); Arbejdstilsynet (1988); ILO (1991); Health and Safety Executive (1992); US Occupational Safety and Health Administration (OSHA) (1992); US National Institute for Occupational Safety and Health (NIOSH) (1992); American Conference of Governmental Industrial Hygienists (ACGIH) (1993); Deutsche Forschungsgemeinschaft (1993); Institut National de Recherche et de Sécurité (1993); Työministeriö (1993); UNEP (1993); TWA, time-weighted average; STEL, short-term exposure limit; TLV, threshold limit value; PEL, permissible exposure level; REL, recommended exposure level; skin, absorption through the skin may be a significant source of exposure

^aSubstance identified by other sources as a suspected or confirmed human carcinogen

2. Studies of Cancer in Humans

2.1 Case reports

Hogstedt *et al.* (1979b) reported three cases of haematopoietic cancer that had occurred between 1972 and 1977 in workers at a Swedish factory where 50% ethylene oxide and 50% methyl formate had been used since 1968 to sterilize hospital equipment. Attention had been drawn to the case cluster by the factory safety committee. One woman with chronic myeloid leukaemia and another with acute myelogenous leukaemia had worked in a storage hall where they were exposed for 8 h per day to an estimated 20 ± 10 (SD) ppm [36 ± 18 mg/m³] ethylene oxide. The third case was that of a man with primary macroglobulinaemia (morbus Waldenström) who had been manager of the plant since 1965 and had been exposed to ethylene oxide for an estimated 3 h per week. [The Working Group noted that Waldenström's macroglobulinaemia is classified in ICD 10 as a malignant immunoproliferative disease.]

2.2 Cohort studies

Two hundred and three workers employed for at least one year at the above factory were subsequently followed up for mortality (Hogstedt *et al.*, 1986). During 1978–82, five deaths occurred (4.9 expected), of which four were from cancer (1.6 expected). Two of the deaths were from lymphatic and haematopoietic cancer (0.13 expected), but one of these decedents had been part of the original case cluster that had prompted the study.

In a second study, Hogstedt *et al.* (1979a, 1986) and Hogstedt (1988) examined workers at a Swedish chemical plant where ethylene oxide had been produced by the chlorohydrin process. The cohort comprised men who had taken part in a medical survey in 1960–61 and included 89 operators with regular exposure to ethylene oxide, 86 maintenance staff with intermittent exposure and 66 unexposed men. All of the men had been exposed or employed

for at least one year. Average exposures to ethylene oxide during 1941–47 were estimated to have been below 25 mg/m³ but with occasional peaks above the odour threshold of 1300 mg/m³. During the 1950s and through to 1963, an average concentration of 10–50 mg/m³ was estimated. In 1963, production of ethylene oxide ceased, but the compound continued to be used in manufacturing processes, and random samples showed ethylene oxide concentrations in the range 1–10 mg/m³, with occasional higher values. Other exposures in the plant included chloroform, chlorinated acetals, chloral, DDT, ethylene glycol, surfactants, cellulose ethers, ethylene, ethylene chlorhydrin, ethylene dichloride, bis(2-chlorethyl)ether and propylene oxide. The cohort was followed from January 1961 to December 1985. With no adjustment for any latency from first exposure, there were 34 deaths from all causes among the ethylene oxide operators (25.0 expected), including 14 cancer deaths (6.1 expected) of which five were due to stomach cancer (0.6 expected) and two to leukaemia (0.2 expected). There was no overall excess mortality from cancer among the maintenance staff with intermittent exposure or among the unexposed workers; however, four of the maintenance men had died of stomach cancer (0.6 expected) and one from leukaemia (0.2 expected).

The above reports also describe a second cohort of Swedish workers exposed to ethylene oxide in a plant where the process used was based on direct oxidation of ethylene (Hogstedt *et al.*, 1986; Hogstedt, 1988). The cohort comprised 128 workers employed in the production of ethylene oxide or ethylene glycol, who had almost pure exposure to ethylene oxide; 69 workers employed in the processing of ethylene oxide and propylene oxide to nonionic surfactants and polyols, whose principal exposure was to ethylene oxide and propylene oxide but who had also been exposed to various amines, sodium nitrate, formaldehyde and 1,2-butene oxide; and 158 maintenance and technical personnel with multiple exposures including ethylene oxide. Analyses of air samples and interviews with experienced staff indicated 8-h TWA exposures to ethylene oxide of 1–8 ppm [1.8–14.4 mg/m³] during 1963–76, which fell to 0.4–2 ppm [0.7–3.6 mg/m³] during 1977–82. Expected numbers of cancers and deaths were calculated from five-year age-, sex- and calendar year-specific rates for the national population. During follow-up from 1964 to 1981, eight deaths were observed in the entire cohort as compared with 11.6 expected; one man in the maintenance and repair group died of chronic myeloid leukaemia, but no additional incident cases of leukaemia were recorded. The expected number of incident leukaemia cases was 0.16. During extended follow-up to 1985, a fatal case of reticular-cell sarcoma was recorded among the production workers [expected number not given]. [The Working Group noted that the cohort was not defined precisely.]

Hogstedt (1988) summarized the findings of the three cohort studies described above. After exclusion of the three cases in the initial cluster at the sterilizing plant, seven lymphatic and haematological malignancies were observed during follow-up for cancer incidence to 1983 (2.2 expected) [standardized incidence ratio [SIR], 3.2; 95% confidence interval [CI], 1.3–6.6], including five cases of leukaemia (0.8 expected) [SIR, 6.3; 95% CI, 2.0–15.0].

Morgan *et al.* (1981) reported a retrospective cohort study of 767 men employed at a chemical plant in eastern Texas, USA, between 1955 and 1977 where ethylene oxide was produced. All of the men had worked at the factory for at least five years and were 'potentially exposed' to the compound. Potential exposure to ethylene oxide was determined

by personnel at the company on the basis of work histories. In an industrial hygiene survey in 1977, all samples taken in the ethylene oxide production area contained less than 10 ppm [18 mg/m³]. Vital status was ascertained for more than 95% of cohort members from a combination of plant records, 'personal knowledge' and telephone follow-up. Altogether, 46 deaths were recorded, whereas 80 were expected on the basis of US vital statistics. Death certificates were obtained for 42 of the 46 deceased subjects. Eleven deaths were from cancer (15.2 expected), and nonsignificant excesses were seen of cancers of the pancreas (3/0.8) and brain and central nervous system (2/0.7) and of Hodgkin's disease (2/0.4); no death from leukaemia was found. [The Working Group noted that details were missing on the nature of the manufacturing process, on the extent to which exposure readings were representative of earlier conditions in the plant and on potential confounding exposures.]

The results of an extended follow-up of this cohort to 1985 were presented at a meeting and reported by Shore *et al.* (1993) as part of a meta-analysis of cohort studies on ethylene oxide. The follow-up rate was 99.7%. Three deaths were observed from brain cancer (1.1 expected), three from lymphatic and haematopoietic cancer (3.0 expected), none from leukaemia (1.1 expected) and none from stomach cancer [expected number not given].

Thiess *et al.* (1981b) examined the mortality of 602 active and former male employees of a company in western Germany who had worked for at least six months in an area of alkylene oxide production. Until 1965, ethylene oxide had been made from ethylene chlorohydrin, but thereafter it was produced by direct oxidation of ethylene. Propylene oxide had been made since 1959 by a propylene chlorohydrin process. Industrial hygiene measurements in 1978 showed that the average concentration of ethylene oxide was < 4 ppm [7.2 mg/m³], but no earlier measurement was available. Discussions with long-standing employees indicated that exposures in the past would have been higher. Other potential exposures included propylene oxide, butylene oxide, dioxane, epichlorohydrin, dichloropropane, ethylene chlorohydrin, propylene chlorohydrin, aniline, piperazine, cyclohexylamine, cyclohexane, formaldehyde, isobutyraldehyde, ethylene-imine, hydrocyanic acid, hydrogen sulfide, aluminium chloride, benzene, phenol, cyanuric acid, acrylic acid and acetylene alcohols. The first worker was employed in 1928, and follow-up was from that year until 30 June 1980. Follow-up of former German employees was 97.6% successful, but 30/66 non-German ex-employees were lost to follow-up. The expected numbers of deaths in the cohort were calculated for each five-year age group by the person-years method, using mortality rates for the populations of Ludwigshafen and Rhinehessia-Palatinate during 1970-75 and of Germany during 1971-74 as reference. In addition, an internal comparison group of 1662 persons employed in a styrene production facility on the same site was used. During follow-up, 56 deaths were recorded in the exposed cohort, whereas the expected numbers were 71.5 (Ludwigshafen), 73.4 (Rhinehessia-Palatinate), 76.6 (Germany) and 57.9 (styrene cohort). Fourteen of the deaths were due to cancer, whereas 16.6 were expected from national statistics. The deaths from cancer included one case of myeloid leukaemia and one case of lymphatic sarcoma. [The Working Group noted that no indication is given of the completeness with which the cohort was ascertained, and the methods of follow-up are not stated. It is not clear how losses to follow-up were handled in the analysis.]

Most of the above cohort was included in a larger study of employees from six chemical companies in western Germany (Kiesselbach *et al.*, 1990). The 2658 cohort members had

been exposed to ethylene oxide for at least 12 months before 31 December 1982. The year of their first exposure ranged from 1928 to 1981, but most had first been exposed after 1950. Other possible exposures included benzene, 4-aminobiphenyl and 2-naphthylamine, but no information was given on the extent of exposure to those substances. Subjects who had left employment were traced through local registries and, in the case of foreigners who had returned home, by letter or by asking fellow countrymen who were still working in the plant. Of the cohort members, 97.6% were traced successfully to 31 December 1982. For those who had died, the cause of death was ascertained from death certificates (27.6% of all deaths), lay statements, the physician who last treated the patient or hospital reports. Mortality was compared with that expected from five-year age-, sex- and calendar period-specific rates in the national population; no statistics were available for periods before 1951, so the rates for 1951 were used. Altogether, 268 deaths were observed, with 307.6 expected. There were 68 cancer deaths (69.9 expected), including three from oesophageal cancer (1.5 expected), 14 from stomach cancer (10.2 expected) and five from lymphatic and haematopoietic cancer (5.0 expected). Two deaths were ascribed to leukaemia (2.4 expected). When expected numbers were calculated on the basis of rates in the states in which each plant was situated, the findings were much the same. Mortality ratios based on calculations in which the first 10 years of exposure for each subject were ignored were similar to those in the main analysis. It was possible to classify the ethylene oxide exposure of 67.2% of subjects as 'weak', 'medium' or 'high'. The excess of stomach cancer was greatest in those with weak or medium exposure and with less than 15 years of total exposure. When foreign workers were excluded from the analysis, there was no change in the observed number of deaths and mortality ratios were only slightly increased. [The Working Group noted that the definition of the cohort was imprecise, no data were given on likely levels of exposure to ethylene oxide or on the nature of the processes on which subjects worked, and calculation of expected numbers from death certificate data may have been a source of bias since certificates were available for only about one-quarter of deaths in the cohort.]

Gardner *et al.* (1989) studied 2876 workers in four British chemical companies where ethylene oxide or its derivatives had been manufactured and in eight hospitals where ethylene oxide had been used as a sterilant. In one company, ethylene oxide had been produced by the chlorohydrin process during 1950–60 and by direct oxidation of ethylene from 1959 onwards; in the second company, the chlorohydrin process was used during 1955–70 and direct oxidation thereafter; in the third company, ethylene oxide was produced during 1960–81 only by direct oxidation; in the fourth company, ethylene oxide had been used in the manufacture of derivatives since 1959. The eight hospitals had started using ethylene oxide between 1962 and 1972. The cohort comprised all workers at each factory and hospital with likely exposure to ethylene oxide during specified periods for which employment records were complete. Sixteen subjects had to be excluded because information about them was incomplete. Jobs held by cohort members at the factories were classified as having involved definite, probable or possible exposure to ethylene oxide. At the hospitals, jobs were classed as involving continual, intermittent or possible exposure. Environmental and personal monitoring since 1977 had shown a TWA concentration of < 5 ppm [9 mg/m³] in almost all jobs, but with occasional peaks of exposure up to several hundred parts per million as a result of operating difficulties in the chemical plants and

during loading and unloading of sterilizers in the hospitals. Exposures were thought to have been higher in earlier years, and peak exposures above the odour threshold of 700 ppm were reported both by the chemical manufacturers and at the hospitals. Cohort members at the manufacturing plants were potentially exposed to many other chemicals, including chlorohydrin, propylene oxide, styrene and benzene; some of the hospital workers had occasionally been exposed to formaldehyde and carbon tetrachloride. The cohort was followed up to 1987 through National Health Service and Social Security records, and tracing was 98% successful. Expected numbers of deaths were calculated from national sex-, age- and five-year calendar period-specific rates. Among the 1471 factory employees (all but one were male), there were 157 deaths from all causes (172.0 expected) and 53 deaths from cancer (46.6 expected). The latter included three cases of stomach cancer (4.3 expected), two of non-Hodgkin's lymphoma (1.0 expected) and three of leukaemia (1.3 expected). Two of the leukaemias were acute myeloid and the other was lymphatic unspecified. All three of the leukaemia cases were classed as having had definite exposure to ethylene oxide (0.86 expected), and in each case the death occurred after a latency of at least 20 years from first exposure. On the basis of their job histories, none was thought likely to have been exposed to benzene. Among the 1405 hospital employees (394 men and 1011 women), there were 69 deaths from all causes (86.9 expected) and 32 from cancer (30.0 expected). These included two deaths from stomach cancer (1.7 expected), two from non-Hodgkin's lymphoma (0.6 expected) and none from leukaemia (0.8 expected). Adjustment for local differences in mortality rates had little effect on the expected numbers of leukaemia in the cohort. In the cohort as a whole, there were slight excesses of oesophageal cancer (5/2.2), lung cancer (29/24.6) and bladder cancer (4/2.0), but these were not significant.

A series of studies was carried out on a cohort of 2174 male employees at two chemical plants in West Virginia, USA, where ethylene oxide had been produced and used (Greenberg *et al.*, 1990; Benson & Teta, 1993; Teta *et al.*, 1993). It was produced by the chlorohydrin process during 1925–57 and by direct oxidation from 1937–71. After 1971, the plants continued to use ethylene oxide brought in from elsewhere. The cohort comprised men employed at the plants during 1940–78 and assigned at any time before 1979 to a chemical production department in which ethylene oxide was judged to have been manufactured or used at the time of the assignment. The first large-scale environmental monitoring project at the plant began in 1976. The 8-h TWA concentration of ethylene oxide in departments where it was used was less than 1 ppm [1.8 mg/m³] but ranged up to 66 ppm [120 mg/m³]. The authors estimated that the 8-h TWA concentration in ethylene oxide production by direct oxidation in the 1960s ranged from 3 to 20 ppm and that exposures during production by the chlorohydrin process were probably rather higher. Departments were classified as having high, medium or low exposure concentrations according to the operations carried out, and the classification was validated by reference to reported incidents of acute exposure. The cohort was followed to the end of 1988, and vital status was ascertained for more than 98% of subjects. Death certificates were obtained for 99% of decedents, and expected numbers of deaths were calculated on the basis of national five-year age- and calendar period-specific rates in white males.

A total of 278 men had worked in a chlorohydrin unit which primarily produced ethylene chlorohydrin, with ethylene dichloride and bischloroethyl ether as by-products (Benson &

Teta, 1993). For part of the time, propylene chlorohydrin was also made. Ethylene oxide was handled only sporadically and in small volumes. Of these men, 147 died, with 140.8 expected. The deaths included 40 from cancer (30.8 expected), eight from lymphatic and haematopoietic cancer (2.7 expected) and eight from pancreatic cancer (1.6 expected). In a comparison with workers from other plants in the same locality, the risks for cancers of all types, for lymphatic and haematopoietic cancer, leukaemia and pancreatic cancer increased with duration of assignment to the chlorohydrin unit.

Among the 1896 men who had never been assigned to the chlorohydrin unit, there were 431 deaths, whereas 547.7 were expected (Teta *et al.*, 1993). The numbers of observed and expected deaths were 110/128.1 for cancer at any site, 8/5.0 from stomach cancer, 4/6.6 from pancreatic cancer, 6/4.0 from cancers of the brain and nervous system, 7/11.8 from lymphatic and haematopoietic cancer, 2/2.0 from lymphosarcoma and reticulosarcoma (ICD9 200), 5/4.7 from leukaemia and aleukaemia and 0/1.2 from Hodgkin's disease. No significant excess mortality was observed for any cause of death. There were no excesses of leukaemia or stomach cancer among men who had spent two or more years in high-exposure departments. Comparison with death rates of workers from plants in the same location who had never been assigned to ethylene oxide production or use showed no significant trend with duration of assignment for all cancer, leukaemia or pancreatic, brain or stomach cancers; but a two- to three-fold increase in risk for leukaemia (based on three cases) was observed among workers with more than 10 years of assignment to ethylene oxide departments. These studies confirmed and amplified the findings of an earlier case-control study at the same plants (Ott *et al.*, 1989).

Steenland *et al.* (1991) followed up 18 254 employees at 14 US industrial plants where ethylene oxide had been used to sterilize medical supplies or spices or in the testing of sterilizing equipment. The plants were selected because they held adequate records on personnel and exposure and their workers had accumulated at least 400 person-years at risk before 1978. Only workers with at least three months of exposure to ethylene oxide were included in the cohort. Forty five per cent of the cohort were male, 79% were white, 1222 were sterilizer operators and 15 750 were employed before 1978. Analysis of 627 8-h personal samples indicated that average exposure during 1976-85 was 4.3 ppm [7.7 mg/m³] for sterilizer operators; the average level for other exposed workers, on the basis of 1888 personal samples, was 2.0 ppm [3.6 mg/m³]. Many companies began to install engineering controls in 1978, and exposures before that year were thought to have been higher. There was no evidence of confounding exposure to other occupational carcinogens. The cohort was followed to 1987 through the national death index and records of the Social Security Administration, the Internal Revenue Service and the US Postal Service, and 95.5% were traced successfully. The expected numbers of deaths were calculated from rates in the US population, stratified according to age, race, sex and calendar year. In total, 1177 cohort members had died (1454.3 expected), including 40 for whom no death certificate was available. There were 343 deaths from cancer (380.3 expected). The observed and expected numbers of deaths were 36/33.8 from all lymphatic and haematopoietic cancer, including 8/5.3 from lymphosarcoma-reticulosarcoma [ICD9 200], 4/3.5 from Hodgkin's disease, 13/13.5 from leukaemia, 8/6.7 from non-Hodgkin's lymphoma [ICD9 202] and 3/5.1 from myeloma; 6/11.6 from cancer of the brain and nervous system; 11/11.6 from cancer of the

stomach; 16/16.9 from cancer of the pancreas; 8/7.7 from cancer of the oesophagus; and 13/7.2 from cancer of the kidney. Mortality ratios for subjects first exposed before 1978 were virtually identical to those for the full cohort. No significant trend in mortality was observed in relation to duration of exposure, but the mortality ratios for leukaemia (1.79 based on five deaths) and non-Hodgkin's lymphoma (1.92 based on five deaths) were higher after allowance for a latency of more than 20 years. Among the sterilizer operators, mortality ratios (and observed numbers of deaths) were 2.78 (two) for leukaemia and 6.68 (two) for lymphosarcoma/reticulosarcoma; no death from stomach cancer was seen.

In a further analysis of the same study (Stayner *et al.*, 1993), a regression model was used to estimate individual exposures to ethylene oxide at 13 of the facilities studied; information about the other facility was inadequate. Mortality from lymphatic and haematopoietic cancer was greatest in the highest category of cumulative exposure to ethylene oxide (> 8500 ppm-days) (standardized mortality ratio [SMR], 124; 95% CI, 66–213; 13 deaths), but the trend across three categories of cumulative exposure was weak (χ^2 , 0.97; $p = 0.32$). A similar pattern was observed for non-Hodgkin's lymphoma, but not for leukaemia. The Cox proportional hazards model was also used to examine cumulative exposure (ppm-days), average exposure (ppm), maximal exposure (ppm) and duration of exposure (days) to ethylene oxide. A significant positive trend in risk with increasing cumulative exposure to ethylene oxide was observed for all neoplasms of the lymphatic and haematopoietic tissues ($p < 0.05$, two-tailed). This trend was strengthened when analysis was restricted to neoplasms of lymphoid cell origin (lymphocytic leukaemia, ICD9 204; non-Hodgkin's lymphoma, ICD9 200, 202). The exposure-response relationship between cumulative exposure to ethylene oxide and leukaemia was positive but nonsignificant. The regression coefficients for neoplasms of the lymphatic and haematopoietic tissues for duration, average and maximal exposure were either weakly positive or negative. Rate ratios for neoplasms of the lymphatic and haematopoietic tissues corresponding to a working lifetime (45 years) of exposure to ethylene oxide at a level of 1 ppm were also estimated. The results given in Table 9 are shown for the best fitting regression models, in which exposures were 'lagged' from 5 to 10 years. Lagging was used in order to discount exposures occurring in previous years that might not be etiologically relevant to the occurrence of the disease. Significantly increased rate ratios of about 1.2 were found for all neoplasms of the lymphatic and haematopoietic tissues, non-Hodgkin's lymphoma and neoplasms of lymphoid cell origin. In this analysis, no significant increase was found for cancers of the stomach, pancreas, brain or kidney.

Wong and Trent (1993) subsequently reported a separate analysis of mortality in much the same population (Steenland & Stayner, 1993), with similar results. The cohort comprised 18 728 employees, and follow-up was to the end of 1988. [The Working Group noted that this report adds little useful information to that provided by Steenland *et al.* (1991).]

Hagmar *et al.* (1991) studied employees at two Swedish plants where disposable medical equipment sterilized with ethylene oxide was produced. In plant A, a 50:50 mixture of ethylene oxide and methyl formate had been used since 1970. In 1973, personal sampling for two packers indicated an exposure to ethylene oxide of 24 ppm [43 mg/m³]. After 1981, monitoring carried out annually over one to three days for sterilizers and packers showed a continuous decrease in exposure such that, after 1985, only sterilizers were exposed to

Table 9. Results from Cox proportional hazards models for mortality due to lymphatic and haematopoietic neoplasms in which cumulative exposures to ethylene oxide were lagged

Neoplasm	Lag period (years)	β	Standard error	χ^2	Rate ratio for 45 ppm-years	95% CI
All haematopoietic cancers	10	1.12×10^{-5}	4.24×10^{-6}	4.96	1.20	1.05-1.38
Leukaemia	10	1.29×10^{-5}	7.73×10^{-6}	2.07	1.24	0.96-1.58
Non-Hodgkin's lymphoma	10	1.29×10^{-5}	5.36×10^{-6}	3.98	1.24	1.04-1.47
Lymphoid	5	1.20×10^{-5}	3.31×10^{-6}	8.44	1.22	1.09-1.35

From Stayner *et al.* (1993). The results presented are those from models including a lag period that maximizes the goodness of fit (i.e. minimizes the $-2 \log$ likelihood). Results from all models were controlled for calendar year, age at risk, sex and race. CI, confidence interval. Confidence intervals for the rate ratios were estimated by computing the upper and lower bound estimates of the regression coefficients ($\beta \pm SE$) and substituting those bounds into the rate ratio formula. Rate ratios for a particular exposure level were estimated from the formula: $\exp(\beta, \chi)$, where χ is the cumulative exposure in ppm-days. For example, the rate ratio for all haematopoietic neoplasms corresponding to 45 years of exposure at 1 ppm is $\exp[(1.2 \times 10^{-5})(45 \text{ ppm-years})(365 \text{ days/year})]$.

concentrations greater than 0.2 ppm [0.4 mg/m³] (the limit of detection of the method used). In plant B, a 50:50 mixture of ethylene oxide and methyl formate was used from 1964 but was replaced by an ethylene oxide:carbon dioxide mixture in 1978. In 1975, personal monitoring indicated exposures of 4-5 ppm [7-9 mg/m³] ethylene oxide for four packers. After 1985, the 8-h TWA concentration was < 0.2 ppm [0.4 mg/m³] for all employees except sterilizers and store workers. The authors estimated that sterilizers were exposed to up to 75 ppm [135 mg/m³] in the earliest years of operation at this plant. On the basis of estimates of exposures in different job categories and time periods, the authors calculated individual cumulative exposures for 97% of subjects at plant A and 89% at plant B. The cohort comprised 594 men and 557 women who had been employed at plant A for at least 12 months between 1970 and 1985 and who were still working after 1 June 1975, and 267 men and 752 women employed at plant B for at least 12 months between 1964 and 1985 and still working after 1 January 1972. These subjects were followed to 1986 for mortality and from 1972 to 1985 for cancer registration. None was lost to follow-up. Expected mortality was calculated on the basis of calendar year-, sex- and five-year age-specific rates (censored at age 80) for the county in which the plants were situated, and expected cancer incidence from corresponding registration rates in the same area. Fifteen deaths were observed (25.7 expected), including eight from cancer (9.0 expected), two from gastrointestinal cancer (2.1 expected) and one from haematopoietic and lymphatic cancer (1.0 expected). The observed and expected numbers of incident cancers were 21/26.8 cancers at any site, no case of stomach cancer (0.5 expected), 1/1.6 for brain cancer, 2/1.3 for lymphoma and myeloma and one case of polycythaemia vera with 0.7 cases of leukaemia, polycythaemia vera and myelofibrosis expected. Among subjects with more than 1 ppm-year of cumulative exposure to ethylene oxide, there were two cases of cancer (3.3 expected) and none of lymphatic or haematopoietic cancer (0.2 expected).

Bisanti *et al.* (1993) studied a cohort comprising all 1971 male chemical workers in the Lombardy and Piedmont regions of Italy who had held a licence to handle ethylene oxide for at least one year during 1938–84; 637 had held licences for ethylene oxide only and 1334 for other toxic gases as well. Some workers may have been exposed to ethylene oxide before getting a licence. The cohort was followed from 1 January 1940 to 31 May 1984, and vital status was ascertained at the census office at each subject's place of residence. Sixteen subjects (0.8%) who were lost to follow-up were considered to be still living. Expected numbers of deaths were calculated from five-year age-, sex- and calendar period-specific rates for the regional (Lombardy) population. Seventy-six deaths were recorded (98.8 expected), including 43 from cancer (33.0 expected). The observed and expected numbers of deaths were 5/4.1 from stomach cancer, 3/1.2 from cancer of the pancreas, 1/0.6 from cancer of the kidney, 4/0.6 from lymphosarcoma and reticulosarcoma and 2/1.0 from leukaemia. The two deaths from leukaemia occurred among men with fewer than five years' exposure and after a latency of fewer than 10 years since first exposure to ethylene oxide. Among the men who had held licences only for ethylene oxide, there were 27 deaths (30.1 expected), 15 from cancer (10.5 expected), including one from stomach cancer (1.3 expected), three from lymphosarcoma and reticulosarcoma (0.2 expected) and two from leukaemia (0.3 expected). Results obtained with national mortality rates as the basis for expected numbers were similar. [The Working Group noted that no data were available on levels of exposure to ethylene oxide or on exposure to other chemicals.]

Epidemiological findings on ethylene oxide are summarized in Table 10.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

Rat: Groups of 50 female Sprague-Dawley rats, about 100 days old, were administered ethylene oxide (purity, 99.7%) at 7.5 or 30 mg/kg bw in a commercial vegetable oil [composition unspecified] by gastric intubation twice weekly for 107 weeks (average total dose, 1186 or 5112 mg/kg bw, respectively). Control groups consisted of 50 untreated female rats and 50 female rats treated with vegetable oil alone. The survival rate of rats in the high-dose group was lower than that of the control groups. Treatment with ethylene oxide resulted in a dose-dependent increase in the incidence of forestomach tumours, which were mainly squamous-cell carcinomas. Such tumours were not found in the untreated or vehicle controls. In total, 31/50 treated animals developed malignant tumours of the stomach; 29 were squamous-cell carcinomas of the forestomach and two were fibrosarcomas, one of which was located in the glandular stomach. In addition, 4/50 had carcinomas *in situ* and 11/50 had papillomas, hyperplasia or hyperkeratosis of the squamous epithelium of the forestomach. In the low-dose group, 8/50 animals developed squamous-cell carcinomas, four had carcinomas *in situ* and nine had papillomas, hyperplasia or hyperkeratosis in the forestomach. Of the 37 squamous-cell carcinomas found in the two dose groups, 10 metastasized or grew invasively into neighbouring organs. There was no increase in the incidence of tumours at other sites in the treated animals over that in controls (Dunkelberg, 1982).

Table 10. Summary of epidemiological findings on ethylene oxide

Reference (country)	Type of plant; study period; number of subjects; minimal period employed; follow-up	No. of deaths	No. of cancers	RR	95% CI	Site	Comments
Hogstedt <i>et al.</i> (1986); Hogstedt (1988) (Sweden)	Production of sterilized supplies; 1978-82; 203 subjects; 1 year; 100% follow-up	5	4 2	[2.5] [15]	[0.68-6.4] [1.9-56]	All neoplasms L&H	Estimated average past exposure in storage room was 20 ppm; one leukaemia was part of a cluster which had originally prompted the study.
Hogstedt <i>et al.</i> (1979a, 1986); Hogstedt (1988) (Sweden)	Ethylene oxide production plant (one facility); 1961-85; 241 subjects, of which 89 'full-time operators'; 1 year; 100% follow-up	34	14 5 2	[2.3] [8.3] [10]	[1.3-4.8] [2.9-21] [1.2-36]	All neoplasms Stomach Leukaemia	Estimated average exposure before 1963, 5-25 ppm; mortality rates shown only for 'full-time operators' (high-exposure group); no overall excess tumour mortality among workers with intermittent exposure or those unexposed; excess mortality from stomach cancer (4 deaths, SMR, 6.67) and from leukaemia (1 death; 0.2 expected) among workers with intermittent exposure
Hogstedt <i>et al.</i> (1986) (Sweden)	Ethylene oxide production (one plant); 1964-81; 355 subjects; 1 year; 100% follow-up	8	1 ^a	-	-	Leukaemia	The one case of leukaemia (0.16 expected) was in a maintenance worker with multiple exposures; average exposure in 1963-76, 1-8 ppm; after 1977, 0.4-2 ppm
Morgan <i>et al.</i> (1981) (USA)	Production of ethylene oxide; 1955-77; 767 men; 5 years; around 95% follow-up	46	11 2 0	0.72 5.7 0	0.36-1.3 0.64-21 0-5.2	All neoplasms Hodgkin's disease Leukaemia	High percentage of deaths of unknown cause (9%); limited information on manufacturing processes and exposure concentrations; exposures probably below 10 ppm with occasional peaks to 6000 ppm; nonsignificant excess risks from cancer of the pancreas and cancers of the central nervous system
Divine (unpublished); reported by Shore <i>et al.</i> (1993) (USA)	Updating of Morgan <i>et al.</i> (1981); 1955-85; 99.7% follow-up	Not applicable	3 0	[1.0] [0]	[0.21-2.9] [0.0-3.4]	Hodgkin's disease Leukaemia	
Kiesselbach <i>et al.</i> (1990) (Germany)	Chemical plants (8 facilities); 1928-82; 2658 men; 1 year; 97.6% follow-up	268	68 14 5 2	0.97 1.4 1.0 0.85	0.76-1.2 0.75-2.3 0.32-2.3 0.10-3.1	All neoplasms Stomach L&H Leukaemias	No information on exposure concentrations or on nature of production processes; most of study population of Thiess <i>et al.</i> included.

Table 10 (contd)

Reference (country)	Type of plant; study period; number of subjects; minimal period employed; follow-up	No. of deaths	No. of cancers	RR	95% CI	Site	Comments
Gardner <i>et al.</i> (1989) (UK)	Production or use of ethylene oxide (4 facilities); 1956-87; 1471 subjects; no minimal employment; around 98%	157	53 3 3 2	1.1 0.7 2.3 [1.9]	[0.85-1.5] [0.15-2.1] [0.47-6.6] [0.23-7.0]	All neoplasms Stomach Leukaemia Non-Hodgkin's lymphoma	Average exposure after 1977 was to less than 5 ppm (< 1 ppm in many jobs), with occasional peak exposures of several hundred ppm; highest mortality from leukaemia among subjects with definite exposure to ethylene oxide; risk increased with latency of exposure; non-significant excess risks for cancers of the oesophagus, lung and bladder.
	Hospital sterilization units (8 hospitals); 1964-87; 1405 subjects; no minimal exposure; around 98%	69	32 2 0 2	1.1 1.2 0 [3.5]	0.73-1.5 0.15-4.3 0-4.9 [0.42-13]	All neoplasms Stomach Leukemia Non-Hodgkin's lymphoma	
Benson & Teta (1993) (USA)	Work in a chlorohydrin unit and potential exposure to ethylene oxide (2 facilities); 1940-88; 278 men; no minimal employment; 98%	147	40 1 8 4	1.3 [0.7] 2.9 [3.5]	0.93-1.8 0.02-3.9 1.3-5.8 0.96-8.9	All neoplasms Stomach L&H Leukaemia	Updating of study by Greenberg <i>et al.</i> (1990), including only workers ever employed in the chlorohydrin department; excess of pancreatic cancer (8 deaths, SMR, 4.9; 95% CI, 1.6-11).
Teta <i>et al.</i> (1993) (USA)	Production or use of ethylene oxide (2 facilities); 1940-88; 1896 men; no minimal employment; 99%	431	110	0.86	0.71-1.0	All neoplasms	Average exposure in production departments < 1 ppm, but occasionally up to 66 ppm 8-h TWA. Updating of study by Greenberg <i>et al.</i> (1990), excluding workers ever employed in the chlorohydrin department; in an internal comparison with workers in the same complex, a two- to three-fold increase in leukaemia risk was observed for workers exposed for more than 10 years to ethylene oxide.
			8	1.6	0.69-3.2	Stomach	
			7	0.59	0.24-1.2	L&H	
Steenland <i>et al.</i> (1991); Stayner <i>et al.</i> (1993) (USA)	Production of sterilized medical supplies and spices (14 facilities); 1943-87; 18 254 subjects; 3 months; 95.5%	1117	343	0.90	0.81-1.0	All neoplasms	Recent average exposure of sterilizer operators was 4.3 ppm, that of other workers was 2.0 ppm; no significant trend in mortality from L&H with duration of exposure; mortality from L&H increased with latency (SMR at ≥ 20 years since first exposure, 1.8 [95% CI, 0.94-3.0]); test for linear trend, $p = 0.03$; increased risk for L&H with cumulative exposure (for results by cumulative exposure, see Table 9); mortality from kidney cancer was also elevated (SMR, 1.8, 13 deaths) and increased with latency
			11	0.95	0.45-1.7	Stomach	
			36	1.06	0.75-1.5	L&H	
			13	0.97	0.52-1.7	Leukaemia	
		[16]	[1.3]	[0.76-2.2]	[Non-Hodgkin's lymphoma; ICD9 200, 202]		

Table 10 (contd)

Reference (country)	Type of plant; study period; number of subjects; minimal period employed; follow-up	No. of deaths	No. of cancers	RR	95% CI	Site	Comments
Hagmar <i>et al.</i> (1991) (Sweden)	Production of disposable medical equipment (2 facilities); 1964-86; 2170 subjects; 1 year; 98.2%	15	21 ^a 3 0	0.78 1.5 0	0.49-1.2 0.32-4.5 0-7.4	All neoplasms L&H Stomach	Average estimated exposure of sterilizers, around 40 ppm in 1970-72, less than 1 ppm in 1985; packers, around 35-40 ppm in 1970-72, less than 0.2 after 1985; no trend in risk with increasing cumulative exposure but only 0.2 expected cases of L&H in 'high' exposure group (> 1 ppm-year).
Bisanti <i>et al.</i> (1993) (Italy)	Workers licenced to handle ethylene oxide; 1940-84; 1971 men; 1 year with licence; 99.2%	76	43 6 2 4 5	1.3 2.5 1.9 6.8 1.2	0.94-1.8 0.91-5.5 0.23-7.0 1.9-17 0.40-2.9	All neoplasms L&H Leukaemias Lympho- and reticulosarcoma Stomach	Increased mortality from all types of cancer; no increase in risk for L&H with latency or duration of exposure; risk for L&H highest among workers licenced only for ethylene oxide (5 deaths; SMR, 7.0; 95% CI, 2.3-16); no information on exposure levels

RR, risk estimate: standardized mortality ratio, SMR, unless otherwise specified; CI, confidence interval; L&H, neoplasms of the lymphatic and haematopoietic tissues

^aCancer cases, standardized incidence ratio

3.2 Inhalation

3.2.1 Mouse

Groups of 50 male and 50 female B6C3F1 mice, eight weeks of age, were exposed by inhalation to 0, 50 or 100 ppm (0, 92 or 183 mg/m³) ethylene oxide (> 99% pure) for 6 h per day on five days per week for up to 102 weeks, at which time the experiment was terminated. Mean body weights of treated males and females were similar to those of controls. At the end of the study, 28/50 control males, 31/50 low-dose males and 34/50 high-dose males, and 25/50 control females, 24/50 low-dose females and 31/50 high-dose females were still alive. The incidences of alveolar/bronchiolar carcinomas in male mice were 6/50 control, 10/50 low-dose and 16/50 high-dose ($p = 0.017$, incidental tumour test for trend). A slight increase in the incidence of alveolar/bronchiolar adenomas also occurred. The combined incidences of lung tumours were 11/50 control, 19/50 low-dose and 26/50 high-dose ($p = 0.002$, incidental tumour test for trend). In females, the incidences of alveolar/bronchiolar adenomas (2/49 control, 4/48 low-dose and 17/49 high-dose) and alveolar/bronchiolar carcinomas (0/49 control, 1/48 low-dose and 7/49 high-dose) and the combined incidence of lung tumours (2/49 control, 5/48 low-dose and 22/49 high-dose) were all significantly increased ($p < 0.001$, incidental tumour test for trend). The incidence of papillary cystadenoma of the Harderian gland increased significantly in animals of each sex (males: 1/43 control, 9/44 low-dose and 8/42 high-dose; females: 1/46 control, 6/46 low-dose and 8/47 high-dose; $p < 0.05$, incidental tumour test for trend). In addition, one papillary cystadenocarcinoma of the Harderian gland was observed in a high-dose male mouse and one in a low-dose female mouse. In females, the incidences of malignant lymphomas were 9/49 control, 6/48 low-dose and 22/49 high-dose ($p = 0.023$, life-table test for trend). An increase in the incidence of uterine adenocarcinomas was observed: 0/49 control, 1/47 low-dose and 5/49 high-dose ($p = 0.019$, incidental tumour test for trend). In females, the incidences of mammary gland carcinomas were 1/49 control, 8/48 low-dose ($p = 0.012$, incidental pair-wise tumour test) and 6/49 high-dose ($p = 0.087$, incidental pair-wise tumour test) (US National Toxicology Program, 1987).

In a screening assay based on increased multiplicity and incidence of lung tumours in a strain of mice highly susceptible to development of this neoplasm, groups of 30 female strain A/J mice, eight to ten weeks of age, were exposed by inhalation to ethylene oxide (at least 99.7% pure) at 0, 70 or 200 ppm (0, 128 or 366 mg/m³) for 6 h per day on five days per week for up to six months in two independent experiments; in the second experiment, the 70 ppm group was omitted. Two groups of 30 female mice were exposed to room air and served as negative controls, and two groups of 20 animals received a single intraperitoneal injection of urethane (1000 mg/kg bw) and served as positive controls for both experiments. At the end of the sixth month, the survivors were killed and examined for pulmonary adenomas. In the first experiment, survival was 30/30 (0 ppm), 28/30 (70 ppm), 29/30 (200 ppm) and 19/20 (urethane); that in the second was 29/30 (0 ppm), 28/30 (200 ppm) and 19/20 (urethane). The numbers of animals with pulmonary adenomas among survivors in the first experiment were: untreated controls, 8/30 (0.46 ± 0.38 adenomas/mouse); low-dose, 16/28 (0.86 ± 0.45); high-dose, 25/29 (2.14 ± 0.49); and urethane-treated, 19/19 (20.1 ± 1.77); the numbers in the second experiment were: untreated controls, 8/29 (0.22 ± 0.38); ethylene oxide-treated,

12/28 (0.73 ± 0.98); and urethane-treated, 19/19 (23.5 ± 6.49). In the first experiment, the number of lung tumour-bearing animals increased significantly in a dose-dependent manner [$p < 0.0001$ Cochran-Armitage trend test]; in the second, a slight, nonsignificant increase was observed, although the high dose was the same as that used in the first experiment. The number of tumours per surviving mouse increased significantly in each experiment ($p < 0.05$, Duncan's new multiple-range test) (Adkins *et al.*, 1986).

3.2.2 Rat

Groups of 120 male and 120 female Fischer 344 rats, eight weeks of age, were exposed by inhalation to ethylene oxide (purity, $> 99.9\%$) vapour at 10, 33 or 100 ppm (18, 59 or 180 mg/m^3) for 6 h per day on five days per week for two years. Two control groups, each of 120 male and 120 female rats, were exposed in inhalation chambers to room air. All animals that died or were killed when moribund and those killed at scheduled intervals of 6, 12, 18 and 24–25 months were examined. During month 15 of exposure, mortality increased in both treated and control groups due to a viral sialodacryoadenitis. Mortality was higher in the groups inhaling 33 and 100 ppm ethylene oxide than in the other groups and was more frequent in females than in males near the fifteenth month. Up to 18 months of exposure, no significant increase in tumour incidence was observed. In treated rats killed after 18 months, the incidence of tumours in the brain classified as 'gliomas, malignant reticulosis and granular-cell tumours' was increased for animals of each sex. The incidences of glioma among rats killed at 18 and 24–25 months were: males: 1/181 (controls), 0/92 (10 ppm), 3/86 (33 ppm) and 6/87 (100 ppm) ($p < 0.05$, trend analysis and Fisher's exact test for high dose *versus* control); and females: 0/187 (controls), 1/94 (10 ppm), 2/90 (33 ppm) and 2/78 (100 ppm) ($p < 0.05$, trend analysis). In females killed after 24 months of exposure, mononuclear-cell leukaemia was found in 5/60 (control I), 6/56 (control II), 11/54 (10 ppm), 14/48 (33 ppm) and 15/26 (100 ppm) animals; the incidence of leukaemia was reported by the authors to be significantly increased in the 100-ppm group ($p < 0.001$) and in a mortality-adjusted trend test ($p < 0.005$). In males, mononuclear-cell leukaemia was found in 5/48 (control I), 8/49 (control II), 9/51 (10 ppm), 12/39 (33 ppm) and 9/30 (100 ppm) animals ($p < 0.05$ in a mortality-adjusted trend test). Peritoneal mesotheliomas originating in the testicular serosa were found in 1/48 (control I), 1/49 (control II), 2/51 (10 ppm), 4/39 (33 ppm) and 4/30 (100 ppm) males ($p < 0.005$ trend test). The incidence of subcutaneous fibromas in male rats of the high-dose group was also significantly increased: 1/48 (control I), 2/49 (control II), 9/51 (10 ppm), 1/39 (33 ppm) and 11/30 (100 ppm) ($p < 0.001$) (Snellings *et al.*, 1984a; Garman *et al.*, 1985, 1986).

Groups of 80 male weanling Fischer 344 rats were exposed by inhalation to ethylene oxide (purity, 99.7%) vapour at 0 (control; filtered air), 50 or 100 ppm (92 or 180 mg/m^3) for approximately 7 h per day on five days per week for two years. The mortality rate was increased in the two treated groups over that in controls, and the increase was significant for the high-dose group ($p < 0.01$). Mononuclear-cell leukaemia was observed in 24/77 control rats, 38/79 exposed to 50 ppm ethylene oxide and 30/76 exposed to 100 ppm. The overall increase in the incidence of mononuclear-cell leukaemia was significant ($p = 0.03$) in the low-dose group, but the increase could not be ascertained in the high-dose group owing to excessive mortality. Peritoneal mesotheliomas in the region of the testis developed in 3/78

control, 9/79 low-dose and 21/79 high-dose rats; the increase was significant for the high-dose group ($p = 0.002$). Gliomas were found in 0/76 control, 2/77 low-dose and 5/79 high-dose animals ($p < 0.05$, pair-wise comparison for the high dose). Focal proliferation of glial cells, termed 'gliosis', was observed in two rats exposed to 50 ppm and in four rats exposed to 100 ppm ethylene oxide. The incidences of other neoplasms were comparable in the control and treated groups and were not associated with exposure to ethylene oxide. A high incidence of proliferative lesions described as 'multifocal cortical hyperplasia' and 'cortical nodular hyperplasia' was observed in the adrenal cortex of animals exposed to ethylene oxide (Lynch *et al.*, 1984a).

3.3 Skin application

Mouse: Thirty female ICR/Ha Swiss mice, eight weeks of age at the start of treatment, were painted with about 100 mg of a 10% solution of ethylene oxide (purity, 99.7%) in acetone per application on the clipped dorsal skin three times per week for life. The median survival time was 493 days. No skin tumour was observed (Van Duuren *et al.*, 1965).

3.4 Subcutaneous administration

Mouse: Groups of 100 female NMRI mice, six to eight weeks old, received subcutaneous injections of ethylene oxide (purity, 99.7%) in tricapylin at 0.1, 0.3 or 1.0 mg/mouse once per week for 95 weeks (mean total dose, 7.3, 22.7 and 64.4 mg/mouse). Groups of 200 untreated and 200 tricapylin-treated mice served as controls. The survival rate of the group given the highest dose was reduced. Ethylene oxide induced a dose-dependent increase in the incidence of tumours at the injection site: 0/200 untreated controls, 4/200 animals treated with tricapylin alone, and 5/100 (0.1 mg), 8/100 (0.3 mg) and 11/100 (1 mg) ethylene oxide-treated animals [$p < 0.001$, Cochran-Armitage test for trend]. No significant increase in the incidence of tumours at other sites was observed (Dunkelberg, 1981).

3.5 Induction of enzyme-altered foci in a two-stage liver system

Rat: Groups of male and female Sprague-Dawley rats, three to five days of age, were exposed by inhalation to ethylene oxide [purity unspecified] at 0 ppm (5 male and 9 female rats), 33 ppm (60 mg/m³, 10 females), 55 ppm (100 mg/m³, 4 males and 7 females) or 100 ppm (183 mg/m³, 4 males and 8 females) for 8 h per day on five days per week for three weeks. One week later, the rats were administered 10 mg/kg bw Clophen A 50 (a mixture of polychlorinated biphenyls [not otherwise specified]) orally by gavage twice a week for up to eight additional weeks (promotion), at which time the experiment was terminated. The livers were examined for ATPase-deficient and γ -glutamyltranspeptidase (GGT)-positive foci. In females receiving the two highest doses, but not in males, the number and total area of ATPase-deficient foci increased significantly ($p < 0.05$, t test) in comparison with the controls receiving Clophen A 50 only. There was no significant difference between controls and animals given the high dose of ethylene oxide in the number or total area of GGT-positive foci (Denk *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

(a) Ethylene oxide

Ethylene oxide is readily taken up by the lungs. A study on workers exposed to ethylene oxide revealed an alveolar retention of 75–80%, calculated from hourly determinations of ethylene oxide concentrations in environmental air ranging from 0.2 to 22.5 mg/m³ [0.11–12.3 ppm] and in alveolar air from 0.05 to 7 mg/m³ [0.03–3.8 ppm] (Brugnone *et al.*, 1985, 1986). At steady state, therefore, 20–25% of inhaled ethylene oxide reaching the alveolar space is exhaled as unchanged compound and 75–80% is taken up by the body and metabolized. Blood samples taken from workers 4 h after the work shift and later gave venous blood:alveolar air coefficients of 12–17 and venous blood:environmental air coefficients of 2.5–3.3. The difference from the value of 90 determined for the blood:air partition coefficient *in vitro* was explained by incomplete saturation of tissues and limitation of the metabolic rate by the lung uptake rate (Brugnone *et al.*, 1986).

The data set of Brugnone *et al.* (1986) was used by two groups to estimate the elimination rate constant for ethylene oxide. A value of 3/h, corresponding to a 14-min half-life of ethylene oxide in the body, was calculated by Osterman-Golkar and Bergmark (1988) on the basis of an alveolar retention of 80%, a venous blood:environmental air coefficient of 3.3 (see above) and the following assumptions: (i) steady-state conditions; (ii) elimination according to first-order kinetics; (iii) equal distribution of ethylene oxide within the body; and (iv) alveolar ventilation of 0.2 L/min per kg bw. Filser *et al.* (1992) calculated a 42-min half-life of ethylene oxide in the body, corresponding to an elimination rate constant of 1/h, by fitting an exponential function to the time dependence of the mean alveolar air:environmental air ratio given for ethylene oxide by Brugnone *et al.* (1986). The procedure chosen by Filser *et al.* (1992) relied on two assumptions: (i) steady-state conditions; and (ii) elimination according to first-order kinetics.

Using data obtained by Filser and Bolt (1984) in studies of rats, the half-life of ethylene oxide has been calculated as 3.3 h (Beliles & Parker, 1987) and 39 min (Filser *et al.*, 1992) on the basis of allometric scaling with body surface factors (two-thirds body weight). The difference is due to the scaling methods: the lower value was calculated on the basis of the scaled elimination clearance (Filser, 1992), and the higher value was scaled directly from the half-life. Using the latter method for data obtained in studies on dogs (Martis *et al.*, 1982), Beliles and Parker (1987) estimated a half-life of 2.4 h.

Pharmacokinetic data obtained in animals have been used to calculate the internal dose of ethylene oxide in man derived from daily exposure. For a man exposed for 8 h to ethylene oxide at an air concentration of 1.8 µg/L [1 ppm], the area under the concentration–time curve in blood plasma was estimated to be 18.8 µg×h/ml on the basis of data for rats and 14.3 µg×h/ml on the basis of data for dogs (Beliles & Parker, 1987).

The pharmacokinetics of ethylene oxide as a metabolite of ethylene are summarized in the monograph on ethylene.

(b) *Metabolites*

Ethylene oxide is eliminated metabolically by hydrolysis and by conjugation with glutathione. Blood concentrations of ethylene glycol were determined at the end of day 3 of a normal working week in sterilization personnel exposed to ethylene oxide. TWA concentrations determined over 8 h ranged from 0.3 to 52 ppm [0.55–95.2 mg/m³] (overall mean, 4.2 ppm [7.7 mg/m³]). The mean concentrations of ethylene glycol in blood of exposed subjects were twice as high (90 mg/L) as those in unexposed ones (45 mg/L) (Wolfs *et al.*, 1983).

The concentration of thioethers excreted in urine collected at the end of sterilization processes was found to be twice as high in nonsmoking personnel (10.2 mmol/mol creatinine) exposed to peak concentrations of 1–200 ppm ethylene oxide [1.83–366 mg/m³] as in unexposed workers (5.46 mmol/mol creatinine). The concentration of ethylene oxide in air was not monitored routinely (Burgaz *et al.*, 1992).

The disappearance of ethylene oxide was investigated in the gas phase of closed vials containing glutathione and cytoplasm of erythrocytes obtained from a study population ($n = 36$ [not further specified]). Ethylene oxide was eliminated three to six times faster in samples from the three-quarters of the population who were so-called conjugators (defined by a standardized conjugation of methyl bromide and glutathione) than in those from the remaining quarter, in whom disappearance did not differ from that of controls (Hallier *et al.*, 1993).

(c) *Binding to haemoglobin and DNA*

Ethylene oxide, an electrophilic agent, alkylates nucleophilic groups in biological macromolecules. Haemoglobin (Hb) adducts have been used to monitor tissue doses of ethylene oxide (Calleman *et al.*, 1978; Farmer *et al.*, 1987; Osterman-Golkar, 1988; Ehrenberg, 1991; Ehrenberg & Törnqvist, 1992). Sensitive methods involving gas chromatography–mass spectrometry, gas chromatography–electron capture detection and radioimmunoassay have been developed for the determination of *N*-2(hydroxyethyl) (HOEt) adducts at histidine-N^T (HOEtHis) and at N-terminal valine (HOEtVal) in Hb of humans occupationally exposed to ethylene oxide (Farmer *et al.*, 1986; Mowrer *et al.*, 1986; Törnqvist *et al.*, 1986; Wraith *et al.*, 1988; Kautiainen & Törnqvist, 1991; Törnqvist *et al.*, 1992).

HOEtHis levels were investigated in workers engaged for 1–14 years in ethylene oxide manufacture. Concentrations measured between 1974 and 1981 were generally below the detection limit of 0.05 ppm [0.09 mg/m³], with occasional transient concentrations of up to 8 ppm [14.6 mg/m³]. Without taking smoking habits into consideration, van Sittert *et al.* (1985) determined a mean HOEtHis level of 2080 pmol/g globin, which did not differ significantly from the level of 1590 pmol/g globin found in unexposed controls (van Sittert *et al.*, 1985).

Higher concentrations of HOEtVal adducts were measured in workers occupationally exposed to ethylene oxide than in controls, and workers in loading operations had higher concentrations than those in manufacture (van Sittert & van Vliet, 1994).

In workers exposed to low, intermediate and high concentrations of ethylene oxide [not further specified], the HOEtHis levels (pmol/g Hb) were 550–1000, 2000 and 5300, and 2000 and 8000, while the HOEtVal levels (pmol/g Hb) were 20–410, 980 and 4600, and 1500 and

7700. Hb adduct concentrations in controls were 530–1600 pmol/g Hb for HOEtHis and 30–930 pmol/g Hb for HOEtVal. A linear correlation was found between HOEtHis and HOEtVal, with a slope of 1; HOEtHis levels were on average 600 pmol/g Hb higher than the corresponding HOEtVal levels (Farmer *et al.*, 1986; Bailey *et al.*, 1987). The reason for the high background level of HOEtHis is unknown. Two speculations seem reasonable: trace amounts of HOEtHis might be formed during the analytical procedure; alternatively, during protein synthesis, HOEtHis may be incorporated as such (Farmer *et al.*, 1986).

Workers at one plant who were exposed daily to ethylene oxide at concentrations of $< 8\text{--}312 \text{ ppm}\times\text{h}$ [$14.6\text{--}571 \text{ mg/m}^3\times\text{h}$] had concentrations of HOEtHis ranging from 400 to 14 300 pmol/g Hb. In two other plants, where daily exposures were estimated to be $8\text{--}16 \text{ ppm}\times\text{h}$ [$14.6\text{--}29.2 \text{ mg/m}^3\times\text{h}$], HOEtVal concentrations were 700 to about 10 000 pmol/g Hb. These data were used to estimate the first-order rate constant of ethylene oxide elimination from the organism. Taking into consideration the life-time of erythrocytes, the reaction constant of ethylene oxide with histidine-N^τ in Hb and the HOEtHis concentrations, the elimination rate constant was calculated to be $< 2.6\text{--}54/\text{h}$ [corresponding half-lives, > 16 and 0.8 min]. Values obtained using the HOEtVal concentrations and the reaction constant of ethylene oxide with the N-terminal valine in Hb were $\sim 1\text{--}\leq 8.8/\text{h}$ [corresponding half-lives, ~ 42 and ≥ 4.7 min] (Osterman-Golkar & Bergmark, 1988).

Background levels of HOEtVal in nonsmokers not exposed to ethylene oxide have been reported to be 11–188 pmol/g Hb (see monograph on ethylene, p. 54). Högstedt *et al.* (1990) investigated HOEtVal levels in two groups of workers in the same factory. One group, referred to as sterilizers, was exposed during 8-h shifts to atmospheric ethylene oxide at a concentration of about 2 ppm [3.7 mg/m^3]; the other group assembled electronic equipment about 100 m from the sterilizer. The HOEtVal concentrations were 1900–10 000 pmol/g Hb in the sterilizers and 850–2300 pmol/g Hb in the assemblers; the concentration in a group exposed only to propylene oxide was 20–870 pmol/g Hb. The results did not indicate an influence of smoking.

A cohort of workers exposed to ethylene oxide at 0.02, 0.1 and > 0.2 ppm [$0.037, 0.18$ and $> 0.37 \text{ mg/m}^3$], estimated by personal air monitoring, had HOEtVal concentrations of 50, 230 and 1380 pmol/g Hb. On the basis of the previous finding that HOEtVal is formed at a rate of 2400 pmol/g Hb per ppm of exposure to ethylene oxide during a work shift of 8 h, the measured HOEtVal concentrations were used to estimate the corresponding concentrations of ethylene oxide at the workplace, after correction for smoking habits and for a background level of 20 pmol/g Hb in controls. The predicted values, 0.02, 0.1 and 0.7 ppm, were in agreement with the ranges estimated from personal air monitoring (Hagmar *et al.*, 1991).

HOEtVal concentrations were determined in three groups of nonsmokers exposed occupationally to ethylene oxide. One group was exposed once or twice a week for about 10 min to ethylene oxide at air concentrations ranging from 22 to 72 ppm [$40\text{--}132 \text{ mg/m}^3$]; the other groups were exposed to an average concentration of about 17 ppm [31 mg/m^3] ethylene oxide either daily or occasionally. The mean concentrations of HOEtVal increased from 32 pmol/g Hb in matched controls to 80 pmol/g Hb in subjects in the first group and from 32 pmol/g Hb in matched controls to 13 200 pmol/g Hb in subjects exposed daily and to 2720 pmol/g Hb in subjects exposed occasionally. On the basis of the relationship between

adduct and exposure levels used by Hagmar *et al.* (1991), the 40-h TWA concentration of ethylene oxide was calculated as 0.025 ppm [0.046 mg/m³] at the low dose and as 5 ppm [9.2 mg/m³] at the high dose. These values were several times lower than those measured in air samples. The use of gas masks was discussed as a possible reason for this discrepancy (Tates *et al.*, 1991a, 1992).

An 8-h TWA exposure concentration of about 0.046 ppm [0.084 mg/m³] was estimated by air monitoring for plant workers exposed to ethylene oxide. The HOEtVal concentration in nonsmokers in the group (about 139 µmol/mol Hb [2160 pmol/g Hb]) differed significantly from that in controls (45 µmol/mol Hb [700 pmol/g Hb]). An 8-h TWA exposure concentration of 0.008 ppm [0.015 mg/m³] was estimated for the control group by personal monitoring (Mayer *et al.*, 1991). The values for HOEtVal are one order of magnitude higher than would have been expected from the relationship between adduct level and ethylene oxide concentration. The discrepancy is due to the use of d₄-hydroxyethylvaline instead of d₄-hydroxyethylated globin as internal standard. A 10-fold lower yield from the free amino acid was noted in a subsequent inter-laboratory comparison of methods (Törnqvist *et al.*, 1992).

At nine US and one Mexican hospital, workers exposed to ethylene oxide were divided according to four-month cumulative exposures of > 0–32 ppm×h [> 0–59 mg/m³×h] and > 32 ppm×h [> 59 mg/m³×h]. The mean exposures were estimated to be 12.8 and 105.2 ppm×h [23.4 and 193 mg/m³×h] in the US hospitals and 10.5 and 349.1 ppm×h [19.2 and 639 mg/m³×h] in the Mexican hospital. The corresponding 8-h TWA concentrations, weighted by duration of each job task, were estimated to be 0.04 and 0.16 ppm [0.07 and 0.29 mg/m³] and 0.02 and 0.54 ppm [0.037 and 1 mg/m³]. After adjustment for confounding factors, including smoking habits, the mean concentrations of HOEtVal were determined by radioimmunoassay to be 90 and 160 pmol/g Hb in the USA and 60 and 160 pmol/g Hb in the Mexican workers. In a US hospital control group, the mean background level of HOEtVal was found to be 60 pmol/g Hb; the level in one Mexican worker not exposed to ethylene oxide was 140 pmol/g Hb. A significant correlation was seen between cumulative dose of ethylene oxide and HOEtVal concentration in both groups of workers (Schulte *et al.*, 1992).

In a study of workers at an ethylene oxide producing plant, concentrations of HOEtVal were determined during three successive annual health assessments (van Sittert *et al.*, 1993). The median increments in HOEtVal concentration were determined by radioimmunoassay and gas chromatography–mass spectrometry to be 145, 238 and 53 pmol/g Hb. Using the relationship between adduct and exposure levels of Hagmar *et al.* (1991), the median four-month 8-h TWA concentrations of ethylene oxide were estimated to be 0.056, 0.1 and 0.02 ppm [0.1, 0.18 and 0.037 mg/m³]. These results are consistent with measurements made during normal plant operations which showed 8-h airborne ethylene oxide concentrations to be below 0.5 ppm. The authors concluded that determination of HOEtVal in Hb is a sensitive method for monitoring low, time-integrated levels of ethylene oxide.

In the population of 36 subjects, of whom 27 were characterized as ‘conjugators’ and nine as ‘non-conjugators’ (see p. 105) in terms of the enzymic conjugation of ethylene oxide with glutathione in erythrocytes (Hallier *et al.*, 1993), blood was taken from three individuals in each group and incubated at 37 °C over 4 h with ¹⁴C-ethylene oxide. Radioactivity bound to blood plasma and erythrocytes was determined in the low-relative-molecular-mass

fractions (< 10 kDa), containing glutathione, and in the high-relative-molecular-mass fractions (> 10 kDa), containing proteins, such as albumin and Hb. Counts in blood from conjugators were significantly higher in both low-relative-molecular-mass fractions and significantly lower in the high-relative-molecular-mass fraction of erythrocytes than in blood from non-conjugators. No significant difference between conjugators and non-conjugators was seen in the amount of radioactivity associated with the high-relative-molecular-mass fraction of blood plasma. Radioactivity counts in lymphocytes, ascribed to DNA adducts of ^{14}C -ethylene oxide, were similar in the two groups (Föst *et al.*, 1991; Gansewendt *et al.*, 1991).

4.1.2 Experimental systems

(a) Ethylene oxide

The permeation rate of a solution of 1% ethylene oxide in water (w/v) through excised human skin at 30 °C was determined to be 0.125 mg/(cm²×h) (Baumbach *et al.*, 1987).

The pharmacokinetics of inhaled ethylene oxide have been investigated in male Sprague-Dawley (Filser & Bolt, 1984) and Fischer 344 rats (Krishnan *et al.*, 1992). The studies were carried out in closed exposure chambers of 6.4 and 9.5 L occupied by two and three rats, respectively. The initial concentrations of ethylene oxide vapour in the chamber atmospheres were up to about 1100 ppm [2000 mg/m³]. Filser and Bolt (1984) showed that ethylene oxide is rapidly taken up by the lungs, as the clearance due to uptake, reflecting the rate of transfer of ethylene oxide from the atmosphere into the organism, was 11 100 ml/h (185 ml/min) for two Sprague-Dawley rats of 500 g bw. Johanson and Filser (1992) calculated a value of 58 ml/min for one animal of 250 g bw by allometric scaling, according to the method of Filser (1992). This value represents 50% of the alveolar ventilation (117 ml/min; Arms & Travis, 1988), indicating that about 50% of the amount inhaled into the lung is exhaled again without becoming systemically available via the bloodstream. A possible explanation for this finding is that there is a 'wash in-wash out' effect in the upper airways (Johanson & Filser, 1992), which may be more effective in rodents than in humans (Filser *et al.*, 1993). The maximal accumulation of ethylene oxide in the body of Sprague-Dawley rats, determined as the thermodynamic partition coefficient whole body:air, was 30. Owing to fast metabolic elimination, the concentration ratio at steady-state whole body:air, calculated for two animals of 500 g bw, was only 1.52 over the entire dose range. A recalculation of this parameter according to Filser (1992) for one rat weighing 250 g bw yielded a value of 1.88, which is similar to the coefficient for venous blood:environmental air found in workers exposed to ethylene oxide under steady-state conditions (see above).

An almost uniform distribution of ethylene oxide within the body was concluded from the similar tissue:air partition coefficients for organs of male Fischer 344 rats determined *in vitro*: fat, 44.1; muscle, 48.3; brain, 58.7; lung, 60.9; liver, 61.6; blood, 64.1; testes, 83 (Krishnan *et al.*, 1992).

Elimination of ethylene oxide was described by first-order kinetics over the whole concentration range examined, in both Sprague-Dawley (Filser & Bolt, 1984) and Fischer 344 rats (Krishnan *et al.*, 1992). At steady state, the clearance due to metabolism in relation to the concentration in the atmosphere (Cl_{tot} of Filser & Bolt, 1984) was 10 600 ml/h (177 ml/min) for two Sprague-Dawley rats weighing 500 g bw. Recalculation for one rat of

250 g bw according to the method of Filser (1992) gives a value of almost 55 ml/min. This indicates that at steady state about 95% of systemic ethylene oxide is eliminated unchanged by metabolism and only 5% by exhalation, as calculated on the basis of values for clearance due to uptake and clearance due to metabolism in relation to the concentration in the atmosphere. On the basis of the finding that clearance due to metabolism in relation to the concentration in the atmosphere is nearly identical to clearance due to uptake, uptake of ethylene oxide by inhalation was concluded to be the rate-limiting step for metabolism of this compound. The alveolar retention in one Sprague-Dawley rat of 250 g bw was calculated as 47% on the basis of the ventilation rate of 117 ml/min (see above) and the clearance of metabolism in relation to the concentration in the atmosphere of 55 ml/min. The half-life was reported in two animals weighing 500 g bw to be 6 min (Bolt & Filser, 1987). Recalculation for one Sprague-Dawley rat of 250 g bw according to the method of Filser (1992) gives a similar value.

In male Fischer 344/N rats exposed by nose only for 60 min to 5 ppm [9.2 mg/m³] ethylene oxide, a steady-state blood level of about 60 ng/g was reached after 15 min (Maples & Dahl, 1993).

(c) *Metabolites*

After intraperitoneal injection of ethylene oxide labelled uniformly with ¹⁴C (2 mg/kg bw) to male Sprague-Dawley rats, 9% of the radioactivity was excreted in urine as *S*-(2-hydroxyethyl)cysteine and 33% as *N*-acetyl-*S*-(2-hydroxyethyl)cysteine within 18 h; 1.5% of the dose was exhaled as ¹⁴CO₂ and 1% as unchanged ethylene oxide within 6 h (Jones & Wells, 1981).

Exposure of male Sprague-Dawley rats for 6 h by inhalation to ethylene oxide at concentrations of 1–200 ppm [1.83–366 mg/m³] resulted in urinary excretion of *N*-acetyl-*S*-(2-hydroxyethyl)cysteine. The amounts excreted within 24 h correlated linearly with the concentration of ethylene oxide in air; the average amount was 0.27 μmol/ppm [0.15 μmol/mg per m³] for a rat weighing 200 g bw (Gérin & Tardif, 1986). A value of 0.21 μmol/ppm [0.11 μmol/mg per m³] can be calculated from the clearance of metabolism in relation to the concentration in the atmosphere (55 ml/min per 250 g bw = 44 ml/min per 200 g bw) (Filser & Bolt, 1984), a molar gas volume of 25 L, and the finding that 33% is excreted as *N*-acetyl-*S*-(2-hydroxyethyl)cysteine in urine (Jones & Wells, 1981).

After intravenous injection of 1 and 10 mg/kg ethylene oxide to male Sprague-Dawley rats, *N*-acetyl-*S*-(2-hydroxyethyl)cysteine was excreted as a constant percentage of the dose: about 30% from 0 to 12 h and 5% from 12 to 24 h. With 100 mg/kg ethylene oxide, the equivalent percentages were 16% and 5%. These results indicate that at the high dose the capacity for glutathione conjugation could have been exceeded within the first 12 h (Gérin & Tardif, 1986).

Ethylene glycol, 2-hydroxymercapturic acid, 2-methylthioethanol and 2-mercaptoethanol were identified as metabolites in the urine of male Wistar rats exposed for 6 h to ethylene oxide at 500 ppm [915 mg/m³] (Koga *et al.*, 1987). The amounts of ethylene glycol in the urine of male Wistar rats collected during 6-h exposures to ethylene oxide at 50, 100, 200, 300 and 500 ppm [91.5, 183, 366, 549 and 915 mg/m³] and up to 20 h thereafter were 0.2, 0.35, 1.0, 2.5 and 4.2 mg (means read from a figure), thus increasing disproportionately to the

exposure concentrations (Koga *et al.*, 1985). The findings might indicate a relative decrease in glutathione conjugation.

The pattern of excretion of ethylene oxide metabolites in mice, rats and rabbits was investigated in urine collected 24 h after treatment with ethylene oxide, either intravenously (20 and 60 mg/kg) or by inhalation for 6 h (about 200 ppm [366 mg/m³]). Marked species differences were seen (Table 11), as metabolites resulting from conjugation of ethylene oxide with glutathione were found in the urine of male Swiss CD-1 mice and male Sprague-Dawley rats but not in that of rabbits [strain not given]. *N*-Acetyl-*S*-(2-hydroxyethyl)cysteine was excreted in the urine of mice and rats, but *S*-(2-hydroxyethyl)cysteine and *S*-(carboxymethyl)cysteine were present only in the urine of mice. Ethylene glycol, the reaction product of the hydrolytic pathway of ethylene oxide, was found in the urine of animals of all three species (Tardif *et al.*, 1987).

Table 11. Urinary excretion of ethylene oxide metabolites within 24 h after treatment intravenously or by inhalation of mice, rats and rabbits with ethylene oxide

Treatment	Urinary metabolites ($\mu\text{mol}/100 \text{ g bw}$) (mean values)			
	<i>N</i> -Acetyl- <i>S</i> -(2-hydroxyethyl)cysteine	<i>S</i> -(2-Hydroxyethyl)cysteine	<i>S</i> -(Carboxymethyl)cysteine	Ethylene glycol
20 mg/kg intravenously				
Mouse	3.75	2.62	0.85	1.48
Rat	14.00	ND	ND	2.68
Rabbit	ND	ND	ND	0.95
60 mg/kg intravenously				
Mouse	9.53	6.80	4.30	3.55
Rat	32.28	ND	ND	8.59
Rabbit	ND	ND	ND	3.76
200 ppm, 6 h inhalation [366 mg/m ³]				
Mouse	4.63	2.62	2.83	0.77
Rat	19.61	ND	ND	1.84
Rabbit	ND	ND	ND	2.56

Adapted from Tardif *et al.* (1987)

ND, not detected

(c) *Glutathione depletion*

Treatment of animals with ethylene oxide lowered the concentration of glutathione in various tissues. Immediately after a 4-h exposure of male Swiss-Webster mice and male Fischer 344 rats to ethylene oxide at atmospheric concentrations of 100, 400 and 900 ppm [183, 732 and 1647 mg/m³] (mice) and 100, 600 and 1200 ppm [183, 1098 and 2196 mg/m³]

(rats), there were concentration-related decreases in glutathione levels in kidney, heart, lung, brain, stomach, spleen, testis and liver of both species, in blood of mice but not of rats, and in bone marrow which was examined in rats only. In both species, the glutathione levels were reduced more in liver, lung and stomach than in other organs. After exposure to the highest concentrations, glutathione levels in the tissue were depressed to 20–30% of the control values (McKelvey & Zemaitis, 1986).

The concentrations of glutathione in hepatic cytosol of male Wistar rats decreased to 37% of that of controls after a single exposure (4 h) to 500 ppm [915 mg/m³], to 10% after exposure to 1500 ppm [2745 mg/m³] (Katoh *et al.*, 1990), to 10% after exposure to 1300 ppm [2379 mg/m³] (Katoh *et al.*, 1991) and to 5% after exposure to 2500 ppm [4575 mg/m³] (Nakashima *et al.*, 1987). Immediately after the last of a series of repeated exposures of male Wistar rats (6 h/day, three days per week, six weeks) to 500 ppm [915 mg/m³] ethylene oxide, the hepatic glutathione concentration was diminished by 50%. Control values were reached again 12 h thereafter (Katoh, T. *et al.*, 1989).

(d) *Binding to haemoglobin and DNA*

Binding of ethylene oxide to Hb and DNA has been reviewed (European Chemical Industry Ecology and Toxicology Centre, 1989; Walker *et al.*, 1990; Uziel *et al.*, 1992).

¹⁴C-Ethylene oxide was reacted *in vitro* (30 min, 37 °C, pH 7.4) with Hb in washed erythrocytes obtained from CBA mice, Fischer rats and humans. The second-order rate constants (Table 12) were about the same for N²-valine, N^π-histidine and N^τ-histidine and did not differ between the three species; however, large species differences were seen with respect to S-cysteine.

Table 12. Second-order rate constants for in-vitro binding of ethylene oxide to S-cysteine, N²-valine, N^π-histidine and N^τ-histidine in human, mouse and rat haemoglobin

Species	Rate constant [L/(g Hb) per h] (mean values)			
	S-Cysteine	N ² -Valine	N ^π -Histidine	N ^τ -Histidine
Man	0.06 × 10 ⁻⁴	0.45 × 10 ⁻⁴	0.38 × 10 ⁻⁴	0.37 × 10 ⁻⁴
Mouse	0.70 × 10 ⁻⁴	0.32 × 10 ⁻⁴	0.37 × 10 ⁻⁴	0.21 × 10 ⁻⁴
Rat	10 × 10 ⁻⁴	0.46 × 10 ⁻⁴	0.62 × 10 ⁻⁴	0.27 × 10 ⁻⁴

From Segerbäck (1990)

After male Sprague-Dawley rats had been exposed for several hours to a constant concentration of ethylene oxide in air, a correlation was seen between estimated dose taken up and 7-(2-hydroxyethyl)guanine (7-HOEtGua) in hepatic DNA. In DNA extracted from blood of untreated rats, the mean background level of HOEtGua was 5600 pmol/g DNA (Föst *et al.*, 1989).

Comparative studies were performed in male B6C3F1 mice and male Fischer 344 rats in order to investigate the applicability of Hb adducts for monitoring DNA adducts in various tissues. Rats were killed after a 6-h exposure by nose only to atmospheric ¹⁴C-ethylene oxide

at concentrations of 1, 10 and 33 ppm [1.83, 18.3 and 60.4 mg/m³], and hydroxyethyl adducts were determined at S-cysteine, N-terminal valine and N^π- and N^τ-histidine of Hb and at 7-guanine of DNA from brain, lung, liver, spleen, kidney or testis. Linear relationships were seen between formation of hydroxyethyl adducts in both Hb and DNA and the exposure concentration (Table 13). The mean ratios of the hydroxyethyl adducts to S-cysteine : N-terminal valine : N^π-histidine : N^τ-histidine were 16 : 1.6 : 1.9 : 1.0. Alkylation frequencies determined in DNA were similar in all tissues studied, except for testis in which they were 60% lower. There was no evidence of saturation kinetics (Potter *et al.*, 1989).

Table 13. Hydroxyethyl adducts to N^π-histidine of Hb and 7-guanine of DNA of rats exposed to atmospheric ethylene oxide for 6 h

Ethylene oxide ppm (mg/m ³)	N ^π -(Hydroxyethyl)- histidine (pmol/g Hb)	7-(2-Hydroxyethyl)guanine (pmol/g DNA)	
		In testis	In other tissues ^a
1 (1.83)	136	65	79-118
10 (18.3)	1030	466	777-964
33 (60.4)	4640	2000	3030-3660

Adapted from Potter *et al.* (1989)

^aRanges in brain, lung, liver, spleen and kidney

Male B6C3F1 mice and male Fischer 344 rats were exposed repeatedly (6 h/day, five days/week, four weeks) to atmospheric concentrations of 0, 3, 10, 33 and 100 ppm ethylene oxide [5.5, 18.3, 60.4 and 183 mg/m³] and rats also to 300 ppm [549 mg/m³]. In both species, HOEtVal concentrations in Hb after the end of exposure (Table 14) increased linearly with exposure concentration up to 33 ppm [60.4 mg/m³], with an identical slope (mean) of about 1100 pmol HOEtVal/g globin per ppm ethylene oxide for these conditions of exposure. At concentrations between 33 and 100 ppm [60.4 and 183 mg/m³] (mice) and 33 and 300 ppm [60.4 and 549 mg/m³] (rats), the mean slopes were higher, at about 1440 and 1330 pmol HOEtVal/g globin per ppm per h ethylene oxide, calculated from the figures presented by Walker *et al.* (1992a). The authors compared their results with those of Osterman-Golkar *et al.* (1983), who investigated the concentrations of HOEtHis in Hb of male Fischer 344 rats exposed repeatedly (6 h/day, five days/week, two years) to atmospheric ethylene oxide at concentrations of 0, 10, 33 and 100 ppm [18.3, 60.4, 183 mg/m³]. Similar adduct levels were found in the two studies up to 33 ppm [60.4 mg/m³] ethylene oxide. The adduct levels determined by Osterman-Golkar *et al.* (1983) were, however, almost directly proportional to the 6-h exposure concentrations up to 100 ppm [183 mg/m³], with a slope of about 1000 pmol HOEtVal/g globin per ppm per h ethylene oxide, calculated from published data. After cessation of exposure to the highest concentrations (300 ppm in rats; 100 ppm in mice), the initial loss of HOEtVal was faster than expected on the basis of the normal erythrocyte life span (Walker *et al.*, 1992a). It was suggested that these findings indicate removal of older, more heavily alkylated populations of erythrocytes, accompanied by a burst of erythropoiesis.

Table 14. Hydroxyethyl adducts to N-terminal valine of haemoglobin of rats and mice exposed repeatedly (6 h/day, 5 days/week, 4 weeks) to atmospheric ethylene oxide

Ethylene oxide (ppm)	HOEtVal (pmol/g Hb)	
	Rat	Mouse
0	42	58
1	3 500	3 400
10	11 200	11 100
33	33 400	37 900
100	133 000	144 000
300	397 000	-

Adapted from Walker *et al.* (1992a)

Male B6C3F1 mice and male Fischer 344 rats were exposed repeatedly (6 h/day, five days/week, four weeks) to atmospheric ethylene oxide at concentrations of 0–100 ppm [0–183 mg/m³] (mice) and 0–300 ppm [0–549 mg/m³] (rats), as described above. 7-HOEtGua in DNA was determined in various tissues immediately after the end of exposure. Similar adduct levels were found among the tissues, the lowest values being found in testis and the highest in lung. After equivalent exposures to ethylene oxide, the 7-HOEtGua levels were two- to three-fold lower in mice than in rats (Table 15). In order to allow a comparison between species, the data were expressed as picomoles per micromole guanine, taking into account differences in the guanine content of DNA in mouse and rat tissues (28% and 22%, respectively). The slopes of the curves representing the levels of 7-HOEtGua in DNA in various tissues in relation to ethylene oxide exposure concentration increased with increasing concentration, as was observed for HOEtVal levels in Hb (see above) (Walker *et al.*, 1992b).

Removal of 7-HOEtGua from tissue DNA was investigated in mice and rats exposed repeatedly (6 h/day, five days/week, four weeks) to ethylene oxide at concentrations of 100 ppm [183 mg/m³] and 300 ppm [549 mg/m³], respectively. It disappeared slowly from DNA of mouse kidney (half-life, 6.9 days) and rat brain and lung (half-lives, 5.4–5.8 days). The authors suggested that the disappearance rate was consistent with a loss due mainly to chemical depurination and that the more rapid removal in other tissues from mice (liver, testis, spleen, brain, lung; half-lives, 1.0–2.3 days) and rats (spleen, white blood cells, kidney, liver, testis; half-lives, 2.9–4.8 days) indicated DNA repair in addition to depurination. Two further DNA adducts of ethylene oxide were found in tissues of rats exposed to 300 ppm ethylene oxide [549 mg/m³]: O⁶-HOEtGua in brain, kidney, lung and spleen and 3-(2-hydroxyethyl)adenine in spleen, which reached a steady-state level of about 1000 pmol/g DNA, 250- to 300-fold less than the corresponding level of 7-HOEtGua (Walker *et al.*, 1992b).

Walker *et al.* (1993) presented a comparison of their results on adduct formation of ethylene oxide to Hb and DNA. On the basis of the observation in laboratory animals that the relationships between HOEtVal in Hb and 7-HOEtGua in DNA vary with length of

exposure, interval since exposure, species and tissue, the authors concluded that the HOEtVal adduct in human Hb was unlikely to provide accurate predictions of DNA adducts in tissues under conditions in which the actual exposure concentration of ethylene oxide is unknown.

Table 15. Hydroxyethyl adducts to 7-guanine in DNA of various tissues from mice and rats exposed repeatedly (6 h/day, 5 days/week, 4 weeks) to an atmospheric ethylene oxide concentration of 100 ppm [183 mg/m³]

Tissue	Mean 7-HOEtGua (pmol/ μ mol guanine [nmol/g DNA])	
	Mouse	Rat
Lung	38 [34]	105 [75]
Brain	38 [34]	87 [62]
Spleen	33 [30]	81 [58]
Kidney	33 [30]	55 [39]
Liver	31 [28]	49 [35]
Testis	21 [19]	44 [31]

Adapted from Walker *et al.* (1992b); 7HOEtGua, 7-(2-hydroxyethyl)guanine

The effects of different rates of exposure (300 ppm [549 mg/m³] for 1 h, 150 ppm [275 mg/m³] for 2 h, 75 ppm [137 mg/m³] for 4 h) to [1,2-³H]ethylene oxide on incorporation of radioactivity in Hb and DNA of testis were studied in (C3H/R1 \times BI10/R1)F₁ hybrid male mice. Animals were killed 90 min and one, three and six days after the end of the exposures. The radioactivity count in Hb (averaged over the four time points) was 1.5 times higher after the high exposure rate than after the lowest. A clear effect of exposure rate on radioactivity counts in DNA of testis was observed only 90 min after the end of exposure: incorporation of radioactivity was 2.9-fold higher after the highest exposure rate than after the lowest. The concentration of 7-HOEtGua in DNA of testis showed a first-order decline with a half-life of 2.8 days after exposure to 300 ppm for 1 h (Sega *et al.*, 1991).

A physiologically based pharmacokinetic model has been developed for dosimetry of inhaled and intravenously injected ethylene oxide in rats (Krishnan *et al.*, 1992). The model makes it possible to describe tissue distribution, metabolic pathways, i.e. hydrolysis by epoxide hydrolase and conjugation with glutathione by glutathione *S*-transferase, depletion of hepatic and extrahepatic glutathione and binding of ethylene oxide to Hb and DNA. The biochemical parameters used in the model were obtained by fitting data obtained after inhalation of ethylene oxide in closed chambers (see above) to data on tissue glutathione concentrations (McKelvey & Zemaitis, 1986) and on levels of hydroxyethyl adducts in Hb and tissue DNA (Potter *et al.*, 1989). The model was validated by comparing simulated and published data on urinary excretion of *N*-acetyl-*S*-(2-hydroxyethyl)cysteine after inhalation and intravenous administration of ethylene oxide (Gérin & Tardif, 1986; Tardif *et al.*, 1987)

and on levels of hydroxyethyl adducts in Hb and tissue DNA after exposure (6 h) to 300 ppm [549 mg/m³] ethylene oxide (Walker *et al.*, 1990, 1992a). The second-order rate constants obtained for the binding of ethylene oxide to amino acid residues in Hb are similar to those published by Segerbäck (1990). According to the model, adduct formation in Hb and DNA accounted for 0.25% and 0.001% of the inhaled dose, respectively. After exposure to atmospheric concentrations of up to 500 ppm [915 mg/m³] ethylene oxide, the model predicted first-order kinetics for whole-body elimination but nonlinearity in individual metabolic pathways and exhalation. Comparison of the predictions for low and 500-ppm exposures indicated that the share of glutathione conjugation decreased from 38 to 27%, whereas the share of hydrolysis increased from 31 to 36% and that of exhalation from 23 to 28% (Krishnan *et al.*, 1992).

4.2 Toxic effects

The toxicology of ethylene oxide has been reviewed (European Chemical Industry Ecology and Toxicology Centre, 1984; US Occupational Safety and Health Administration, 1984; WHO, 1985; US Environmental Protection Agency, 1985; Golberg, 1986; Henschler, 1993).

4.2.1 Humans

(a) Acute effects

Burns on the hands were attributed to gloves containing residual traces of ethylene oxide used for sterilization (Fisher, 1988). Eye and skin irritation in sterilizer operators were associated with personal exposures to ethylene oxide in air at concentrations up to 10.7 ppm [19.6 mg/m³] (Bryant *et al.*, 1989). Five sterilizer operators were exposed accidentally to atmospheric ethylene oxide at concentrations high enough to be smelt (odour threshold: 700 ppm [1280 mg/m³]) for periods up to 0.5 h. Two of the subjects were moderately intoxicated, with headache and diarrhoea as acute symptoms, which disappeared after about 70 h. More severe intoxication was seen in the three other subjects, who had a variety of immediate clinical symptoms including irritation of eyes and throat, mouth dryness, pruritus, headache, vertigo and myasthenia. Indigestion appeared on the day after exposure. All of these symptoms had disappeared by day 21. Haemolysis diagnosed on days 9–11 lasted until day 16 (Deleixhe *et al.*, 1986). Following accidental exposure (4 h/day, four days) to concentrations of ethylene oxide high enough to be smelt, one worker out of five developed persistent nonimmunological asthma, probably induced by extensive epithelial injury which led finally to fibrosis (Deschamps *et al.*, 1992).

(b) Chronic effects

In two studies of workers engaged in ethylene oxide manufacture for at least six months and between one and 14 years, respectively, no significant differences in selected immunological, haematological and biochemical parameters were observed when comparison was made with matched control personnel unexposed to ethylene oxide (Currier *et al.*, 1984; van Sittert *et al.*, 1985). In a cohort of workers exposed to TWA concentrations of ethylene oxide in air that were generally below 10 ppm and mostly below 1 ppm [18.3 and 1.83 mg/m³], the

prevalence of proteinuria was increased significantly (Currier *et al.*, 1984). In a cohort exposed to ethylene oxide at air concentrations generally below 0.05 ppm [0.09 mg/m³] but transiently up to 8 ppm [14.6 mg/m³], a differential white blood cell count revealed that duration of employment was correlated positively with the percentage of neutrophils and negatively with the percentage of lymphocytes. The values remained within the limits of a control population and were therefore considered to have no significance for health (van Sittert *et al.*, 1985).

People working in a sterilization unit were exposed for 0.6–13 years to ethylene oxide in air at mean concentrations of < 0.25–9.2 ppm [< 0.46 –16.8 mg/m³] measured during seven working days. No haematological, hepatological, nephrological or immunological abnormalities were observed (Wagner & Kollorz, 1987).

In an epidemiological study, the toxicity of ethylene oxide to the lens was investigated in sterilizer operators exposed to atmospheric concentrations varying from 0.06 ppm [0.11 mg/m³] for 97 min to 39 ppm [70 mg/m³] for 2.5 min. The prevalence of cataract (but not of lens opacities in the absence of reduced visual acuity) was significantly higher in exposed (aged over 45) than in unexposed, matched subjects. There was, however, no correlation with concentration of ethylene oxide (Deschamps *et al.*, 1990a,b). A regression analysis showed that cumulative exposure to ethylene oxide (years employed \times working hours per week \times ppm ethylene oxide) was associated with decreased numbers of white blood cells (Deschamps *et al.*, 1990b).

(c) Sensitization

The sensitizing effects of ethylene oxide have been reviewed (Bommer & Ritz, 1987; Bousquet & Michel, 1991).

A broad spectrum of IgE-mediated allergic symptoms, including anaphylactic reaction, has been observed among dialysis patients, which is due to the use of ethylene oxide for sterilization of dialysis equipment (Bommer *et al.*, 1985; Röckel *et al.*, 1985; Piazzolo & Brech, 1986; Kessler *et al.*, 1988; Röckel *et al.*, 1989; Lemke *et al.*, 1990). In these patients, IgE and IgG antibodies were found to be directed against ethylene oxide–human serum albumin conjugates (Marshall *et al.*, 1984; Caruana *et al.*, 1985; Grammer *et al.*, 1985a,b; Marshall *et al.*, 1985; Rumpf *et al.*, 1985; Nicholls, 1986; Grammer & Patterson, 1987; Lemke, 1987; Pearson *et al.*, 1987; Rumpf *et al.*, 1987; Wass *et al.*, 1988).

Exposure to residual ethylene oxide in fluid administration sets induced IgE antibodies against ethylene oxide–human serum albumin conjugate in a few donors undergoing repeated plateletpheresis or plasmapheresis (Leitman *et al.*, 1986; Muylle *et al.*, 1986; Dolovich *et al.*, 1987; Strobel *et al.*, 1988). Cases of allergic asthma have been observed among nurses in haemodialysis centres, who may show a combined IgE-dependent sensitization to ethylene oxide after handling ethylene oxide-sterilized equipment (Balland *et al.*, 1990; Meurice *et al.*, 1990; Dugue *et al.*, 1991; Jacson *et al.*, 1991).

(d) Neurotoxicity

In several studies, chronic occupational exposure of sterilizer operators to ethylene oxide has been associated with symptoms of peripheral and central neurotoxicity (Schröder *et al.*, 1985; Fukushima *et al.*, 1986; Estrin *et al.*, 1987; Crystal *et al.*, 1988; Estrin *et al.*, 1990;

Klees *et al.*, 1990; Grober *et al.*, 1992). Exposures over 0.5–20 years were characterized by a few daily short-term peaks of air concentrations of 250–700 ppm [458–1281 mg/m³] ethylene oxide. Eight-hour TWA concentrations ranged from < 1 to 4.7 ppm [< 1.83–8.6 mg/m³] ethylene oxide. The symptoms and pathological features found in cases of peripheral neuropathy include numbness in the feet and fingers, muscular weakness in the lower limbs, reduction in sural nerve velocity, nerve fibre degeneration and demyelination. Toxic effects were concluded to have occurred on the central nervous system on the basis of personality dysfunction or cognitive impairment.

4.2.2 Experimental systems

(a) Acute effects

The acute effects of a 4-h exposure to ethylene oxide were investigated in male and female B6C3F1 mice exposed at air concentrations up to 1600 ppm [2928 mg/m³]. At 800 ppm [1464 mg/m³], all males and four of five females died within six days; at 1600 ppm [2928 mg/m³], all animals died within 4 h. Lachrymation and dyspnoea occurred at 800 ppm [1464 mg/m³] and semiconsciousness, severe dyspnoea and diarrhoea at 1600 ppm [2928 mg/m³] (US National Toxicology Program, 1987).

(b) Subchronic effects

Subchronic effects of ethylene oxide in animals are summarized in Table 16. Reductions in erythrocyte lifespan and increased erythrocyte fragility have been noted (Popp *et al.*, 1986; Mori *et al.*, 1989, 1990a), which may explain the rapid elimination of Hb adducts in ethylene oxide-exposed animals (Walker *et al.*, 1992a).

(c) Chronic effects

In a chronic study, male Wistar rats and male cynomolgus monkeys were exposed (7 h/day, five days/week, two years) to air concentrations of 50 and 100 ppm [91.5 and 183 mg/m³] ethylene oxide. Exposed rats had higher incidences of inflammatory lesions of the lungs, nasal cavities, trachea and internal ear than controls. Furthermore, proliferative and degenerative lesions of the adrenal cortex were found which were characterized by vacuolation and hyperplasia or hypertrophy of the cells of the zona fascicularis. Skeletal myopathy consisting of multifocal areas of atrophy and degeneration without neural changes was observed at 100 ppm [183 mg/m³] ethylene oxide. In exposed monkeys, the incidence of cataracts was elevated. Decreased nerve conduction velocity was measured in two of 12 monkeys exposed to the higher concentration. Neuropathological examination of two animals in each group revealed demyelination in the very distal portion of the fasciculus gracilis in one animal in each exposure group (Lynch *et al.*, 1984a,b).

Exposure (6 h/day, five days/week, 102 weeks) of male and female B6C3F1 mice to atmospheric concentrations of ethylene oxide up to 100 ppm [183 mg/m³] did not result in treatment-related clinical signs (US National Toxicology Program, 1987).

Table 16. Subchronic effects in rodents exposed to atmospheric ethylene oxide

Species	Exposure	Effects	Reference
General toxicity			
Wistar rats, males	0, 500 ppm [915 mg/m ³] 6 h/day, 3 days/week, 13 weeks	Decrease in glutathione reductase in liver and brain, increase in lipid peroxidation (malondialdehyde level) in liver Disturbance of porphyrin-haem metabolism, decrease in hepatic cytochrome P450, decrease in haemoglobin concentration, normocytic and normochromic anaemia Decrease in glutathione reductase and glutathione in erythrocytes Decrease in glutathione reductase in lens	Katoh <i>et al.</i> (1988, 1989) Fujishiro <i>et al.</i> (1990a) Mori <i>et al.</i> (1990a) Fujishiro <i>et al.</i> (1991)
Wistar rats, males and females	0, 250 ppm [458 mg/m ³] 6 h/day, 5 days/week, 17 weeks	Males: decrease in hepatic cytochrome P450; females: increase in hepatic NADPH-cytochrome c reductase Females: increase in liver weight; males and females: decrease in glutathione reductase and increase in glutathione-S-transferase in the liver; males: increase in hepatic glutathione peroxidase	Fujishiro <i>et al.</i> (1990b) Mori <i>et al.</i> (1990b)
B6C3F1 mice, males and females	0-250 ppm [0-458 mg/m ³] 6 h/day, 5 days/week, 10 (males) and 11 (females) weeks	100 ppm [183 mg/m ³]: decrease in spleen weight in females; 250 ppm [458 mg/m ³]: decrease in spleen weight, increase in relative liver weight in females, decrease in absolute testicular weight and slight decrease in haemoglobin concentration and erythrocyte count	Snellings <i>et al.</i> (1984b)
B6C3F1 mice, males and females	0-600 ppm [1098 mg/m ³] 6 h/day, 5 days/week, 14 weeks	Dose-related epithelial damage in the nasal portion of the respiratory tract; 100-400 ppm [183-732 mg/m ³]: renal tubular degeneration; 200-600 ppm [366-1098 mg/m ³]: rhinitis of nasal cavity; 600 ppm [1098 mg/m ³]: renal tubular necrosis; lymphocytic necrosis of thymus and spleen in males	US National Toxicology Program (1987)
C57BL/6J mice, males	0, 255 ppm [467 mg/m ³] 6 h/day up to 16 days; 6 h/day, 5 days/week, 4-10 weeks	Haematological damage: general depression of cellularity in blood and bone marrow, with large fluctuations, however; transient increase in granulocytes	Popp <i>et al.</i> (1986)
ddY mice, males	0, 400 ppm [732 mg/m ³] 6 h/day, 3 days/week, 13 weeks	Macrocytic anaemia; hepatic cytochrome P450 increased two fold; increase in ferricyanide reductase; decrease in glutathione reductase and glutathione peroxidase in liver; increase in hepatic glutathione-S-transferase	Fujishiro <i>et al.</i> (1992)

Table 16 (contd)

Species	Exposure	Effects	Reference
Neurotoxicity			
B6C3F1 mice, males and females	0–250 ppm [0–58 mg/m ³] 6 h/day, 5 days/week, 10 (males) and 11 (females) weeks	Dose-related trend in reduction in locomotor activity and in abnormal reflexes; no microscopic findings	Snellings <i>et al.</i> (1984b)
Wistar rats, males	0, 250 ppm [458 mg/m ³] 6 h/day, 5 days/week, 9 months	Preferential distal axonal degeneration of myelinated fibres in sural nerves and gracile fascicles	Ohnishi <i>et al.</i> (1986)
Wistar rats, males and females	0, 250 ppm [458 mg/m ³] 6 h/day, 5 days/week, 17 weeks	Paresis of hindlegs; degeneration of myelinated fibres in the peroneal nerve, in the nerve of the soleus muscle and in gracile fascicles; no sex difference	Mori <i>et al.</i> (1990c)
Wistar rats, males	0, 500 ppm [915 mg/m ³] 6 h/day, 3 days/week, 4–13 weeks	Ataxic gait after six weeks; preferential distal axonal degeneration of myelinated fibres in hindleg nerves and gracile fascicles; decrease in creatine kinase activity in serum, brain and spinal cord after four weeks	Ohnishi <i>et al.</i> (1985); Matsuoka <i>et al.</i> (1990, 1993)

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

Hemminki *et al.* (1982) reported the results of a retrospective study of all female sterilizing staff employed in hospitals in Finland in 1980. Nursing supervisors from approximately 80 hospitals identified the study participants and the exposure status of each with regard to specific sterilizing agents, which included ethylene oxide, glutaraldehyde and formaldehyde. The 1443 pregnancy outcomes that occurred between the early 1950s and 1981 were categorized as 'exposed' or 'unexposed' on the basis of the work history at the beginning of each pregnancy, established by answers to questionnaires from the study participants. A control group was established, consisting of 1179 pregnancies among female nursing auxiliaries who had had no exposure to sterilizing agents, anaesthetic gases or X-rays. The rates of spontaneous abortion were adjusted for age, parity, decade of pregnancy, coffee consumption, alcohol consumption and smoking habits. The most marked increase was observed for women who had been exposed during pregnancy to ethylene oxide alone: 16.1% of 82 exposed *versus* 7.8% of 1068 unexposed ($p < 0.01$) and 10.5% of 1179 controls. The rates of spontaneous abortion among women exposed to glutaraldehyde and formaldehyde were similar to those among unexposed women. Similar results were obtained in a comparison of pregnancy outcomes identified from hospital discharge registries for sterilizing staff and controls in Finland in 1973–79. In a subsequent analysis (Hemminki *et al.*, 1983), the authors applied a stricter age adjustment and restricted attention among controls to pregnancies that began during hospital employment. The rates were 11.3% of 721 pregnancies for the controls and 20.4% ($p < 0.05$) for exposure to ethylene oxide alone; the rate was also increased for women exposed to glutaraldehyde but not for those exposed to formaldehyde.

4.3.2 *Experimental systems*

The reproductive and prenatal effects of ethylene oxide have been reviewed (Kimmel *et al.*, 1984), and only the most important papers published up to that date are highlighted. All papers published after 1984 are reviewed here. The reproductive toxicity of ethylene oxide has been studied in mice, rats and rabbits following oral, intravenous and inhalational exposure.

In CD-1 mice, intravenous administration of 0, 75 or 150 mg/kg bw ethylene oxide in 5% dextrose solution on days 4–6, 6–8, 8–10 or 10–12 of gestation significantly increased the incidences of craniofacial defects and of fusions of vertebrae in high-dose animals exposed on days 6–8 (19.3%) and 10–12 (9.5%). The incidence ranged from 0 to 2.3% in the control groups. The high-dose level resulted in maternal mortality after treatment on days 4–6, 8–10 and 10–12 (LaBorde & Kimmel, 1980).

Female Sprague-Dawley rats were exposed by inhalation for 7 h per day on five days per week on days 7–16 of gestation, on days 1–16 of gestation or for three weeks prior to mating and then daily until day 16 of gestation to 150 ppm (measured concentration was within 10% of target) ethylene oxide (99.7% pure) vapour. An increased incidence of resorptions (13.6% *versus* 5.4% in controls) was reported in the third group. Pregestational exposure appeared to be important, as similar effects were not found in females exposed during gestation only.

Maternal weight gain and fetal growth were reduced in all groups (Hackett *et al.*, 1982; Hardin *et al.*, 1983).

Male and female Fischer 344 rats were exposed by inhalation to 10, 33 or 100 ppm [18, 60.4 or 183 mg/m³] ethylene oxide vapour for 6 h per day on five days per week for 12 weeks and then mated; exposure was continued during mating on seven days per week, and females continued to be exposed through to day 19 of gestation. Fewer implantation sites per female, a smaller ratio of fetuses born to number of implants, a decreased number of pups born per litter and a tendency for longer gestation were observed only in animals exposed to 100 ppm. No treatment-related effect was found at the two lower dose levels. It was not determined whether the effects seen were due to treatment of the males or females or both. When lactating females were subsequently exposed from day 5 to 21 of lactation, no adverse effect was seen on pup growth rate or survival (Snellings *et al.*, 1982a).

Snellings *et al.* (1982b) also reported a study of teratogenic effects in Fischer 344 rats exposed to ethylene oxide (> 99.9% pure). Groups of 22 pregnant rats were exposed to 10, 33 or 100 ppm [18, 60.4 or 183 mg/m³] ethylene oxide for 6 h per day on days 6–15 of gestation; two control groups were exposed to air only. Fetuses were delivered for examination on day 20. All were examined grossly, and then the control group and that exposed to the highest dose were examined for visceral and skeletal defects. No toxicity was observed in the dams and no treatment-related adverse effect was observed, except for a small but significant reduction in fetal weight at the highest dose. There was no evidence of any teratogenic effect.

Exposure of rabbits by inhalation to 150 ppm [274.5 mg/m³] ethylene oxide (99.7% pure) vapour for 7 h per day on days 7–19 or 1–19 of gestation resulted in no evidence of maternal toxicity, embryotoxicity or teratogenicity (Hackett *et al.*, 1982; Hardin *et al.*, 1983).

It has been believed for a long time that chemicals cannot induce congenital malformations during the preimplantation period of development. Exposure at that time either results in cell death or allows the remaining, undamaged cells to go on to produce a normal embryo: the concept of 'totipotency' of the cells. A series of publications by Generoso and his coworkers has demonstrated, however, that mutagens can induce fetal malformations and death when administered around the time of fertilization.

Generoso *et al.* (1987) first demonstrated that exposure of (C3H×C57Bl)F1 or (SEC×C57Bl)F1 female mice mated with (C3H×C57Bl)F1 males to ethylene oxide gas (1200 ppm [2196 mg/m³] for 1.5 h) could produce different results, depending on the timing of exposure. Females were exposed 1, 6, 9 or 25 h after carefully timed 30-min matings, these intervals corresponding, respectively, to time of sperm penetration, early pronuclear stage (before DNA synthesis), pronuclear DNA synthesis and early two-cell stage. Exposure at 1 or 6 h increased the number of midgestational and late fetal deaths, but few such effects were seen after exposure at 9 h and none after 25 h. A large proportion of fetuses that survived after exposure at 6 h had a range of congenital malformations, including omphalocele, hydropia, open thorax and limb and tail defects (37% versus 2% in controls). Malformations were also seen in fetuses exposed at 1 h but not in those exposed at 9 or 25 h. In a later study (Rutledge & Generoso, 1989), with identical exposure protocols but more detailed fetal examination, an increased incidence of malformations was found after exposure at 1, 6, 9 and 25 h. Other females exposed to ethylene oxide for up to 14 days before mating had mainly an

increase in early embryonic death around the time of implantation, probably as a result of dominant lethal mutations. In a subsequent publication (Generoso *et al.*, 1988), ethyl methane-sulfonate (EMS), which has mutagenic activity, was shown to produce similar effects on midterm and late fetal deaths and malformations in mice after exposure 6 h after mating.

The mechanism involved in the induction of fetal malformations so early in gestation was further investigated by Katoh, M. *et al.* (1989) in mated female (C3H×C57Bl)F1 mice exposed to ethylene oxide at 1200 ppm [2196 mg/m³] for 1.5 h or to 250 mg/kg bw EMS intraperitoneally beginning 6 h after the end of the 30-min mating period. Reciprocal zygote transfer to treated or untreated recipient mice 3–9 h after treatment with EMS or buffer resulted in midterm and late fetal deaths and malformations only when the donor females had received EMS, so that the effect was mediated on the zygote and was not secondary to effects on the dam. Analysis of chromosomes of exposed embryos from the late zygote and two-cell stages through to 14 days (only the 10-day embryos had been exposed to ethylene oxide, the others to EMS) showed no increase in either numerical or structural aberrations in the early embryonic stages, nor evidence of aneuploidy in the later embryos. Since the effect is on the zygote but is not associated with chromosomal aberrations, it may be a consequence of gene mutation or reflect an epigenetic effect on gene expression. Postnatal survival of live-born fetuses to weaning was also reduced (79% versus 94% in controls) in (SEC×C57Bl)F1 mice treated with 1200 ppm [2196 mg/m³] ethylene oxide for 1.5 h starting 1 or 6 h after mating. The surviving males were tested for heritable translocations by examining them for sterility or semisterility; none was found in 131 offspring tested (Rutledge *et al.*, 1992).

Mori *et al.* (1991) exposed groups of six male Wistar rats to 50, 100 or 250 ppm [91.5, 183 or 457.5 mg/m³] ethylene oxide for 6 h per day on five days per week for 13 weeks; there were 12 unexposed controls. In the 250-ppm dose group, epididymal but not testicular weight was reduced, there was slight degeneration in some seminiferous tubules, reduced sperm count in the body and tail but not the head of the epididymis and an increase in sperm head abnormalities, due mainly to the presence of immature sperm. An increase in malformed sperm heads unrelated to dose was observed in all treated groups over that in controls (15% versus 2%).

4.4 Genetic and related effects

The mutagenicity of ethylene oxide has been reviewed (Dellarco *et al.*, 1990).

4.4.1 Humans

(a) DNA adducts

The background level of 7-HOEtGua in DNA of peripheral lymphocytes from eight people not occupationally exposed to ethylene or ethylene oxide was 8.5 ± 5.7 nmol/g DNA. The sources of the adduct were not discussed (Föst *et al.*, 1989). No data were available on the formation of DNA adducts in humans exposed to ethylene oxide.

(b) *Mutation and allied effects* (see also Tables 17 and 18)

A review of the mutagenicity of ethylene oxide (Dellarco *et al.*, 1990) contains a section on cytogenetic studies of the somatic cells of humans exposed to ethylene oxide. Detailed summaries of many of the studies considered in that analysis are given in section 4.4.2.

Many studies have been carried out to evaluate the effect of exposure to ethylene oxide on the incidences of chromosomal aberrations (including micronuclei) and sister chromatid exchange in peripheral blood lymphocytes of workers exposed occupationally to ethylene oxide. These include workers at hospital and factory sterilization units and those working at ethylene oxide manufacturing and processing plants. The results, summarized in Table 17, show that ethylene oxide induces chromosomal damage in exposed humans. In general, the degree of damage is correlated with the level and duration of exposure. The induction of sister chromatid exchange appears to be more sensitive to exposure to ethylene oxide than is the formation of adducts, chromosomal aberrations or micronuclei. Alkali-labile sites and DNA single-strand breaks (Table 18) were not observed in lymphocytes of sterilization workers, but the induction of DNA cross-linking was reported in one study.

Concentrations of ethylene oxide are often reported as 8-h TWA levels, which do not necessarily reflect the actual concentration to which workers are exposed. During certain operations (e.g. unloading sterilizers), workers may be exposed to short bursts of ethylene oxide at concentrations as high as 400 ppm [720 mg/m³], while the 8-h TWA may be as low as 5 ppm [9 mg/m³] (Tates *et al.*, 1991a). Furthermore, the length of time that an individual is exposed to ethylene oxide may be an important factor in determining the relationship between genetic effects and exposure (Thiess *et al.*, 1981a).

Four informative studies (Yager *et al.*, 1983; Stolley *et al.*, 1984; Galloway *et al.*, 1986; Tates *et al.*, 1991a) of genetic end-points in exposed workers are described in detail below.

Yager *et al.* (1983) reported an increased incidence of sister chromatid exchange in peripheral blood lymphocytes of 14 hospital sterilization workers exposed to 1 ppm ethylene oxide (8-h TWA) over that in 13 unexposed controls. In order to evaluate the relationship between exposure and sister chromatid exchange induction, workers were divided into a high-exposure group (five subjects) and a low-exposure group (nine subjects) on the basis of a six-month cumulative dose of ethylene oxide determined by measuring air concentrations during specified tasks and multiplying this value by the number of times each task was performed. The high-exposure group, which received an average cumulative dose of 501 mg ethylene oxide, showed a significant increase in sister chromatid exchange frequency over that in controls and in the low-exposure group (average cumulative dose, 13 mg ethylene oxide). Sister chromatid exchange frequency did not differ significantly between the low-exposure group and the controls.

Tates *et al.* (1991a) compared the frequencies of sister chromatid exchange in nine hospital workers and 15 workers from factory sterilization units occupationally exposed to ethylene oxide and in two respective control groups matched for age, sex and smoking habits (eight donors from administrative personnel working in the neighbourhood and 15 from the same factory). Exposure was measured by gas chromatography in the sterilization rooms (20–25 ppm) and in front of the sterilizer after opening (mean, 50 ppm) for the hospital workers and was monitored during four months (period covering the erythrocyte lifespan) for

the factory workers. Additionally, HOEtVal concentrations were determined in two laboratories. Sister chromatid exchanges were analysed in independent cultures in two different laboratories. The mean frequency of sister chromatid exchange was significantly elevated by 20% in the hospital workers and by almost 100% in the factory workers; moreover, the frequency was clearly greater in daily than in occasionally exposed workers in the factory population.

Other investigators have also reported increased incidences of sister chromatid exchange in lymphocytes of workers exposed to ethylene oxide in hospital sterilization units (Garry *et al.*, 1979; Abrahams, 1980; Laurent *et al.*, 1984; Sarto *et al.*, 1984a,b, 1987, 1991; Lerda & Rizzi, 1992; Schulte *et al.*, 1992). The results from two studies (Högstedt *et al.*, 1983; Hansen *et al.*, 1984) showed that sister chromatid exchanges were not induced in workers who were exposed to less than 1 ppm ethylene oxide.

In a longitudinal study (Stolley *et al.*, 1984), 61 sterilization workers from three work sites were evaluated for induction of sister chromatid exchange at 6, 12 and 24 months. At work site I there was low exposure (0.5 ppm TWA), at work site II there was moderate exposure (5–10 ppm) and at worksite III there was high exposure (5–20 ppm at the time of sampling; action had been taken six months previously to reduce the TWA from 50–200 ppm). Workers at each site were further divided with regard to low and high potential for exposure on the basis of job classification and proximity to sterilizer operations and controls. Controls were primarily randomly selected site personnel (53) considered to have no exposure; community controls (29) were also included. Initial exposures were confirmed by measurements of ethylene oxide in breathing zones. After the initial sampling, blood was taken at each of three sampling times at the work sites and at 6 and 18 months for community controls. The effects on sister chromatid exchange frequency of age, sex, smoking habits and cytogenetic scorer were taken into account. The results showed no increase in sister chromatid exchange frequency for any exposure at work site I or for the workers with potentially low exposure at work site II. Pair-wise comparisons between groups at work site II indicated that the group with potentially high exposure had significantly higher mean frequencies of sister chromatid exchange than the group with potentially low exposure initially ($p = 0.003$), at 12 months ($p < 0.001$) and at 24 months ($p = 0.023$). Similarly, the differences in mean sister chromatid exchange frequency between the group with potentially high exposure and control groups were increased significantly initially and at 12 and 24 months ($p = 0.011$, $p < 0.001$ and $p = 0.018$, respectively). At work site III, the mean sister chromatid exchange frequency in the group with potentially low exposure differed significantly from those in the work site control group at the initial ($p = 0.024$) and six-month ($p = 0.008$) testings, but not subsequently. Subjects at work site III did not continue to receive exposure after the initial blood samples were taken. These results indicate that the induction of sister chromatid exchange in workers exposed to ethylene oxide is related to the concentration of ethylene oxide at the workplace and that it persists up to six months after cessation of exposure.

Galloway *et al.* (1986) evaluated chromosomal aberration frequencies in lymphocytes from the same group studied by Stolley *et al.* (1984). The results showed no increase in chromosomal aberration frequencies at work sites I or II in any of the samples. Frequencies were significantly elevated in two samples from the group with potentially high exposure at

work site III in comparison with controls and with the group with potentially low exposure taken at 6 and 24 months. Significance was achieved for total structural aberrations only at 24 months ($p = 0.018$) and when data were pooled over time ($p = 0.003$). The 24-month samples from the group with potentially low exposure at work site III had significantly higher numbers of chromosomal aberrations than those from the community controls but not those from the site controls. The authors indicate that the work site III controls may have been exposed accidentally to low levels of ethylene oxide during a leak in 1980, which would account for the higher levels of aberrations than in the other work site controls. The effects of possible confounding variables on the control aberration frequencies were analysed: There was no detectable effect of sex; smoking and age had small but significant effects on the frequencies of chromatid aberrations and chromosomal exchanges, respectively. Exposures at levels of 5 ppm [9 mg/m^3] or above (8-h TWA) are thus required for ethylene oxide to induce chromosomal aberrations in peripheral blood lymphocytes. Similar results were reported from other studies (Pero *et al.*, 1981; Sarto *et al.*, 1984b; Mayer *et al.*, 1991; Tates *et al.*, 1991a).

Tates *et al.* (1991a) reported a significant increase in the frequency of micronuclei in lymphocytes from factory workers exposed to ethylene oxide at concentrations ranging from 14 to 400 ppm [$25\text{--}720 \text{ mg/m}^3$]; the 40-h TWA was estimated to be 5 ppm on the basis of measurements of Hb adducts. Four other studies (Högstedt *et al.*, 1983; Mayer *et al.*, 1991; Sarto *et al.*, 1991; Schulte *et al.*, 1992) showed no significant increase in the incidence of micronuclei in lymphocytes from workers exposed to ethylene oxide. Högstedt *et al.* (1983) did show, however, that micronuclei were induced in erythroblasts and polychromatic erythrocytes in bone-marrow samples from factory workers who had been exposed to less than 1 ppm ethylene oxide for six months to eight years. Sarto *et al.* (1990) showed increased frequencies of micronucleated exfoliated nasal mucosa cells in two of three workers who had been acutely exposed to ethylene oxide during accidental leakage.

Associations between different genetic end-points were analysed in two studies. Galloway *et al.* (1986) reported a weak overall association between the frequencies of chromosomal aberration and sister chromatid exchange in 61 employees in three work sites and in 304 unexposed controls. The correlation was significant ($p < 0.001$) in potentially exposed groups but not in control groups, and, for any individual, one observation could not be used to predict the other. Tates *et al.* (1991a) confirmed the correlation ($p < 0.001$) between chromosomal aberration and sister chromatid exchange frequencies in pooled data for 9 hospital and 15 factory workers. Additionally, sister chromatid exchange frequencies were shown to correlate better with HOEtVal levels than with chromosomal aberration frequencies which, in turn, correlated better with HOEtVal levels than with micronucleus formation frequency.

hprt Mutations were found in circulating lymphocytes of factory workers exposed to ethylene oxide in a single study (Tates *et al.*, 1991b). The sensitivity of this end-point is considered to be lower than that of Hb adducts and cytogenetic end-points.

Table 17. Cytogenetic observations in people occupationally exposed to ethylene oxide

No. exposed	No. of referents	Exposure time (years)		Ethylene oxide in air (ppm [mg/m ³])		Cytogenetic effects ^a			Reference
		Range	Mean	Range	Mean (TWA)	CA	MN	SCE	
12	8			0-36 ^b				+	Garry <i>et al.</i> (1979)
75	41				≤ 50			+	Abrahams (1980)
12	11	1-8	4	0.5-1				-	Pero <i>et al.</i> (1981)
5	11	0.8-3	1.6	5-10				+	
9	13 (low-dose task)				13 ^c			-	Yager <i>et al.</i> (1983)
5	13 (high-dose task)				501 ^c			+	
18	11 (factory I)	0.5-8	3.2		< 1	+	+ ^d	-	Högstedt <i>et al.</i> (1983)
10	9 (factory II)	0.5-8	1.7		< 1	+	-	-	
13	12 (work site I)		3.2	0.5 ^e		-		-	Stolley <i>et al.</i> (1984);
22 (21) ^f	19 (20) (work site II)		3.1	5-10 ^e		-		(+)	Galloway <i>et al.</i> (1986)
26 (25)	22 (21) (work site III)		4	5-20 ^e		(+)		+	
10	15 (nonsmokers)	0.5-10	5.7	[36-225]				+	Laurent <i>et al.</i> (1984)
15	7 (smokers)	0.5-10	4.5					+	
14	14			< 0.07-4.3 ^e				-	Hansen <i>et al.</i> (1984)
22	22 (low exposure)	1-4	3	0.2-0.5 ^e	0.35	(+)		+	Sarto <i>et al.</i> (1984b)
10	10 (moderate exposure)			0-9.3 ^e	1.84			+	Sarto <i>et al.</i> (1987)
19	19 (high exposure)	1.5-15	6.8	3.7-20 ^e	11	+		+	Sarto <i>et al.</i> (1984b)
56	141	1-10		1-40 ^e		+		+	Richmond <i>et al.</i> (1985)
36	35	1-14		0.1-8	0.05	-			van Sittert <i>et al.</i> (1985)
18	10 (sterilization unit)			0-2.7		+			Karelová <i>et al.</i> (1987)
21	20 (factory workers)			0-4		+			
14	10 (laboratory workers)			0-5		+			
11	10 (laboratory workers)			0-2.4		-			
9	27	0.5-12	5	0.025-0.38 ^e				-	Sarto <i>et al.</i> (1990)
3	27			> 0.38 ^g				+	

Table 17 (contd)

No. exposed	No. of referents	Exposure time (years)		Ethylene oxide in air (ppm [mg/m ³])		Cytogenetic effects ^a			Reference
		Range	Mean	Range	Mean (TWA)	CA	MN	SCE	
5	10	0.1-4	2		0.025		-	-	Sarto <i>et al.</i> (1991)
5	10	4.11	8.6	< 1-4.4	0.38		-	+	
9	8 (hospital workers)	2-6	4	20-25 [36-45]	0.125 ^h	+	-	+	Tates <i>et al.</i> (1991a)
15	15 (factory workers)	3-27	12	17-33 [30-60]	5 ^h	+	+	+	
34	23		8	0.008-2.4 ^e	< 0.3	-	-	+	Mayer <i>et al.</i> (1991)
32	8		5.1	0-0.3 ^e	0.04		-	+	Schulte <i>et al.</i> (1992)
11	8		9.5	0.13-0.3 ^e	0.16		-	+	
10	10		3	60-69		+		+	Lerda & Rizzi (1992)
47	47				< 1			-	Tomkins <i>et al.</i> (1993)

Blanks, not studied

^aCA, chromosomal aberrations; MN, micronuclei; SCE, sister chromatid exchange

^bMaximal concentration measured during purge cycle

^cAverage six-month cumulative dose in mg ethylene oxid

^dPositive for erythroblasts and polychromatic erythrocytes; negative for peripheral blood lymphocytes

^eTime-weighted average (TWA)

^fNumbers in parentheses are for chromosomal aberrations evaluated by Galloway *et al.* (1986)

^gExposed acutely from sterilizer leakage

^hEstimated 40-h TWA based on haemoglobin adducts

4.4.2 *Experimental systems* (see also Table 18)

(a) *DNA adducts*

The reaction of ^{14}C -ethylene oxide *in vitro* (3 h, 37 °C, pH 7.4) with calf thymus DNA yielded 7-HOEtGua as the main product; O^6 -HOEtGua and 3-(2-hydroxyethyl)adenine occurred as 0.5 and 4.4% of the amount of 7-HOEtGua (Segerbäck, 1990).

After 50 mmol ethylene oxide had been incubated (10 h, 37 °C, 0.05 mol/L phosphate buffer, pH 7–7.5) in screw-cap flasks with calf thymus DNA (3 mg/ml), several adducts were found: 7-HOEtGua at 330 nmol/mg DNA, 3-(2-hydroxyethyl)adenine at 39 nmol/mg DNA, N^6 -(2-hydroxyethyl)adenine at 6.2 nmol/mg DNA, 3-(2-hydroxyethyl)cytosine at 3.1 nmol/mg DNA, 3-(2-hydroxyethyl)thymine at 2 nmol/mg DNA and 3-(2-hydroxyethyl)uracil at 0.8 nmol/mg DNA. 3-(2-Hydroxyethyl)deoxyuridine was formed from 3-(2-hydroxyethyl)-cytosine by hydrolytic deamination of the imino group at C4 (Li *et al.*, 1992).

7-Guanine has commonly been identified and quantified as a hydroxylated base *in vivo*, and many studies have also used 7-HOEtGua as a measure of tissue dose (Segerbäck, 1983). [The Working Group calculated the covalent binding index—(μmol adduct per mol DNA nucleotide)/(mmol chemical per kg bw)—from the data of Segerbäck (1983) in order to evaluate DNA binding potency. Five hours after intraperitoneal injection of ethylene oxide to mice, a covalent binding index of 6.4 was calculated for liver DNA.] For example, it has been observed that exposure of male Sprague-Dawley rats over several hours to a constant atmospheric concentration of ethylene oxide results in a correlation between estimated uptake and 7-HOEtGua in hepatic DNA. A mean background level of 5600 pmol/g DNA was found in DNA extracted from blood of untreated rats (Föst *et al.*, 1989). In studies of tissue dose, Hb adducts have frequently been used as a surrogate for DNA adducts, so that when tissue dose was the objective, studies of DNA and Hb were described in section 4.1.2.

In mouse kidney and rat brain and lung, there is a slow loss of 7-HOEtGua from DNA, with half-lives of 5.4–6.9 days; there is somewhat more rapid loss from other tissues, with half-lives of 1.0–2.3 days in mice and 2.9–4.8 days in rats (Walker *et al.*, 1992b). The authors concluded that the differences were due to the extent of DNA repair in the various tissues superimposed upon chemical depurination.

Other adducts identified *in vivo* in rats exposed to 300 ppm [549 mg/m^3] ethylene oxide are O^6 -HOEtGua in brain, kidney, lung and spleen and 3-(2-hydroxyethyl)adenine in spleen. Steady-state levels of about 1000 pmol/g DNA were attained, which were 250- to 300-fold lower than the corresponding levels of 7-HOEtGua (Walker *et al.*, 1992b).

(b) *Mutations and allied effects*

Ethylene oxide caused DNA damage and gene mutation in bacteria. It induced gene conversion in yeast and gene mutation in yeast and fungi. In plants, it caused gene mutation and chromosomal aberrations. Ethylene oxide induced somatic cell and sex-linked recessive lethal mutations and heritable translocations in *Drosophila melanogaster*. Gene mutation, micronuclei, chromosomal aberrations and cell transformation were induced in rodent cells *in vitro*. Ethylene oxide caused unscheduled DNA synthesis and sister chromatid exchange in human lymphocytes, gene mutation and sister chromatid exchange in human fibroblasts and chromosomal aberrations in transformed human amniotic cells *in vitro*.

Exposure to ethylene oxide *in vivo* induced *hprt* locus mutation in mouse spleen T lymphocytes, sister chromatid exchange in rat, rabbit and cynomolgus monkey lymphocytes, in mouse and rat bone marrow and in rat spleen, micronuclei in mouse and rat bone-marrow cells and chromosomal aberrations in mouse and rat bone-marrow cells and in cynomolgus monkey but not rat lymphocytes. Aneuploidy was not induced in cells from 10-day-old mouse fetuses from dams exposed to ethylene oxide for 1.5 h beginning 6 h after mating.

Ethylene oxide induced alkali-labile sites and DNA single-strand breaks in mouse sperm and spermatids, as measured by alkaline elution of DNA from polycarbonate filters. It also induced dominant lethal effects in mice and rats, chromosomal aberrations in mouse spermatocytes and heritable translocations in mice. In two studies on offspring of male mice exposed to ethylene oxide by inhalation, under similar exposure conditions but using different mating regimens and examining different genetic events, no significant increase in the frequency of specific locus mutations was seen in one study (Russell *et al.*, 1984), while dominant visible and electrophoretically detected mutations were observed in another (Lewis *et al.*, 1986).

(c) *Mutational spectra*

The mutational spectrum of *hprt* locus mutants was studied in B6C3F1 mice given intraperitoneal injections of ethylene oxide from day 12 after birth on alternate days until eight weeks after the first treatment (Walker & Skopek, 1993). After selection of splenic T-lymphocytes for 6-thioguanine resistance, DNA was extracted and the exon 3 region of *hprt* was sequenced. Of the 123 *hprt*⁻ mutants analysed, 18 were located in exon 3; 11 of the 18 mutants were base-pair substitutions at eight different sites. Four AT transversions, three AT transitions, two GC transversions and two GC transitions were observed. Three of the substitutions (two AT → CG, one AT → GC) occurred at a single base (203) in a single mouse. The remaining seven mutations, isolated from four different mice, had the same +1 frameshift mutation in a run of six consecutive guanine bases (207–212) in exon 3. Thus, ethylene oxide mutagenesis in mice involves both modified guanine and adenine bases.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Ethylene oxide has been produced since the early 1900s, originally by the reaction of ethylene chlorohydrin with base and in recent years more commonly by catalytic oxidation of ethylene. It has been used as a chemical intermediate in the production of ethylene glycol, glycol ethers, nonionic surfactants and other industrial chemicals. Although much smaller amounts are used in sterilizing medical instruments and supplies in hospitals and industrially and for the fumigation of spices, it is during these uses that the highest occupational exposure levels have been measured.

Table 18. Genetic and related effects of ethylene oxide

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BRD, <i>Bacillus subtilis</i> , differential toxicity	(+)	0	480.0000	Tanooka (1979)
BPF, Bacteriophage, forward mutation	-	0	14500.0000	Cookson <i>et al.</i> (1971)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	220.0000	Pfeiffer & Dunkelberg (1980)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	0.0000	De Flora (1981)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	1.0000 ^c	Simmon (1981)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	0	+	125.0000 ^d	Hughes <i>et al.</i> (1987)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	0.0900 ^c	Victorin & Ståhlberg (1988)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	132.0000	Agurell <i>et al.</i> (1991)
SA2, <i>Salmonella typhimurium</i> TA102, reverse mutation	0	+	500.0000 ^d	Hughes <i>et al.</i> (1987)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	0	220.0000	Pfeiffer & Dunkelberg (1980)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	+	0.0000	De Flora (1981)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	0	1.0000 ^c	Simmon (1981)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	0	132.0000	Agurell <i>et al.</i> (1991)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	0	2200.0000	Pfeiffer & Dunkelberg (1980)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	0.0000	De Flora (1981)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	0.0000	De Flora (1981)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	0	2200.0000	Pfeiffer & Dunkelberg (1980)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	0.0000	De Flora (1981)
ECF, <i>Escherichia coli</i> KMBL 3835, forward mutation	+	0	220.0000	Kolman (1985)
ECW, <i>Escherichia coli</i> WP2 <i>uvrA</i> , reverse mutation	+	0	440.0000	Kolman & Näslund (1987)
EC2, <i>Escherichia coli</i> WP2, reverse mutation	+	0	440.0000	Kolman & Näslund (1987)
ECR, <i>Escherichia coli</i> WU36-10-89, reverse mutation	+	0	220.0000	Kolman & Näslund (1983)
ECR, <i>Escherichia coli</i> WU36-10 and WU-10-89, reverse mutation	+	0	220.0000	Kolman (1984)
ECR, <i>Escherichia coli</i> WP6 (<i>polA</i>), reverse mutation	+	0	220.0000	Kolman & Näslund (1987)
ECR, <i>Escherichia coli</i> WU36-10, reverse mutation	+	0	440.0000	Kolman <i>et al.</i> (1989a)
BSM, <i>Bacillus subtilis</i> , multigene test	(+)	0	580.0000 ^c	Jones & Adams (1981)
SCG, <i>Saccharomyces cerevisiae</i> D7, gene conversion	+	0	880.0000	Agurell <i>et al.</i> (1991)

Table 18 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ANG, <i>Aspergillus nidulans</i> , genetic crossing-over	-	0	88000.0000	Morpurgo (1963)
SCR, <i>Saccharomyces cerevisiae</i> D7, reverse mutation	+	0	880.0000	Agurell <i>et al.</i> (1991)
SGR, <i>Streptomyces griseoflavus</i> , reverse mutation	-	0	9.0000	Mashima & Ikeda (1958)
AZF, <i>Schizosaccharomyces pombe</i> , forward mutation	+	+	22.0000	Migliore <i>et al.</i> (1982)
ANF, <i>Aspergillus nidulans</i> , forward mutation	(+)	0	88000.0000	Morpurgo (1963)
NCR, <i>Neurospora crassa</i> , reverse mutation	+	0	1100.0000	Kølmark & Westergaard (1953)
NCR, <i>Neurospora crassa</i> , reverse mutation	+	0	6170.0000	Kilbey & Kølmark (1968)
NCR, <i>Neurospora crassa</i> , reverse mutation	+	0	66.0000	Kølmark & Kilbey (1968)
HSM, <i>Hordeum</i> species, chlorophyll mutation	+	0	0.5300 ^c	Ehrenberg <i>et al.</i> (1956)
HSM, <i>Hordeum</i> species, chlorophyll mutation	+	0	1200.0000	Ehrenberg & Gustafsson (1957)
HSM, <i>Hordeum</i> species, chlorophyll mutation	+	0	750.0000	Ehrenberg <i>et al.</i> (1959)
HSM, <i>Hordeum</i> species, waxy mutation	+	0	0.1800 ^c	Šulovská <i>et al.</i> (1969)
PLM, <i>Oryza sativa</i> , gene mutation	+	0	900.0000	Jana & Roy (1975)
PLM, Soya beans, gene mutation	+	0	500.0000	Sichkar (1980)
HSC, <i>Hordeum</i> species, chromosomal aberrations	+	0	900.0000	Moutschen-Dahmen <i>et al.</i> (1968)
TSC, <i>Tradescantia</i> species, chromosomal aberrations	+	0	14.0000 ^c	Smith & Lotfy (1954)
DMM, <i>Drosophila melanogaster</i> , somatic mutation	+		5000.0000	Fahmy & Fahmy (1970)
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutation	+		100000.0000	Rapoport (1948)
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutation	+		441.0000, inj.	Fahmy & Fahmy (1956)
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutation	+		5000.0000, inj.	Bird (1952)
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutation	+		4000.0000, inj.	Nakao & Auerbach (1961)
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutation	+		4000.0000	Watson (1966)
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutation	+		880.0000	Zijlstra & Vogel (1988)
DMH, <i>Drosophila melanogaster</i> , heritable translocation	+		4000.0000, inj.	Nakao & Auerbach (1961)
DMH, <i>Drosophila melanogaster</i> , heritable translocation	+		4000.0000	Watson (1966)
GCO, Gene mutation, Chinese hamster ovary cells, <i>hprt</i> locus	+	+	88.0000	Tan <i>et al.</i> (1981)
GCO, Gene mutation, Chinese hamster ovary cells, <i>hprt</i> locus	+	0	12.0000 ^c	Zamora <i>et al.</i> (1983)
G9H, Gene mutation, Chinese hamster V79 cells, <i>hprt</i> locus	+	0	2.2000 ^c	Hatch <i>et al.</i> (1986)

Table 18 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
G90, Gene mutation, Chinese hamster V79 cells, ouabain resistance	+	0	2.2000 ^c	Hatch <i>et al.</i> (1986)
G5T, Gene mutation, mouse L5178Y cells, <i>tk</i> locus	+	0	0.0000 ^e	Krell <i>et al.</i> (1979)
MIA, Micronucleus formation, Chinese hamster V79 cells	+	0	22.0000 ^c	Zhong <i>et al.</i> (1992)
CIC, Chromosomal aberrations, Chinese hamster V79 cells <i>in vitro</i>	+	0	6.0000 ^c	Zhong <i>et al.</i> (1992)
TCM, Cell transformation, mouse C3H10T1/2 cells	+	0	110.0000	Kolman <i>et al.</i> (1989b)
TCM, Cell transformation, mouse C3H10T1/2 cells	+	0	110.0000	Kolman <i>et al.</i> (1990)
T7S, Cell transformation, SA7/SHE cells	+	0	1.1000 ^c	Hatch <i>et al.</i> (1986)
UHL, Unscheduled DNA synthesis, human lymphocytes <i>in vitro</i>	+	0	44.0000	Pero <i>et al.</i> (1981)
GIH, Gene mutation, human fibroblasts <i>in vitro</i>	+	0	110.0000	Kolman <i>et al.</i> (1992)
GIH, Gene mutation, human fibroblasts <i>in vitro</i>	+	0	200.0000	Bastlová <i>et al.</i> (1993)
SHF, Sister chromatid exchange, human fibroblasts <i>in vitro</i>	+	0	36.0000	Star (1980)
SHL, Sister chromatid exchange, human lymphocytes <i>in vitro</i>	+	0	10.0000	Garry <i>et al.</i> (1982)
SHL, Sister chromatid exchange, human lymphocytes <i>in vitro</i>	+	0	70.0000 ^c	Tucker <i>et al.</i> (1986)
SHL, Sister chromatid exchange, human lymphocytes <i>in vitro</i>	+	0	110.0000	Agurell <i>et al.</i> (1991)
SHL, Sister chromatid exchange, human lymphocytes <i>in vitro</i>	+ ^f	0	10.0000	Hallier <i>et al.</i> (1993)
CHT, Chromosomal aberrations, transformed human amniotic cells <i>in vitro</i>	+	0	220.0000	Poirier & Papadopoulo (1982)
DVA, DNA single strand breaks, mouse spermatids <i>in vivo</i>	+		190.0000 inhal. 1 h	Sega <i>et al.</i> (1988)
DVA, DNA single strand breaks, mouse sperm <i>in vivo</i>	+		25.0000 × 1 ip	Sega & Generoso (1988)
GVA, Gene mutation, mouse spleen T-lymphocytes, <i>hprt</i> locus <i>in vivo</i>	+		100.0000 × 2 ip	Walker & Skopek (1993)
SLP, Mouse specific locus, postspermatogonia <i>in vivo</i>	-		160.0000, inhal. 6 h/d ^g	Russell <i>et al.</i> (1984)
SLO, Mouse specific locus, other stages <i>in vivo</i>	-		160.0000, inhal. 6 h/d ^g	Russell <i>et al.</i> (1984)
SLO, Mouse specific locus, other stages <i>in vivo</i>	+		125.0000, inhal. 6 h/d ^h	Lewis <i>et al.</i> (1986)
SVA, Sister chromatid exchange, rabbit lymphocytes <i>in vivo</i>	+		40.0000, inhal. 6 h/d, 12 w	Yager & Benz (1982)
SVA, Sister chromatid exchange, rat lymphocytes <i>in vivo</i>	+		19.0000, inhal. 6 h	Kligerman <i>et al.</i> (1983)
SVA, Sister chromatid exchange, monkey lymphocytes <i>in vivo</i>	+		14.0000, inhal. 7 h/d ⁱ	Lynch <i>et al.</i> (1984c)
SVA, Sister chromatid exchange, rabbit lymphocytes <i>in vivo</i>	+		26.0000, inhal. 0.5 h/d ^j	Yager (1987)
SVA, Sister chromatid exchange, monkey lymphocytes <i>in vivo</i>	+		14.0000, inhal. 7 h/d ⁱ	Kelsey <i>et al.</i> (1988)
SVA, Sister chromatid exchange, mouse bone-marrow cells <i>in vivo</i>	+		30.0000, ip × 1	Farooqi <i>et al.</i> (1993)
SVA, Sister chromatid exchange, rat bone-marrow cells <i>in vivo</i>	+		38.0000, 6 h/d, 3 mo	Ong <i>et al.</i> (1993)
SVA, Sister chromatid exchange, rat spleen <i>in vivo</i>	+		38.0000, 6 h/d, 3 mo	Ong <i>et al.</i> (1993)

Table 18 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVM, Micronucleus formation, mouse bone-marrow cells <i>in vivo</i>	+		100.0000 × 1 iv	Appelgren <i>et al.</i> (1978)
MVM, Micronucleus formation, mouse bone-marrow cells <i>in vivo</i>	+		10.0000 × 2 ip	Conan <i>et al.</i> (1979)
MVM, Micronucleus formation, mouse bone-marrow cells <i>in vivo</i>	+		150.0000 × 1 ip	Jensen & Ramel (1980)
MVM, Micronucleus formation, mouse bone-marrow cells <i>in vivo</i>	+		30.0000 mmol/kg ip × 1	Farooqi <i>et al.</i> (1993)
MVR, Micronucleus formation, rat bone-marrow cells <i>in vivo</i>	+		38.0000, inhal. 6 h/d ^k	Hochberg <i>et al.</i> (1990)
MVR, Micronucleus formation, rat bone-marrow cells <i>in vivo</i>	+		100.0000 × 1 iv	Appelgren <i>et al.</i> (1978)
CBA, Chromosomal aberration, rat bone-marrow cells <i>in vivo</i>	+		9.0000 × 1 po	Strekalova (1971)
CBA, Chromosomal aberrations, rat bone-marrow cells <i>in vivo</i>	+		26.0000, inhal. 6 h/d × 2	Fomenko & Strekalova (1973)
CBA, Chromosomal aberrations, rat bone-marrow cells <i>in vivo</i>	+		1.0000, inhal. 66 d	Strekalova <i>et al.</i> (1975)
CBA, Chromosomal aberrations, mouse bone-marrow cells <i>in vivo</i>	+		127.0000, inhal. 6 h/d	Ribeiro <i>et al.</i> (1987a)
CBA, Chromosomal aberrations, mouse bone-marrow cells <i>in vivo</i>	+		30.0000 × 1 ip	Farooqi <i>et al.</i> (1993)
CLA, Chromosomal aberrations, rat lymphocytes <i>in vivo</i>	-		170.0000, inhal. 6 h/d × 3	Kligerman <i>et al.</i> (1983)
CLA, Chromosomal aberrations, monkey lymphocytes <i>in vivo</i>	+		28.0000, inhal. 7 h/d ⁱ	Lynch <i>et al.</i> (1984c)
CCC, Chromosomal aberrations, mouse spermatocytes treated <i>in vivo</i> , spermatocytes observed	+		127.0000, inhal. 6 h/d	Ribeiro <i>et al.</i> (1987a)
DLM, Dominant lethal mutation, mouse <i>in vivo</i>	-		100.0000 × 1 iv	Appelgren <i>et al.</i> (1977)
DLM, Dominant lethal mutation, mouse <i>in vivo</i>	+		150.0000 × 1 ip	Generoso <i>et al.</i> (1980)
DLM, Dominant lethal mutation, mouse <i>in vivo</i>	+		160.0000, inhal. 6 h/d ^l	Generoso <i>et al.</i> (1983)
DLM, Dominant lethal mutation, mouse <i>in vivo</i>	+		190.0000, inhal. 6 h/d × 4	Generoso <i>et al.</i> (1986)
DLM, Dominant lethal mutation, mouse <i>in vivo</i>	+		130.0000, inhal. 6 h/d ^m	Generoso <i>et al.</i> (1990)
DLR, Dominant lethal mutation, rat <i>in vivo</i>	+		1.0000, inhal. 66 d	Strekalova <i>et al.</i> (1975)
DLR, Dominant lethal mutation, ras <i>in vivo</i>	+		250.0000, inhal. 4 h	Embree <i>et al.</i> (1977)
MHT, Mouse heritable translocation	+		30.0000 × 1 ip	Generoso <i>et al.</i> (1980)
MHT, Mouse heritable translocation	+		100.0000, inhal. 6 h/d ^m	Generoso <i>et al.</i> (1990)
AVA, Aneuploidy, mouse fetus <i>in vivo</i>	-		228.0000, inhal. 1.5 h	Katoh <i>et al.</i> (1989)
DVH, DNA strand breaks, human lymphocytes <i>in vivo</i>	-		0.0700	Mayer <i>et al.</i> (1991)
DVH, DNA cross-links, human lymphocytes <i>in vivo</i>	+		0.0000	Popp <i>et al.</i> (1992)
UVH, Unscheduled DNA synthesis, human lymphocytes <i>in vivo</i>	(+)		0.5000	Pero <i>et al.</i> (1981)

Table 18 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
*, Gene mutation, human lymphocytes <i>in vivo</i> , <i>hprt</i> locus	+		1.2000	Tates <i>et al.</i> (1991b)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	+		9.0000 ⁿ	Garry <i>et al.</i> (1979)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	+		0.0000 ⁿ	Abrahams (1980)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	(+)		0.0000	Lambert & Lindblad (1980)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	-		0.2500 ⁿ	Högstedt <i>et al.</i> (1983)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	+		0.0600 ⁿ	Yager <i>et al.</i> (1983)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	-		1.0000 ⁿ	Hansen <i>et al.</i> (1984)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	+		0.2000 ⁿ	Laurent <i>et al.</i> (1984)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	+		0.1000 ⁿ	Sarto <i>et al.</i> (1984b)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	+		1.2500 ⁿ	Stolley <i>et al.</i> (1984)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	+		0.0000 ⁿ	Richmond <i>et al.</i> (1985)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	+		0.4000 ⁿ	Sarto <i>et al.</i> (1987)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	+		0.0000	Laurent (1988)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	+		0.0700	Mayer <i>et al.</i> (1991)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	?		0.0800 ⁿ	Sarto <i>et al.</i> (1991)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	+		0.0030 ⁿ	Tates <i>et al.</i> (1991a)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	+		20.0000 ⁿ	Lerda & Rizzi (1992)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	+		0.0400 ⁿ	Schulte <i>et al.</i> (1992)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	-		1.2500 ⁿ	Tomkins <i>et al.</i> (1993)
MVH, Micronucleus formation, human bone-marrow cells <i>in vivo</i>	+		0.2500 ⁿ	Högstedt <i>et al.</i> (1983)
MVH, Micronucleus formation, human nasal cells <i>in vivo</i>	+ ^o		0.0000	Sarto <i>et al.</i> (1990)
MVH, Micronucleus formation, human buccal and nasal cells <i>in vivo</i>	-		0.0000	Sarto <i>et al.</i> (1990)
MVH, Micronucleus formation, human lymphocytes <i>in vivo</i>	-		0.0700	Mayer <i>et al.</i> (1991)
MVH, Micronucleus formation, human buccal cells and lymphocytes <i>in vivo</i>	-		0.0800 ⁿ	Sarto <i>et al.</i> (1991)
MVH, Micronucleus formation, human lymphocytes <i>in vivo</i>	+		1.2000 ⁿ	Tates <i>et al.</i> (1991a)
MVH, Micronucleus formation, human lymphocytes <i>in vivo</i>	-		0.0400 ⁿ	Schulte <i>et al.</i> (1992)
CLH, Chromosomal aberration, human lymphocytes <i>in vivo</i>	+		0.0000 ⁿ	Abrahams (1980)
CLH, Chromosomal aberration, human lymphocytes <i>in vivo</i>	(+)		0.5000	Pero <i>et al.</i> (1981)
CLH, Chromosomal aberration, human lymphocytes <i>in vivo</i>	+		0.0000	Thiess <i>et al.</i> (1981a)
CLH, Chromosomal aberration, human lymphocytes <i>in vivo</i>	-		0.2500 ⁿ	Högstedt <i>et al.</i> (1983)

Table 18 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CLH, Chromosomal aberration, human lymphocytes <i>in vivo</i>	(+)		0.1000 ⁿ	Sarto <i>et al.</i> (1984b)
CLH, Chromosomal aberration, human lymphocytes <i>in vivo</i>	-		0.0020 ⁿ	Clare <i>et al.</i> (1985)
CLH, Chromosomal aberration, human lymphocytes <i>in vivo</i>	+		0.0000 ⁿ	Richmond <i>et al.</i> (1985)
CLH, Chromosomal aberration, human lymphocytes <i>in vivo</i>	-		0.0300 ⁿ	van Sittert <i>et al.</i> (1985)
CLH, Chromosomal aberration, human lymphocytes <i>in vivo</i>	+		1.2000 ⁿ	Galloway <i>et al.</i> (1986)
CLH, Chromosomal aberration, human lymphocytes <i>in vivo</i>	+		0.0000	Karelová <i>et al.</i> (1987)
CLH, Chromosomal aberration, human lymphocytes <i>in vivo</i>	+		0.5000, inhal. 8 h/d	Högstedt <i>et al.</i> (1990) ^p
CLH, Chromosomal aberration, human lymphocytes <i>in vivo</i>	-		0.0700	Mayer <i>et al.</i> (1991)
CLH, Chromosomal aberration, human lymphocytes <i>in vivo</i>	+		0.0030 ⁿ	Tates <i>et al.</i> (1991a)
CLH, Chromosomal aberration, human lymphocytes <i>in vivo</i>	+		20.0000 ⁿ	Lerda & Rizzi (1992)
BID, Binding (covalent) to calf thymus DNA <i>in vitro</i>	+	0	590.0000	Segerbäck (1990)
BID, Binding (covalent) to calf thymus DNA <i>in vitro</i>	+	0	88000.0000	Li <i>et al.</i> (1992)
BIP, Binding (covalent) to haemoglobin <i>in vitro</i>	+	0	590.0000	Segerbäck (1990)
BVD, Binding (covalent) to mouse DNA <i>in vivo</i>	+		0.2000 inhal. 2 h	Ehrenberg <i>et al.</i> (1974)
BVD, Binding (covalent) to rat DNA <i>in vivo</i>	+		0.9000 × 1 ip	Osterman-Golkar <i>et al.</i> (1983)
BVD, Binding (covalent) to mouse DNA <i>in vivo</i>	+		2.0000 × 1 ip	Segerbäck (1983)
BVD, Binding (covalent) to rat DNA <i>in vivo</i>	+		4.0000, inhal. 6 h	Potter <i>et al.</i> (1989)
BVD, Binding (covalent) to mouse DNA <i>in vivo</i>	+		32.0000, inhal. 1 h	Sega <i>et al.</i> (1991)
BVD, Binding (covalent) to mouse DNA <i>in vivo</i>	+		63.0000, inhal. 6 h/d ^q	Walker <i>et al.</i> (1992b)
BVD, Binding (covalent) to rat DNA <i>in vivo</i>	+		12.5000 inhal. 6 h/d ^q	Walker <i>et al.</i> (1992b)
Protein binding				
BVP, Binding (covalent) to mouse haemoglobin <i>in vivo</i>	+		2.0000 × 1 ip	Segerbäck (1983)
BVP, Binding (covalent) to rat haemoglobin <i>in vivo</i>	+		4.0000, inhal. 6 h	Potter <i>et al.</i> (1989)
BVP, Binding (covalent) to mouse haemoglobin <i>in vivo</i>	+		32.0000, inhal. 1 h	Sega <i>et al.</i> (1991)
BVP, Binding (covalent) to mouse haemoglobin <i>in vivo</i>	+		6.3000, inhal. 6 h/d ^q	Walker <i>et al.</i> (1993)
BVP, Binding (covalent) to rat haemoglobin <i>in vivo</i>	+		3.8000, inhal. 6 h/d ^q	Walker <i>et al.</i> (1993)
BHP, Binding (covalent) to human haemoglobin <i>in vivo</i>	+		23.0000 ⁿ	Calleman <i>et al.</i> (1978)
BHP, Binding (covalent) to human haemoglobin <i>in vivo</i>	+		0.0000	Farmer <i>et al.</i> (1986)
BHP, Binding (covalent) to human haemoglobin <i>in vivo</i>	+		0.0000	Hagmar <i>et al.</i> (1991)
BHP, Binding (covalent) to human haemoglobin <i>in vivo</i>	+		0.0000	Mayer <i>et al.</i> (1991)

Table 18 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BHP, Binding (covalent) to human haemoglobin <i>in vivo</i>	+		0.0050 ⁿ	Sarto <i>et al.</i> (1991)
BHP, Binding (covalent) to human haemoglobin <i>in vivo</i>	+		0.0400 ⁿ	Schulte <i>et al.</i> (1992)
SPM, Sperm morphology, mouse <i>in vivo</i>	+		127.000, inhalation 6 h/d × 5	Ribeiro <i>et al.</i> (1987b)

*Not on profile

^a+, positive; (+), weak positive; -, negative; 0, not tested; ?, inconclusive (variable response within several experiments within an adequate study)

^bIn-vitro tests, µg/ml; in-vivo tests, mg/kg bw

^cAtmospheric concentration in exposure chamber (µg/ml)

^dIncubated in Tedlar bags

^eCells cultured in ethylene oxide-sterilized polycarbonate flasks

^fSingle concentration, positive only for non-conjugators of glutathione

^gSixty days total over a five-month period

^hFive days/week; six to seven months; mating started 7th week of exposure and continued throughout exposure period

ⁱFive days/week; two years (study group from Lynch *et al.*, 1984b)

^jFive days/week; 16 days

^kFive days/week; three, six and nine months

^lFive days/week; two or 11 weeks

^mFive days/week; six weeks then daily 2.5 weeks

ⁿInhalation; dose based on time-weighted average (TWA) concentration in work area

^oPositive in two of three workers exposed by accidental leakage

^pNo controls (not on profile)

^qFive days/week; four weeks

5.2 Human carcinogenicity data

In epidemiological studies of exposure to ethylene oxide, the most frequently reported association has been with lymphatic and haematopoietic cancer. The populations studied fall into two groups—people using ethylene oxide as a sterilant and chemical workers manufacturing or using the compound. In general, people involved in sterilization are less likely to have occupational exposure to other chemicals.

Of the studies of sterilization personnel, the largest and most informative is that conducted in the USA. Overall, mortality from lymphatic and haematopoietic cancer was only marginally elevated, but a significant trend was found, especially for lymphatic leukaemia and non-Hodgkin's lymphoma, in relation to estimated cumulative exposure to ethylene oxide. For exposure at a level of 1 ppm [1.8 mg/m³] over a working lifetime (45 years), a rate ratio of 1.2 was estimated for lymphatic and haematopoietic cancer. Three other studies of workers involved in sterilization (two in Sweden and one in the United Kingdom) each showed nonsignificant excesses of lymphatic and haematopoietic cancer.

In a study of chemical workers exposed to ethylene oxide at two plants in the USA, the mortality rate from lymphatic and haematopoietic cancer was elevated, but the excess was confined to a small subgroup with only occasional low-level exposure to ethylene oxide. Six other studies in the chemical industry (two in Sweden, one in the United Kingdom, one in Italy, one in the USA and one in Germany) were based on fewer deaths. Four found excesses of lymphatic and haematopoietic cancer (which were significant in two), and in two, the numbers of such tumours were as expected from control rates.

Because of the possibility of confounding occupational exposures, less weight can be given to the positive findings from the studies of chemical workers. Nevertheless, they are compatible with the small but consistent excesses of lymphatic and haematopoietic cancer found in the studies of sterilization personnel.

Some of the epidemiological studies of workers exposed to ethylene oxide show an increased risk for cancer of the stomach, which was significant only in one study from Sweden.

5.3 Animal carcinogenicity data

Ethylene oxide was tested for carcinogenicity in one experiment by oral administration in rats, in two experiments by inhalation in mice and two experiments by inhalation in rats. It was also tested in single studies in mice by skin application and by subcutaneous injection.

In the experiment by intragastric intubation in rats, ethylene oxide produced tumours of the forestomach, which were mainly squamous-cell carcinomas. In one study in mice, inhalation of ethylene oxide resulted in increased incidences of alveolar/bronchiolar lung tumours and tumours of the Harderian gland in animals of each sex and of uterine adenocarcinomas, mammary carcinomas and malignant lymphomas in females. In a bioassay of pulmonary tumours in strain A mice, inhalation of ethylene oxide increased the number of pulmonary adenomas per mouse. In the two experiments in which rats of one strain were exposed by inhalation, ethylene oxide increased the incidences of mononuclear-cell leukaemia and brain tumours in animals of each sex and of peritoneal mesotheliomas in the

region of the testis and subcutaneous fibromas in males. Ethylene oxide produced local sarcomas in mice following subcutaneous injection. In a limited study in mice treated by skin application, no skin tumours were observed.

5.4 Other relevant data

Inhaled ethylene oxide is readily taken up in man and rat, and aqueous ethylene oxide solutions can penetrate human skin. Ethylene oxide is uniformly distributed throughout the body of rats. Its half-life has been estimated as between 14 min and 3.3 h in the human body and about 6 min in rats. Exposure of rats to 5 ppm [9 mg/m³] resulted in steady-state ethylene oxide levels in blood of 60 ng/g. Whole-body elimination of ethylene oxide from rats is described by first-order kinetics. It is excreted mainly in the urine as thioethers; at high doses, the proportion of thioethers is reduced, while the proportion of ethylene glycol increases. Rats conjugate ethylene oxide with glutathione to a greater extent than mice, while rabbits do not appear to be capable of this reaction.

Ethylene oxide was not teratogenic to rats or rabbits exposed by inhalation to concentrations up to 150 ppm [270 mg/m³]. It was teratogenic to mice after intravenous injection in a single study. Surprisingly, brief exposure of dams around the time of fertilization to a high concentration (1200 ppm [2160 mg/m³]) of ethylene oxide by inhalation induced teratogenic effects in mice. The effect was shown to be due to a direct action on the zygote.

Ethylene oxide forms adducts with proteins in both man and experimental animals and with DNA in experimental animals. Haemoglobin adducts have been used for biomonitoring, as there is a significant correlation between cumulative exposure over four months and levels of N-terminal hydroxyethyl valine in haemoglobin of exposed workers. The increment of hydroxyethyl valine adduct formed is about 3.5 pmol/g haemoglobin per ppm-h ethylene oxide. Higher proportions of hydroxyethyl histidine are formed. Hydroxyethyl haemoglobin adducts are also found in the absence of known exposure to ethylene oxide. Greater numbers of haemoglobin and DNA adducts occur per unit of exposure in rats and mice at high concentrations (> 33 ppm) than at lower concentrations. 7-Hydroxyethyl guanine is quantitatively the most important DNA adduct formed. Its half-life varies from 1.0 to 6.9 days in mouse and rat tissues.

Studies of workers exposed to ethylene oxide in hospital and factory sterilization units and in ethylene oxide manufacturing and processing plants consistently showed chromosomal damage in peripheral blood lymphocytes, including chromosomal aberrations in 11 of 14 studies, sister chromatid exchange in 20 of 23 studies, micronuclei in three of eight studies and gene mutation in one study. Micronuclei were induced in the bone marrow of exposed workers in one study. In general, the degree of damage is correlated with level and duration of exposure. The induction of sister chromatid exchange appears to be more sensitive to exposure to ethylene oxide than is that of either chromosomal aberrations or micronuclei. In one study, chromosomal aberrations were observed in the peripheral lymphocytes of workers two years after cessation of exposure to ethylene oxide, and sister chromatid exchanges six months after cessation of exposure.

Chromosomal aberrations and sister chromatid exchange were induced in cynomolgus monkeys exposed to ethylene oxide. Ethylene oxide also induced gene mutation, specific

locus mutation, sister chromatid exchange, chromosomal aberrations, micronuclei, dominant lethal mutation and heritable translocation in rodents treated *in vivo*. It induced unscheduled DNA synthesis, gene mutation, sister chromatid exchange and chromosomal aberrations in human cells and gene mutation, micronuclei, chromosomal aberrations and cell transformation in rodent cells *in vitro*.

Analogous genetic and related effects were observed in nonmammalian systems.

5.5 Evaluation¹

There is *limited evidence* in humans for the carcinogenicity of ethylene oxide.

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

In making the overall evaluation, the Working Group took into consideration the following supporting evidence. Ethylene oxide is a directly acting alkylating agent that:

- (i) induces a sensitive, persistent dose-related increase in the frequency of chromosomal aberrations and sister chromatid exchange in peripheral lymphocytes and micronuclei in bone-marrow cells of exposed workers;
- (ii) has been associated with malignancies of the lymphatic and haematopoietic system in both humans and experimental animals;
- (iii) induces a dose-related increase in the frequency of haemoglobin adducts in exposed humans and dose-related increases in the numbers of adducts in both DNA and haemoglobin in exposed rodents;
- (iv) induces gene mutations and heritable translocations in germ cells of exposed rodents; and
- (v) is a powerful mutagen and clastogen at all phylogenetic levels.

Overall evaluation

Ethylene oxide *is carcinogenic to humans (Group I)*.

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¹For definition of the italicized terms, see Preamble, pp. 27-30.

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PROPYLENE

This substance was considered by a previous Working Group, in February 1978 (IARC, 1979). 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.: 115-07-1

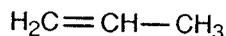
Replaced CAS Reg. No.: 676-63-1; 33004-01-2

Chem. Abstr. Name: 1-Propene

IUPAC Systematic Name: Propene

Synonyms: Methylethylene; 1-propylene

1.1.2 Structural and molecular formulae and relative molecular mass



C_3H_6

Relative molecular mass: 42.08

1.1.3 Chemical and physical properties of the pure substance

- (a) *Description:* Colourless gas (American Conference of Governmental Industrial Hygienists, 1991; Lide, 1991)
- (b) *Boiling-point:* $-47.4\text{ }^\circ\text{C}$ (Lide, 1991)
- (c) *Melting-point:* $-185.2\text{ }^\circ\text{C}$ (Lide, 1991)
- (d) *Density:* 1.9149 g/L at $25\text{ }^\circ\text{C}$, 760 mm Hg [101.3 kPa] (gas) (Eisele & Killpack, 1993); 0.5193 at $20\text{ }^\circ\text{C}/4\text{ }^\circ\text{C}$ (liquid under pressure) (Lide, 1991)
- (e) *Spectroscopy data:* Infrared [prism, 6403] and mass spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991).
- (f) *Solubility:* Soluble in acetic acid and ethanol (Lide, 1991)
- (g) *Volatility:* Vapour pressure, 10 atm [1013 kPa] at $19.8\text{ }^\circ\text{C}$ (Lide, 1991); relative vapour density (air = 1), 1.49 (Eisele & Killpack, 1993)
- (h) *Stability:* Lower explosive limit (in air), 2.0 vol% (35 g/m^3) at 750 mm Hg [100 kPa] and $20\text{ }^\circ\text{C}$ (Eisele & Killpack, 1993)

- (i) *Octanol-water partition coefficient (P)*: log P, 1.77 (Hansch & Leo, 1979)
- (j) *Conversion factor*: $\text{mg/m}^3 = 1.72 \times \text{ppm}^a$

1.1.4 *Technical products and impurities*

Propylene is available commercially in refinery, chemical and polymer grades (Anon., 1992). The grades of lower purity are generally used in close proximity to where they are produced, and the polymer grade dominates the commercial market. Polymer-grade propylene typically has a minimum purity of 99.5–99.8% and contains the following impurities: propane, methane, ethane, ethylene, propyne, butenes, propadiene, methylacetylene, butadiene, acetylene, diolefins, carbonyl sulfide, hydrogen, carbon monoxide, carbon dioxide, oxygen, nitrogen, water and sulfur (Dow Chemical Co., 1989; Phillips 66 Co., 1992; Eisele & Killpack, 1993; Exxon Chemical Co., undated a). Chemical-grade propylene typically has a minimum purity of 92.0–95.0% (Amoco Chemical Co., 1993; Eisele & Killpack, 1993; Exxon Chemical Co., undated b). Refinery-grade propylene generally contains 50–70% propylene admixed with other low relative molecular mass hydrocarbons (Eisele & Killpack, 1993).

1.1.5 *Analysis*

Atmospheric hydrocarbons, including propylene, can be detected and measured by capillary column gas chromatography and a flame ionization detector. The lower limit of measurement is 10 ppb by volume; this can be extended to 0.1 ppb by concentrating 100 ml of a gas sample in a freeze trap (Locke *et al.*, 1989). A variation on this method consists of preconcentration in a two-stage cryotrap system and an aluminium oxide-coated column; the limit of detection was 3 ppt (Schmidbauer & Oehme, 1985) or 2 pg (Matuska *et al.*, 1986). A similar method is based on sample enrichment on a solid sorbent, a zeolite, at room temperature, followed by heat desorption for gas chromatographic separation and flame ionization detection (Persson & Berg, 1989). Another method involves use of solid sorbent tubes in series (Tenax TA + Carbosphere S) and analysis by gas chromatography with electron capture detection in parallel with a tandem photoionization and flame ionization system; the limit of detection for propylene was 12 ppt (Reineke & Bächmann, 1985).

Methods have been developed for monitoring occupational exposures to propylene by determining a haemoglobin (Hb) adduct, *N*-(2-hydroxypropyl)valine (HOPrVal) of the metabolite, propylene oxide, using gas chromatography-mass spectrometry (Törnqvist *et al.*, 1986) and gas chromatography-electron capture detection (Kautiainen & Törnqvist, 1991).

1.2 **Production and use**

1.2.1 *Production*

Propylene is produced primarily as a by-product of petroleum refining and of ethylene production by steam cracking of hydrocarbon feedstocks (Schoenberg *et al.*, 1982). In

^aCalculated from: $\text{mg/m}^3 = (\text{relative molecular mass}/24.45) \times \text{ppm}$, assuming normal temperature (25 °C) and pressure (101.3 kPa)

refinery production, propylene is formed as a by-product of catalytic cracking (and to a lesser extent thermal cracking) of gas oils. In steam cracking, a mixed stream of hydrocarbons ranging from ethane to gas oils is pyrolysed with steam. The product can be changed to optimize production of ethylene, propylene or other alkenes by altering feedstock, temperature and other parameters (Schoenberg *et al.*, 1982; Mannsville Chemical Products Corp., 1987; Eisele & Killpack, 1993).

Propylene has also been obtained by the catalytic dehydrogenation of propane in situations in which the propane value is low and derivatives are limited to local markets (Mannsville Chemical Products Corp., 1987).

Worldwide production of propylene in a number of countries is shown in Table 1. The percentage (by region) of worldwide production of propylene for use as a chemical intermediate is presented in Table 2. Production in 1990 by the countries of the European Union was 8005 thousand tonnes (European Commission, 1993).

Table 1. Worldwide production of propylene (thousand tonnes)

Country or region	Year					
	1982	1984	1986	1988	1990	1992
Canada	693	660	628	669	765	753
China	314	368	442	708	963	NR
France	1033	1203	1036	1496	1432	1795
Germany (western)	1478	1800	1403	1540	1827	2041
Italy	445	564	NR	NR	NR	NR
Japan	2565	2981	3167	3682	4215	4536
Mexico	158	208	232	281	NR	NR
Republic of Korea	NR	NR	307	351	608	1482
Taiwan	225	341	441	441	398	401
United Kingdom	769	974	862	1354	750	832
USA	5686	7058	7494	9627	9909	10 248 ^a

From Scientific & Technical Information Research Institute of the Ministry of Chemical Industry of China (1984); Anon. (1985, 1987, 1989a, 1991, 1993); China National Chemical Information Centre (1993); Japan Petrochemical Industry Association (1993); NR, not reported

^aPreliminary

Information available in 1991 indicated that propylene was produced by 24 companies in the USA, 16 in Japan, nine in Germany, four each in Brazil, Canada and France, three each in Belgium, China, the Netherlands and the United Kingdom, two each in the Republic of Korea, Spain and the former Yugoslavia, and one each in Argentina, Australia, Austria, Bulgaria, the former Czechoslovakia, Finland, India, Italy, Norway, Singapore, South Africa, Thailand and Venezuela (Chemical Information Services Ltd, 1991).

1.2.2 Use

Propylene is a major chemical intermediate. The most important derivatives of chemical and polymer grade propylene are polypropylene, acrylonitrile, propylene oxide, isopropanol

Table 2. Production of propylene for use as a chemical intermediate

Region	Production (%)		
	1980	1984	1990
North America	36.5	34.2	33.0
South America	3.5	4.3	4.8
Western Europe	31.3	31.9	30.2
Eastern Europe	9.1	10.1	8.9
Japan	14.2	13.2	13.9
Asia - Pacific	5.0	6.1	7.9
Others	0.4	0.2	1.3

From Eisele & Killpack (1993)

and cumene. Other commercial derivatives include acrylic acid and esters, oxo alcohols and aldehydes, epichlorohydrin, synthetic glycerine and ethylene-propylene copolymers. Use of polypropylene in plastics (injection moulding) and fibres (carpets) accounts for over one-third of US consumption; other applications are in wire insulating, film and blow moulding. Until the mid 1970s, polypropylene accounted for less than 25% of propylene consumption. Acrylonitrile, produced from chemical-grade propylene and ammonia and used as a precursor for fibres, plastics and nitrile rubber, makes up about 15% of US demand. Use patterns of propylene for several years in the USA are presented in Table 3; similar patterns are seen throughout the world (Schoenberg *et al.*, 1982; Anon., 1983, 1986; Mannsville Chemical Products Corp., 1987; Anon., 1989b, 1992; Eisele & Killpack, 1993). The pattern of use of propylene as a chemical intermediate in Japan in 1992 was: 48% for polypropylene, 15% for acrylonitrile, 9% for butanol and octanol (oxo alcohols), 7% for isopropanol, acetone and phenol, 6% for propylene oxide, 1% for ethylene-propylene rubber and 14% for other uses and exports (Japan Petrochemical Industry Association, 1993).

Refinery production accounts for about 20% of the chemical industry's consumption of propylene in Europe and for more than 40% in the USA. Refineries in the USA use about 75% of their propylene production in nonchemical applications, to prepare alkylates (octane enhancers) in gasoline production, to produce liquefied petroleum gas and as refinery heating gas (Eisele & Killpack, 1993).

1.3 Occurrence

Propylene in the environment arises from both anthropogenic and natural sources: as a natural product from vegetation, as a product of the combustion of organic matter (biomass burning, motor vehicle exhausts and tobacco smoke) and releases during the production and use of propylene (Altshuller, 1983; Tille *et al.*, 1985; Löfroth *et al.*, 1989; Rudolph *et al.*, 1989).

Table 3. Patterns of use of propylene as a chemical intermediate in the USA

Form of propylene	Use (%)			
	1983	1986	1989	1992
Polypropylene	27	36	38	39
Acrylonitrile	18	16	14	14
Propylene oxide	10	11	11	11
Cumene	8	8	10	10
Oxo alcohols	7	8	8	8
Isopropanol	7.5	6	6	7
Propylene oligomers	7	6	5	5
Acrylic acid	4	3	3	3
Miscellaneous ^a	12	6	5	3

From Anon. (1983, 1986, 1989b, 1992)

^aIncludes allyl chloride, ethylene/propylene elastomers, acrolein and exports

Annual US emissions of propylene from natural and anthropogenic sources have been calculated to range between 440 and 600 thousand tonnes (Altshuller, 1983; Middleton *et al.*, 1990). Annual emissions of propylene in western Europe between 35° and 50° N latitude have been calculated on the basis of data on source strength and emissions at 10 600 thousand tonnes and 2300 thousand tonnes, respectively (Tille *et al.*, 1985).

1.3.1 Natural occurrence

Propylene has been identified in emissions from vegetation. In a study of C₂–C₇ hydrocarbon emissions from 12 forest species near Baton Rouge, LA, USA, propylene was emitted from ash, elm, cypress and hackberry trees at levels ranging from 5 to 20 µg/kg foliage per h (Khalil & Rasmussen, 1992). The average emission rate of propylene from Chinese rice fields was estimated to be 0.4 µg/m² per h (Khalil *et al.*, 1990).

1.3.2 Occupational exposure

The National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health in the USA between 1981 and 1983 indicated that 7300 US employees were potentially exposed to propylene at work (US National Institute for Occupational Safety and Health, 1993). Of this number, 88% were estimated to be exposed to propylene and 12% to materials containing propylene. The estimate is based on a survey of US companies and did not involve measurements of actual exposures.

Few data are available on levels of exposure to propylene in the workplace. In a study on exposures of firefighters, samples taken during the 'knockdown' phase of a fire contained a concentration of 8 ppm [13.8 mg/m³] propylene; none was detected during the 'overhaul' phase (Jankovic *et al.*, 1991). In a laboratory study, propylene was identified as a thermal degradation product of polypropylene but was not detected in area samples taken in the

vicinity of injection moulding, extrusion and welding machines in four plants in which polypropylene was processed (Frostling *et al.*, 1984; Purohit & Orzel, 1988).

1.3.3 Air

Propylene concentrations measured in ambient air at rural and remote sites worldwide ranged from 0.02 to 8.3 $\mu\text{g}/\text{m}^3$ (Anlauf *et al.*, 1985; Colbeck & Harrison, 1985; Kanakidou *et al.*, 1989; Lightman *et al.*, 1990; Hov *et al.*, 1991; Mowrer & Lindskog, 1991; Satsumabayashi *et al.*, 1992).

In urban and polluted air, propylene concentrations typically range from 0.6 to 55 $\mu\text{g}/\text{m}^3$. Levels measured as monthly means in a number of cities around the world were: 0.4–18.7 ppb [0.7–32.3 $\mu\text{g}/\text{m}^3$] in Bombay, India (Netravalkar & Rao, 1984; Rao & Pandit, 1988); 0.3 ppb [0.5 $\mu\text{g}/\text{m}^3$] as an average of 192 samples in 1981 in Tokyo, Japan (Uno *et al.*, 1985); 9.9 ppb as carbon (ppbC) [5.7 $\mu\text{g}/\text{m}^3$] in northwest England (Colbeck & Harrison, 1985); a range of 7–32 ppb by volume (ppbv) [12–55 $\mu\text{g}/\text{m}^3$] in Los Angeles, CA, USA (Grosjean & Fung, 1984) and 0.6–4.7 $\mu\text{g}/\text{m}^3$ in Chicago, IL, USA (Aronian *et al.*, 1989). Seinfeld (1989) reported a median propylene level of 7.7 ppbC [4.4 $\mu\text{g}/\text{m}^3$] for 39 US cities.

Although propylene is not a fuel component, it is present in motor vehicle exhaust as a result of fuel-rich combustion of hydrocarbon fuels (Stump *et al.*, 1989). Propylene accounted for 5% w/v of the non-methane hydrocarbon emissions from 67 Australian vehicles tested (Nelson & Quigley, 1984). In 1983, propylene emissions from petrol exhaust in the United Kingdom were estimated at 13 300 tonnes (Bailey *et al.*, 1990a,b). The following levels of propylene were determined in air samples representative of various traffic emissions in Sweden: 28 and 26 $\mu\text{g}/\text{m}^3$ (urban intersection), 6.5 and 3.8 $\mu\text{g}/\text{m}^3$ (fast suburban traffic) and 24 $\mu\text{g}/\text{m}^3$ (cold starts at a garage exit) (Löfgren & Petersson, 1992). Propylene concentrations of 13–160 $\mu\text{g}/\text{m}^3$ were determined in the Tingstad Tunnel in Göteborg, Sweden (Barrefors & Petersson, 1992).

Industrial emissions of propylene to the air in the USA in 1991 were reported to amount to 10 400 tonnes (US National Library of Medicine, 1993).

Cigarette smoke is also a significant source of exposure to propylene, as 1.3–1.4 mg propylene are released per cigarette (Persson *et al.*, 1988). Propylene concentrations of 40 and 70 $\mu\text{g}/\text{m}^3$ were found in two studies of tavern air during normal smoking conditions. The corresponding outdoor air concentration at the time was 6 $\mu\text{g}/\text{m}^3$ in both studies (Löfroth *et al.*, 1989).

A mean propylene concentration of 120 ppbC [69 $\mu\text{g}/\text{m}^3$] was determined in the indoor air of rural Nepali houses, where biomass combustion is the main source of energy (Davidson *et al.*, 1986).

The average atmospheric lifetime of propylene is estimated to be less than one day at low latitudes (Rudolph *et al.*, 1989). Propylene is subject to photochemical degradation by reaction with OH radicals (Tille *et al.*, 1985).

1.4 Regulations and guidelines

Propylene has been classified in several countries as an asphyxiant, because its presence at high concentrations in air lowers the available oxygen concentrations. Countries in which

it is classified as an asphyxiant include Australia, Belgium, Canada, Hungary, the Netherlands, the United Kingdom and the USA. Nevertheless, the major hazard is due to its inflammable and explosive character. No exposure limits have been established in most countries, but Switzerland established a time-weighted average occupational exposure limit of 17 500 mg/m³ (about one-half the lower explosive limit) in 1987 (ILO, 1991; American Conference of Governmental Industrial Hygienists, 1993; UNEP, 1993).

The US Food and Drug Administration (1993) has established regulations for the use of monomers, polymers, homopolymers and copolymers of propylene in products in contact with food.

2. Studies of Cancer in Humans

After the observation of an apparent cluster of colorectal cancers at a polypropylene manufacturing plant, Acquavella *et al.* (1988) carried out a cohort study of men who had been at the plant for six months or longer during 1960–85, allowing a 10-year induction period from first exposure. Seven incident colorectal cancers were ascertained (1.3 expected). Subsequently, a case–control study of adenomatous polyps and carcinoma *in situ* of the large bowel was carried out in the same workforce (Acquavella *et al.*, 1991). The occupational exposures of 24 cases identified in a screening programme were compared with those of 72 controls who had been found to be free of polyps. Propylene was handled at the plant, along with various other chemicals, but neither of the reports classified subjects according to whether they would have been exposed to propylene. They do not allow assessment of risk in relation to propylene exposure specifically.

3. Studies of Cancer in Experimental Animals

3.1 Inhalation

3.1.1 Mouse

Groups of 49–50 male and 50 female B6C3F1 mice, 9–10 weeks old, were exposed by inhalation to air containing 0 (control), 5000 or 10 000 ppm (8600 or 17 200 mg/m³) propylene (purity, > 99%) for 6 h per day on five days per week for 103 weeks. The highest concentration of propylene was limited to 10 000 ppm because of the risk of explosion. Animals were killed at 104 weeks or when moribund. Survival of exposed and control mice was comparable. After week 59 of the study, the mean body weights of high-dose male mice were slightly lower (5%) than those of controls; the weights of animals in other exposed groups were comparable with those of the controls. Haemangiosarcomas were found in one low-dose male mouse (liver), two high-dose male mice (spleen) and three high-dose female mice (subcutis, spleen and uterus). Haemangiomas were found in one low-dose and one high-dose female mice (liver). In female mice, haemangiomas or haemangiosarcomas at all sites (combined) occurred in 0/50 controls, 1/49 low-dose and 4/50 high-dose mice ($p = 0.024$, incidental tumour test for trend). The overall historical incidence in female

controls was 90/2537 (3.5%; range, 0–10%). The occurrence of uterine endometrial stromal polyps in female mice showed a positive trend (control, 0/47; low-dose, 0/47; and high-dose, 3/48; $p = 0.044$, Cochran-Armitage test); the mean historical control rate was 22/2411 (0.9%; range, 0–6%). In male mice, the incidence of alveolar/bronchiolar adenomas or carcinomas (combined) showed a negative trend (control, 16/50; low-dose, 4/49; and high-dose, 7/50; $p < 0.05$, Cochran-Armitage trend test). The historical incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in male mice was 397/2380 (16.7%; range, 2–34%) (Quest *et al.*, 1984; US National Toxicology Program, 1985).

Groups of 100 male and 100 female Swiss mice, seven weeks old, were exposed by inhalation to air containing 0 (control), 200, 1000 or 5000 ppm (344 mg/m³, 1720 mg/m³ or 8600 mg/m³, respectively) propylene (purity: 97.0% propylene, 2.9% [propane], 6.5 ppm ethylene, 2.5 ppm ethane, 1 ppm methane) for 7 h per day on five days per week for 78 weeks. All animals were kept under observation until spontaneous death [times unspecified]. No difference in body weight was observed between treated groups and controls. A slightly increased mortality rate was observed among male mice exposed to 5000 ppm [exact numbers and toxic effects unspecified]. No difference was reported in the incidences of tumours among the different groups (Ciliberti *et al.*, 1988). [The Working Group noted the incomplete reporting.]

3.1.2 Rat

Groups of 50 male and 50 female Fischer 344/N rats, 9–10 weeks old, were exposed by inhalation to air containing 0 (control), 5000 or 10 000 ppm (8600 or 17 200 mg/m³) propylene (purity, > 99%) for 6 h per day on five days per week for 103 weeks. The highest concentration of propylene was limited to 10 000 ppm because of the risk of explosion. Animals were killed at 102 weeks or when moribund. Survival of exposed and control rats was comparable. Throughout most of the study, the mean body weights of exposed male and female rats were slightly lower (0–5%) than those of the controls, but the decrements were not related to concentration. An increased incidence of squamous metaplasia of the nasal cavity was observed in treated female rats (control, 0/49; low-dose, 15/50; high-dose, 6/50) and in low-dose male rats (control, 2/50; low-dose, 19/50; high-dose, 7/50). The incidence of epithelial hyperplasia of the nasal cavity was increased in high-dose female rats (control, 0/49; low-dose, 4/50; high-dose, 9/50). Inflammation of the nasal cavity occurred at increased incidences in low- and high-dose male and in high-dose female rats. No treatment-related increase in tumour incidence was observed (Quest *et al.*, 1984; US National Toxicology Program, 1985).

Groups of 100 or 120 male and 100 or 120 female Sprague-Dawley rats, 17 weeks old, were exposed by inhalation to air containing 0 (control), 200, 1000 or 5000 ppm (0, 344, 1720 or 8600 mg/m³) propylene (purity: 97.0% propylene, 2.9% [propane], 6.5 ppm ethylene, 2.5 ppm ethane, 1 ppm methane) for 7 h per day on five days per week for 104 weeks. All the animals were kept under observation until spontaneous death [times unspecified]. A slightly increased mortality rate was observed among mid- and high-dose male rats [not otherwise specified]. No difference was found in the incidences of tumours among the different groups (Ciliberti *et al.*, 1988). [The Working Group noted the incomplete reporting.]

3.2 Carcinogenicity of metabolites

See the monograph on propylene oxide

4. Other Data Relevant for an Evaluation of Carcinogenicity and Its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

No data were available on the absorption, distribution or excretion of propylene in humans.

Hb adducts of the metabolic propylene intermediate propylene oxide (see below) have been used to monitor internal dose (see the monograph on propylene oxide, p. 193). In nonsmokers not exposed occupationally to propylene, the background level of the 2-hydroxypropyl adduct to the N-terminal valine (HOPrVal) of Hb was found to be about 2 pmol/g Hb. It was estimated that smoking 10 cigarettes per day would induce an increment of 2 pmol/g globin (Törnqvist & Ehrenberg, 1990). Occupational exposure to propylene at 1 ppm [1.72 mg/m³] (40-h time-weighted average) was assumed to be associated with an increment of 5 pmol/g Hb (Kautiainen & Törnqvist, 1991).

4.1.2 Experimental systems

(a) Propylene

Propylene was oxidized to epoxypropane (propylene oxide), yielding about equal amounts of the two enantiomers (R, 43–50%, S, 50–57%), in incubates (37 °C, pH 7.4) containing human liver microsomes, an NADPH-regenerating system and trichloropropylene oxide to inhibit epoxide hydrolase activity (Wistuba *et al.*, 1989).

The pharmacokinetics of inhaled propylene have been investigated in male Sprague-Dawley rats (Golka *et al.*, 1989) and CBA mice (Svensson & Osterman-Golkar, 1984) in closed exposure chambers, in which the atmospheric concentration–time course was measured after injection of a single dose into the chamber atmosphere. The uptake of propylene into the body of rats has been found to be low. Clearance due to uptake, reflecting the rate of transfer of propylene from the atmosphere into the organism, was 19 ml/min for one rat weighing 250 g. This value represents only 16% of the alveolar ventilation (117 ml/min; Arms & Travis, 1988). Most propylene inhaled into the lungs is exhaled again and does not reach the blood to become systemically available. Maximal accumulation of propylene in the same rat, determined as the thermodynamic partition coefficient for whole body:air ($K_{eq} = \text{Conc.}_{\text{animal}}/\text{Conc.}_{\text{air}}$), was only 1.6. At concentrations below 50 ppm [86 mg/m³], the concentration ratio at steady-state whole body:air was even smaller (0.7), owing to metabolic elimination. At these concentrations, first-order kinetics were found, and clearance due to metabolism in relation to the concentration in the atmosphere was calculated to be 11 ml/min for a 250-g rat. Thus, at steady state, about 58% of systemically

available propylene is eliminated metabolically and 42% is eliminated by exhalation as the unchanged substance. No corresponding data have been published for mice. In rats and mice, the rate of propylene metabolism showed saturation kinetics, with maximal metabolic rates (V_{\max}) of $0.17 \mu\text{mol}/(\text{min} \times 250 \text{ g bw})$ [$1.7 \text{ mg}/(\text{h} \times \text{kg bw})$] and $8 \text{ mg}/(\text{h} \times \text{kg bw})$ and atmospheric concentrations at $V_{\max}/2$ of 260 ppm [$447 \text{ mg}/\text{m}^3$] and 800 ppm [$1376 \text{ mg}/\text{m}^3$], respectively.

Dahl *et al.* (1988) investigated the rates of propylene uptake in male Fischer 344/N rats exposed by nose only to a constant atmospheric concentration of 100 ppm [$172 \text{ mg}/\text{m}^3$]. Normalized values of the rates of metabolism determined after 60 min of exposure in two independent experiments were 1.3 and 1.9 $\text{nmol}/(\text{kg bw} \times \text{min} \times \text{ppm})$ [3.3 and $4.8 \mu\text{g}/(\text{kg bw} \times \text{h} \times \text{ppm})$]. Under steady-state conditions, the amount taken up equals the amount metabolized. Consequently the two values are equivalent to the rates of metabolism at steady state and are in agreement with a value for male Sprague-Dawley rats of $4.4 \mu\text{g}/(\text{kg bw} \times \text{h} \times \text{ppm})$, which can be calculated on the basis of the clearance of metabolism reported by Golka *et al.* (1989).

(b) Metabolites

Propylene oxide (see monograph, p. 193) is produced during the first step of propylene metabolism. Epoxidation by a reconstituted cytochrome P450_{LM2} system including cytochrome P450 reductase proceeded with a concomitant, stereospecific (*trans* to the alkyl group) hydrogen/deuterium exchange from the aqueous phase when *trans*-1-deuterio-propylene in H₂O or propylene in D₂O (pH 7.4, 4 °C) was used (Groves *et al.*, 1986).

Both enantiomers of propylene oxide were found after incubation (37 °C, pH 7.4) of propylene with an NADPH-regenerating system and microsomes prepared from livers of male Wistar rats (control and phenobarbital-pretreated animals) and male NMRI/HAN mice (control, phenobarbital- and benzo[*a*]pyrene-pretreated animals). Epoxide hydrolase activity was inhibited in those incubates which contained trichloropropylene oxide. In incubations with liver microsomes of rats, about 30% of R and about 70% of S enantiomer were formed, whereas with mouse microsomes the amounts of the two enantiomers were nearly equal: R, 42–55%; S, 45–58%, depending on the pretreatment (Wistuba *et al.*, 1989).

After male Fischer 344/N rats were exposed by nose only to atmospheric propylene concentrations of 6 and 600 ppm [10.3 and $1032 \text{ mg}/\text{m}^3$], propylene oxide was determined in blood by gas chromatography–mass spectrometry (Maples & Dahl, 1991). Abrupt increases in the concentrations of propylene oxide in blood were reported to occur only a few minutes after beginning of exposure to either 6 or 600 ppm propylene. The concentrations of propylene oxide decreased rapidly after 10 min of exposure, to values about 200 times lower, and remained relatively constant over a further 50 min of exposure. The cytochrome P450 content was measured in microsomes prepared from liver, nasal ethmoturbinates and maxilloturbinates of rats after 0, 20 and 360 min of exposure. After 20 min of exposure to 600 ppm propylene, hepatic and nasal cytochrome P450 activities were reduced to about 70 and 50%, respectively, but returned to the control value (nasal cavity) or to even higher values (liver) after 360 min of exposure. It was speculated that a propylene-specific cytochrome P450 isozyme might be rapidly deactivated during exposure to propylene, resulting in reduced formation of propylene oxide (Maples & Dahl, 1991). In another study, mice were

exposed over 6–7 h to propylene at concentrations of 230, 680, 22 100 and 30 000 ppm [396, 1170, 38 000 and 51 600 mg/m³], and the amounts of the metabolically formed propylene oxide were calculated, taking into account the saturable metabolism of propylene. The Hb adduct levels were then determined and plotted against the calculated amounts of propylene oxide, resulting in a linear curve. In further experiments, Hb adduct levels were measured after intraperitoneal administration of propylene oxide at doses of 0.065, 0.1 and 0.19 mg/kg bw. The plot of Hb adduct level *versus* administered dose of propylene oxide resulted in a linear curve with a slope almost identical to that obtained in the former plot (Svensson *et al.*, 1991).

In male Sprague-Dawley rats, propylene metabolism becomes increasingly saturated at concentrations above 50 ppm [86 mg/m³] in the atmosphere, whereas propylene oxide metabolism is not saturable, at least up to 3000 ppm [5160 mg/m³]. Kinetic data obtained following exposure to propylene and propylene oxide were used to calculate that the theoretical maximal body burden of propylene oxide gas from propylene is 71 nl/ml body tissue [0.16 mg/kg bw]. These calculations do not take into account detoxification in the cell that metabolizes propylene to propylene oxide (Golka *et al.*, 1989).

(c) *Haemoglobin adducts*

Hb adducts of propylene oxide in male CBA mice exposed to ¹⁴C-propylene have been found at cysteine, histidine and N-terminal valine (Svensson & Osterman-Golkar, 1984; Svensson *et al.*, 1991). Exposure to 20 000 ppm propylene [34 400 mg/m³] for 4 h/day on eight consecutive days resulted in an alkylation rate of 2200 pmol N^T-(2-hydroxypropyl)-histidine/g Hb per h and an estimated rate of formation of propylene oxide of 11 mg/(h × kg bw) (Svensson & Osterman-Golkar, 1984). A linear relationship was reported between the concentration of HOPrVal (240–1620 pmol/g Hb) and the amount of propylene oxide (0.3–1.3 mmol/kg bw [17.4–75.4 mg/kg bw]) formed in mice after they had inhaled propylene (230–30 000 ppm × 7 h [2770–361 200 mg × h/m³]). This relationship had almost the same slope as that obtained after intraperitoneal injection of ¹⁴C-propylene oxide in saline (0.065–0.19 mmol/kg bw [3.8–11 mg/kg bw]). It was concluded that propylene oxide is the major primary metabolic product of propylene (Svensson *et al.*, 1991). [The Working Group noted, furthermore, that there is effective biotransformation of propylene to propylene oxide throughout the exposure period.]

HOPrVal in Hb was determined in female Fischer 344 rats and male and female Syrian hamsters exposed for six months to gasoline and diesel exhausts (mean atmospheric concentrations of propylene, < 0.1–0.72 ppm [< 0.17 – 1.24 mg/m³]). Background values for HOPrVal were 9 pmol/g Hb in rats and 6 pmol/g Hb in hamsters. In hamsters, the levels of HOPrVal increased almost linearly with exposure dose and were higher in females than in males. HOPrVal adduct increments at the highest dose were similar in female rats (44 pmol/g Hb) and hamsters (47 pmol/g Hb) (Törnqvist *et al.*, 1988).

After a 4-h exposure of phenobarbital-pretreated male Sprague-Dawley rats to propylene (40% in air [688 g/m³]), abnormal porphyrins were found to have accumulated in the liver. It was concluded that the porphyrins resulted from alkylation of the prosthetic haem group of cytochrome P450 during the oxidation of propylene that led to inactivation of the enzyme. An N-(2-hydroxypropyl) adduct at the pyrrole ring D was identified by nuclear

magnetic resonance analysis of alkylated porphyrins. *In vitro*, an NADPH-dependent reduction of total cytochrome P450 by 32% was measured in propylene-exposed (5% in air [86 g/m³], 30 min) liver microsomes isolated from phenobarbital-pretreated male Sprague-Dawley rats (Kunze *et al.*, 1983).

Exposure of male Sprague-Dawley CD rats to 50 000 ppm [86 g/m³] propylene for 4 h after treatment with phenobarbital, β -naphthoflavone or a combination of the two resulted in a decrease of about 40% in the control values for hepatic cytochrome P450 content. Similar exposure of polychlorinated biphenyls (Aroclor 1254)-treated rats to propylene led to a 60% reduction in the hepatic cytochrome P450 content for at least 24 h after the start of exposure. Furthermore, in liver microsomes prepared from these animals, a 20% reduction in hepatic microsomal aniline hydroxylase activity was observed in comparison with controls. In experiments *in vitro*, liver microsomes obtained from pretreated but not propylene-exposed animals were incubated with propylene (20% in air [344 g/m³], 20 min, 37 °C) in the presence of NADPH. This treatment decreased the cytochrome P450 content to 70–80% of control values. In non-pretreated animals, propylene had no effect on cytochrome P450 concentrations (Osimitz & Conolly, 1985).

4.2 Toxic effects

The toxicology of propylene has been reviewed (Gibson *et al.*, 1987).

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

(a) Acute toxicity

Inhaled propylene was not toxic to male Sprague-Dawley CD rats exposed for 4 h to an atmospheric concentration of 50 000 ppm [86 g/m³]; however, hepatotoxic effects were seen in propylene-exposed (50 000 ppm, 4 h) animals which had been pretreated with Aroclor 1254. Hepatotoxicity was manifested by an increase in the liver:body weight ratio, by the presence of macroscopic focal areas of haemorrhage and by elevated activities of serum sorbitol dehydrogenase and alanine leucine transaminase. SKF-525A, an inhibitor of cytochrome P450-dependent metabolism, abolished these effects when given before propylene exposure. Reduction of the glutathione content by fasting did not influence the hepatotoxicity of propylene in Aroclor-pretreated rats. It was concluded that Aroclor might have induced a specific cytochrome P450 isozyme, or the same isozymes as those induced by phenobarbital or β -naphthoflavone, but to a greater extent. In either case, sufficient propylene may have been metabolized to cause hepatotoxicity. Alternatively, Aroclor may predispose the liver to damage caused by an otherwise innocuous level of propylene metabolism (Osimitz & Conolly, 1985).

(b) Subchronic toxicity

Exposure of male and female Fischer 344/N rats and male and female B6C3F1 mice for 14 days or 14 weeks to atmospheric propylene concentrations ranging from 625 to

10 000 ppm [1075 to 17 200 mg/m³] did not induce toxic effects (US National Toxicology Program, 1985).

(c) *Chronic toxicity*

Male and female Sprague-Dawley rats were exposed to propylene at concentrations of 200, 1000 or 5000 ppm [344, 1720 or 8600 mg/m³] for 7 h/day on five days/week for 104 weeks and male and female Swiss mice for 7 h/day on five days/week for 78 weeks. The mortality rate of male rats increased slightly after exposure to 1000 and 5000 ppm [1720 and 8600 mg/m³] and that of male mice after exposure to 5000 ppm [8600 mg/m³] (Ciliberti *et al.*, 1988).

In another study, male and female Fischer 344/N rats and male and female B6C3F1 mice were exposed for 6 h/day on five days/week for 103 weeks to atmospheric propylene concentrations of 5000 and 10 000 ppm [8600 and 17 200 mg/m³]. Squamous metaplasia of the nasal cavity developed in females at both exposure concentrations (not dose dependent) and in males at the low exposure concentration, and epithelial hyperplasia developed in females exposed to the high concentrations. In males, inflammatory changes were seen in the submucosa and the lumen of the nasal cavity. According to the authors, all of the nasal cavity lesions reflected changes due to local irritation. No change was observed in the nasal cavity of mice of either sex, but the incidences of chronic focal renal inflammation were increased in both exposure groups (Quest *et al.*, 1984; US National Toxicology Program, 1985).

4.3 Reproductive and prenatal 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 4 and Appendices 1 and 2)

Propylene did not induce gene mutation in *Salmonella typhimurium* TA100 exposed for 7 h to 20% propylene in air in either the presence or absence of an exogenous metabolic activation system. Exposure of L5178Y mouse lymphoma cells to 20–50% propylene in air for 4 h did not induce mutations at the *tk* locus in the absence of metabolic activation but did produce inconclusive results in the presence of an exogenous metabolic activation system from rat liver.

Alkylation of DNA at the N7 position of guanine was investigated in male CBA mice exposed to atmospheric propylene or uniformly labelled ¹⁴C-propylene. The adduct levels were related to the concentration of propylene oxide (0.88 mmol/kg bw), calculated from the rate of propylene metabolism. Immediately after exposure to 107 MBq uniformly labelled ¹⁴C-propylene (18.1 MBq/mmol propylene) for 7 h in a closed exposure chamber, in which the atmospheric concentration–time course was measured after injection of a single dose

Table 4. Genetic and related effects of propylene

Test system	Results ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	336.0000 ^c	Victorin & Ståhlberg (1988)
G5T, Gene mutation, mouse L5178Y cells, <i>tk</i> locus	-	?	840.0000 ^c	McGregor <i>et al.</i> (1991)
BVD, Binding (covalent) to mouse DNA <i>in vivo</i>	+		0.0000	Svensson <i>et al.</i> (1991)
Protein binding				
BVP, Binding (covalent) to mouse haemoglobin <i>in vivo</i>	+		8000.0000, inhal. 4 h/d × 8 d	Svensson & Osterman-Golkar (1984)
BVP, Binding (covalent) to mouse protein <i>in vivo</i>	+		480.0000 inhal. 7 h	Svensson <i>et al.</i> (1991)

^a+, positive; -, negative; ?, inconclusive (variable response within several experiments within an adequate study)

^bIn-vitro tests, µg/ml; in-vivo tests, mg/kg bw

^cAtmospheric concentration in exposure chamber (µg/ml)

into the chamber atmosphere, the mice were killed and 2-hydroxypropyl-DNA adducts were measured. The values were: liver, 3000 pmol/g DNA; kidney, 3000 pmol/g DNA; and spleen, 2000 pmol/g DNA (Svensson *et al.*, 1991). [The Working Group noted that the results were based on low counts of radioactivity.]

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Propylene is a major chemical intermediate, produced by catalytic or thermal cracking of hydrocarbons or as a by-product of petroleum refining. It is used mainly in the preparation of alkylates for gasoline and in the production of polypropylene, acrylonitrile, propylene oxide and a number of other industrial chemicals. Propylene is introduced into the atmosphere from natural and man-made sources, including emissions from vegetation, burning of organic material and incomplete combustion of fossil fuels, and from its production and use. Few data are available on levels of occupational exposure.

5.2 Human carcinogenicity data

No relevant data were available to the Working Group.

5.3 Animal carcinogenicity data

Propylene was tested by inhalation in two studies in mice and in two studies in rats. A slight increase in the incidence of vascular tumours was observed in female mice in one study. In one study in rats, no treatment-related increase in tumour incidence was observed. In two studies in mice and rats exposed by inhalation, insufficient information was provided to allow an assessment of carcinogenicity.

5.4 Other relevant data

In rats exposed to 50 ppm propylene, about one-sixth of the inhaled material is absorbed, of which almost one-half is exhaled again, unchanged. The remainder is eliminated metabolically, through oxidation to propylene oxide, which is subsequently either conjugated with glutathione or, to a smaller extent, hydrated by epoxide hydrolase. Oxidation is a saturable reaction mediated by cytochrome P450 enzymes, whereas no saturation concentration has been identified for the hydration of propylene oxide. There is, therefore, a maximal attainable tissue concentration of propylene oxide in rats. Oxidation of propylene can occur in the rat nasal epithelium, where irritation, hyperplasia and metaplasia have been described after chronic exposure.

No data were available on the genetic and related effects of propylene in humans.

Alkylation products of the metabolite, propylene oxide, were found in haemoglobin and in DNA from mice exposed to propylene by inhalation. Although insufficient data are

available to evaluate the genetic and related effects of propylene, its major metabolite, propylene oxide, is genotoxic in a broad range of assays.

5.5 Evaluation¹

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

There is *inadequate evidence* in experimental animals for the carcinogenicity of propylene.

Overall evaluation

Propylene is not classifiable as to its carcinogenicity to humans (Group 3).

6. References

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¹For definition of the italicized terms, see Preamble, pp. 27–30.

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PROPYLENE OXIDE

This substance was considered by previous Working Groups, in February 1976 (IARC, 1976), June 1984 (IARC, 1985) and March 1987 (IARC, 1987). 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

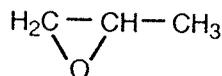
Chem. Abstr. Serv. Reg. No.: 75-56-9

Chem. Abstr. Name: Methyloxirane

IUPAC Systematic Name: Propylene oxide

Synonyms: Epoxypropane; 1,2-epoxypropane; 2,3-epoxypropane; methyloxacyclopropane; propene oxide; propylene epoxide; 1,2-propylene oxide

1.1.2 Structural and molecular formulae and relative molecular mass



$\text{C}_3\text{H}_6\text{O}$

Relative molecular mass: 58.08

1.1.3 Chemical and physical properties of the pure substance

- (a) *Description:* Colourless liquid (Kahlich *et al.*, 1993)
- (b) *Boiling-point:* 34.3 °C (Lide, 1991)
- (c) *Melting-point:* -112.1 °C (Beratergremium für umweltrelevante Altstoffe, 1993); -111.93 °C (Kahlich *et al.*, 1993)
- (d) *Density:* 0.859 at 0 °C/4 °C (Lide, 1991); 1.44 g/L at 20 °C (vapour) (Kahlich *et al.*, 1993)
- (e) *Spectroscopy data:* Infrared [prism, 387; grating, 15270], nuclear magnetic resonance, ultraviolet and mass spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991).
- (f) *Solubility:* Soluble in water (40.5 wt% at 20 °C), acetone, benzene, carbon tetrachloride, diethyl ether and ethanol (American Conference of Governmental Industrial Hygienists, 1991; Lide, 1991; Kahlich *et al.*, 1993)

- (g) *Volatility*: Vapour pressure, 58.8 kPa at 20 °C (Kahlich *et al.*, 1993)
- (h) *Stability*: Lower explosive limit (in air), 2.3%; highly inflammable; inflammable concentrations of vapour form readily at room temperature (Kahlich *et al.*, 1993)
- (i) *Reactivity*: May polymerize violently; very reactive, particularly with chlorine, ammonia, strong oxidants and acids (WHO, 1985; Kahlich *et al.*, 1993)
- (j) *Octanol/water partition coefficient (P)*: log P, 0.03 (Sangster, 1989)
- (k) *Conversion factor*: $\text{mg/m}^3 = 2.38 \times \text{ppm}^a$

1.1.4 Technical products and impurities

Propylene oxide exists in two optical isomers, and commercial propylene oxide is a racemic mixture. The purity of the commercial product is > 99.9% (Kahlich *et al.*, 1993). Typical product specifications are: water, 500 mg/kg max.; aldehydes (total of acetaldehyde and propionaldehyde), 100 mg/kg max.; chlorides (as chlorine), 40 mg/kg max.; acidity (as acetic acid), 20 mg/kg max.; and specific gravity (20 °C/20 °C), 0.829–0.831 (Dow Chemical Co., 1993).

1.1.5 Analysis

Propylene oxide is detected and measured in workplace air by packed column gas chromatography with a flame ionization detector. The sample is adsorbed on charcoal and desorbed with carbon disulfide. This method (NIOSH Method 1612) has an estimated limit of detection of 0.01 mg per sample (Eller, 1985).

Biological monitoring of occupational exposure to propylene oxide by analysis of blood has been reported. These methods involve the determination of the haemoglobin (Hb) adducts *N*-(2-hydroxypropyl)cysteine, *N*-(2-hydroxypropyl)histidine and *N*-(2-hydroxypropyl)valine by gas chromatography–mass spectrometry or capillary gas chromatography with selective ion monitoring mass spectrometry (Osterman-Golkar *et al.*, 1984; Bailey *et al.*, 1987; Kautiainen & Törnqvist, 1991).

Residues of propylene oxide in ethoxylated surfactants and demulsifiers have been determined by headspace gas chromatography with flame ionization detection (Dahlgran & Shingleton, 1987).

1.2 Production and use

1.2.1 Production

The selection of a production route for propylene oxide is influenced by the application and market potential of co-products and by the availability of raw materials and possibilities for by-product management. The techniques so far developed can be divided into: chlorohydrin processes, indirect oxidation processes and direct oxidation processes. Only the chlorohydrin and indirect oxidation processes are practised currently on an industrial scale. It

^aCalculated from: $\text{mg/m}^3 = (\text{relative molecular mass}/24.45) \times \text{ppm}$, assuming normal temperature (25 °C) and pressure (101.3 kPa)

has not been possible so far to produce propylene oxide in a technically and economically satisfactory manner by direct gas-phase oxidation (with silver catalysts), in analogy with the process used for ethylene oxide. Common to all current propylene oxide process techniques is the fact that significant amounts of co-products are always generated. In the conventional chlorohydrin process, up to 2 mol of salt (sodium or calcium chloride) in the form of dilute brine solution are generated per mole of propylene oxide. In the case of the direct oxidation methods, complex organic mixtures arise which must then be separated by distillation and either sold, further converted or incinerated. Indirect oxidation techniques also involve the formation of some by-products (Kahlich *et al.*, 1993).

Commercial production of propylene oxide began in the early 1900s, by the chlorohydrin process involving the dehydrochlorination of propylene chlorohydrin with a base. The chlorohydrin process is carried out in two steps: synthesis of propylene chlorohydrin from propylene and chlorine in water and subsequent dehydrochlorination of propylene chlorohydrin to propylene oxide (Kahlich *et al.*, 1993).

Several reaction routes and process alternatives for the production of propylene oxide by indirect oxidation have been reported, but only a few are practised on an industrial scale. Indirect oxidation is a two-step process: (i) formation of hydrogen peroxide or an organic peroxide from a suitable alkane, aldehyde or acid; and (ii) use of the peroxide as a selective oxidizing agent in the epoxidation of propylene to propylene oxide, with formation of a commercially useful co-product, the corresponding alcohol or acid (Kahlich *et al.*, 1993).

In one indirect oxidation process, ethylbenzene is reacted with oxygen to form ethylbenzene hydroperoxide. This product is subsequently used to epoxidize propylene to propylene oxide and methyl benzyl alcohol. In an alternative process, isobutane is substituted for ethylbenzene and, through analogous chemistry, *tert*-butanol is the co-product. Methyl benzyl alcohol and *tert*-butanol can then be dehydrated to styrene monomer and isobutylene, respectively (ARCO Chemical Co., 1993).

Information available in 1991 indicated that propylene oxide was produced by seven companies in Japan, six in Germany, four in China and the USA and one each in Brazil, Canada, France, Italy, the Netherlands, the Republic of Korea, Spain and the former Yugoslavia (Chemical Information Services Ltd, 1991). Production figures available for Germany, Japan and the USA are presented in Table 1; worldwide capacity for the production of propylene oxide is presented in Table 2.

1.2.2 Use

Propylene oxide is an important basic chemical intermediate. Nearly all of the propylene oxide produced is converted into derivatives, often for applications similar to those of ethylene oxide derivatives. Propylene oxide is used primarily to produce polyether polyols, propylene glycols and propylene glycol ethers. It is certified for use as a package fumigant for dried fruits and as a bulk fumigant for foodstuffs such as cocoa, spices, processed nutmeats, starch and gums; it may be used alone or in mixtures with carbon dioxide. Use patterns for propylene oxide in the USA, which are typical of those for the world market, are presented in Table 3 (Kirk & Dempsey, 1982; Kahlich *et al.*, 1993).

Table 1. Reported production of propylene oxide in selected countries

Country	Production (thousand tonnes)					
	1982	1984	1986	1988	1990	1992
Germany	NR	491	533	549	591	556
Japan	NR	210	225	288	336	323
USA	753	NR	NR	NR	NR	NR

From Mannville Chemical Products Corp. (1984); Japan Chemical Week (1985); Anon. (1987a, 1989); Japan Chemical Week (1987, 1989, 1991); Anon. (1993a); Japan Petrochemical Industry Association (1993); NR, not reported

Table 2. World capacity for production of propylene oxide in 1991

Country or region	Process	Capacity (thousand tonnes/year)
Americas		1735
Brazil	Chlorohydrin, with lime	150
Canada	Chlorohydrin, with NaOH	60
USA	Styrene	270
	<i>tert</i> -Butyl alcohol	550
	Chlorohydrin, with NaOH	700
	Direct oxidation	5
Asia		563
China	Chlorohydrin, with lime	68
India	Chlorohydrin, with lime	24
Japan	Styrene	140
	Chlorohydrin, with lime	216
Republic of Korea	Styrene	100
Taiwan	Chlorohydrin, with lime	15
Europe		1614
Bulgaria	Chlorohydrin, with lime	12
Former Czechoslovakia	Chlorohydrin, with lime	5
France	<i>tert</i> -Butyl alcohol	200
Germany	Chlorohydrin, with NaOH	420
	Chlorohydrin, with lime	290
Italy	Chlorohydrin, with lime	60
Netherlands	<i>tert</i> -Butyl alcohol	250
	Styrene	140
Poland	Chlorohydrin, with lime	20
Romania	Chlorohydrin, with lime	72

Table 2 (contd)

Country or region	Process	Capacity (thousand tonnes/year)
Europe (contd)		
Russia	Chlorohydrin, with lime	25
	Styrene	50
Spain	Styrene	50
Former Yugoslavia	Chlorohydrin, with lime	20
Total		3912

From Kahlich *et al.* (1993)

Table 3. Use patterns (%) for propylene oxide as a chemical intermediate in the USA

Use	Year				
	1981	1984	1987	1990	1993
Urethane polyether polyols	54	59	60	60	65 ^a
Propylene glycol	21	21	20	20	25
Glycol ethers	–	–	3	3	5
Dipropylene glycol	2	2	2		–
Miscellaneous ^b	13	9	6	5	5
Exports	10	9	9	12	–

From Anon. (1981, 1984, 1987b, 1990, 1993b)

^aFlexible foams, 75%; rigid foams, 15%; non-foam uses, 10%

^bIncludes glycol ethers, industrial polyglycols, glycerine, surfactants and isopropanolamines (unless otherwise noted elsewhere)

Most of the propylene oxide produced is used as an intermediate for polyether polyols, which are used mainly in the manufacture of polyurethanes. Polyurethanes can be prepared so as to have a wide range of hardness, rigidity and density characteristics. Flexible polyurethane foams, used in furniture and automobile seating, bedding and carpet underlay, are made from polyols with relative molecular masses > 3000. Polyols with lower relative molecular masses result in rigid foams for such applications as thermal insulation. Homo- and copolymerized polyethers are used as surface-active agents in detergents, textiles, defoamers, hair-care preparations, brake fluids and lubricants (ARCO Chemical Co., 1993; Kahlich *et al.*, 1993).

Propylene glycols constitute the second largest application for propylene oxide. Monopropylene glycol is the direct reaction product of propylene oxide with water. Di-, tri- and higher propylene glycols are co-produced by the reaction of monopropylene glycol with propylene oxide. Propylene glycol is used mainly as the raw material for unsaturated polyester resins, especially in the textile and construction industries. It is also used as a humectant and as a solvent and emollient in food, drugs and cosmetics. Further uses are in coatings,

plasticizers, heat transfer and hydraulic fluids, antifreezes and aircraft deicing fluid (ARCO Chemical Co., 1993; Kahlich *et al.*, 1993).

Propylene glycol ethers are formed by the reaction of propylene oxide with alcohols, usually methanol, ethanol, propanol or butanol. Certain traditional ethylene glycol ethers and their acetates are being replaced by their propylene oxide-based analogues. The demand for propylene oxide-based glycol ethers and acetates is therefore growing rapidly and now constitutes the third largest market for propylene oxide. Typical applications are as a solvent in coatings, paints, inks, resins, cleaners, waxes and electronic circuit board lamination. Glycol ethers are also found in heat-transfer fluids and anti-icing agents for jet fuel (Kahlich *et al.*, 1993).

Various special organic compounds can be derived from propylene oxide, including allyl alcohol (used in glycerol synthesis), propylene carbonate (a special solvent for organic and some inorganic compounds), mono-, di- and tri-isopropanolamines (detergent raw materials) and hydroxypropylated cellulose (Kahlich *et al.*, 1993). Hydroxypropyl starch ethers, produced by treating starch with propylene oxide, are used as additives in salad dressings, pie fillings and other food thickening applications (Whistler & Daniel, 1983). Propylene oxide is also used as a reactive diluent in preparations for embedding tissues for transmission electron microscopy (McDowell, 1978).

1.3 Occurrence

1.3.1 *Natural occurrence*

Propylene oxide is not known to occur as a natural product.

1.3.2 *Occupational exposure*

The National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health in the USA between 1981 and 1983 indicated that 421 000 US employees were potentially exposed to propylene oxide at work (US National Institute for Occupational Safety and Health, 1993). Of this number, 2% were estimated to be exposed to propylene oxide and 98% were estimated to be exposed to materials containing propylene oxide. The estimate is based on a survey of US companies and did not involve measurements of actual exposures.

Occupational exposure to propylene oxide has been measured in the chemical industry, during its production and the production of its derivatives, and in the starch industry where it is used to produce hydroxypropyl starch derivatives (Table 4). In the chemical industry, high exposures have been reported only during brief periods such as maintenance operations (Thiess *et al.*, 1981a; Flores, 1983). Hogstedt *et al.* (1979) reported that exposure to propylene oxide during its production by the chlorohydrin process in a Swedish plant was in the range of 10–25 mg/m³ and occasionally 125–150 mg/m³. Personal sampling in industrial starch production indicated that jobs such as mechanic and operator in the reaction area may entail 8-h time-weighted average (TWA) exposures above 50 mg/m³ (McCammon *et al.*, 1990).

Table 4. Concentrations of propylene oxide in various industries and operations

Industry, operations	No. of samples	Concentration range (mg/m ³)	Year of measurement	Country	Reference
Production of propylene oxide	NR	< 0.1-6.5 (8-h TWA)	1978-81	Netherlands	de Jong <i>et al.</i> (1988)
Production of propylene oxide	NR	0.5-4.8 (8-h TWA) 24-9025 (peak) ^a	1979	USA	Flores (1983)
Production of propylene oxide derivatives	7	< 0.4-3.6 (6-h TWA)	1978	USA	Oser <i>et al.</i> (1978)
Production of propylene oxide derivatives			1979	USA	Oser <i>et al.</i> (1979)
Polymer polyol unit	4	0.5-5.9 (6-h TWA)			
Oxide adducts unit	6	< 0.6-1.2 (6-h TWA)			
Flexible polyol unit	4	< 0.5 (6-h TWA)			
Industrial starch production	5 workers	1.4-29 (TWA for 25-75% of working time) Up to 2400 (peak)	1981	Sweden	Pero <i>et al.</i> (1982)
Industrial starch production			1988-89	USA	Hills (1990a)
Personal samples	42	0.2-14 (8-h TWA)			
Area samples	15	< 0.2-26 (5 min-11 h)			
Industrial starch production			1989	USA	McCammon <i>et al.</i> (1990)
Personal samples	26	< 0.3-1500 (45 min-4 h TWA)			
Area samples	40	< 1-5600 (45 min-8 h TWA)			
Industrial starch production			1988	USA	Hills (1990b)
Area samples	89	0.2-14 (45 min-12 h)			
Personal samples					
Operators	15	0.2-1.7 (8-h TWA)			
Laboratory technician	11	< 0.2-1.0 (8-h TWA)			

NR, not reported; TWA, time-weighted average. Values are converted to mg/m³, when necessary.

^aBefore implementation of engineering controls

1.3.3 Environment

Annual industrial emissions of propylene oxide into air in the USA, reported by industrial facilities to the US Environmental Protection Agency, decreased from 1600 tonnes in 1987 to 500 tonnes in 1991 (US National Library of Medicine, 1993).

It has been suggested that propylene oxide may also be introduced into the atmosphere from combustion exhausts of sources that burn hydrocarbons. In the atmosphere, propylene oxide reacts slowly with photochemically produced hydroxyl radicals, with an atmospheric half-life of 3–20 days (Grosjean, 1990; Kahlich *et al.*, 1993).

In water, propylene oxide is hydrolysed to propylene glycol with an estimated half-life of 11.6 days at 25 °C and pH 7. The chloride ions in salt water accelerate the chemical degradation to a half-life of 4.1 days (Kahlich *et al.*, 1993).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for propylene oxide in a number of countries are presented in Table 5.

The US Food and Drug Administration (1993) has approved the use of not more than 25% propylene oxide, by itself or in combination with various sources of active chlorine and/or oxygen, for etherification in the production of modified food starch; the residual propylene chlorohydrin should not exceed 5 ppm (21 CFR 172.892). It has also established regulations for use of propylene oxide in products in contact with food, including defoaming agents and slimicides used in the manufacture of paper and paperboard (21 CFR 176.210, 176.300); and as a reactant in the production of lubricants with incidental food contact (21 CFR 178.3570).

Residues of propylene oxide are exempt from the requirement of a tolerance when used as a stabilizer in pesticide formulations applied to growing crops or to raw agricultural commodities after harvest or applied to animals (US Environmental Protection Agency, 1992a).

Propylene oxide, either alone or in mixtures with carbon dioxide, is permitted as a package fumigant in or on dried prunes and glacé fruit [residue tolerance, 700 ppm (as propylene glycol)] or as a bulk fumigant in or on cocoa, gums, processed spices, starch and processed nutmeats (except peanuts) [residue tolerance, 300 ppm as propylene glycol] (US Environmental Protection Agency, 1992b).

2. Studies of Cancer in Humans

2.1 Cohort studies

Several of the cohort studies described in the monograph on ethylene oxide included some workers who were also exposed to propylene oxide (Hogstedt *et al.*, 1979, 1986; Thiess *et al.*, 1981b; Hogstedt, 1988; Gardner *et al.*, 1989). No conclusion could be drawn about the risk for cancer in relation to exposure to propylene oxide specifically.

Table 5. Occupational exposure limits and guidelines for propylene oxide

Country	Year	Concentration (mg/m ³)	Interpretation
Argentina	1991	50	TWA; potential carcinogen
Australia	1983	50	TWA; probable human carcinogen
Austria	1982	120	TWA
Belgium	1984	50	TWA
Canada	1986	50	TWA
Denmark	1988	12	TWA; suspected carcinogen; skin
Finland	1993	12	TWA; suspected of having carcinogenic potential
France	1993	50	TWA
Germany	1993	None	Carcinogenic in animals
Indonesia	1978	240	TWA
Italy	1978	240	TWA
Mexico	1989	50	TWA
Netherlands	1986	240	TWA
Norway	1990	2	TWA; carcinogen
Romania	1975	100	Average
		200	Maximum
Sweden	1991	12	TWA; suspected of having carcinogenic potential; skin
		25	STEL (15 min)
Switzerland	After 1987	6	TWA; suspected carcinogen
United Kingdom	1992	50	TWA
		240	STEL (10 min)
USA			
ACGIH (TLV)	1994	48	TWA ^a
OSHA (PEL)	1992	240	TWA
NIOSH	1992	20	LOQ ^b
Venezuela	1978	240	TWA
		360	Ceiling

From Cook (1987); Direktoratet for Arbeidstilsynet (1990); ILO (1991); US National Institute for Occupational Safety and Health (NIOSH) (1992); US Occupational Safety and Health Administration (OSHA) (1992); American Conference of Governmental Industrial Hygienists (ACGIH) (1993); Deutsche Forschungsgemeinschaft (1993); Institut National de Recherche et de Sécurité (1993); Työministeriö (1993); UNEP (1993)

TWA, time-weighted average; STEL, short-term exposure limit; TLV, threshold limit value; PEL, permissible exposure level; LOQ, limit of quantification; skin, absorption through the skin may be a significant source of exposure

^aSubstance identified by other sources as a suspected or confirmed human carcinogen

^bNIOSH has not established a specific numerical recommended exposure level for propylene oxide but has recommended reduction of worker exposures to the lowest feasible concentration and that the substance be recognized as a potential occupational carcinogen.

2.2 Case-control study

Risk estimates for exposure to propylene oxide and 20 other chemicals were derived in a nested case-control study carried out at two large chemical manufacturing facilities and a research and development centre in the USA (Ott *et al.*, 1989). The study was carried out because an earlier cohort study of the same population (Rinsky *et al.*, 1988) had shown elevated death rates from lymphatic and haematopoietic cancer. The cases (52 of non-Hodgkin's lymphoma, 20 of multiple myeloma, 39 of non-lymphatic leukaemia and 18 of lymphatic leukaemia) were identified from underlying and contributory causes of death for male members of the cohort who died during 1940-78. Controls were selected from the total cohort in a ratio of 5:1 and were matched to cases by sex, decade of first employment and survival to the start of the same five-year period. Exposures were inferred from recorded job histories up to the beginning of the survival period of the case. Odds ratios for men ever *versus* never exposed to propylene oxide (and the numbers of exposed cases) were as follows: non-Hodgkin's lymphoma, 1.5 (four); multiple myeloma, 3.4 (three); non-lymphatic leukaemia, 1.3 (three); lymphatic leukaemia, 0 (zero). None of these associations was significant. The associations between non-Hodgkin's lymphoma and exposure to propylene oxide were similar for men with less than five (odds ratio, 1.7) and at least five years' exposure (odds ratio, 1.3). [The Working Group noted that no information was given on levels of exposure to propylene oxide or on possible confounding effects of other exposures.]

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

Rat: Groups of 50 female Sprague-Dawley rats, about 100 days old, were administered 0 (control), 15 or 60 mg/kg bw propylene oxide (purity, 99%) in a commercial vegetable oil [composition unspecified] by gastric intubation twice a week for 109.5 weeks (average total dose, 2714 or 10 798 mg/kg bw, respectively) and observed for life. Another untreated control group consisting of 50 females was also available. Survival rates in rats treated with propylene oxide were comparable to those of controls. Treatment with propylene oxide resulted in a dose-dependent increase in the incidence of forestomach tumours, which were mainly squamous-cell carcinomas. The incidences of squamous-cell carcinomas of the forestomach were 0/50 and 0/50 in control groups, 2/50 in the low-dose group and 19/50 in the high-dose group; one additional animal in the high-dose group had a carcinoma *in situ*, and a further animal had an adenocarcinoma of the glandular stomach. In addition, 7/50 low-dose and 17/50 high-dose animals developed papillomas, hyperplasia and hyperkeratosis of the forestomach. The incidences of tumours at other sites in treated animals were no greater than those in controls (Dunkelberg, 1982).

3.2 Inhalation

3.2.1 Mouse

Groups of 50 male and 50 female B6C3F1 mice, seven to nine weeks old, were exposed by inhalation to 0 (control), 200 or 400 ppm (474 or 948 mg/m³) propylene oxide (purity,

> 99.9%) vapour for 6 h per day on five days per week for 103 weeks. Fewer treated than control animals survived to the end of the experiment: males—control, 42/50; low-dose, 34/50; high-dose, 29/50 ($p = 0.006$); females—control, 38/50; low-dose, 29/50; high-dose, 10/50 ($p < 0.001$). One squamous-cell carcinoma and one papilloma of the nasal cavity occurred in two high-dose male mice, and two high-dose female mice had adenocarcinomas of the nasal cavity. The (combined) incidences of haemangiomas and haemangiosarcomas in the nasal cavity were: males—0/50 control, 0/50 low-dose and 10/50 high-dose ($p < 0.001$, Fisher exact test); females—0/50 control, 0/50 low-dose and 5/50 high-dose ($p = 0.028$, Fisher exact test). Propylene oxide caused inflammation of the respiratory epithelium of the nasal turbinates; squamous metaplasia was observed in one low-dose male and in two high-dose female mice. Three high-dose males and three high-dose females had focal angiectasis of the submucosal turbinate vessels (US National Toxicology Program, 1985; Renne *et al.*, 1986).

3.2.2 Rat

Groups of 80 male weanling Fischer 344 rats were exposed by inhalation to filtered air containing 0 (control), 100 or 300 ppm (237 or 711 mg/m³) propylene oxide (purity, 98%) vapour for about 7 h per day on five days per week for 104 weeks. Increased mortality over that in controls was observed in the two groups of rats exposed to propylene oxide, which was significant in the high-dose group ($p < 0.01$). Rats exposed to 100 or 300 ppm propylene oxide had an increased incidence of inflammatory lesions of the respiratory system and of a 'complex epithelial hyperplasia' in the nasal cavity, which was dose dependent (control, 0/76; low-dose, 2/77; high-dose, 11/78). Two rats in the high-dose group developed adenomas in the nasal cavity, which were not seen in controls. Adrenal phaeochromocytomas developed in 8/78 controls, 25/78 rats of the low-dose group and 22/80 rats of the high-dose group ($p < 0.05$, χ^2 test). A slight, nonsignificant increase in the incidence of peritoneal mesotheliomas was also found in the exposed groups (control, 3/78; low-dose, 8/78; high-dose, 9/80) (Lynch *et al.*, 1984a).

Groups of 50 male and 50 female Fischer 344/N rats, seven to eight weeks of age, were exposed by inhalation to 0 (control), 200 or 400 ppm (474 or 948 mg/m³) propylene oxide (purity, > 99.9%) vapour for 6 h per day on five days per week for 103 weeks. Survival of rats exposed to propylene oxide was comparable to that of controls; terminal body weights were lower in high-dose males and high-dose females than in controls. Suppurative inflammation, epithelial hyperplasia and squamous metaplasia of the respiratory epithelium and underlying submucosal glands of the nasal turbinates were observed in exposed rats. Papillary adenomas of the nasal cavity occurred in 0/50 control, 0/50 low-dose and 3/50 high-dose females ($p = 0.037$, Cochran-Armitage trend test) and in 0/50 control, 0/50 low-dose and 2/50 high-dose males ($p > 0.05$). In historical controls, the incidence of nasal cavity tumours was 3/1523 in females and 1/1477 in males. In female rats, the combined incidences of C-cell adenomas and C-cell carcinomas of the thyroid were increased (control, 2/45; low-dose, 2/35; high-dose, 7/37; $p = 0.023$, Cochran-Armitage trend test). The incidence in historical controls was 122/1472 ($8.3 \pm 4.3\%$ (SD)) (US National Toxicology Program, 1985; Renne *et al.*, 1986).

Groups of 50 male Sprague-Dawley rats, 11–12 weeks old, were exposed by inhalation to 435 or 870 ppm (1031 or 2062 mg/m³) propylene oxide (purity, > 95%) vapour in air for 6 h per day on five days per week for 30 days. A group of 50 rats was exposed to 1740 ppm (4124 mg/m³) propylene oxide for only eight days because of high mortality [exact numbers unspecified]. A control group of 98 male rats was exposed to air alone. All animals were observed for life. Median lifespans were: control, 613 days; low-dose, 655 days; mid-dose, 635 days; and high-dose, 519 days. No nasal tumour was observed in any group receiving propylene oxide; two mid-dose animals had adenomas of the lung. Control animals developed no tumour in any part of the respiratory tract (Sellakumar *et al.*, 1987). [The Working Group noted the short exposure period.]

Groups of 100 male and 100 female Wistar rats, 34–38 days old, were exposed by inhalation to 0 (control), 30, 100 or 300 ppm (71.1, 237 or 711 mg/m³) propylene oxide (purity, > 99.99%) vapour for 6 h per day on five days per week for 124 weeks (males) and 123 weeks (females). The body weights of exposed males in the high-dose group were lower than those of the controls throughout the study and lower than those of treated females only during the first year. After 12, 18 and 24 months, 10 rats of each sex from each group were killed. By week 115, mortality of male and female rats in the high-dose group was higher than that in controls; at week 119, the mortality of females in the mid-dose group was also higher than that in controls. The incidences of mammary gland tumours were significantly higher in high-dose females: fibroadenoma—control, 32/69; low-dose, 30/71; mid-dose, 39/69; high-dose, 47/70 ($p < 0.04$); tubulopapillary adenocarcinoma—control, 3/69; low-dose, 6/71; mid-dose, 5/69; high-dose, 8/70 ($p < 0.01$; Cox's test, adjusted for time of tumour appearance). The mean numbers of benign mammary tumours per tumour-bearing rat were: control, 1.3; low-dose, 2.1; mid-dose, 2.2; and high-dose, 2.4. Exposure to propylene oxide increased the incidences of degenerative and hyperplastic changes in the nasal mucosa in all of the treatment groups over that in controls. Three malignant tumours were found in the nasal cavity of treated males: one tumour described as an 'ameloblastic fibrosarcoma' in a low-dose male, one squamous-cell carcinoma in a low-dose male and one in a high-dose male. Four males in the high-dose group had a carcinoma in the larynx or pharynx, trachea or lungs; no such tumour was seen in any of the controls or low-dose males (Kuper *et al.*, 1988).

3.3 Subcutaneous administration

3.3.1 Mouse

Groups of 100 female NMRI mice, six to eight weeks old, received subcutaneous injections of propylene oxide (purity, 99%) in tricapylin at 0.1, 0.3, 1.0 or 2.5 mg/mouse once a week for 95 weeks (mean total dose, 6.8, 21.7, 72.8 or 165.4 mg/mouse, respectively). Groups of 200 untreated and 200 tricapylin-treated mice served as controls. Survival rates in the animals treated with propylene oxide were comparable to those in controls. The incidences of sarcomas at the site of injection were: untreated control, 0/200; tricapylin control, 4/200; 0.1-mg, 3/100; 0.3-mg, 2/100; 1.0-mg, 12/100; and 2.5-mg, 15/100 [$p < 0.001$, Cochran-Armitage test for trend]. No increase in tumour incidence at other sites was found (Dunkelberg, 1981).

3.3.2 Rat

Of 12 rats [age, sex and strain unspecified] given a total of 1500 mg/kg bw propylene oxide [purity unspecified] in arachis oil by subcutaneous injection over a period of 325 days [dosing schedule unspecified], eight developed local sarcomas after 507–739 days. In a similar experiment, in which 1500 mg/kg bw propylene oxide in water were injected subcutaneously, 3/12 rats developed a local sarcoma after 158 days and two developed local sarcomas after 737 days (Walpole, 1958). [The Working Group noted the inadequate reporting of the experiment.]

4. Other Data Relevant for an Evaluation of Carcinogenicity and Its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

No data were available on absorption or distribution of propylene oxide in humans.

Propylene oxide is an electrophilic compound which directly alkylates macromolecules, introducing a hydroxypropyl group at nucleophilic centres. The adducts of propylene oxide at N^T-histidine (HOPrHis) and at the N-terminal valine (HOPrVal) of Hb have been used to monitor dose in humans. Workers (all smokers) exposed to propylene oxide during the production of alkylated starch had HOPrHis adduct levels of 180–10 200 pmol/g Hb; unexposed people (38% smokers) had levels of < 100–380 pmol/g Hb. A negative correlation ($r = -0.64$) was reported between HOPrHis level and DNA repair proficiency, measured as unscheduled DNA synthesis in leukocytes after a challenge with *N*-acetoxy-2-acetylaminofluorene *in vitro*. The authors suggested that the effect was due to inactivation of DNA repair enzymes or of enzymes involved in purine or pyrimidine metabolism (Pero *et al.*, 1985).

In a study in a starch alkylation factory, the concentrations of HOPrVal were determined to be below 20 pmol/g Hb in controls, including smokers and nonsmokers, and from 230 to 3500 pmol/g Hb in exposed workers. The adduct levels correlated with the mean concentrations in the breathing zones, which varied between 0.33 and 11.4 ppm [0.8 and 27.5 mg/m³] during sampling periods of 2–4 h (Högstedt *et al.*, 1990). The steady-state background concentration of HOPrVal adducts in Hb was 2 pmol/g Hb in unexposed people (Törnqvist & Ehrenberg, 1990). The increment in workers exposed for 40 h/week to 1 ppm [2.4 mg/m³] propylene oxide (TWA) was estimated to be about 500 pmol/g Hb (Kautiainen & Törnqvist, 1991).

Hydroxylpropyl valine adduct concentrations in Hb were found to be slightly higher in propylene oxide manufacturing and loading workers than in controls, after correction for smoking (van Sittert & van Vliet, 1994).

4.1.2 Experimental systems

The presence of propylene oxide did not inhibit styrene oxide metabolism catalysed by microsomal epoxide hydrolase isolated from human liver, indicating that epoxide hydrolase

has little or no affinity for propylene oxide as a substrate (Oesch, 1974). The action of glutathione *S*-transferase on 1-chloro-2,4-dinitrobenzene (1 mmol/L) in human erythrocytes was reduced to 57% of the control value by the presence of propylene oxide (3 mmol/L) in incubations containing 1 mmol/L glutathione (pH 6.5, 25 °C). This value, measured *in situ*, was similar to the I_{50} value of 3.1 mmol/L obtained with purified anionic glutathione from human erythrocytes (Ansari *et al.*, 1987). Purified human plasma α_1 -proteinase inhibitor was inactivated by very high concentrations (> 100 mmol/L) of propylene oxide (Ansari *et al.*, 1988).

The inhalation pharmacokinetics of propylene oxide in male Sprague-Dawley rats was studied by gas uptake in closed exposure chambers, in which the atmospheric concentration-time course was measured after injection of a single dose into the chamber atmosphere. A single injection of propylene oxide into the atmosphere of the chambers resulted in an initial air concentration of up to 3000 ppm [7230 mg/m³]. The uptake of propylene oxide into the body was rather high: clearance due to uptake, reflecting the transfer rate of propylene oxide from the atmosphere into the body of one rat weighing 250 g, was 75 ml/min, representing 64% of the alveolar ventilation (117 ml/min; Arms & Travis, 1988). Maximal accumulation of propylene oxide in a single rat, determined as the thermodynamic partition coefficient for whole body:air ($K_{eq} = \text{Conc.}_{\text{animal}}/\text{Conc.}_{\text{air}}$), was 45. In the same rat, the concentration ratio at steady-state whole body:air was only 1.2 because of rapid metabolic elimination of the inhaled propylene oxide. Most (96%) of the propylene oxide taken up by the rat was metabolized, and only a small amount (3%) was exhaled unchanged. On the basis of the pharmacokinetic parameters derived, the body burden of propylene oxide gas in rats exposed under steady-state conditions to 100 ppm was calculated by the authors to be 124 nl/ml tissue (Golka *et al.*, 1989).

In male Fischer 344/N rats exposed by nose only for 60 min to 14 ppm [33.7 mg/m³] propylene oxide, the blood concentrations increased during the first 10 min, reaching a plateau at about 3 ng/g blood (Maples & Dahl, 1993).

As determined *in vitro*, propylene oxide is metabolized via two pathways: conjugation with glutathione and hydrolysis to 1,2-propanediol. Propylene oxide (10 mmol/L) was reacted at pH 6.5 with glutathione (10 mmol/L) in the presence of glutathione *S*-transferase isolated from livers of male Sprague-Dawley rats. Adducts were identified only indirectly with ninhydrin after thin-layer chromatography (Fjellstedt *et al.*, 1973). Propylene oxide (1 mmol/L) was hydrolysed to 1,2-propanediol by two forms of epoxide hydrolase obtained from liver microsomes of male Sprague-Dawley rats which had been pretreated with phenobarbital. Incubations were carried out at pH 8.7. The metabolic rate of propylene oxide hydrolysis was reported to be lower than that of other epoxides tested, 1 and 4 nmol/min per mg protein or 0.058–0.232 $\mu\text{g}/\text{min}$ per mg protein in two fractions (Guengerich & Mason, 1980).

The inhibitory effectiveness of a series of epoxides, including propylene oxide, was compared *in vitro* with microsomal membrane-bound and purified epoxide hydrolase obtained from livers of phenobarbital-pretreated male Fischer 344 rats; styrene-7,8-oxide and benzo[*a*]pyrene-4,5-epoxide were used as substrates. The inhibition constants (IC_{50}) for propylene oxide were higher (62.5 mmol/L for styrene-7,8-oxide and 450 mmol/L for benzo[*a*]pyrene-4,5-epoxide) than those for the other epoxides. The authors concluded that

propylene oxide is a poor substrate for microsomal epoxide hydrolase (Dent & Schnell, 1981).

Non-enzymic hydrolysis of propylene oxide at pH 1 in 0.1 mol/L hydrochloric acid (37 °C) resulted in a half-life similar to that in the acidic conditions of the stomach and was reported to be about 1 min (reviewed by Ehrenberg & Hussain, 1981).

1,2-Propanediol is further metabolized to lactic and pyruvic acids and, hence, has served as a nutrient for dogs, cattle, rats and chickens (reviewed by Ruddick, 1972). 1,2-Propanediol can also be excreted in part unchanged in urine after its oral administration to dogs (Lehman & Newman, 1937).

Propylene oxide alkylated the N-terminal valine of Hb when incubated with whole blood or erythrocytes (Törnqvist *et al.*, 1986) or with Hb (Mowrer *et al.*, 1986). HOPrHis and HOPrVal have been used for tissue dosimetry of propylene oxide in animals *in vivo* (Farmer *et al.*, 1982; Bailey *et al.*, 1987; Svensson *et al.*, 1991; Segerbäck *et al.*, 1992). An HOPrHis adduct level of 10.5 ng/g globin was detected by gas chromatography-mass spectrometry (detection limit, 0.2 ng/g globin) in female Wistar rats after exposure to 1300 ppm [3130 mg/m³] propylene oxide over 4 h (Farmer *et al.*, 1982). Concentrations of HOPrVal were measured in male CBA mice 3 and 5 h after intraperitoneal injection of uniformly labelled ¹⁴C-propylene oxide (0.065–0.19 mmol/kg bw [3.8–11 mg/kg bw]). A linear relationship through the origin was found between adduct level and administered dose of propylene oxide, corresponding to about 1300 pmol/g globin per mmol propylene oxide/kg bw [22 pmol/g globin per mg propylene oxide/kg bw] (Svensson *et al.*, 1991).

Species differences in HOPrVal levels in Hb after administration of propylene oxide were investigated in mice, rats and dogs (Table 6). Male B6C3F1 mice and male Fischer 344 rats were exposed to uniformly labelled ¹⁴C-propylene oxide administered intraperitoneally or via inhalation in closed exposure chambers in which the atmospheric concentration-time course was measured after injection of a single dose into the chamber atmosphere. The exposures were terminated after 5 h, when the atmospheric radioactivity had dropped to less than 5% of the initial values. The animals were killed 2 h later and blood was collected. HOPrVal levels were also measured in male and female beagle dogs exposed for about 1 h to 100 or 500 ppm [238 or 1190 mg/m³] propylene oxide through an anaesthetic mask. Blood samples were taken before exposure, immediately after exposure and when the dogs were killed 4 h later. The mean levels of HOPrVal in mice and rats, when normalized to an uptake of propylene oxide of 1 mg/kg bw, were independent of route of administration. The extent of HOPrVal alkylation was similar in rats and dogs (70–90 pmol/g Hb per mg propylene oxide/kg bw) but about 50% lower in mice (30–40 pmol/g Hb per mg propylene oxide/kg bw) (Segerbäck *et al.*, 1992).

In a further study, the rate constants for the reaction of propylene oxide with N-terminal valine in Hb in erythrocytes from different species were determined *in vitro*. The values for rats, dogs and man ($K_{\text{val}} \times 10^4$) were similar: 0.22, 0.28 and 0.25 L/g Hb per h. The value for mice was 0.14 L/g Hb per h. The rate constant for the reaction of propylene oxide with 7-guanine in calf thymus DNA *in vitro* was 0.25 L/g DNA per h, and the value should not differ among species. On the basis of Hb adduct levels measured in workers exposed to propylene oxide and the adduct levels in Hb and DNA measured experimentally *in vivo*, tissue doses were calculated for the purposes of interspecies comparison. The dose in blood

was virtually the same in the three experimental animal species and in man, but an extrapolation based on surface area showed as approximately seven-fold difference between mice and dogs (Segerbäck *et al.*, 1994).

Table 6. Concentrations of propylene oxide–haemoglobin adducts in mice, rats and dogs after treatment with propylene oxide

Species	Dose route	Dose (mg/kg)	HOPrVal adduct concentration (pmol/g Hb)
Mouse		0	5
	Intraperitoneal	3.1	72–86
		7.6	170–210
	Inhalation	6.5	270
18.4		590	
Rat		0	15
	Intraperitoneal	3.1	110–250
		7.6	430–740
	Inhalation	3.8	260–320
		10.9	660–760
15.3		1700	
Dog		0	< 5
	Inhalation	3.8	280

From Segerbäck *et al.* (1992); HOPrVal, *N*-(2-hydroxypropyl)-valine

4.2 Toxic effects

The toxicology of propylene oxide has been reviewed (WHO, 1985; Meylan *et al.*, 1986; US Environmental Protection Agency, 1987; Beratergremium für umweltrelevante Alstoffe, 1993).

4.2.1 Humans

Three cases of corneal burns after exposure to propylene oxide have been described (McLaughlin, 1946).

van Ketel (1979) described the development of hand eczema in a female laboratory analyst who was working with pure propylene oxide or a concentrated solution (50%). Two cases of contact dermatitis from a disposable swab containing 70% isopropyl alcohol and 1% propylene oxide were reported by Jensen (1981).

4.2.2 Experimental systems

(a) Acute toxicity

No organ injury was seen in rats exposed by inhalation to calculated concentrations of 9480 mg/m³ (4000 ppm) propylene oxide vapour for 0.5 h, to 4740 mg/m³ (2000 ppm) for 2 h

or 2370 mg/m³ (1000 ppm) for 7 h. Four of 10 rats died after 4 h of exposure to the highest concentration (Rowe *et al.*, 1956).

A single exposure of female CD1 mice to 20 ppm propylene oxide [48.2 mg/m³] by inhalation for 3 h had no significant effect on lung host defenses, monitored as mortality resulting from streptococcal pneumonia or pulmonary bactericidal activity against inhaled ³⁵S-*Klebsiellae pneumoniae* (Aranyi *et al.*, 1986).

(b) *Subchronic toxicity*

Male Fischer 344 rats were exposed to 0, 10, 20, 50, 150 or 525 ppm [24.1, 48.2, 120.5, 361.5 or 1265 mg/m³] propylene oxide vapour for up to four weeks followed by recovery for up to four weeks, and toxicity and cell proliferation were examined in the nasal cavity. Respiratory epithelial hyperplasia, degeneration of olfactory epithelium and cell proliferation at both sites were dose and time dependent and reversible upon cessation of exposure. No effect was observed at 50 ppm propylene oxide (Eldridge *et al.*, 1994).

Exposure of male Wistar rats to 1500 ppm [3615 mg/m³] propylene oxide (6 h/day, five days/week, seven weeks) by inhalation caused ataxia in the hindlegs without muscular atrophy. A central-peripheral distal axonopathy was verified histologically by the observation of axonal degeneration of myelinated fibres in hindleg nerves and in the fasciculus gracilis (Ohnishi *et al.*, 1988; Ohnishi & Murai, 1993).

(c) *Chronic toxicity*

The chronic toxicity of inhaled propylene oxide was studied in male weanling Fischer 344 rats exposed to 100 or 300 ppm [241 and 723 mg/m³] for 7 h/day on five days/week for 104 weeks. Body weights of rats exposed to either concentration were significantly lower than those of controls, and a significant increase in mortality was observed in both groups of exposed rats over that in controls. All groups developed *Mycoplasma pulmonis* infection about 16 months into the study, and the infection, alone and in combination with the exposures, affected the survival of rats and influenced the development of proliferative lesions in the nasal mucosa. Some changes in organ:body weight ratios were seen in treated rats after two years of the study: small increases were reported for lungs, adrenal glands and brain, and a small reduction for testes. The serum activities of aspartate aminotransferase and sorbitol dehydrogenase were increased only in the low-exposure group. Skeletal muscle atrophy with no sciatic nerve involvement was observed primarily in the high-exposure group and consisted of multifocal areas of atrophy and degeneration. Treated animals had higher incidences than controls of inflammatory lesions in the nasal cavity, trachea, lung and middle ear, which increased with dose in the nasal cavity. A dose-dependent increase, significant at the high dose, was also seen in the incidence of complex epithelial hyperplasia in the nasal mucosa, although an influence of the infection could not be ruled out (Lynch *et al.*, 1984a).

In another study, male and female Fischer 344/N rats and male and female B6C3F1 mice were exposed by inhalation to propylene oxide at concentrations of 200 and 400 ppm [482 and 964 mg/m³] for 6 h/day on five days/week for 103 weeks. During the second year of exposure, the mean body weights of animals of both species at the high dose were lower than those of the controls; survival of rats was comparable to that of controls, but survival of exposed mice was decreased. A dose-related increase in the incidence of acute-chronic

rhinitis was seen in exposed mice (28% in each sex at 200 ppm [482 mg/m³], 76% in males and 36% in females at 400 ppm [964 mg/m³]), with degeneration and necrosis of mucosal epithelium in some areas of severe rhinitis. Suppurative rhinitis was observed in rats, in 18% of male and 6% of female controls, 42% of males and 10.4% of females at 200 ppm [482 mg/m³] and 76% of males and 48% of females at 400 ppm [964 mg/m³]. In rats exposed to the high dose of propylene oxide, there were also significant increases in the incidences of squamous metaplasia and hyperplasia of the respiratory epithelium of the nasal mucosa and the epithelium of the mucosal glands (US National Toxicology Program, 1985; Renne *et al.*, 1986).

After male and female Wistar rats were exposed chronically (6 h/day, five days/week, 28 months) to atmospheric concentrations of 30, 100 and 300 ppm [72.3, 241 and 723 mg/m³] propylene oxide, mortality was increased in animals of each sex at the highest dose and in females also at the medium dose. Body weights were lower than those of the controls throughout the study in males exposed to 300 ppm and during the first year in females exposed to 300 ppm. Increased incidences of degenerative and hyperplastic changes of the nasal mucosa were observed in all exposed groups (Kuper *et al.*, 1988).

4.3 Reproductive and prenatal effects

4.3.1 Humans

No data were available to the Working Group.

4.3.1 Experimental systems

New Zealand white rabbits were exposed by inhalation to 0 (17 animals) or 500 ppm [1205 mg/m³] propylene oxide (purity, 99%) vapour for 7 h per day on gestation days 7–19 (11 animals) or 1–19 (19 animals). Fetuses were examined on day 30. Food consumption, but not maternal body weight, was generally depressed in the treated groups during the periods of exposure. Fertility was low in all groups, but the overall resorption rate was not increased, and no adverse effect was observed in the fetuses in the treated groups. Sprague-Dawley rats were exposed by inhalation to 0 (46 animals) or 500 ppm propylene oxide (purity, 99%) vapour for 7 h per day either from three weeks before gestation to day 16 of gestation (43 animals) or on days 1–16 (41 animals) or 7–16 of gestation (44 animals). Fetuses were examined on day 21. Food consumption was reduced in females that received the pregestational exposure, and maternal weight gain tended to be lower in all treated groups during exposure. Fetal growth was lower in all treated groups than in controls. No major malformation related to treatment was seen, but the incidence of rib dysmorphology (primarily wavy ribs) was increased in all treated groups (Hackett *et al.*, 1982).

Hayes *et al.* (1988) exposed Fischer 344 rats to propylene oxide (> 99.7% pure) by inhalation (whole-body exposure) for two generations. Groups of 30 males and 30 females were exposed to 0, 30, 100 or 300 ppm [72.3, 241 or 723 mg/m³] propylene oxide for 6 h per day on five days per week (seven days per week from mating to end of lactation except for four to five days during parturition) for 14 weeks, and then mated. Groups of offspring (F1) were exposed after weaning to the same levels for 17 weeks and then mated to produce F2

litters. Other than decreased body weight gain at the highest dose level, no adverse effect was seen on fertility, litter size, development or postnatal survival. Gross and histological examination of the high-dose and control pups (F1 and F2) showed no adverse effects.

A teratology study was carried out on groups of 20–23 pregnant Fischer 344 rats exposed (whole-body) to 0, 100, 300 or 500 ppm [241, 723 or 1205 mg/m³] propylene oxide (> 99% pure) for 6 h per day from days 6 to 15 of gestation and killed on day 20. Maternal body weight gain was depressed at the highest dose level, but no adverse effect was observed on litter size, resorptions or fetal weight, and no increase in malformations was seen. The incidence of seventh cervical ribs was increased (13.6% versus 2.8% in controls [$p = 0.026$]) in the highest dose group only (Harris *et al.*, 1989).

4.4 Genetic and related effects (see also Table 7 and Appendices 1 and 2)

4.4.1 Humans

Results from the analysis of chromosomal aberrations and micronuclei in peripheral blood lymphocytes from 20 male workers exposed to propylene oxide during the production of alkylated starch were inconclusive because no data were available on controls (Högstedt *et al.*, 1990).

4.4.2 Experimental systems

(a) DNA adducts

Propylene oxide binds covalently to DNA. Using ³²P-postlabelling, Randerath *et al.* (1981) detected 15 different DNA adducts after incubation (12 h, 37 °C, pH 5.8) of propylene oxide (200 mmol/L) with calf thymus DNA (3 mmol/L).

After incubation (48 h, 37 °C, pH 7.4) of propylene oxide with calf thymus DNA (0.2 mmol propylene oxide per mg DNA), the yields of alkylated nucleosides were: deoxyguanosine (46% reacted) > deoxyadenosine (38% reacted) > deoxycytidine (24% reacted) > deoxythymidine (15% reacted) (Djuric *et al.*, 1986).

After incubation (10 h, 37 °C, pH 7.5) of propylene oxide (2 mol/L) with calf thymus DNA (3 mg/ml), the following 2-hydroxypropyl adducts were found: 7-guanine (133 nmol/mg DNA), 3-adenine (14 nmol/mg DNA), 3-uracil (13 nmol/mg DNA) and N⁶-adenine (1 nmol/mg DNA). The 2-hydroxypropyl adduct at 3-uracil was formed from the corresponding cytosine adduct by hydrolytic deamination of the imino group (Solomon *et al.*, 1988).

7-(2-Hydroxypropyl)guanine was detected in DNA hydrolysates of various organs obtained from male CBA mice 3 h and 10 h after intraperitoneal injection of ¹⁴C-propylene oxide (Svensson *et al.*, 1991). In mice, rats and dogs, the levels of DNA adducts in liver (in pmol/g DNA per mg propylene oxide per kg bw) were 17 in mice, 38 in rats and 17 in dogs after intraperitoneal or intravenous injection and somewhat higher after exposure by inhalation in mice (Segerbäck *et al.*, 1994). [The Working Group calculated a covalent binding index—(μmol adduct/mol DNA nucleotide)/(mmol chemical/kg bw)—of 0.3 for mouse liver DNA 6 h after an intraperitoneal injection.]

DNA-associated radiolabel was determined in the respiratory mucosa of male Fischer 344 rats on completion of exposure for 2 h by nose only to tritiated propylene oxide at concentrations ranging from 6 to 46 ppm [14.5–111 mg/m³]. After exposure to 46 ppm, radioactivity counts were highest in nasal cavities, intermediate in trachea and lowest in lung, reaching 17, 5.8 and 3.3 adducts/10⁶ base, respectively. The persistence of radiolabel in the DNA of the nasal cavities, trachea and lungs of rats exposed to 19.5 ppm [47 mg/m³] was also investigated. Either a small or no decrease in counts was found in the mucosa of lung and trachea, while a biphasic elimination of radiolabel from nasal mucosa was observed, with half-lives of 8 h and 5.3 days (Snyder & Solomon, 1993). [The Working Group noted that the author did not attempt to differentiate between radioactivity incorporated metabolically into DNA and radiolabel covalently bound to DNA; therefore, these figures may not accurately reflect the DNA binding of propylene oxide.]

(b) *Mutation and allied effects*

Propylene oxide induced DNA damage and gene mutation in bacteria. It caused gene mutation in yeast and fungi, and in one study it induced mitotic gene conversion in *Saccharomyces cerevisiae*. Induction of sex-linked recessive lethal mutations in *Drosophila* was reported in a single study. Propylene oxide induced DNA damage, gene mutation, sister chromatid exchange and chromosomal aberrations in mammalian cells *in vitro*. It also induced sister chromatid exchange and chromosomal aberrations in human lymphocytes *in vitro*. Propylene oxide was shown to alkylate calf thymus DNA in one study *in vitro* at a high concentration.

One study showed no significant increase in sister chromatid exchange or chromosomal aberration frequency in peripheral blood lymphocytes of cynomolgus monkeys exposed to 300 ppm propylene oxide for 7 h per day on five days a week for two years. Micronuclei were not induced in bone-marrow cells of mice administered propylene oxide by gavage but were induced in those of mice receiving the compound by intraperitoneal injection. In single studies, chromosomal aberrations and sister chromatid exchange were induced in mouse bone-marrow cells after intraperitoneal injection. Dominant lethal mutations were not induced in mice exposed orally or in rats exposed by inhalation. Propylene oxide did not cause sperm abnormalities in mice treated by inhalation (7 h per day, five days).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Propylene oxide is produced by dehydrochlorination of propylene chlorohydrin or by indirect oxidation of propylene. It is used primarily as a chemical intermediate to produce polyether polyols, propylene glycols and propylene glycol ethers. It is used to a lesser extent in the production of hydroxypropyl starch ethers, as a food additive and as a fumigant for certain dried fruits and nuts.

Occupational exposure occurs during the production of propylene oxide and its derivatives and during production of hydroxypropyl starch ethers.

Table 7. Genetic and related effects of propylene oxide

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, SOS functions, <i>Salmonella typhimurium</i> TA1535/pSK1002	+	+	23.0000 ^c	Ong <i>et al.</i> (1987)
BRD, Bacteria (other), differential toxicity	+	0	500.0000	Bootman <i>et al.</i> (1979)
BPF, Bacteriophage, forward mutation	-	0	39000.0000	Cookson <i>et al.</i> (1971)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	(+)	0	750.0000	Wade <i>et al.</i> (1978)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	50.0000	Bootman <i>et al.</i> (1979)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	0	58.0000	Hemminki & Falck (1979)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	145.0000	Pfeiffer & Dunkelberg (1980)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	239.0000 ^c	Simmon (1981)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	200.0000	Yamaguchi (1982)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	1667.0000	Canter <i>et al.</i> (1986)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	30.0000	Djurić <i>et al.</i> (1986)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	0	+	50.0000 ^d	Hughes <i>et al.</i> (1987)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	5000.0000	Zeiger <i>et al.</i> (1988)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	174.0000	Agurell <i>et al.</i> (1991)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	0.2300 ^c	Castelain <i>et al.</i> (1993)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	(+)	0	500.0000	Wade <i>et al.</i> (1978)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	+	50.0000	Bootman <i>et al.</i> (1979)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	0	145.0000	Pfeiffer & Dunkelberg (1980)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	0	96.0000 ^c	Simmon (1981)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	+	1667.0000	Canter <i>et al.</i> (1986)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	0	10.0000	Djurić <i>et al.</i> (1986)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	+	833.0000	Zeiger <i>et al.</i> (1988)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	0	174.0000	Agurell <i>et al.</i> (1991)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	+	0.1200 ^c	Castelain <i>et al.</i> (1993)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	350.0000	Bootman <i>et al.</i> (1979)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	0	2900.0000	Pfeiffer & Dunkelberg (1980)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	?	?	0.0000	Canter <i>et al.</i> (1986)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	5000.0000	Zeiger <i>et al.</i> (1988)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	1000.0000	Dean <i>et al.</i> (1985)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	350.0000	Bootman <i>et al.</i> (1979)

Table 7 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	0	2900.0000	Pfeiffer & Dunkelberg (1980)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	0.0000	Canter <i>et al.</i> (1986)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	5000.0000	Zeiger <i>et al.</i> (1988)
SAS, <i>Salmonella typhimurium</i> TA97, reverse mutation	+	+	0.0000	Canter <i>et al.</i> (1986)
SAS, <i>Salmonella typhimurium</i> TA97, reverse mutation	-	-	5000.0000	Zeiger <i>et al.</i> (1988)
ECW, <i>Escherichia coli</i> WP2 <i>uvrA</i> , reverse mutation	-	0	58.0000	Hemminki & Falck (1979)
ECW, <i>Escherichia coli</i> WP2 <i>uvrA</i> , reverse mutation	+	+	20.0000	Dean <i>et al.</i> (1985)
EC2, <i>Escherichia coli</i> WP2, reverse mutation	+	+	350.0000	Bootman <i>et al.</i> (1979)
EC2, <i>Escherichia coli</i> WP2, reverse mutation	+	-	20.0000	Dean <i>et al.</i> (1985)
ECR, <i>Escherichia coli</i> B (Arg-) Hs30R, reverse mutation	+	0	58.0000	Kohda <i>et al.</i> (1987)
KPF, <i>Klebsiella pneumoniae</i> , forward mutation	+	0	29.0000	Voogd <i>et al.</i> (1981)
SCG, <i>Saccharomyces cerevisiae</i> , gene conversion	+	0	1740.0000	Agurell <i>et al.</i> (1991)
SCR, <i>Saccharomyces cerevisiae</i> , reverse mutation	+	0	1740.0000	Agurell <i>et al.</i> (1991)
SZF, <i>Schizosaccharomyces pombe</i> , forward mutation	+	+	174.0000	Migliore <i>et al.</i> (1982)
NCR, <i>Neurospora crassa</i> , reverse mutation	+	0	29000.0000	Kølmark & Giles (1955)
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutation	+	0	1.5000 ^c	Hardin <i>et al.</i> (1983)
DIA, DNA single strand breaks, rat hepatocytes <i>in vitro</i>	+	0	1.7000	Sina <i>et al.</i> (1983)
GCO, Gene mutation, Chinese hamster ovary cells, <i>hprt</i> locus	+	0	16.0000 ^c	Zamora <i>et al.</i> (1983)
G5T, Gene mutation, mouse L5178Y cells, <i>tk</i> locus	+	0	1.0000 ^c	McGregor <i>et al.</i> (1991)
SIC, Sister chromatid exchange, Chinese hamster ovary cells <i>in vitro</i>	+	+	5.0000	Gulati <i>et al.</i> (1989)
SIC, Sister chromatid exchange, Chinese hamster V79 cells <i>in vitro</i>	+	0	290.0000	von der Hude <i>et al.</i> (1992)
SIR, Sister chromatid exchange, rat liver cells <i>in vitro</i>	+	0	50.0000	Dean & Hodson-Walker (1979)
CIC, Chromosomal aberrations, Chinese hamster ovary cells <i>in vitro</i>	(+)	+	160.0000	Gulati <i>et al.</i> (1989)
CIR, Chromosomal aberrations, rat liver cells <i>in vitro</i>	+	0	25.0000	Dean & Hodson-Walker (1979)
CIR, Chromosomal aberrations, rat liver cells <i>in vitro</i>	+	0	25.0000	Dean <i>et al.</i> (1985)

Table 7 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SHL, Sister chromatid exchange, human lymphocytes <i>in vitro</i>	+	0	58.0000 ^c	Tucker <i>et al.</i> (1986)
SHL, Sister chromatid exchange, human lymphocytes <i>in vitro</i>	+	0	145.0000	Agurell <i>et al.</i> (1991)
CHL, Chromosomal aberrations, human lymphocytes <i>in vitro</i>	+	0	1.9000	Bootman <i>et al.</i> (1979)
SVA, Sister chromatid exchange, monkey lymphocytes <i>in vivo</i>	-		110.0000, inhal. 7 h/d ^e	Lynch <i>et al.</i> (1984b)
SVA, Sister chromatid exchange, mouse bone-marrow cells <i>in vivo</i>	+		100.0000 × 1 ip	Farooqi <i>et al.</i> (1993)
MVM, Micronucleus formation, mouse bone-marrow cells <i>in vivo</i>	+		300.0000 × 2 ip	Bootman <i>et al.</i> (1979)
MVM, Micronucleus formation, mouse bone-marrow cells <i>in vivo</i>	-		500.0000 × 2 po	Bootman <i>et al.</i> (1979)
MVM, Micronucleus formation, mouse bone-marrow <i>in vivo</i>	+		100.0000 × 1 ip	Farooqi <i>et al.</i> (1993)
CLA, Chromosomal aberrations, monkey lymphocytes <i>in vivo</i>	-		110.0000, inhal. 7 h/d ^e	Lynch <i>et al.</i> (1984b)
CBA, Chromosomal aberrations, mouse bone-marrow cells <i>in vivo</i>	+		100.0000, ip × 1	Farooqi <i>et al.</i> (1993)
DLM, Dominant lethal mutation, mouse <i>in vivo</i>	-		250.0000 × 14 po	Bootman <i>et al.</i> (1979)
DLR, Dominant lethal mutation, rat <i>in vivo</i>	-		29.0000, inhal. 7 h/d × 5	Hardin <i>et al.</i> (1983)
MVH, Micronucleus formation, human lymphocytes <i>in vivo</i>	?		0.0000 ^f	Högstedt <i>et al.</i> (1990)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	?		0.0000 ^f	Högstedt <i>et al.</i> (1990)
BID, Binding (covalent) to calf thymus DNA <i>in vitro</i>	+	0	11600.0000	Randerath <i>et al.</i> (1981)
BID, Binding (covalent) to calf thymus DNA <i>in vitro</i>	+	0	40.0000	Djurić <i>et al.</i> (1986)
BID, Binding (covalent) to calf thymus DNA <i>in vitro</i>	+	0	116000.0000	Solomon <i>et al.</i> (1988)
BVD, Binding (covalent) to mouse DNA <i>in vivo</i>	+		6.0000 × 1 ip	Svensson <i>et al.</i> (1991)
BVD, Binding (covalent) to rat DNA <i>in vivo</i>	+		3.0000 inhal. 2 h	Snyder & Solomon (1993)
BVD, Binding (covalent) to mouse, rat and dog DNA <i>in vivo</i>	+		3.0000 × 1, ip, iv or inhal. 5 h	Segerbäck <i>et al.</i> (1994)
SPM, Sperm morphology, mouse <i>in vivo</i>	-		290.0000, inhal. 7 h/d × 5	Hardin <i>et al.</i> (1983)

Table 7 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Protein binding				
BVP, Binding (covalent) to mouse protein <i>in vivo</i>	+		4.0000 × 1 ip	Svensson <i>et al.</i> (1991)
BVP, Binding (covalent) to mouse, rat and dog haemoglobin <i>in vivo</i>	+		3.0000 × 1 ip, iv or inhal. 5 h	Segerbäck <i>et al.</i> (1994)
BHP, Binding (covalent) to human haemoglobin <i>in vivo</i>	+		0.0000	Pero <i>et al.</i> (1985)

^a+, positive; (+), weak positive; -, negative; 0, not tested; ?, inconclusive (variable response within several experiments within an adequate study)

^bIn-vitro tests, µg/ml; in-vivo tests, mg/kg bw

^cAtmospheric concentration in exposure chamber

^dIncubated in Tedlar bags

^eFive days/week; two years

^fNo controls (not on profile)

5.2 Human carcinogenicity data

One case-control study provides information about cancer risk in relation to exposure to propylene oxide specifically but does not allow any firm conclusion regarding carcinogenicity.

5.3 Animal carcinogenicity data

Propylene oxide was tested by oral gavage in one study in rats, by inhalation in one study in mice and in three adequate studies in rats and by subcutaneous administration in one study in mice and in one study in rats. Propylene oxide administered by oral gavage to rats produced tumours of the forestomach, which were mainly squamous-cell carcinomas. In mice exposed by inhalation, propylene oxide produced haemangiomas and haemangiosarcomas of the nasal cavity and a few malignant nasal epithelial tumours. In a study in rats of each sex exposed by inhalation, papillary adenomas of the nasal cavity were observed in males and females and thyroid adenomas and carcinomas were found in females; in the second study, in males, papillary adenomas of the nasal cavity and an increased incidence of adrenal pheochromocytomas were observed; in the third study, in females, increased incidences of mammary fibroadenomas and adenocarcinomas were observed. Subcutaneous administration of propylene oxide to mice produced local sarcomas; the study in rats was inadequate for evaluation.

5.4 Other relevant data

In rats exposed by inhalation, there is strong uptake of propylene oxide, which is then metabolized extensively and eliminated rapidly. Metabolism occurs predominantly by conjugation with glutathione. Propylene oxide can also be hydrolysed by epoxide hydrolase to 1,2-propanediol, which is subsequently metabolized to lactic and pyruvic acids. Propylene oxide forms adducts with proteins, including haemoglobin, in man, dog, rat and mouse. In mice, the concentration of the N-terminal valine adduct of propylene oxide in haemoglobin is linearly related to the administered dose. The alkylation efficiency in mice exposed by inhalation is about one-half that observed in rats and dogs.

In a seven-week study of rats exposed by inhalation, ataxia in the absence of muscular atrophy was observed, which was due to distal axonopathy in the central and peripheral nervous systems. Chronic and subchronic exposure of rats to propylene oxide by inhalation induced proliferative lesions, irritation and toxicity in the nasal mucosa and respiratory epithelium.

Other than occasional reductions in fetal weight, no adverse effects on reproduction were observed in rats or rabbits exposed to propylene oxide at up to 500 ppm.

DNA adducts of propylene oxide are formed in various organs of mice, rats and dogs. Binding in mouse liver DNA was about one-twentieth that of ethylene oxide.

Dominant lethal mutations were not induced in rats or mice, and sperm abnormalities were not observed in mice exposed to propylene oxide *in vivo*. Micronuclei and, in single studies, chromosomal aberrations and sister chromatid exchange were induced in mouse

bone marrow after intraperitoneal injection of propylene oxide. Neither sister chromatid exchange nor chromosomal aberrations were induced in monkeys exposed by inhalation to 300 ppm. Propylene oxide induced chromosomal aberrations and sister chromatid exchange in human lymphocytes and DNA damage, gene mutation, chromosomal aberrations and sister chromatid exchange in mammalian cells *in vitro*. It caused dominant lethal mutation in *Drosophila* and was mutagenic to yeast, fungi and bacteria.

5.5 Evaluation¹

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

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

Overall evaluation

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

6. References

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¹For definition of the italicized terms, see Preamble, pp. 27–30.

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ISOPRENE

1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 78-79-5

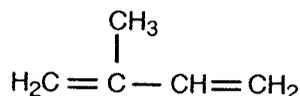
Deleted CAS Reg. No.: 78006-92-5

Chem. Abstr. Name: 2-Methyl-1,3-butadiene

IUPAC Systematic Name: 2-Methyl-1,3-butadiene

Synonyms: Isopentadiene; β -methylbivinylyl; 2-methylbutadiene; 3-methyl-1,3-butadiene

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 liquid or gas with faint aromatic odour (Exxon Chemical Co., 1989; Weitz & Loser, 1989)
- (b) *Boiling-point*: 34 °C (Lide, 1991)
- (c) *Melting-point*: -146 °C (Lide, 1991)
- (d) *Density (liquid)*: 0.6810 at 20 °C/4 °C (Lide, 1991)
- (e) *Spectroscopy data*: Infrared [prism (688); grating (154)], ultraviolet and mass spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991; US National Library of Medicine, 1993a).
- (f) *Solubility*: Insoluble in water (0.029 mol%); soluble in acetone, benzene, diethyl ether and ethanol (Lide, 1991)
- (g) *Volatility*: Vapour pressure, 60.7 kPa at 20 °C (Weitz & Loser, 1989); relative vapour density (air = 1), 2.35 (Exxon Chemical Co., 1989)
- (h) *Stability*: Lower explosive limit, 1-1.5% (28-40 g/m³) (Weitz & Loser, 1989); will ignite readily at ambient temperatures; will polymerize vigorously or decompose with shocks of pressure or temperature (Exxon Chemical Co., 1989)

- (i) *Octanol–water partition coefficient (P)*: log P, 2.30 (US National Library of Medicine, 1993a)
- (j) *Conversion factor*: $\text{mg/m}^3 = 2.79 \times \text{ppm}^a$

1.1.4 *Technical products and impurities*

Isoprene is available commercially at a minimal purity of 99.0%; minor contaminants include acetylenes, cyclopentadiene, peroxides, piperylenes, pentenes and sulfur. It is typically inhibited with *para-tert-butyl* catechol (Exxon Chemical Co., undated). Isoprene feedstock recovered from C₅ hydrocarbon refinery mixtures contains about 20% isoprene (Chevron Chemical Co., undated).

1.1.5 *Analysis*

The US Environmental Protection Agency has proposed methods for the analysis of volatile 'priority pollutants' in water [EPA Method 624] by gas chromatography–mass spectrometry and purge-and-trap techniques. Isoprene, at a detection limit of 3 µg/L, was one of the compounds studied (Spingarn *et al.*, 1982).

A method for the analysis of volatile organic compounds in air samples involves gas chromatography with flame ionization detection and coupled fused silica capillary columns of different polarity and length in order to separate the complex mixtures. Isoprene was detected by this method in atmospheric and alveolar air samples from exposed workers (Clair *et al.*, 1991).

A method for the collection and assay of volatile compounds, including isoprene, in breath involves drawing a sample through a water trap, then through an adsorptive trap of graphitized carbon. The sample is eluted by thermal desorption, concentrated by two-stage cryofocusing, then assayed by gas chromatography with flame ionization and flame photometric detection (Phillips & Greenberg, 1991).

An analytical method for the determination of non-methane hydrocarbons, including isoprene, in air makes use of gas chromatography and simultaneous programming of pressure and temperature on a capillary column. A combination of on-column cryofocusing and gas chromatographic reinjection was used, with a detection limit of 2 pg (Matuška *et al.*, 1986).

1.2 **Production and use**

1.2.1 *Production*

Isoprene was first isolated in 1860 during the pyrolysis of natural rubber. The reverse reaction, polymerization of isoprene to poly(*cis*-1,4-isoprene), which has a structure corresponding to that of natural rubber, was the subject of intensive effort. The first successful attempts were reported in 1954 and 1955 by the Goodrich Gulf (using an

^aCalculated from: $\text{mg/m}^3 = (\text{relative molecular mass}/24.45) \times \text{ppm}$, assuming normal temperature (25 °C) and pressure (101.3 kPa)

aluminium–titanium Ziegler catalyst) and Firestone companies (using an alkyl lithium catalyst). Isoprene was commonly prepared on a laboratory scale by thermolysis of turpentine oil (the so-called isoprene lamp). Pyrolysis of dipentene (limonene) was used in the USA early in the Second World War as a commercial source of isoprene (Bibb process). Isoprene itself was of no commercial importance until after the Second World War, when cost-effective methods were developed for obtaining it from petrochemical sources (Weitz & Loser, 1989).

The first commercial synthesis of isoprene, the Goodyear Scientific Design isoprene synthesis, begins with dimerization of propylene to 2-methyl-1-pentene. This is then isomerized to 2-methyl-2-pentene, which is subsequently cracked with loss of methane to give isoprene. The most frequently used synthetic procedure is acid-catalysed addition of formaldehyde to isobutene (Prins reaction). Production facilities of this type are currently in operation in Japan and the former USSR (Weitz & Loser, 1989).

One-step dehydrogenation of isopentane to isoprene can be carried out according to the Houdry–Catadiene procedure (chromic oxide–aluminium oxide catalyst, about 600 °C, and about 7 kPa), with a yield of 52%. Isoprene is prepared commercially in this way in the former USSR. It has been produced by dehydrogenation of methylbutenes in the USA and the Netherlands (Weitz & Loser, 1989).

The primary source of isoprene today is as a by-product in the production of ethylene by naphtha cracking. A solvent extraction process very similar to that for 1,3-butadiene is used. The yield of isoprene is typically 2–5 wt% based on ethylene, although it may be increased by starting with a heavier raw material such as gas oil. With regard to energy consumption, recovery of isoprene from crack fractions is considerably more efficient than synthesizing the compound chemically. Increasingly heavier raw materials are being used in Europe and the USA for ethylene production in crackers, thereby increasing the quantity of isoprene by-product. The most appropriate solvents for production-scale separation of isoprene from other hydrocarbons include *N*-methylpyrrolidone, dimethylformamide and acetonitrile (Weitz & Loser, 1989; Bouton, 1992).

Information available in 1991 indicated that isoprene was produced by five companies in the USA, three in Japan and one each in the Netherlands and the United Kingdom (Chemical Information Services Ltd, 1991). Isoprene is produced in large quantities at several locations in the former USSR, which has a capacity of about 1000 thousand tonnes per year (Weitz & Loser, 1989). Production of isoprene in the USA was 230 thousand tonnes in 1981, 63 thousand tonnes in 1986 and 214 thousand tonnes in 1991 (US International Trade Commission, 1982, 1987, 1993). Production in Japan was 102 thousand tonnes in 1986 and 76 thousand tonnes in 1992. In western Europe, production in 1992 was approximately 15 thousand tonnes (Smith, 1993).

1.2.2 Use

Isoprene is used primarily for the synthesis of isoprene rubber, poly(*cis*-1,4-isoprene), which is used mostly for the production of vehicle tyres (Weitz & Loser, 1989). The second largest market for isoprene has been the manufacture of block polymers containing styrene (styrene–isoprene–styrene, or SIS, polymers), which are especially useful as thermoplastic rubbers and as pressure-sensitive or thermosetting adhesives. Smaller amounts of isoprene

are used in the production of butyl rubber (isobutene–isoprene copolymer). The distinctive features of butyl rubber include its low gas permeability, so that it is used in the construction of hoses and as a liner in tubeless tyres (Weitz & Loser, 1989).

The only chemical reaction of isoprene of commercial importance (other than polymerization) is its conversion to terpenes, e.g. citral, linalool, ionones, myrcene, L-menthol, *N,N*-diethylnerylamine, geraniol and nerolidols, which are used extensively in flavours and fragrances (Weitz & Loser, 1989).

1.3 Occurrence

Isoprene occurs widely in nature at low concentrations. It has been estimated that isoprene emissions from plants (associated with photosynthesis) amount to one-half of the total non-methane hydrocarbon emissions from the biosphere (Loreto & Sharkey, 1993). It is present in roasted coffee, in the gas phase of tobacco smoke (Graedel *et al.*, 1986; Löfroth *et al.*, 1989; Weitz & Loser, 1989), in gasoline and turbine automobile exhausts (Katzman & Libby, 1975; Hampton *et al.*, 1983; Stump & Dropkin, 1985; Graedel *et al.*, 1986), and can be regarded as a precursor of polycyclic aromatic compounds (Weitz & Loser, 1989). Isoprene is the basic structural unit of countless natural products, including natural rubber and the terpenes, and of biologically active important substances such as vitamins A and K (Saltman, 1981) and the steroid sex hormones. The biosynthesis of rubber and other natural products containing the isoprene skeleton proceeds not via isoprene itself, but rather via mevalonic acid (3,5-dihydroxy-3-methylpentanoic acid) (Stump & Dropkin, 1985).

1.3.1 Natural occurrence

Isoprene is the predominant hydrocarbon emitted by a number of deciduous forest species, including oak, poplar, sycamore and willow (Tingey, 1981; Shaw *et al.*, 1983; Lamb *et al.*, 1984; Isidorov *et al.*, 1985; MacKenzie *et al.*, 1991). It is also found in trace amounts in the emissions of coniferous trees and is a component of the volatile emissions of a number of shrubs, ferns, mosses and grasses (Altshuller, 1983; Winer *et al.*, 1989, 1992). Isoprene emission rates from foliage have been found to be in the range of 2.3–45 µg/g per h. Within forest canopies, the concentrations of isoprene and monoterpenes have been reported to range between [10 and 100 ppb carbon (ppbC)], while concentrations outside the canopy range from [1 to 10 ppbC] (Khalil & Rasmussen, 1992). The mean flux of isoprene from an isolated grove of oak trees was determined in two tracer studies to be 6780 and 8110 µg/m² per h (geometric means) (Lamb *et al.*, 1986).

Isoprene is produced endogenously in humans (see section 4.1).

1.3.2 Occupational exposure

The National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health between 1981 and 1983 indicated that 3700 US employees were potentially exposed occupationally to isoprene (US National Institute for Occupational Safety and Health, 1993). Of this number, 93% were estimated to be exposed to isoprene and 7% to be exposed to materials containing isoprene. The estimate is based on a survey of US companies and did not involve measurements of actual exposures.

Exposures to isoprene may occur during the manufacture of synthetic rubber and elastomers. Only two dated reports of occupational exposures to isoprene have been published. Pigolev (1968) reported area samples averaging 40 mg/m^3 in polymerization and rubber separation shops in Russia. The concentration exceeded 40 mg/m^3 in 48 of 140 air samples, with a maximal level of 52 mg/m^3 . In an earlier investigation in a Russian synthetic rubber plant, air concentrations of isoprene ranged from 17 to 28 mg/m^3 in a raw materials shop (mean, 19 mg/m^3), from 4 to 37 mg/m^3 in the polymerization department (mean, 22 mg/m^3) and from 3 to 18 mg/m^3 in the rubber separation department (mean, 8 mg/m^3) (Faustov, 1972).

1.3.3 Air

Isoprene enters the atmosphere as emissions from vegetation and emissions formed during wood pulping, biomass combustion and rubber abrasion (Graedel *et al.*, 1986). Emission rates and ambient concentrations measured in various field and laboratory studies have been reviewed (Shaw *et al.*, 1983; Graedel *et al.*, 1986; Lamb *et al.*, 1986, 1987).

The global annual emission of isoprene in 1988 was estimated to be 285 million tonnes (Turner *et al.*, 1991). Zimmerman *et al.* (1978) estimated an annual foliar isoprene emission of 350 million tonnes carbon [400 million tonnes isoprene], and Rasmussen and Khalil (1988) estimated a total annual isoprene emission of 450 million tonnes. The annual biogenic emission of isoprene in tropical Australia was estimated to be about 25 million tonnes carbon [28 million tonnes isoprene] (uncertainty range, 6–100 million tonnes carbon) (Ayers & Gillett, 1988). The biogenic isoprene emission rate for the United Kingdom was estimated to be 2000 tonnes per year for the years 1985 and 1989 (Hewitt & Street, 1992). The total rate of emission of isoprene from deciduous forests in the USA has been estimated to be 4.9 million tonnes per year (Lamb *et al.*, 1987), with the greatest emissions in the summer and with the southeast accounting for approximately 48% of the isoprene emitted (Altshuller, 1983). It has been estimated that 15 million tonnes per year of isoprene are emitted from the entire contiguous USA (Altshuller, 1983).

In studies conducted in US cities, the concentrations of isoprene in ambient air ranged from 0.005 to 90 ppbC [$0.003\text{--}50 \text{ }\mu\text{g/m}^3$] (Lonneman *et al.*, 1979; Arnts & Meeks, 1981; Winer *et al.*, 1981; Khalil & Rasmussen, 1992; US National Library of Medicine, 1993a). Natural isoprene concentrations in Japan during average wet and clear seasons were 0.56 and 1.9 ppbC [0.31 and $1.1 \text{ }\mu\text{g/m}^3$], respectively. The atmospheric concentrations of isoprene at Auch, France, were 0.088–0.675 ppb by volume (ppbv) [$0.25\text{--}1.9 \text{ }\mu\text{g/m}^3$] at ground level, 0.017 ppbv [$0.05 \text{ }\mu\text{g/m}^3$] at 1160 m altitude and 0.160 ppbv [$0.45 \text{ }\mu\text{g/m}^3$] at 270 m; it was not detected at 2900 m (Kanakidou *et al.*, 1989).

Isoprene has been detected in emissions from wood stoves and fireplaces at concentrations ranging from 0.003 to 0.130 ppbC/ppm as CO_2 (Edgerton *et al.*, 1986). In another study, it was detected at a concentration of 7 ppb [$20.0 \text{ }\mu\text{g/m}^3$] in woodsmoke (Kleindienst *et al.*, 1986). It was detected at a concentration of 0.46 mg/m^3 in an atmospheric grab sample taken near an oil fire (Perry, 1975; US National Library of Medicine, 1993a) and was detected in five of 10 samples of exhaust from turbojet engines at concentrations ranging from 0.05 to 19.8 ppmC [$0.028\text{--}11 \text{ mg/m}^3$] (Katzman & Libby, 1975). The average airborne yield of isoprene from cigarette smoke has been estimated to be 3.1 mg/cigarette. Isoprene

levels of 85 and 150 $\mu\text{g}/\text{m}^3$ were determined in two studies of tavern air during normal smoking conditions; the corresponding outdoor air concentrations at the time were 2 and $< 1 \mu\text{g}/\text{m}^3$ isoprene (Löfroth *et al.*, 1989).

1.3.4 Other

In a pilot study of pollutants in the breast milk of women living in four urban industrial areas in the USA, isoprene was detected in one of eight samples (Pellizzari *et al.*, 1982). In a pilot study of volatile organic compounds in 224 samples of the exhaled breath of 28 non-smoking volunteers, isoprene was detected in about 50% of the samples at concentrations ranging from 0.2 to 0.6 ng/L (Krotoszynski *et al.*, 1977) (see also p. 221).

1.4 Regulations and guidelines

Most countries have not established standards or guidelines for occupational exposures to isoprene (American Conference of Governmental Industrial Hygienists, 1993; ILO, 1993; UNEP, 1993). The short-term exposure limit in Russia is 40 mg/m^3 , and the time-weighted average concentration is 100 mg/m^3 in Poland and 10 mg/m^3 in Bulgaria (ILO, 1993; UNEP, 1993; US National Library of Medicine, 1993b).

The US Food and Drug Administration (1993) permits use of isoprene in certain polymeric products in contact with food.

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 40 male B6C3F1 mice, six to eight weeks old, were exposed to 0 (control), 70, 220, 700, 2200 or 7000 ppm [194–19 457 mg/m^3] isoprene (purity, $> 99\%$) vapours by inhalation (whole-body exposure) for 6 h per day on five days a week for six months. At the end of the exposure period, 10 mice per group were killed for gross and microscopic examination. The remaining 30 mice per group were maintained for a recovery period of six months without additional exposure to isoprene. Survival at termination of the study (12 months) was 27, 28, 28, 27, 26 and 21 for the control and exposed groups, respectively. Exposure to isoprene was associated with significant increases in the incidences of tumours at four sites, with the following incidences for control and treated groups: alveolar/bronchiolar adenoma or carcinoma: 2, 2, 1, 5, 10 and 9 ($p < 0.001$, logistic regression trend test); Harderian gland adenoma: 2, 6, 4, 14, 13 and 12 ($p < 0.001$, logistic regression trend test);

hepatocellular adenoma or carcinoma: 7, 3, 7, 15, 18 and 17 ($p < 0.001$, logistic regression trend test); forestomach squamous-cell papilloma or carcinoma: 0, 0, 0, 1, 4 and 6 ($p = 0.02$, incidental tumour test). Non-neoplastic lesions related to treatment included forestomach squamous-cell hyperplasia, lung alveolar hyperplasia, nasal olfactory degeneration and spinal cord degeneration (US National Toxicology Program, 1994).

3.1.2 Rat

Groups of 40 male Fischer 344/N rats, six to eight weeks old, were exposed to 0 (control), 70, 220, 700, 2200 or 7000 ppm [194–19 457 mg/m³] isoprene (purity, > 99%) vapours by inhalation (whole-body exposure) for 6 h per day on five days a week for six months. At the end of the six-month exposure period, 10 rats per group were killed. The incidence of interstitial-cell hyperplasia of the testis was significantly increased in the 7000-ppm exposure group ($p < 0.01$) over that in the controls and other exposure groups (1/10, 1/10, 3/10, 1/10, 3/10 and 10/10). The remaining 30 rats per group were maintained for a recovery period of six months without additional exposure to isoprene. There was no treatment-related effect on body weight or survival. Survival at termination of the study (12 months) was 30, 30, 29, 30, 30 and 30 for the control and exposed groups, respectively. In comparison with the incidence in controls, there was a slight increase ($p = 0.02$, Cochran-Armitage trend test) in the incidence of interstitial-cell adenoma of the testis (3/30 controls *versus* 3/30, 4/30, 7/30, 8/30 and 9/30 treated rats). There was no significant treatment-related increase in any other type of tumour (US National Toxicology Program, 1994). [The Working Group noted the short duration of the study and that the design prevented adequate evaluation of carcinogenic potential. It was also noted that the spontaneous incidence of interstitial-cell tumours of the testis in this strain of rat is high at two years.]

4. Other Data Relevant for an Evaluation of Carcinogenicity and Its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

Isoprene is formed endogenously, probably from mevalonic acid (Deneris *et al.*, 1984), a precursor of cholesterol biosynthesis. The endogenous production rate of isoprene was calculated to be 0.15 $\mu\text{mol/kg per h}$ (Hartmann & Kessler, 1990). Concentrations in the blood range between 15 and 70 nmol/L (mean, 37 nmol/L [2.5 ng/ml]) (Cailleux *et al.*, 1992). Isoprene is also found in human breath at concentrations in the range of 10–30 nmol/L (Jansson & Larsson, 1969; DeMaster & Nagasawa, 1978; Cailleux & Allain, 1989). The quantity exhaled per day per individual was estimated to be 2–4 mg (Gelmont *et al.*, 1981).

4.1.2 Experimental systems

The rate of endogenous production of isoprene was determined to be 1.9 $\mu\text{mol/kg per h}$ in rats and 1.9 $\mu\text{mol/kg per h}$ in mice (Peter *et al.*, 1987).

1,2-Epoxy-2-methyl-3-butene was the major metabolite of isoprene formed by mouse liver microsomes; 3,4-epoxy-2-methyl-1-butene was a minor metabolite (20%) (Del Monte *et al.*, 1985; see Figure 1). 3,4-Epoxy-2-methyl-1-butene, but not 1,2-epoxy-2-methyl-3-butene, can be further metabolized to isoprene diepoxide. Microsomes from mice and Syrian hamsters showed a six-fold higher maximal metabolic velocity (V_{\max}) for the latter reaction than those from rats and rabbits: 1.6 *versus* 0.25 nmol diepoxide/mg protein per min. Pretreatment with phenobarbital increased the V_{\max} for this reaction by 3–20 times (Longo *et al.*, 1985).

The maximal metabolic elimination rates estimated from experiments in a closed inhalation system were 130 and 400 $\mu\text{mol/kg}$ per h in male Wistar rats and B6C3F1 mice, respectively. The half-lives of isoprene were 6.8 min in rats and 4.4 min in mice. At atmospheric concentrations above 300 ppm [837 mg/m^3], the rate of metabolism was no longer proportional to concentration (Peter *et al.*, 1987).

Male Fischer 344 rats exposed by nose-only inhalation for 6 h to 8, 260, 1480 and 8200 ppm [23, 738, 4200 and 23 268 mg/m^3] [$4\text{-}^{14}\text{C}$]isoprene retained 19, 9, 6 and 5% of the inhaled radioactivity, respectively. About 75% of the retained isoprene radioactivity was excreted in urine within 66 h. Liver, blood and, especially, fat were the tissues that contained most isoprene and metabolites. In the inhalation phase, respiratory tract tissues contained concentrations of volatile metabolites substantially out of proportion to their masses relative to liver and blood, which was interpreted to indicate metabolism in the respiratory tract. Most of the radioactivity in blood (> 85%) was associated with material of low volatility, probably mostly conjugates or tetrols. Between 0.031% (at 8 ppm) and 0.002% (at 8200 ppm) of the inhaled $4\text{-}^{14}\text{C}$ label was tentatively identified as isoprene diepoxide. Under the assumption that all radioactive material with the volatility of the diepoxide was indeed the diepoxide, blood diepoxide concentrations of 0.37, 7.4, 15 and 17 mmol/L were derived from 6-h exposures to 8, 260, 1480 and 8200 ppm, respectively (Dahl *et al.*, 1987). A species difference was demonstrated in a two-compartment model of isoprene pharmacokinetics. Both rats and mice exhibited saturation kinetics when exposed to isoprene at concentrations above 300 ppm; however, the V_{\max} in mice was determined to be 400 $\mu\text{mol/h}$ per kg, or more than three times that in rats (130 $\mu\text{mol/h}$ per kg), implying that mice are sensitive with regard to isoprene metabolism (Peter *et al.*, 1987).

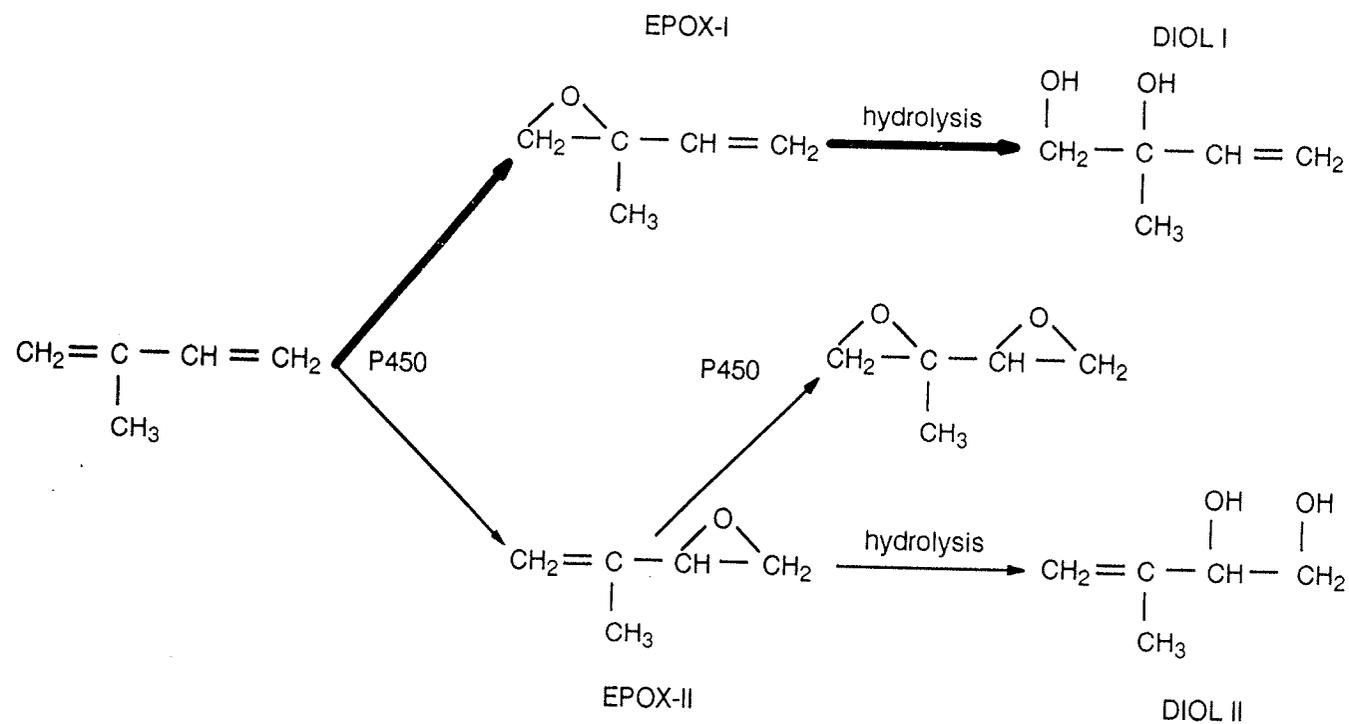
Male B6C3F1 mice exposed to 20, 200 and 2000 ppm [57, 568 and 5675 mg/m^3] ^{14}C -isoprene rapidly reached steady-state levels at which the blood concentrations of isoprene were 24.8, 830 and 6800 ng/ml, respectively (Bond *et al.*, 1991). Radioactivity in haemoglobin, measured 24 h after a single intraperitoneal injection into male Sprague-Dawley rats and male B6C3F1 mice, was linearly related to the administered dose up to 500 $\mu\text{mol/kg}$ and showed the same slope for both species (Sun *et al.*, 1989).

4.2 Toxic effects

4.2.1 Humans

Catarrhal inflammation, subtrophic and atrophic processes in the upper respiratory tract, and deterioration of olfaction were noted in isoprene rubber production workers.

Fig. 1. Microsomal metabolic pathways of isoprene



From Gervasi & Longo (1990)
 EPOX-I, 1,2-epoxy-2-methyl-3-butene; EPOX-II, 3,4-epoxy-2-methyl-1-butene; P450, cytochrome P450
 Major metabolic pathways are indicated by thick arrows and minor pathways by thin arrows.

Prevalence and degree were correlated with increasing length of service (Mitin, 1969; Sandmeyer, 1981).

4.2.2 *Experimental systems*

Exposure of Fischer 344 rats of each sex to 0, 438, 875, 1750, 3500 or 7000 ppm [1222–19 530 mg/m³] isoprene vapours by inhalation for 6 h a day on five days a week for two weeks had no effect on survival, body weight gain, clinical signs, haematological parameters or clinical chemical measurements and did not produce gross or microscopic lesions. Male B6C3F1 mice, however, had reduced body weight gain, atrophy of the thymus and testis (at 7000 ppm only), olfactory epithelial degeneration (at ≥ 1750 ppm), vacuolized liver cytoplasm and forestomach epithelial hyperplasia (at ≥ 438 ppm). The last effect was also seen in female mice. In both male and female mice in all exposure groups, there were reductions in erythrocyte numbers, haemoglobin concentrations and volume of packed erythrocytes; the numbers of reticulocytes and polychromatic erythrocytes were not increased (Melnick *et al.*, 1990).

Exposure of male Fischer 344 rats and male B6C3F1 mice to 0, 70, 220, 700, 2200 or 7000 ppm [195–19 530 mg/m³] isoprene vapours by inhalation for 6 h a day on five days a week for 26 weeks induced exposure-related neoplastic and proliferative lesions in the lung, Harderian gland and forestomach in mice. Interstitial-cell hyperplasia of the testis was induced in rats exposed to 7000 ppm isoprene (US National Toxicology Program, 1994).

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

The developmental toxicity of isoprene was reported in abstracts by Mast *et al.* (1989, 1990). Swiss CD-1 mice and Sprague-Dawley rats were exposed to 0, 280, 1400 or 7000 ppm [781–19 530 mg/m³] isoprene vapour for 6 h per day on seven days per week on days 6–17 (mice) or days 6–19 of gestation (rats). In mice, exposure to 7000 ppm isoprene reduced maternal weight gain, and exposure to any dose reduced fetal body weight. An increase in the incidence of supernumerary ribs was observed at 7000 ppm, but there was no increase in fetal malformations. In rats, there was no adverse effect on the dams or on any reproductive index at any dose level, and there was no increase in the incidence of either fetal malformations or variations, other than reduced ossification of the vertebral centra at 7000 ppm.

4.4 Genetic and related effects (see also Table 1 and Appendices 1 and 2)

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

Isoprene did not induce mutation in *Salmonella typhimurium*.

Exposure of male B6C3F1 mice to isoprene by inhalation produced increases in the frequencies of micronuclei in circulating erythrocytes and of sister chromatid exchange in bone-marrow cells, but no chromosomal aberrations were seen.

Neither the major metabolite in mouse liver microsomes, 1,2-epoxy-2-methyl-3-butene, nor an important minor metabolite, 3,4-epoxy-2-methyl-1-butene, was mutagenic to *S. typhimurium* TA98 or TA100. The latter metabolite can be metabolized further to 2-methyl-1,2:3,4-diepoxybutane, which was mutagenic to both strains. This compound may be responsible for the activity of isoprene *in vivo*; it is an analogue of 1,2:3,4-diepoxybutane, which can induce chromosomal aberrations and sister chromatid exchange in mouse bone marrow (Conner *et al.*, 1983; Walk *et al.*, 1987).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Isoprene is produced by dehydrogenation of isopentane or as a by-product in the production of ethylene via naphtha cracking. It is used mainly in the production of isoprene rubber (used in vehicle tyres), block polymers containing styrene (used as thermoplastic rubbers) and pressure-sensitive adhesives and in butyl rubber used for construction of hoses and tyres. Large quantities of isoprene are produced and released into the atmosphere by vegetation. Few data are available on occupational exposure to isoprene.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Isoprene was tested for carcinogenicity in male mice and male rats exposed by inhalation in one-year studies. In mice, exposure to isoprene resulted in increased incidences 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.

5.4 Other relevant data

Isoprene is formed endogenously and is present in exhaled air from man and rodents. Isoprene concentrations in human blood, from apparently endogenous sources, are approximately one-tenth of the concentrations reached in mouse blood following exposure to 20 ppm [56 mg/m³] in the atmosphere. At low concentrations, less inhaled isoprene is retained in mice than in rats. Haemoglobin adducts of isoprene can be formed in both mice and rats, but at equal retained doses mice form twice as much haemoglobin adduct as rats. Toxic responses have been observed only in mice. Isoprene can be oxidized sequentially by microsomal enzymes to 2-methyl-1,2:3,4-diepoxybutane.

Table 1. Genetic and related effects of isoprene

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Isoprene				
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	680.0000 ^c	de Meester <i>et al.</i> (1981)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	5000.0000	Mortelmans <i>et al.</i> (1986)
SA2, <i>Salmonella typhimurium</i> TA102, reverse mutation	-	0	0.0000	Kushi <i>et al.</i> (1985) (abstr.)
SA3, <i>Salmonella typhimurium</i> TA1530, reverse mutation	-	-	680.0000 ^c	de Meester <i>et al.</i> (1981)
SA4, <i>Salmonella typhimurium</i> TA104, reverse mutation	-	0	0.0000	Kushi <i>et al.</i> (1985) (abstr.)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	680.0000 ^c	de Meester <i>et al.</i> (1981)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	5000.0000	Mortelmans <i>et al.</i> (1986)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	5000.0000	Mortelmans <i>et al.</i> (1986)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	680.0000 ^c	de Meester <i>et al.</i> (1981)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	680.0000 ^c	de Meester <i>et al.</i> (1981)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	5000.0000	Mortelmans <i>et al.</i> (1986)
SVA, Sister chromatid exchange, mouse bone-marrow cells <i>in vivo</i>	+		430.0000, inhal. 6 h × 12	Tice <i>et al.</i> (1988)
MVM, Micronucleus test, mouse peripheral red blood cells <i>in vivo</i>	+		430.0000, inhal. 6 h × 12	Tice <i>et al.</i> (1988)
CBA, Chromosomal aberrations, mouse bone-marrow cells <i>in vivo</i>	-		6900.0000, inhal. 6 h × 12	Tice <i>et al.</i> (1988)
Protein binding				
BVP, Binding (covalent) to mouse haemoglobin <i>in vivo</i>	+		2.0000 × 1 ip	Sun <i>et al.</i> (1989)
BVP, Binding (covalent) to mouse haemoglobin <i>in vivo</i>	+		60.0000, inhal. 6 h	Bond <i>et al.</i> (1991)
BVP, Binding (covalent) to rat haemoglobin <i>in vivo</i>	+		2.0000 × 1 ip	Sun <i>et al.</i> (1989)
1,2-Epoxy-2-methylbutane				
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	0	2600.0000	Gervasi <i>et al.</i> (1985)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	0	2600.0000	Gervasi <i>et al.</i> (1985)

Table 1 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
3,4-Epoxy-2-methyl-1-butene				
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	0	1300.0000	Gervasi <i>et al.</i> (1985)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	0	1300.0000	Gervasi <i>et al.</i> (1985)
2-Methyl-1,2;3,4-diepoxbutane				
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	500.0000	Gervasi <i>et al.</i> (1985)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	+	0	750.0000	Gervasi <i>et al.</i> (1985)

^a+, positive; -, negative; 0, not tested

^bIn-vitro tests, µg/ml; in-vivo tests, mg/kg bw

^cAtmospheric concentration

Exposure of mice and rats to isoprene by inhalation had no adverse effect on reproduction.

No data were available on the genetic and related effects of isoprene in humans. Isoprene induced micronuclei in mouse circulating erythrocytes and sister chromatid exchange but not chromosomal aberrations in mouse bone marrow *in vivo*.

Neither isoprene nor its primary metabolites, 3,4-epoxy-2-methyl-1-butene and 1,2-epoxy-2-methyl-3-butene, were mutagenic to bacteria. 2-Methyl-1,2:3,4-diepoxybutane, a metabolite of 3,4-epoxy-2-methyl-1-butene, was mutagenic to *Salmonella typhimurium*.

5.5 Evaluation¹

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

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

Overall evaluation

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

6. References

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¹For definition of the italicized terms, see Preamble, pp. 27–30.

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STYRENE

This substance was considered by a previous Working Group, in February 1978 (IARC, 1979). 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.: 100-42-5

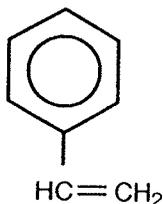
Replaced CAS Reg. No.: 79637-11-9

Chem. Abstr. Name: Ethenylbenzene

IUPAC Systematic Name: Styrene

Synonyms: Cinnamene; phenethylene; phenylethene; phenylethylene; styrol; styrole; styrolene; vinylbenzene; vinylbenzol

1.1.2 Structural and molecular formulae and relative molecular mass



C₈H₈

Relative molecular mass: 104.15

1.1.3 Chemical and physical properties of the pure substance

- (a) *Description:* Colourless, viscous liquid with a pungent odour (WHO, 1983)
- (b) *Boiling-point:* 145.2 °C (Lide, 1991)
- (c) *Melting-point:* -30.6 °C (Lide, 1991)
- (d) *Density:* 0.9060 at 20 °C/4 °C (Lide, 1991)
- (e) *Spectroscopy data:* Infrared [81 (grating)], ultraviolet [94], nuclear magnetic resonance and mass spectral data have been reported (Sadler Research Laboratories, 1991; US National Library of Medicine, 1993a).
- (f) *Solubility:* Slightly soluble in water (30 mg/100 ml at 20 °C); soluble in acetone, carbon tetrachloride, diethyl ether, ethanol and *n*-heptane; very soluble in benzene and petroleum ether (WHO, 1983)

- (g) *Volatility*: Vapour pressure, 867 Pa at 25 °C; relative vapour density (air = 1), 3.6 (WHO, 1983)
- (h) *Stability*: Lower explosive limit, 1.1% by volume in air (WHO, 1983; Dow Chemical Co., 1989)
- (i) *Reactivity*: Polymerizes easily at room temperature in the presence of oxygen and oxidizes on exposure to light and air (WHO, 1983)
- (j) *Octanol-water partition coefficient (P)*: log P, 3.05 (Sangster, 1989)
- (k) *Conversion factor*: $\text{mg/m}^3 = 4.26 \times \text{ppm}^a$

1.1.4 *Technical products and impurities*

Styrene is available as a commercial product with the following specifications: purity, 99.6–99.9% min.; ethylbenzene, 85 ppm max.; polymer content, 10 ppm max.; *para-tert*-butylcatechol (inhibitor), 10–15 ppm or 45–55 ppm; aldehydes (as benzaldehyde), 200 ppm max.; peroxides (as H₂O₂), 0.0015 wt% or 100 ppm max.; benzene, 1 ppm max.; sulfur, 25 ppm max.; chlorides (as chlorine), 50 ppm max. (Dow Chemical Co., 1991; Chevron Chemical Co., 1992; Amoco Chemical Co., 1993)

1.1.5 *Analysis*

(a) *Environmental monitoring*

Styrene in workplace air can be determined by packed capillary column gas chromatography with a flame ionization detector. The sample is adsorbed on charcoal and desorbed with carbon disulfide. This method (NIOSH Method 1501) has an estimated limit of detection of 0.001–0.01 mg per sample (Eller, 1984).

US EPA Method 8240 can be used to determine the concentration of various volatile organic compounds, including styrene, by gas chromatography–mass spectrometry, in a variety of matrices, including groundwater, aqueous sludges, waste solvents, oily wastes, tars, soils, sediments and others. Samples may be analysed using direct injection or the purge-and-trap method (US EPA Method 5030); the practical quantification limits are 5 µg/L for groundwater samples, 5 µg/kg for low-level soil and sediment samples, 250 µg/L for water-miscible liquid waste samples, 625 µg/L for high-level soil and sludge samples and 2500 µg/L for non-water-miscible waste samples (US Environmental Protection Agency, 1986).

(b) *Biological monitoring*

Biological methods for monitoring exposure to styrene have been reviewed (Guillemin & Berode, 1988; American Conference of Governmental Industrial Hygienists, 1991; Lauwerys & Hoet, 1993; Pekari *et al.*, 1993). Generally accepted biological indicators of exposure are mandelic acid (2-hydroxy-2-phenylacetic acid) and phenylglyoxylic acid, the main metabolites of styrene, in urine and styrene in blood. Gas chromatographic procedures

^aCalculated from: $\text{mg/m}^3 = (\text{relative molecular mass}/24.45) \times \text{ppm}$, assuming normal temperature (25 °C) and pressure (101.3 kPa)

have been described for the quantitative determination of urinary phenylglyoxylic and mandelic acids which involve solvent extraction of the acids and their subsequent determination as derivatives by flame ionization detection on packed or capillary columns (Guillemin & Bauer, 1976; Flek & Šedivec, 1980; Bartolucci *et al.*, 1986; Dills *et al.*, 1991). High-pressure liquid chromatography is widely used for the determination of these metabolites. The acids may or may not be solvent extracted, are separated on reverse-phase columns and quantified with an ultraviolet detector (Ogata & Sugihara, 1978; Ogata & Taguchi, 1987, 1988; Chua *et al.*, 1993). Styrene has been determined in blood by gas chromatography with flame ionization or mass selective detection either after solvent extraction (Karbowski & Braun, 1978) or by head-space techniques (Pezzagno *et al.*, 1985; Bartolucci *et al.*, 1986; Brugnone *et al.*, 1993).

Measurement of adducts of styrene-7,8-oxide (see monograph, p. 328) to N-terminal valine in haemoglobin has been proposed for monitoring occupational exposure. After enrichment of adducted globin chains by ion-exchange chromatography, the samples are analysed by gas chromatography-mass spectrometry after Edman degradation (Christakopoulos *et al.*, 1993).

1.2 Production and use

1.2.1 Production

Styrene was first isolated in 1831 by distillation of storax, a natural balsam. Commercial production of styrene via dehydrogenation of ethylbenzene began in Germany in 1925 (Tossavainen, 1978; Lewis *et al.*, 1983; US National Institute for Occupational Safety and Health, 1983).

Styrene is produced mainly by catalytic dehydrogenation of high-purity ethylbenzene in the vapour phase. Typical catalysts are based on ferric oxide with the additives chromia (stabilizer) and potassium oxide (coke retardant) (Lewis *et al.*, 1983). Fractionation of the product results in separation of high-purity styrene, unconverted ethylbenzene and minor reaction by-products such as toluene and benzene (WHO, 1983).

A smaller amount of styrene is produced as a co-product from a propylene oxide process. In this route, ethylbenzene is oxidized to its hydroperoxide and reacted with propylene to yield propylene oxide. The co-product methyl phenyl carbinol is then dehydrated to styrene (Mannsville Chemical Products Corp., 1987; Collins & Richey, 1992). Limited data on production of styrene in Japan, Taiwan and the USA are presented in Table 1. Production levels in western Europe are similar to those in the USA, although specific data are not available. Global production of styrene in 1992 was 14 282 thousand tonnes (European Chemical Industry Council, 1994). Global production in the early 1980s was estimated at 10 000 thousand tonnes per year (WHO, 1983).

Information available in 1991 indicated that styrene was produced by nine companies in Japan, six each in China and the USA, five in France, four in Brazil, three each in Canada, Germany and the Republic of Korea, two each in Argentina, Bulgaria and the United Kingdom and one each in Australia, the former Czechoslovakia, Italy, Mexico, Poland, Saudi Arabia, Spain, the former USSR, Venezuela and the former Yugoslavia (Chemical Information Services Ltd, 1991).

Table 1. Production of styrene in selected regions (thousand tonnes)

Country or region	Year					
	1982	1984	1986	1988	1990	1992
Japan	1086	1421	1402	1733	2161	2182
Taiwan	NR	NR	243	331	355	338
USA	2695	3497	3578	4075	3636	4056 ^a

From Anon. (1985, 1989a, 1993); Japan Petrochemical Industry Association (1993)

NR, not reported

^aPreliminary

1.2.2 Use

Styrene is one of the most important monomers worldwide, and its polymers and copolymers are used in an increasingly wide range of applications. The major uses for styrene are in plastics, latex paints and coatings, synthetic rubbers, polyesters and styrene-alkyd coatings (Collins & Richey, 1992). The broad spectrum of uses includes construction, packaging, automotive and household goods (Mannsville Chemical Products Corp., 1987).

Packaging is the single largest use in which styrene-containing resins, particularly foams, are used as fillers and cushioning. Construction applications include pipes, fittings, tanks, lighting fixtures and corrosion-resistant products. Household goods include synthetic marble, flooring, disposable tableware and moulded furnishings. Transport applications range from tyres to reinforced plastics and automobile body putty (Mannsville Chemical Products Corp., 1987).

Most styrene is converted to polystyrene resins, which are readily moulded and are compatible with a range of colourants, modifiers and fillers. They are used extensively in the fabrication of plastic packaging, disposable beverage tumblers, toys and other moulded goods. Expandable polystyrene beads are used for disposable cups, containers and packaging as well as for insulation. Copolymers and adducts are the second largest family of styrene derivatives. Acrylonitrile-butadiene-styrene (ABS) and styrene-acrylonitrile resins have a variety of applications, including in appliance, automotive, construction, pipes and electronics mouldings (Mannsville Chemical Products Corp., 1987).

A variety of special resins have the styrene functionality. Styrene-butadiene rubber, used for tyres and other elastomer applications, is the largest volume synthetic rubber produced in the USA. Styrene-butadiene latex is used for carpet backing and paper processing. Styrene is the essential co-reactant and solvent in unsaturated polyesters used in reinforced plastic fabrications, including boats, corrosion-resistant tanks and pipes and automobile body parts (Mannsville Chemical Products Corp., 1987). Typical use patterns for styrene in the USA for several years are presented in Table 2. In Japan, the use patterns in 1993 were: polystyrene, 64%; acrylonitrile-butadiene-styrene resins, 10%; styrene-butadiene rubber, 7%; unsaturated polyesters, 5%; styrene-acrylonitrile, 4%; and other applications, 10% (Japan Petrochemical Industry Association, 1993).

Table 2. Use patterns (%) for styrene in the USA

Use	1983	1986	1989	1992
Polystyrene	52	55	55	54
Expandable polystyrene	–	–	–	12
Acrylonitrile–butadiene–styrene resins	9	9	10	10
Styrene–butadiene rubber	7	7	5	7
Styrene–butadiene latexes	6	6	5	6
Unsaturated polyester resins	6	6	5	–
Exports	15	13	13	–
Miscellaneous ^a	5	4	7	11 ^b

From Anon. (1983, 1986, 1989b, 1992)

^aIncludes other copolymers and styrene–acrylonitrile

^bIncluding unsaturated polyester resins, styrenated alkyds and acrylic ester–styrene copolymers

1.3 Occurrence

1.3.1 *Natural occurrence*

Styrene has been identified in trace amounts in the gummy exudate (storax balsam) from the damaged trunks of certain trees, probably from the natural degradation of the cinnamic acid derivatives that occur in large quantities in these exudates (Furia & Bellanca, 1971; Tossavainen, 1978; Duke, 1985).

1.3.2 *Occupational exposure*

The National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health between 1981 and 1983 indicated that 1 112 000 US employees were potentially exposed to styrene at work (US National Institute for Occupational Safety and Health, 1993). The estimate is based on a survey of US companies and did not involve actual measurements of exposure.

Workers may be exposed in a number of industries and operations, including styrene production, production of polystyrene and other styrene-containing polymer resins, plastics and rubber products fabrication, fabrication of reinforced-polyester plastics composites and use of products containing styrene, such as floor waxes and polishes, paints, adhesives, putty, metal cleaners, autobody fillers and varnishes (US National Institute for Occupational Safety and Health, 1983).

(a) *Production of styrene and polystyrene*

Average exposure to styrene in styrene production and polymerization factories has been reported rarely to exceed 20 ppm [85 mg/m³] and is usually due to occasional bursts and leakages of reactors, tubing and other equipment (Tossavainen, 1978). Surveys conducted in US plants engaged in the development or manufacture of styrene-based products between 1962 and 1976 showed that the average exposure of employees in all jobs was below 10 ppm

[43 mg/m³]. Peak concentrations of up to 50 ppm [213 mg/m³] were measured during the drumming of styrene. Batch polymerization of styrene in 1942 produced concentrations up to 88 ppm [375 mg/m³] during filling operations; subsequent continuous polymerization processes generally resulted in exposure levels of 1 ppm [4.26 mg/m³] or below (Ott *et al.*, 1980). In a US plant where styrene was produced and polymerized, the highest levels of styrene were found in polymerization, manufacturing and purification areas (mean, 8–35 ppm [34–149 mg/m³]), while levels of less than 5 ppm [21 mg/m³] occurred in maintenance, laboratory and packaging operations. Urinary mandelic acid and blood styrene were undetectable in most samples taken from these workers at the end of a shift: < 10 mg/g creatinine for mandelic acid (5 ng/ml) and < 2 ng/ml for styrene in blood. The maximal concentrations were 140 mg/g creatinine for mandelic acid and 90 ng/ml blood for styrene (Wolff *et al.*, 1978). In a German styrene production, polymerization and processing plant, samples taken in 1975–76 in various areas of the plant contained none (< 0.01 ppm [0.04 mg/m³]) to 6.8 ppm [29 mg/m³], most values being below 1 ppm [4.3 mg/m³]. In a part of the plant where polystyrene was manufactured, area samples in 1975 contained from none (< 0.01 ppm [0.04 mg/m³]) to 47 ppm [200 mg/m³], most values being below 1 ppm. Of 67 employees engaged in either area of the plant, six had urinary concentrations of mandelic acid above 50 mg/L (Thiess & Friedheim, 1978).

Other substances that may be found in workplace air during the manufacture of styrene and polystyrene include benzene, toluene, ethylbenzene, other alkylbenzene compounds and ethylene (Ott *et al.*, 1980; Lewis *et al.*, 1983; US National Institute for Occupational Safety and Health, 1983). Exposure to benzene was previously a primary concern in these processes. In the US plant described above, the time-weighted average concentration of benzene in styrene monomer manufacture was 0.3–14.7 ppm [1–47 mg/m³] between 1953 and 1972. Samples taken in 1942 during a washing operation in the polymerization plant contained up to 63 ppm [202 mg/m³] (Ott *et al.*, 1980).

(b) *Production of styrene-butadiene rubber and other styrene-based polymers*

Concentrations of styrene in area samples and breathing-zone air measured in 1965 in various plants of a US styrene-butadiene latex manufacturing company (see above) were 4–22 ppm [17–94 mg/m³]. The initial stages of the process, including loading, operating and cleaning of polymerization reactors, involved the most exposure, and operators in these job categories were found to be exposed to concentrations ranging from 3.6 to 7.3 ppm [15.3–31 mg/m³] in 1973 (Ott *et al.*, 1980).

In two adjacent US plants where styrene-butadiene rubber was produced, the time-weighted average concentrations of styrene were 0.94 and 1.99 ppm [4 and 8.5 mg/m³], with an overall range of 0.03–12.3 ppm [0.13–52.4 mg/m³] (Meinhardt *et al.*, 1982). The mean concentration in 159 personal air samples taken in 1979 in various departments in another US styrene-butadiene rubber production plant were usually below 1 ppm [4.3 mg/m³], except for factory service and tank farm workers, for whom the means were 1.69 and 13.67 ppm [7.2 and 58.2 mg/m³], respectively (Checkoway & Williams, 1982). Company data provided by five out of eight US styrene-butadiene rubber plants for the period 1978–83 gave an average styrene level in 3649 samples from all plants of 3.53 ppm [15 mg/m³], with a standard deviation of 14.3 ppm [61 mg/m³] (Matanoski *et al.*, 1993).

In a US plant where acrylic ester-styrene copolymers [wrongly called polystyrene by the authors] were produced, concentrations in the breathing zone in 50 samples ranged from less than 1 ppb [$4.3 \mu\text{g}/\text{m}^3$] to 19.8 ppm [$84 \text{mg}/\text{m}^3$] with an average of [about 600 ppb; $2.5 \text{mg}/\text{m}^3$]; the highest concentrations occurred during styrene unloading operations (Samimi & Falbo, 1982).

The numerous other substances to which workers may be exposed in these processes include 1,3-butadiene, acrylonitrile, acrylates, acrylic acid, α -methylstyrene (*meta*-vinyltoluene, see monograph, p. 373), 4-vinylcyclohexene (see monograph, p. 347), toluene, benzene, ammonia, formaldehyde, colourants and a variety of solvents (Ott *et al.*, 1980; Samimi & Falbo, 1982; US National Institute for Occupational Safety and Health, 1983).

(c) *Processing of styrene-based polymers*

Styrene was measured as a thermal degradation product in the air of a Finnish factory during the processing of polystyrene, impact polystyrene and acrylonitrile-butadiene-styrene resins. The mean concentrations (6 h) were 0.4, 0.1 and $0.06 \text{mg}/\text{m}^3$, respectively (Pfäffli, 1982). Personal 8-h samples taken in 1978, 1979 and 1980 in US companies where acrylonitrile-butadiene-styrene moulding occurred contained < 0.01 – $[5.9] \text{mg}/\text{m}^3$ (Burroughs, 1979; Belanger & Elesh, 1980; Ruhe & Jannerfeldt, 1980).

Styrene is one of the volatile organic compounds produced during extrusion and vulcanization of styrene-butadiene rubber. Rappaport and Fraser (1977) reported styrene at concentrations of 61–146 ppb [0.3 – $0.6 \text{mg}/\text{m}^3$] in the curing area of the press room of a passenger car tyre manufacturing company. Area samples taken in the vulcanization and extrusion areas of shoe-sole, tyre retreading and electrical cable insulation plants contained styrene at concentrations of 2–500 $\mu\text{g}/\text{m}^3$ (vulcanization) and 0–20 $\mu\text{g}/\text{m}^3$ (extrusion) (Cocheo *et al.*, 1983). A more complete description of the work environment encountered in the rubber products manufacturing industry may be found in a previous monograph (IARC, 1982).

(d) *Manufacture of glass fibre-reinforced polyester products*

Occupational exposure to styrene is most extensive, with respect to number of workers and levels of exposure, in the fabrication of objects from glass fibre-reinforced polyester composite plastics, such as boats, tanks, wall panels, bath and shower units and automotive parts (US National Institute for Occupational Safety and Health, 1983). Styrene serves as a solvent and a reactant for the unsaturated polyester resin, in which it constitutes about 40% by weight. In the open mould process, a releasing agent is usually applied to the mould, a first coat containing pigments (gel coat) is applied, then successive layers of chopped and/or woven fibre glass are deposited manually or with a chopper gun at the same time as the resin is sprayed or brushed on, and then the surface is rolled. During lamination and curing, about 10% of the styrene may evaporate into the workplace air (US National Institute for Occupational Safety and Health, 1983; Crandall & Hartle, 1985). Exposure to styrene in this industry has been extensively documented and summarized in several reports (US National Institute for Occupational Safety and Health, 1983; WHO, 1983; Pfäffli & Säämänen, 1993). Table 3 lists levels of occupational exposure to styrene (personal breathing zone samples)

reported in various countries in the larger studies. Table 4 gives the concentrations of the classical biological indicators of exposure from various studies.

Several factors influence the level of styrene in air. The manufacture of objects with large surface areas, such as boats, truck parts, baths and showers, by the open-mould process results in the highest exposure. Data from 28 plants producing reinforced plastics products in the USA showed that the average exposure to styrene in open-mould processes was two to three times higher than that in press-mould processes: 24–82 ppm [102–350 mg/m³] versus 11–26 ppm [47–111 mg/m³] (Lemasters *et al.*, 1985a). In a detailed survey of 12 plants making fibreglass in Washington State, USA, 40% of 8-h samples contained more than 100 ppm [426 mg/m³]. Chopper gun operators had the highest exposure, followed by laminators and gel-coat applicators; boat-building involved higher exposures than any other sector. A relationship was seen between level of exposure and the quantity of resin used by an employee (Schumacher *et al.*, 1981). Essentially similar results were reported by Sullivan and Sullivan (1986) in their survey of 10 plants in Ontario, Canada. They also found that dilution ventilation and often auxiliary fans were used in almost all plants, but there was little use of local exhaust ventilation. This was also the case for boat construction in the USA. Gel coaters have lower exposure because they generally work in ventilated booths (Crandall & Hartle, 1985). The presence of flexible exhaust ventilation hoses was reported to reduce styrene concentrations by a factor of two in a boat construction company in Japan (Ikeda *et al.*, 1982). So-called 'low-styrene emission resins' are in theory promising for reducing exposure, but their potential to do so in the workplace has not been sufficiently validated and they are not widely used (A.D. Little, Inc., 1981; Sullivan & Sullivan, 1986; Säämänen *et al.*, 1993).

Measurement of biological indicators of exposure complements the picture based on air levels because biological levels incorporate the influence of other routes of absorption and of the use of personal protective equipment. Despite early reports that percutaneous absorption of styrene was an important source of exposure, measurement of biological indicators of the exposure of workers who did and did not wear gloves and other forms of protective clothing indicated that absorption through the skin makes a negligible contribution to overall exposure in the manufacture of glass fibre-reinforced polyester products (Brooks *et al.*, 1980; Bowman *et al.*, 1990; Truchon *et al.*, 1992). Wearing a respirator appropriate for organic vapours reduces exposure markedly, but not entirely (Brooks *et al.*, 1980; Ikeda *et al.*, 1982; Bowman *et al.*, 1990; Truchon *et al.*, 1992). Respirators are worn most often by gel-coat and chopper gun operators but not by laminators, who consider that they hinder their work (Truchon *et al.*, 1992). Single-use dust respirators, which provide unsatisfactory protection, were often the only type of protection worn (Schumacher *et al.*, 1981; Sullivan & Sullivan, 1986).

Other substances may be found in workplace air in plants for the production of unsaturated polyester reinforced plastics, although at levels usually considerably lower than that of styrene. These include: solvents, mainly used to clean tools and equipment, such as ketones (e.g. acetone), chlorinated hydrocarbons (e.g. dichloromethane), aliphatic alcohols and esters and aromatic hydrocarbons; organic peroxides used as initiators (e.g. methylethyl ketone peroxide, benzoyl peroxide); styrene oxide and other oxidation products resulting from the reaction of peroxides with styrene; hydroquinone and analogues used as inhibitors (e.g. hydroquinone, quinone, catechol); dusts and fibres originating mainly from filler and

Table 3. Occupational exposure to styrene in the fibre glass-reinforced plastics industry in various countries

Country and year of survey	No. of plants	Job/task	Duration of sampling	No. of samples	Air concentration in personal breathing zone (mg/m ³)		Reference
					Mean	Range	
Canada (Ontario) 1981	10	All jobs	25 min	126	[< 4.3-716] ^a	[< 4.3-1393]	Sullivan & Sullivan (1986)
		Boat laminating		59	[430] GM	[8.1] GSD	
		Non-boat laminating		23	[123] GM	[29.4] GSD	
		Chopper gun use		8	[554] GM	[7.7] GSD	
		Gel-coat spraying		6	[298] GM	[7.7] GSD	
		Filament winding		3	[533] GM	[6.0] GSD	
Canada (Quebec) NR	3	Chopper gun use	8-h	7	564	307-938	Truchon <i>et al.</i> (1992)
		Painting (gel coat)		9	517	280-843	
		Laminating (rollers)		18	502	292-865	
		Foreman		8	97	18-279	
		Cutter		11	75	16-234	
		Warehouse work		19	35	9-187	
		Finishing		31	34	8-110	
		Mould repair		8	28	8-147	
Denmark 1955-70 1971-80 1981-88	30	NR	1-60 min	227	714	10-4700	Jensen <i>et al.</i> (1990)
	97	NR		1117	274	4-1905	
	129	NR		1184	172	1-4020	
Italy 1978-90	87	Hand laminating	Variable	1028	227		Galassi <i>et al.</i> (1993)
		Spray laminating		166	134		
		Rolling		40	163		
		Semi-automatic process operators		71	85		
		Non-process work		159	71 (38 GM)	3.8 GSD	
Italy NR	10	NR	8 h	64 subjects	113.6 GM	8-770.4	Gobba <i>et al.</i> (1993)

Table 3 (contd)

Country and year of survey	No. of plants	Job/task	Duration of sampling	No. of samples	Air concentration in personal breathing zone (mg/m ³)		Reference
					Mean	Range	
Japan NR	5	Boat fabrication	4 h				Ikeda <i>et al.</i> (1982)
		Hull lamination		25	[507] GM	[145-1091]	
		Hull lamination with local exhaust ventilation		9	[277] GM	[196-383]	
		Lamination of hold walls		25	[537] GM	[371-916]	
Switzerland NR	10	NA	Full shift	90	[201]	[8-848]	Guillemin <i>et al.</i> (1982)
Netherlands NR	4	Filament winding	4 h	18	NR	134-716	Geuskens <i>et al.</i> (1992)
		Spraying		62	NR	48-602	
		Hand laminating		180	NR	18-538	
USA NR	7	Boat fabrication	Full shift				Crandall & Hartle (1985)
		Hull lamination		168	[331]	[7-780]	
		Deck lamination		114	[313]	[52-682]	
		Small parts lamination		70	[193]	[34-554]	
		Gel coating		45	[202]	[23-439]	
Europe (5 countries) ^f		Lamination	Variable				Bellander <i>et al.</i> (1994)
		Boat fabrication		1703	[332]		
				2993	[234]		
		Containers		437	[247]		
				1098	[187]		
		Panels and construction		401	[213]		
				846	[145]		
		Small pieces		486	[251]		
				629	[158]		
		Hand lamination		3205	[281]		
		Spray lamination		414	[132]		
		Non-manual lamination		231	[68]		
		1955-90					

Table 3 (contd)

Country and year of survey	No. of plants	Job/task	Duration of sampling	No. of samples	Air concentration in personal breathing zone (mg/m ³)		Reference
					Mean	Range	
USA 1967-78	30	Spray-up/lay-up	8 h TWA	NR	[256] ^c	[21-511]	A.D. Little, Inc. (1981)
		Gel-coating			[192]	[43-256]	
		Winding			[170]	[64-362]	
		Sheet-moulding compound production			[170]	[43-341]	
		Foaming			[128]	[64-213]	
		Mixing			[107]	[9-341]	
		Casting			[85]	[21-192]	
		Cut, press and weigh			[64]	[21-341]	
Other jobs ^d	[≤ 43]	[0-213]					

GM, geometric mean; GSD, geometric standard deviation; NR, not reported

^aRange of arithmetic means for different plants

^bItalian plants reported by Galassi *et al.* (1993) and Finland, Norway, Sweden and the United Kingdom

^cTypical level

^dIncludes general and non-production, finish and assembly, store and ship, office and other, injection moulding, field service, preform production and pultrusion

Table 4. Occupational exposure to styrene in the fibre glass-reinforced plastics industry

Country and year of survey	No. of plants	Job/task	No. of samples	Concentrations at end of shift						Reference
				Mandelic acid in urine (mg/g creatinine)		Phenylglyoxylic acid in urine (mg/g creatinine)		Styrene in blood (mg/L)		
				Mean	SD	Mean	SD	Mean	SD	
Canada (Québec) NR	3	Chopper gun operation	7	[980]	[980]					Truchon <i>et al.</i> (1992)
		Painting (gel coat)	9	[750]	[310]					
		Laminating (rolling)	18	[1690]	[605]					
		Foreman	8	[350]	[470]					
		Cutting	11	[320]	[380]					
		Warehouse worker	19	[70]	[70]					
		Finishing	31	[110]	[120]					
		Mould repair	8	[30]	[50]					
Germany 1980, 1983	1	Boat industry	11	816-1660 ^a		200-342 ^a		0.70-0.92 ^a		Triebig <i>et al.</i> (1989)
Italy NR	4	Refrigerating containers	6	493	434	121	96	0.32	0.42	Bartolucci <i>et al.</i> (1986)
		Flooring tiles	6	428	248	72	22	0.42	0.16	
		Fibre-glass canoes	5	270	54	62	24	0.52	0.32	
		Fibre-glass tanks	3	323	129	132	41	NR		
Italy 1978-90	118	Hand lamination	2386	450 GM	2.75 GSD					Galassi <i>et al.</i> (1993)
		Spray lamination	250	211 GM	3.3 GSD					
		Rolling	63	182 GM	3.08 GSD					
		Semiautomatic process operation	121	154 GM	2.59 GSD					
		Non-process work	762	94 GM	3.27 GSD					
Switzerland NR	10	NR	88	1004	1207	339	360			Guillemin <i>et al.</i> (1982)
United Kingdom 1979	1	Boat industry	27	[780]	555			[0.72]	[0.43]	Cherry <i>et al.</i> (1980)

NR, not reported; GM, geometric mean; GSD, geometric standard deviation

^aRange of means for different days

reinforcement materials (e.g. glass fibres, silica, asbestos); foaming agents such as isocyanates; and cobalt salts and amines used as accelerators (Pfäffli *et al.*, 1979; A.D. Little, Inc., 1981; Makhlof, 1982; Högstedt *et al.*, 1983; US National Institute for Occupational Safety and Health, 1983; Coggon *et al.*, 1987; Jensen *et al.*, 1990; Bellander *et al.*, 1994). Table 5 gives the concentrations of such substances measured in studies covering several plants in various countries.

Acetone, used extensively as a cleaning solvent, is the major concurrent exposure with styrene. In the study of A.D. Little, Inc. (1981), involving industrial hygiene surveys in 30 US plants, maximal levels of various typical concomitant exposures were reported for five of the plants (Table 5). The following substances were not detected or were reported as zero concentration: benzene, hydrogen fluoride, arsine, phosphine, stibine, formaldehyde, acetaldehyde, acetic acid, hydrochloric acid, free silica and cresol. The concomitant exposures generally occurred at concentrations orders of magnitude lower than those permitted by the US Occupational Safety and Health Administration. Benzene was not reported in any of 2528 air samples containing styrene in the Danish work environment (Jensen *et al.*, 1990).

(e) *Miscellaneous operations*

In a study of exposures of firefighters, samples taken during the 'knockdown' phase of a fire contained styrene at a concentration of 1.3 ppm [5.5 mg/m³]; none was detected during the 'overhaul' phase (Jankovic *et al.*, 1991). During working operations at a US hazardous waste site in 1983, a mean styrene concentration of 235 µg/m³ was associated with heavy exposure; 100 µg/m³ were measured near use of heavy equipment, but none was detected in 45 other samples (maximum, 678 µg/m³) (Costello, 1983). During the manufacture of polyester paints, lacquers and putties in Finland, occasional high exposure to styrene was recorded, with 5% of measurements above 20 ppm [85 mg/m³]; use of the same products resulted in exposures below 1 ppm [4.3 mg/m³] (Säämänen *et al.*, 1991). Application of polyester putty during cable splicing operations for a US telephone company resulted in short-term levels (3–16 min) ranging from 2 to 16 ppm [8.5–68 mg/m³] in four samples (Kingsley, 1976). In a Japanese plant where plastic buttons were manufactured from polyester resins, the 8-h time-weighted average concentration of styrene for 34 workers was 7.1 ppm [30 mg/m³], with a maximum of 28 ppm [119 mg/m³] (Kawai *et al.*, 1992).

Four 100-min air samples taken in 1982 at a US college during a sculpture class in which polyester resins were used contained concentrations ranging from 0.8 to 1.2 ppm [3.4–5.1 mg/m³]; two personal samples contained 2.8 and 3.0 ppm [11.9 and 12.8 mg/m³]. The concentration of methyl ethyl ketone peroxide was below the detection limit (< 0.02 ppm) (Reed, 1983).

Taxidermists who used polyester resins during specimen preparation were shown to be exposed for short periods (2–15 min) to concentrations of styrene ranging from 21 to 300 mg/m³ (11 samples) (Kronoveter & Boiano, 1984a,b).

In two US cooking ware manufacturing companies where styrene-based resins were used, the 8-h time-weighted concentrations of styrene ranged from 0.2 to 81 ppm [0.9–

Table 5. Concentrations of substances other than styrene in workplace air in the reinforced plastics industry

Country	No. of plants	Substance	Concentration	Reference
Denmark	256 (2528 samples)	Acetone	Mean, 131 mg/m ³ (in 90% of samples)	Jensen <i>et al.</i> (1990)
		Dichloromethane	Mean, 51 mg/m ³ (in 8% of samples)	
		Xylene	Mean, 49 mg/m ³ (in 6% of samples)	
		Toluene	Mean, 113 mg/m ³ (in 5% of samples)	
		Tetrachloroethylene	Mean, 7 mg/m ³ (in 2% of samples)	
		Trichloroethylene	Mean, 5 mg/m ³ (in 1% of samples)	
		Isododecane	Mean, 4 mg/m ³ (in 2% of samples)	
Switzerland	10	Acetone	10-300 ppm [24-720 mg/m ³]	Guillemin <i>et al.</i> (1982)
		Peroxides	'Background'	
		Dust	0.5-2.5 mg/m ³ (peak, 12 mg/m ³)	
		Glass fibres	0.05 fibres/ml (peak, 1 fibre/ml)	
USA	5	Acetone	ND-59 ppm [ND-142 mg/m ³] max (5 plants)	A.D. Little, Inc. (1981)
		Dichloromethane	ND-15 ppm [ND-52 mg/m ³] max (2 plants)	
		Toluene	1.5-35 ppm [5.7-132 mg/m ³] max (2 plants)	
		Vinyl toluene	< 0.02-70 ppm [$< 0.1-338$ mg/m ³] max (2 plants)	
		Carbon monoxide	0- < 5 ppm [0- < 5.7 mg/m ³] max (2 plants)	
		Xylene	2 ppm [8.7 mg/m ³] max (1 plant)	
		Mineral spirits	30 ppm max (1 plant)	
		Hexane	10 ppm [32 mg/m ³] max (1 plant)	
		Benzyl chloride	0.7 ppm [3.6 mg/m ³] max (1 plant)	
		Cyclohexanone	5 ppm [20 mg/m ³] max (1 plant)	
		Quinone	0.03 ppm [0.13 mg/m ³] max (1 plant)	
		Epichlorohydrin	Trace (1 plant)	
		Toluene diisocyanate	0.007 ppm [0.05 mg/m ³] max (1 plant)	
		Methylene diisocyanate	0.0009 ppm [0.004 mg/m ³] max (1 plant)	
		Nuisance dust	4.5- < 6 mg/m ³ max (2 plants)	
		Chromium	0.01 mg/m ³ max (1 plant)	
Cobalt	0.001 mg/m ³ max (1 plant)			
Finland	10	Acetone	78 ppm (3-565 ppm) [187 (7.2-1356 mg/m ³)]	Pfäffli <i>et al.</i> (1992)
		Peroxides	0.05 ppm	
		Dimethylphthalate	0.1 mg/m ³	
		Total dust	< 5 mg/m ³	
Finland	2	Acetophenone	0.47 ppm [2.3 mg/m ³]	Pfäffli <i>et al.</i> (1979)
		Benzaldehyde	0.48 ppm [2.1 mg/m ³]	
		Styrene oxide	< 1 mg/m ³	

ND, not detected

See mono-
graph,
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345 mg/m³]; two short-duration samples (24 min) contained 142 and 186 ppm (605 and 792 mg/m³) (Fleeger & Almaguer, 1988; Barsan *et al.*, 1991).

1.3.3 Air

Styrene has been detected in the atmosphere in many locations. Its presence in air is due principally to emissions from industrial processes involving styrene and its polymers and copolymers. Other sources of styrene in the environment include vehicle exhaust, cigarette smoke and other forms of combustion and incineration of styrene polymers (WHO, 1983).

Styrene emissions reported to the European Union by member countries (Bouscaren *et al.*, 1987) are shown in Table 6. Air emissions in the USA, reported to the US Environmental Protection Agency by industrial facilities, declined from 15 580 tonnes in 1989 to 12 900 tonnes in 1991 (US National Library of Medicine, 1993b). Ambient air levels of styrene sampled in the vicinity of seven reinforced plastic processors in three US states ranged from 0.29 to 2934 µg/m³, and those in communities near the processors from not detected (< 0.15 µg/m³) to 23.8 µg/m³ (McKay *et al.*, 1982). Styrene levels of 1.1–6.6 µg/m³ were measured in air samples from the Pennsylvania Turnpike Allegheny Mountain Tunnel in 1979. The mean concentration in the tunnel intake air was < 0.1 µg/m³ (Hampton *et al.*, 1983).

Table 6. Estimated emissions of styrene in member countries of the European Union (thousand tonnes/year)

Country	Source	
	Road (gasoline)	Chemical industry
Belgium	0.5	0.75
Denmark	0.28	NR
France	2.9	3.4
Germany	2.9	3.4
Greece	0.5	N
Ireland	0.19	NR
Italy	3.0	3.5
Luxembourg	0.02	0.03 (other sources)
Netherlands	0.7	1.45
Portugal	0.5	0.3
Spain	2.0	1.2
United Kingdom	3.0	3.7
Total	16.0	18.0

From Bouscaren *et al.* (1987); NR, not reported

Except in highly polluted areas, styrene concentrations in outdoor air are generally < 1 µg/m³. In indoor air, e.g. mobile homes, the mean concentrations are frequently somewhat higher (< 1–6 µg/m³), smoking making a significant contribution (Connor *et al.*,

1985; Wallace *et al.*, 1987a,b; Shah & Singh, 1988; Weschler *et al.*, 1992). The styrene content of cigarette smoke has been reported to be 18–48 µg/cigarette (WHO, 1983). Off-gassing of styrene from some styrene-containing household products may also contribute to indoor air levels (Knöppel & Schauenburg, 1989).

Styrene levels in ambient air were determined in a survey of 18 sites (mostly urban) in Canada in 1988–90. The mean concentrations in 586 24-h samples ranged from 0.09 to 2.35 µg/m³. In a national survey of styrene levels in indoor air in 757 single-family dwellings and apartments, representative of the homes of the general population of Canada in 1991, the mean 24-h concentration was < 0.48 µg/m³ (limit of detection); individual values ranged up to 129 µg/m³ (average, 0.28 µg/m³) (Newhook & Caldwell, 1993).

Thermal degradation of styrene-containing polymers also releases styrene into ambient air (Hoff *et al.*, 1982; Lai & Locke, 1983; Rutkowski & Levin, 1986). Gurman *et al.* (1987) reported that styrene monomer is the main volatile product of the thermal decomposition of polystyrene, comprising up to 100% of the volatiles.

In order to illustrate the relative significance of various sources of exposure to styrene, Fishbein (1992) approximated exposure levels in several environments and compared nominal daily intakes from those sources (Table 7).

Table 7. Estimated daily intake of styrene from different sources of exposure

Source	Estimated concentration	Nominal daily intake ^a
Reinforced plastics industry	200 000 µg/m ³	2 g
Styrene polymerization	10 000 µg/m ³	100 mg
Within 1 km of a production unit	30 µg/m ³	600 µg
Polluted urban atmosphere	20 µg/m ³	400 µg
Urban atmosphere	0.3 µg/m ³	6 µg
Indoor air	0.3–50 µg/m ³	6–1000 µg
Polluted drinking-water (2 L/day)	1 µg/L	2 µg
Cigarette smoke (20 cigarettes/day)	20–48 µg/cigarette	400–960 µg

From Fishbein (1992)

^aCalculated on the assumption of a daily respiratory intake of 10 m³ at work and 20 m³ at home or in an urban atmosphere

1.3.4 Water

Although styrene has been detected occasionally in estuaries and inland waters and in drinking-water, its presence is usually traceable to an industrial source or to improper disposal (WHO, 1983; Law *et al.*, 1991). In surveys of Canadian drinking-water supplies, the frequency of detection of styrene was low; when detected, it was generally at a concentration of < 1 µg/L (Newhook & Caldwell, 1993).

1.3.5 Food

Polystyrene and its copolymers have been used widely as food packaging materials, and residual styrene monomer can migrate into food from the packaging (WHO, 1983). Analysis

of styrene in 133 plastic food containers from retail food outlets in the United Kingdom showed concentrations ranging from 16 to 1300 mg/kg; 73% of containers had styrene concentrations of 100–500 mg/kg, and only five containers had levels exceeding 1000 mg/kg. The food in the containers had levels of monomer ranging from < 1 to 200 µg/kg, although 77% of the foods had levels below 10 µg/kg and 26% had levels below 1 µg/kg (Gilbert & Startin, 1983).

Styrene has been detected as a natural constituent of a wide range of foods and beverages, the highest measured levels occurring in cinnamon (Maarse, 1992a,b; Steele, 1992). Enzymatic degradation of cinnamic acid derivatives was proposed as a possible source (Oliviero, 1906; Ducruet, 1984).

1.4 Regulations and guidelines

1.4.1 Occupational exposure limits

Occupational exposure limits and guidelines for styrene are presented in Table 8. A tolerable daily intake of 7.7 µg/kg bw for styrene has been established by WHO (1993), with a guideline value of 20 µg/L in drinking-water. The US Environmental Protection Agency (1993) has set the drinking-water standard for styrene at 0.1 ppm (100 µg/L).

Table 8. Occupational exposure limits and guidelines for styrene

Country or region	Year	Concentration (mg/m ³)	Interpretation
Argentina	1991	215	TWA
		425	STEL (15 min)
Australia	1983	215	TWA
		425	STEL
Austria	1982	420	TWA
Belgium	1984	215	TWA; skin
		425	
Brazil	1978	328	TWA
Bulgaria	1984	5	TWA
Canada	1986	215	TWA; skin
		425	STEL
Chile	1983	172	TWA
China	1981	40	TWA
Czechoslovakia	1985	200	TWA
		1000	STEL
Denmark	1988	105	TWA
Finland	1993	85	TWA
		420	STEL
France	1990	215	TWA
Germany	1993	85	TWA; substance with systemic effects (onset < 2 h) ^a
Hungary	1978	50	TWA; suspected of having carcinogenic potential; irritant
		100	Ceiling
India	1983	420	TWA
		525	STEL

Table 8 (contd)

Country or region	Year	Concentration (mg/m ³)	Interpretation
Indonesia	1978	420	Ceiling
Italy	1978	300	TWA
Japan	1983	210	TWA
Mexico	1984	215	TWA
		425	STEL
Netherlands	1986	420	TWA
Poland	1982	100	TWA
Romania	1975	250	Average
		350	Maximum
Sweden	1991	90	TWA; skin
		200	STEL (15 min)
Switzerland	1987	215	TWA; provisional ^a
Taiwan	1981	420	TWA
United Kingdom	1992	420	TWA; maximum exposure limit
		1050	STEL (10 min)
USA			
ACGIH (TLV)	1994	213	TWA; skin
		426	STEL
OSHA (PEL)	1992	426	TWA
		852	Ceiling
		2556	AMP
NIOSH (REL)	1992	215	TWA
		425	Ceiling
Venezuela	1978	420	TWA
		525	Ceiling

From Cook (1987); Arbejdstilsynet (1988); ILO (1991); US Occupational Safety and Health Administration (OSHA) (1992); US National Institute for Occupational Safety and Health (NIOSH) (1992); American Conference of Governmental Industrial Hygienists (ACGIH) (1993); Deutsche Forschungsgemeinschaft (1993); Työministeriö (1993); UNEP (1993)

TWA, time-weighted average; STEL, short-term exposure limit; TLV, threshold limit value; AMP, acceptable maximum peak above the acceptable ceiling concentration for an 8-h shift for 5 min in any 3 h; PEL, permissible exposure level; REL, recommended exposure level; skin, absorption through the skin may be a significant source of exposure

^aThere is no reason to fear a risk of damage to the developing embryo or fetus when exposure limits are adhered to (Group C).

The US Food and Drug Administration (1993) has established regulations for the use of polymers and copolymers of styrene in products in contact with food. For styrene and methyl methacrylate copolymers as components of paper and paperboard in contact with fatty foods, the monomer content in the copolymer is limited to 0.5%.

1.4.2 Reference values for biological monitoring of exposure

The relationship between external (air concentrations) and biological measures of exposure has been studied more extensively for styrene than for most other organic compounds in the occupational environment. Correlations between the concentration of

styrene in air and in venous blood and with mandelic acid and phenylglyoxylic acid in urine have been described by a number of investigators and reviewed by Guillemin and Berode (1988), the American Conference of Governmental Industrial Hygienists (1993), Lauwerys and Hoet (1993) and Pekari *et al.* (1993).

For example, the concentration of mandelic acid in urine that corresponds to inhalation of 50 ppm styrene (213 mg/m^3) for 8 h would be 800–900 mg/g creatinine at the end of a shift and 300–400 mg/g creatinine the following morning (Droz & Guillemin, 1983; Guillemin & Berode, 1988; Pekari *et al.*, 1993). The phenylglyoxylic acid concentration in urine that corresponds to an 8-h exposure to 50 ppm styrene would be expected to be 200–300 mg/g creatinine at the end of a shift and about 100 mg/g creatinine the following morning (American Conference of Governmental Industrial Hygienists, 1991; Pekari *et al.*, 1993). The styrene concentration in blood that corresponds to an 8-h exposure at 50 ppm styrene would be expected to be 0.5–1 mg/L at the end of a shift and about 0.02 mg/L in blood the following morning (Guillemin & Berode, 1988; American Conference of Governmental Industrial Hygienists, 1991).

Each year, the American Conference of Governmental Industrial Hygienists (1993) and the Deutsche Forschungsgemeinschaft (1993) publish biological reference values for use in interpreting the results of biological monitoring for styrene in the workplace. The results must be interpreted in relation to the different definitions of those reference values. Biological exposure indices are reference values intended for use as guidelines for evaluating potential health hazards in the practice of industrial hygiene. The indices represent the levels of the determinants that are most likely to be observed in specimens collected from healthy workers exposed by inhalation to air concentrations at the level of the threshold limit value (American Conference of Governmental Industrial Hygienists, 1993). The biological tolerance value for a working material is defined as the maximal permissible quantity of a chemical compound or its metabolites, or any deviation from the norm of biological parameters induced by those substances in exposed humans. According to current knowledge, these conditions generally do not impair the health of an employee, even if exposure is repeated and of long duration. Biologische arbeitsstofftoleranzwerte (BAT) values are defined as ceiling values for healthy individuals (Deutsche Forschungsgemeinschaft, 1993). Biological monitoring reference values for exposure to styrene, based on styrene metabolite levels in urine or styrene in blood, are given in Table 9.

2. Studies of Cancer in Humans

2.1 Case reports

Cases of leukaemia and lymphoma were identified among workers engaged in the production of styrene–butadiene rubber (Lemen & Young, 1976), in the manufacture of styrene–butadiene (Block, 1976) and in the manufacture of styrene and polystyrene (Nicholson *et al.*, 1978). A total of 19 cases of leukaemia and eight of lymphoma were reported in these studies. Exposure to benzene, butadiene, ethyl benzene and other chemicals could also have occurred in these operations.

Table 9. Reference values for biological monitoring of exposure to styrene

Determinant	Sampling time	Biological exposure index ^a	BAT ^b
Mandelic acid in urine	End of shift	800 mg/g creatinine ^c	2000 mg/L
	Prior to next shift	300 mg/g creatinine ^c	Does not apply
Phenylglyoxylic acid in urine	End of shift	240 mg/g creatinine ^c	Does not apply
	Prior to next shift	100 mg/g creatinine ^c	Does not apply
Mandelic acid plus phenylglyoxylic acid in urine	End of shift	Does not apply	2500 mg/L
Styrene in venous blood	End of shift	0.55 mg/L ^d	
	Prior to next shift	0.02 mg/L ^d	Does not apply

^aAmerican Conference of Governmental Industrial Hygienists (1993)

^bBiologische arbeitsstofftoleranzwerte; Deutsche Forschungsgemeinschaft (1993)

^cNonspecific, as it is also observed after exposure to other chemicals such as ethylbenzene

^dSemiquantitative, because of short half-life of styrene in blood

2.2 Cohort studies

2.2.1 Styrene-butadiene rubber manufacture

The mortality of workers engaged in the rubber industry in the USA, where there is potential exposure to styrene, among other chemicals, has been investigated in a number of studies (Andjelkovic *et al.*, 1976; McMichael *et al.*, 1976; Monson & Nakano, 1976; Andjelkovic *et al.*, 1977; Matanoski *et al.*, 1990). Several showed elevated standardized mortality ratios (SMRs) for cancers at various sites. All of these studies are described in detail in previous *IARC Monographs* (IARC, 1979, 1982, 1986, 1987a, 1992). In the only study (McMichael *et al.*, 1976) in which exposure to styrene-butadiene was specified, an association (relative risk [RR], 6.2; 99.9% confidence interval [CI], 4.1–13) between lymphatic and haematopoietic malignancies and work in the styrene-butadiene rubber manufacture department of a rubber plant was suggested.

A retrospective cohort study (Meinhardt *et al.*, 1982) was conducted in two plants where styrene-butadiene rubber was produced (plants A and B) in eastern Texas, USA, which included 1662 (plant A) and 1094 (plant B) white men who had been employed for at least six months (total, 53 929 person-years at risk). Follow-up was from 1943 to 1976 (plant A) and from 1950 to 1976 (plant B). The study was conducted after the initial finding of two cases of lymphatic and haematopoietic cancer at one of the plants. Environmental samples were taken only at the time of the study: mean exposures to styrene were 0.94 ppm at plant A (4 mg/m³) and 1.99 ppm (8.5 mg/m³) at plant B; those for 1,3-butadiene were 1.24 ppm (2.74 mg/m³) and 13.5 ppm (30 mg/m³), respectively. Traces of benzene were also detected in plant A. The SMR for all causes of death was 80 [95% CI, 70–90] for workers in plant A, and nine deaths from cancers of the lymphatic and haematopoietic tissues occurred between January 1943 and March 1976 (5.79 expected), giving an SMR of 155 [95% CI, 71–295]. Further analysis showed that these nine men had been employed between January 1943 and December 1945 during operation of a batch process, for which period the expected number

was 4.3, giving an SMR of 212 [95% CI, 97–402]. The SMR for leukemias was 278 [95% CI, 90–648]. In plant B no significant excess mortality from any cancer was found: 11 cancer deaths were observed and 21 expected; there were two deaths from neoplasms of lymphatic and haematopoietic tissues (2.6 expected).

Matanoski *et al.* (1990) studied a cohort of 12 110 male workers in the USA and Canada who had worked for a minimum of one year in any of eight plants where styrene–butadiene rubber was manufactured. Limited information on exposure was available: the average exposure to styrene in five of the eight plants ranged between 0.29 and 6.66 ppm [1.23–28.4 mg/m³] (mean, 3.53 ppm [15 mg/m³]) for the period 1978–83 (Matanoski *et al.*, 1993). The subcohorts at each plant were followed from various times between 1943 and 1982, for a total of 251 431 person-years of follow-up. Vital status was known for 96.6% of the workers, and by the end of the study 20% of the cohort had died. SMRs were calculated from country- and race-specific mortality rates. The SMR for all cancers was 0.85 (95% CI, 0.78–0.93), and no significant excess for cancer at any site was observed for the total cohort. An examination of specific causes of death in the subset of workers involved in production (adjusted for age, ethnicity and calendar year) showed an excess of all lymphatic and haematopoietic neoplasms in the subgroup of 371 black production workers (SMR, 5.1; 95% CI, 1.9–11). Three of the malignancies were leukaemias (SMR, 6.6; 95% CI, 1.4–19). These results were questioned (Cole *et al.*, 1993), because in one plant no records were available for active workers and in another plant ethnicity was not designated on all records (Matanoski *et al.*, 1993). When those two plants were omitted, the SMR for leukaemia in black production workers was 8.3 (Matanoski *et al.*, 1993).

A case–control study of 59 cases of lymphohaematopoietic cancer (Santos-Burgoa *et al.*, 1992) was conducted within the US cohort studied by Matanoski *et al.* (1990). A total of 193 controls were matched to cases by plant, age, year of employment, duration of employment and survival of the case. Each job was assigned an estimated exposure to styrene and 1,3-butadiene by a panel of experts in the rubber industry, who ranked jobs from low to high. Analysis showed an association between leukaemia and exposure to 1,3-butadiene (odds ratio, 9.4; 95% CI, 2.1–23) and a nonsignificant association with exposure to styrene (odds ratio, 3.1; 95% CI, 0.84–11). When exposures to both styrene and 1,3-butadiene were included in a conditional logistic regression model, the odds ratio for 1,3-butadiene remained elevated (7.4; 95% CI, 1.3–41), but the estimated association of leukaemia with styrene was decreased (odds ratio, 1.1; 95% CI, 0.23–5.0). Because of criticism about the use of controls matched for duration of work, a new set of three controls per case was selected and matched on all previously matched variables except duration of work (Matanoski *et al.*, 1993). Exposure to styrene was not significantly associated with lymphatic and haematopoietic cancers, whereas the association with exposure to 1,3-butadiene remained significant.

2.2.2 Styrene manufacture and polymerization

A study by Frentzel-Beyme *et al.* (1978) of 1960 German workers engaged in the manufacture of styrene and polystyrene between 1931 and 1976 for a minimum of one month showed no significant excess mortality from cancer. The cohort had accumulated only 20 138 person-years. Follow-up was 93% of the German workers but only 29% of non-German

workers. There were 74 deaths (96.5 expected) available for analysis. One death from lymphatic and haematopoietic cancer was observed, with less than one expected. In 1975, concentrations of styrene in the plant were generally below 1 ppm [4.3 mg/m³], but higher concentrations were occasionally recorded (Thiess & Friedheim, 1978). [The Working Group noted that insufficient information was provided to assess the risk for cause-specific deaths by exposure period or duration of exposure.]

Ott *et al.* (1980) studied a cohort assembled from four US plants where styrene-based products were developed and produced. Exposure to styrene varied by process. During batch polymerization in 1942, the styrene concentrations were 5–88 ppm [21–375 mg/m³]; in continuous polymerization and extrusion units, the concentrations were below 10 ppm [43 mg/m³] and generally below 1 ppm in 1975 and 1976. Cohorts from each plant had been exposed between 1937 and 1960 and were followed from 1940 to 1975. Other potential exposures included benzene, acrylonitrile, 1,3-butadiene, ethylbenzene, dyes and pigments. Age- and race-specific US mortality rates were used to calculate the expected numbers of deaths. A total of 2904 workers with a minimum of one year of employment were followed, of whom 303 had died (425.0 expected). A total of 58 cancer deaths were observed, whereas 76.5 were expected. Seven were from lymphatic and haematopoietic cancers, except leukaemia (SMR, 132 [95% CI, 58–272]), and six were from leukaemia (SMR, 176 [95% CI, 64–383]). Bond *et al.* (1992) updated the study, adding a further 11 years of observations. A total of 687 deaths were reviewed; again, mortality from all causes and all neoplasms was lower than expected (overall SMR, 76 [95% CI, 70–82]; cancer SMR, 81; 95% CI, 69–95), and 28 of the deaths were due to lymphatic and haematopoietic malignancies (SMR, 144; 95% CI, 95–208). There were five cases of lymphatic and haematopoietic cancer (3.9 expected) among workers in the styrene monomer and finishing areas and five multiple myelomas (1.7 expected), six leukaemias (3.6 expected) and four non-Hodgkin's lymphomas (2.9 expected) in the polymerization, colouring and extrusion department.

Hodgson and Jones (1985) reported on 622 men who had worked for at least one year in the production, polymerization and processing of styrene at a plant in the United Kingdom between 1945 and 1978. Of these, 131 men were potentially exposed to styrene in laboratories and 491 in production of styrene monomer, polymerization of styrene or manufacture of finished products. No measurements of exposure were provided, but many other chemicals were present in the working environment. Expected numbers of deaths were calculated on the basis of national rates. There were 34 deaths (43.1 expected) among the 622 exposed workers. A significant excess of deaths from lymphoma (3 observed, 0.56 expected) was observed. An analysis of cancer registrations for this population revealed an additional case of lymphatic leukaemia, giving a total of four incident cases of lymphatic and haematopoietic cancer, whereas 1.6 would have been expected from local cancer registration rates [standardized incidence ratio (SIR), 250; 67–640]. Additionally, three incident cases of laryngeal cancer were found (0.5 expected).

2.2.3 Use in reinforced plastics

Okun *et al.* (1985) studied 5021 workers who had been employed in two reinforced-plastic boat-building facilities in the USA for at least one day between 1959 and 1978. On the basis of industrial hygiene surveys, 2060 individuals were classified as having had high

exposure to styrene, with means in the two facilities of 42.5 ppm and 71.7 ppm [181 and 305 mg/m³]. Of these, 48% had worked for one month to one year and only 7% for more than five years. There were 47 deaths in the high-exposure group (41.5 expected); no case of lymphatic or haematopoietic cancer was observed (approximately one expected).

Coggon *et al.* (1987) studied a cohort of 7949 men and women who had been employed during 1947–84 at eight British facilities where glass-reinforced plastics were manufactured. Of these, 5434 had worked in jobs entailing exposure to styrene, but only 2458 had done so for at least one year. Hand laminators (the most highly exposed) were estimated to have had 8-h time-weighted exposures to styrene of 40–100 ppm [170–426 mg/m³]. The main analysis was restricted to seven facilities where a satisfactory proportion of the cohort had been traced (average, 96.7%). A total of 100 deaths from cancer were observed (SMR, 93 [95% CI, 76–113]), including one from Hodgkin's disease (SMR, 78), one from myeloma (SMR, 89), one from leukaemia (SMR, 33) and 51 from cancers of the lung, pleura and mediastinum (SMR, 126 [94–166]). No information was available on smoking habits. No death was recorded from non-Hodgkin's lymphoma in exposed subjects. The excess of lung cancer was concentrated particularly among workers who had had one to nine years of exposure to styrene, but risk did not increase with time since first exposure. Follow-up of this cohort was later extended to 1990 as part of an international collaborative study (Kogevinas *et al.*, 1994a) (see below). By the time of that analysis, the previous deficit of lymphatic and haematopoietic cancer had largely disappeared (13 deaths; SMR, 88; 95% CI, 47–151) and the excess of lung cancer was less marked (77 deaths; SMR, 106; 95% CI, 84–132).

Wong (1990) and Wong *et al.* (1994) reported on a cohort of 15 826 male and female employees who had worked at one of 30 reinforced-plastics plants in the USA for at least six months between 1948 and 1977. Workers were followed until 1989; vital status was determined using Social Security Administration files, the National Death Index and the records of credit agencies. A total of 307 932 person-years at risk were accumulated. Expected numbers of deaths were based on national age-, gender-, cause- and year-specific death rates for whites, as no information was available on race. Exposure to styrene was calculated in a job-exposure matrix that included work history and current and past time-weighted average exposures. A total of 1628 (10.3%) members of the cohort were found to have died, and death certificates were obtained for 97.4% of them. The overall mortality rate was 108 (95% CI, 103–113), and the mortality rate from all cancers was 116 (95% CI, 105–127). Mortality from cancers at a number of sites was increased significantly: oesophagus: 198, 14 observed, 95% CI, 105–322; bronchus, trachea and lung: 141, 162 observed, 95% CI, 120–164; cervix uteri: 284, 10 observed, 95% CI, 136–521; and other female genital organs: 202, 13 observed, 95% CI, 107–345. No excess was observed for lymphohaematopoietic cancers (82; 31 observed; 95% CI, 56–117). For workers involved in open-mould processing with high exposure to styrene, the SMR for lymphatic and haematopoietic cancers was 141 (four observed cases). For the highest cumulative exposure (> 100 ppm×years) and > 20 years of latency, the SMR was 134 (5–373). The data were analysed by Cox regression with age, gender, length of exposure and cumulative exposure included in the model. Neither cumulative exposure nor length of exposure was significant in the model for lymphatic and haematopoietic cancer. No positive dose-response relationship was found for any other cancer that occurred in excess. [The Working Group noted that the

possibility that the two exposure variables included in the regression model were correlated may have reduced the likelihood of accurate assessment.]

Kogevinas *et al.* (1994a,b) described a historical cohort mortality study of 40 688 workers employed in 660 plants of the reinforced plastics industry and enrolled in eight subcohorts in Denmark, Finland, Italy (two), Norway, Sweden and the United Kingdom (two). Exposure to styrene was reconstructed from job and production records, environmental measurements and, in Italy, biological monitoring. An exposure database was constructed on the basis of about 16 500 personal air samples and 18 695 measurements of styrene metabolites in urine. Styrene exposure levels decreased considerably during the study period. The data from Denmark were considered to be representative of all six countries: They showed exposures of about 200 ppm [852 mg/m³] in the 1950s, about 100 ppm [426 mg/m³] in the late 1960s and about 20 ppm [85 mg/m³] in the late 1980s. The 40 688 workers accumulated 539 479 person-years at risk and were followed for an average of 13 years. Workers lost to follow-up and those who emigrated constituted 3.0% of the total cohort, and in no individual cohort did the proportion exceed 8.0%; 60% of the cohort had less than two years' exposure and 9% had more than 10 years' exposure. The WHO mortality data bank was used to compute national mortality reference rates by sex, age (in five-year groups) and calendar year. No excess was observed for mortality from all causes [2196 deaths; SMR, 96; 95% CI, 92–100] or from all neoplasms (550 deaths; SMR, 91; 83–98). The mortality rate in exposed workers for neoplasms of the lymphatic and haematopoietic tissues was not elevated (50 deaths; SMR, 96; 95% CI, 71–127) and was not associated with length of exposure. Evaluation of risk by job type also showed no significant pattern. In an analysis by country, one of the cohorts in the United Kingdom and that from Denmark had moderate increases in mortality from lymphatic and haematopoietic cancer (Table 10). An increased risk for those cancers was observed in Poisson regression models for years since first exposure ($p = 0.012$) and for average exposure ($p = 0.019$) but not for cumulative exposure (see Table 11). Within the models there was an increasing trend in risk for lymphatic and haematopoietic cancer with average intensity of exposure, culminating in an RR of 3.6 (95% CI, 1.0–13) for the highest category, > 200 ppm; for more than 20 years since first employment, the RR was 4.0 (95% CI, 1.3–12). Although there were no increased risks for cancers of the pancreas, kidney or oesophagus, nonsignificant increases in risks at these sites were seen with time since first exposure or cumulative exposure to styrene.

Kolstad *et al.* (1994) evaluated the incidence of malignancies in lymphohaematopoietic tissues among male Danish workers, some 12 800 of whom were included in the mortality study of Kogevinas *et al.* (1994a,b). Using national pension fund records from 1964 onwards, they identified 36 525 'exposed' male workers who had been employed in 386 industries that were identified by industry experts as ever having produced reinforced plastics; however, the 36 525 workers may not all have been exposed to styrene. The mean annual levels of styrene calculated for 128 of these companies reflect the exposures measured in the industry, which range from 180 ppm [767 mg/m³] in 1964–70 to 43 ppm [183 mg/m³] in 1976–88. Duration of employment was calculated from pension fund payments made beginning in 1964–89. SIRs were calculated on the basis of national rates standardized for sex, age and year of diagnosis, and 95% CIs were calculated on the basis of the Poisson distribution. Within the cohort,

Table 10. Mortality from lymphatic and haematopoietic malignancies in the international study on European workers exposed to styrene, by subcohort

Subcohort	No. of deaths among exposed workers	SMR	95% CI
Denmark ^a	24	122	78-181
Finland ^b	3	106	22-310
Italy 1	1	99	3-552
Italy 2	2	65	8-234
Norway	1	34	1-187
Sweden	3	58	12-168
United Kingdom 1 ^c	13	88	47-151
United Kingdom 2	3	121	25-355

From Kogevinas *et al.* (1994a). SMR, standardized mortality ratio; CI, confidence interval

^aAlso included by Kolstad *et al.* (1994)

^bExtended follow-up of part of a previous study (Härkönen *et al.*, 1984a)

^cExtended follow-up of Coggon *et al.* (1987)

there were 112 malignancies of the lymphatic and haematopoietic system, with 93.7 expected (SIR, 1.2; 95% CI, 0.98-1.4). In workers with > 10 years since first employment, the SIR for leukaemia was 157 (107-222). The excess was due to cases in workers who had had less than one year of employment. For those employed in 1964-70, the SIR was 1.3 (1.0-1.7). The SIR for leukaemia more than 10 years after first short-term employment (2.3; 1.4-3.6) was the only significant increase; for workers with more than one year of employment, the corresponding SIR was 1.0 (0.52-1.7). For workers with less than 10 years since first employment, the only significant increase was for lymphomas (21 observed; 1.7; 1.0-2.5), with similar increases for short- and long-term employees. For all lymphatic and haematopoietic cancers, a similar risk differential was seen with duration of exposure.

Table 12 summarizes the characteristics of cohort and nested case-control studies on mortality from neoplasms of the lymphatic and haematopoietic tissues among workers exposed to styrene.

2.3 Case-control studies

Flodin *et al.* (1986) conducted a matched case-control study of 59 cases of acute myeloid leukaemia and 354 controls in Sweden to assess potential risk factors, which included radiation, medications and various occupational exposures. Cases were aged 20-70 years and were identified at hospitals in Sweden between 1977 and 1982. Two series of controls were drawn from a population register: one was matched to cases for sex, age (within five years) and location, and the other was a random population sample. Information on exposure was obtained through a questionnaire mailed to subjects. Reported exposure to styrene was

Table 11. Mortality from neoplasms of the lymphatic and haematopoietic tissues (ICD8 200–208) by cumulative exposure (ppm × years), time since first exposure and average exposure (ppm) to styrene

	No. of cases observed	RR	95% CI
Cumulative exposure (years)			
< 75	20	1	
75–99	8	0.98	0.43–2.3
200–499	10	1.2	0.57–2.7
≥ 500	9	0.84	0.35–2.0
Test for linear trend (<i>p</i> value)		0.65	
Time since first exposure (years)			
< 10	13	1	1
10–19	25	2.9	1.3–6.5
> 20	9	4.0	1.3–12
Test for linear trend (<i>p</i> value)		0.012	
Average exposure (years) ^a			
< 60	7	1	
60–99	9	1.7	0.59–4.8
100–119	10	3.1	1.1–9.1
120–199	13	3.1	1.0–9.1
≥ 200	8	3.6	0.98–13
Test for linear trend (<i>p</i> value)		0.019	

From Kogevinas *et al.* (1994a). Calculated on the basis of exposure model A, no lag; Poisson regression analysis. Models for cumulative and average exposure are adjusted by age, sex, country, calendar period and time since first exposure. RR, relative risk; CI, confidence interval

^aFor malignant lymphomas, the RR and 95% CI are for data from five countries, excluding Finland, because the model comprising the full data set did not converge

found to be a risk factor (standardized rate ratio, 1.9; 95% CI, 1.9–357), but the number of exposed subjects (3/59 cases and 1/354 controls) was small.

A population-based case-control study of cancer comprised 3730 histologically confirmed male cases of cancer at 11 major sites (including non-Hodgkin's lymphoma) newly diagnosed between 1979 and 1986 among residents of Montréal, Canada, aged 35–70 and ascertained in 19 major hospitals (Siemiatycki, 1991). The exposure of each subject to 293 occupational agents was evaluated by a group of chemists on the basis of jobs held, and cases of cancer at each site were compared with those in the rest of the study population, after adjustment for age, ethnic group, alcohol drinking and tobacco smoking. One percent of subjects were classified as ever having been exposed to styrene. The only significant increase in risk was seen for cancer of the rectum (odds ratio, 1.7; 90% CI, 0.8–3.8; six cases). A higher risk (odds ratio, 4.1; 90% CI, 1.4–11.8, five cases) was seen for subjects with 'substantial' exposure to styrene (subjects with exposure to styrene at medium or high concentration and frequency and with at least five years accumulated duration of exposure, up to five years before onset of disease).

Table 12. Characteristics of cohort and nested case-control studies of mortality from neoplasms of the lymphatic and haematopoietic tissues (L&H) among workers exposed to styrene

Reference (country)	Type of plant; study period; number of subjects; minimal period employed; follow-up	No. of deaths/ cancer deaths	Results ^a				Comments
			N	SMR	95% CI	Site	
Frentzel-Beyme <i>et al.</i> (1978) (Germany)	Styrene and polystyrene manufacture (one facility); 1931-76; 1960 subjects; 1 month; 93% German, 29% non-German	74/11	1	-	-	Lymphoma	Malignant neoplasm of the spleen; less than one death expected from neoplasms of the L&H
Ott <i>et al.</i> (1980) (USA)	Dow Chemical workers in development or production of styrene-based products; 1940-75; 2904 men; 1 year; 97.0%	303/58	13 21	149 161	80-256 100-246	L&H, mortality L&H, incidence	Excess incidence of lymphatic leukaemia (SIR, 427; 95% CI, 172-879; 7 cases); highest risks in workers exposed to styrene, ethylbenzene and other fumes, solvents and colourants
Bond <i>et al.</i> (1992) (USA)	Dow Chemical workers in development or production of styrene-based products (updating of Ott <i>et al.</i> , 1980); 1940-86; 2904 men; 1 year; 96.7%	687/162	28	144	95-208	L&H, mortality	Elevated incidences of multiple myeloma and Hodgkin's disease
Okun <i>et al.</i> (1985) (USA)	Reinforced plastics boat building (two facilities); 1959-78; 5201 subjects; 1 day; 98.1%	176/36	0	-	-	-	Less than one death expected from neoplasms of the L&H
Hodgson & Jones (1985) (United Kingdom)	Production, polymerization and processing of styrene; 1945-78; 622 men; 1 year; 99.7% exposed, 99.4% referents	34/10	3 0 4	[536 - 250]	110-1566 - 68-640]	Lymphomas, mortality Leukaemias, mortality L&H, incidence	
Coggon <i>et al.</i> (1987) (United Kingdom)	Production of glass-reinforced plastics (8 facilities) ^b ; 1947-84; 7949 subjects; no minimal employment; variable, 99.7-61.9%	693/181	6	[40	15-88]	L&H, mortality	Only one of six deaths among subjects with high exposure to styrene; extended follow-up of this cohort included by Kogevinas <i>et al.</i> (1994a,b)
Wong <i>et al.</i> (1994) (USA)	Reinforced plastics manufacturing plants (30 facilities); 1948-89; 15 826 subjects; 6 months; 83.9%	1628/425	425 31 11	116 82 74	105-127 56-117 37-133	All neoplasms L&H Leukaemia	Higher risk of L&H among workers with cumulative exposure > 100 ppm-years at > 20 years since first exposure (SMR, 134; 5 deaths), and among workers in open-mould processing (SMR, 141; 4 deaths)

Table 12 (contd)

Reference (Country)	Type of plant; study period; number of subjects; minimal period employed; follow-up	No. of deaths/ cancer deaths	Results ^a				Comments
			N	SMR	95% CI	Site	
Kolstad <i>et al.</i> (1994) (Denmark)	Production of glass-reinforced plastics and other plastics (552 facilities); 1970–89; 53 720 subjects (36 525 exposed to styrene); no minimal employ- ment; 98.2%	NR/1915 ^d	112	120	98–144	L&H, incidence	Significantly higher incidence of leukaemia (SIR, 157) at ≥ 10 years since first exposure; incidence of leukaemia higher among short-term workers with estimated higher expo- sure to styrene; part of this cohort included by Kogevinas <i>et al.</i> (1994a,b)
			42	122	88–165	Leukaemia, incidence	
Kogevinas <i>et al.</i> (1994a,b) (six European countries)	Production of glass-reinforced plastics (660 facilities); 1945– 91; varies between cohorts; 40 688 workers; 97%	2714/686	550	91	83–98	All neoplasms	Mortality shown for exposed workers only; risk for L&H increased with latency (<i>p</i> for linear trend = 0.012) and with average exposure (<i>p</i> for linear trend = 0.019); risk did not increase with duration of exposure or cumula- tive exposure; excess risk observed for cancer of the pancreas
			50	96	71–127	L&H	
			12	77	40–134	Non-Hodgkin's lymphoma	
			7	111	45–229	Hodgkin's disease	
			23	104	66–157	Leukaemia	
Meinhardt <i>et al.</i> (1982) (USA)	Styrene-butadiene rubber plants (2 facilities); 1943–76; 2756 men; 6 months, 96.8%	332/56	11	[132	66–236]	L&H	Highest risk (SMR, 212, 9 deaths) among workers in plant A first employed during operation of batch process
McMichael <i>et al.</i> (1976) (USA)	Rubber workers in styrene- butadiene synthetic plant (one facility); 1964–73; 6678 men; no minimal employment; NR	NR	NR	6.2 3.9	4.1–12.5 2.6–8.0	L&H Lymphatic leukaemia	Nested case-control study; odds ratios are for workers in styrene- butadiene plant <i>versus</i> other workers; 99.9% CI
Santos-Burgoa <i>et al.</i> (1992) (USA)	Styrene-butadiene rubber manufacture (8 facilities); 1943–82; 12 110 men; one year; 96.6%	NR	26 18	1.1 0.9	0.2–5.0 0.2–5.5	Leukaemia Multiple myeloma and other lymphoma (ICD 202–203)	Nested case-control study of 59 cases of L&H cancer and 193 controls; odds ratios for exposure to styrene adjusted for concomitant exposure to 1,3-butadiene; unadjusted RR for leukaemia was 2.9 (0.8–10.3)

NR, not reported

^aN, number of deaths/cases; SMR, standardized mortality ratio; CI, confidence interval^bIncludes one company in which only a low proportion of subjects could be traced.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F1 mice, six weeks old, received daily administrations of 150 or 300 mg/kg bw styrene (purity, 99.7%) in corn oil by gastric intubation on five days per week for 78 weeks. Control groups of 20 male and 20 female mice received corn oil alone. The study was terminated at 91 weeks. Body weights of treated females were slightly reduced, and survival was slightly reduced in high-dose males (20/20, 46/50, 39/50) and females (18/20, 40/50, 38/50). The combined incidences of adenoma and carcinoma of the lung in male mice were: 0/20 (control), 6/44 (low-dose) and 9/43 (high-dose) ($p = 0.02$; Cochran-Armitage test for trend). The mean incidence among untreated controls at the laboratory was 12%, the highest incidence being 20% in two control groups. There was a significant ($p = 0.034$; Cochran-Armitage) increasing trend in the incidence of hepatocellular adenoma in female mice (control, 0/20; low-dose, 1/44; high-dose, 5/43) (US National Cancer Institute, 1979a).

Groups of 50 male and 50 female B6C3F1 mice, six weeks old, received daily administrations of 203 or 406 mg/kg bw styrene in a mixture (solution of 70% styrene and 30% β -nitrostyrene) in corn oil by gastric intubation on three days per week for 78 weeks. Control groups of 20 male and 20 female mice received corn oil only. The study was terminated at 92 weeks. Body weights of high-dose female mice were slightly reduced. Survival among males was 18/20 (control), 43/50 (low-dose) and 33/50 (high-dose), and that among females was 17/20, 47/50 and 38/50, respectively. The combined incidences of adenoma and carcinoma of the lung in males were 0/20 (control), 11/44 (low-dose; $p = 0.016$, Fisher's exact test) and 2/43 (high-dose) (US National Cancer Institute, 1979b). [The Working Group noted the high β -nitrostyrene content of the mixture.]

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344/N rats, six weeks old, received daily administrations of 1000 or 2000 mg/kg bw styrene (purity, 99.7%) in corn oil by gastric intubation on five days a week for 78 weeks. Control groups of 20 male and 20 female rats received corn oil only. Because of high mortality in the high-dose groups by week 23, additional groups of 50 male and 50 female rats administered 500 mg/kg styrene in corn oil by gastric intubation on five days per week for 103 weeks were included in the study, and additional groups of 20 male and 20 female rats receiving only corn oil were included as vehicle controls for these animals. All surviving rats were killed at 104–105 weeks. Body weights of the mid- and high-dose male rats were slightly reduced. Survival was poor in both high-dose males (17/20, 18/20, 44/50, 47/50, 6/50) and females (15/20, 18/20, 46/50, 46/50, 7/50) in comparison with controls ($p < 0.001$, Cox's test). There was no treatment-related increase in the incidence of any type of tumour in male or female rats (US National Cancer Institute, 1979a).

Groups of 40 male and 40 female Sprague-Dawley rats, 13 weeks old, were administered 0 (control), 50 or 250 mg/kg bw styrene (purity, > 99%) in olive oil by gastric intubation daily on four to five days a week for 52 weeks. The study was terminated [duration unspecified] when the last animal died. There was no treatment-related effect on body weight; survival of female rats receiving the high dose was reduced [data not provided]. No treatment-related increase in the incidence of tumours was reported (Conti *et al.*, 1988). [The Working Group noted the incomplete reporting and the short duration of treatment.]

Groups of 50 male and 70 female Sprague-Dawley rats, seven weeks old, were administered 125 or 250 ppm [mg/L] styrene (purity, 98.9%) daily in the drinking-water for 104 weeks. Control groups of 76 male and 104 female rats received drinking-water without styrene. At 52 weeks, 10 rats per sex and group were removed from the study for a scheduled interim kill. There was a significant reduction in water consumption among treated male and female rats and a significant reduction in body weight among high-dose females ($p < 0.05$, Student's *t* test). There was no treatment-related effect on survival and no evidence of chronic toxicity or carcinogenicity (Beliles *et al.*, 1985). [The Working Group noted the low level of exposure.]

Groups of 50 male and 50 female Fischer 344/N rats, six weeks old, received daily administrations of 350 and 700 mg/kg bw (males) or 175 and 350 mg/kg bw (females) styrene in a mixture (solution of 70% styrene and 30% β -nitrostyrene) in corn oil by gastric intubation on three days a week for 79 weeks. Control groups of 20 male and 20 female rats received corn oil only. The study was terminated at 108 weeks. The body weights of male rats were slightly reduced. There was no effect on survival in male (16/20, 34/50, 31/50) or female (12/20, 33/50, 31/50) rats, and there was no treatment-related increase in the incidence of any type of tumour (US National Cancer Institute, 1979b). [The Working Group noted the high β -nitrostyrene content of the mixture.]

3.2 Prenatal exposure followed by postnatal oral administration

3.2.1 Mouse

A group of 29 pregnant O20 mice received a single administration of 1350 mg/kg bw styrene (purity, 99%) in olive oil by gastric intubation on day 17 of gestation. A control group of nine pregnant mice received olive oil alone. Preweaning mortality was 43% among offspring of dams receiving styrene and 22% among offspring of controls. Groups of 45 male and 39 female progeny from the dams that received styrene were administered 1350 mg/kg bw styrene in olive oil by gastric intubation once a week from weaning until 16 weeks of age. Control groups of 20 male and 22 female mice with no prenatal exposure received olive oil alone. Administration of styrene was stopped at 16 weeks because of high mortality related to treatment (64% survival at 20 weeks). Centrilobular necrosis of the liver was frequent in mice that died within the first 20 weeks. The experiment was terminated at 120 weeks. At the time of observation of the first tumour, 19 control males and 23 treated males and 21 female mice and 32 treated females were still alive. In the progeny that received weekly administrations of styrene, the combined incidence of lung adenomas and carcinomas was significantly ($p < 0.01$) increased over that in vehicle controls: males, 8/19 *versus* 20/23, and females, 14/21 *versus* 32/32. There was no treatment-related difference in the incidences of

tumours at other sites in the progeny and no treatment-related difference in tumour incidences between control and styrene-treated dams (Ponomarkov & Tomatis, 1978). [The Working Group noted the high mortality in treated mice early in the study.]

A group of 15 pregnant C57Bl mice received a single administration of 300 mg/kg bw styrene (purity, 99%) in olive oil by gastric intubation on day 17 of gestation. A control group of five pregnant mice received olive oil only. There was no treatment-related effect on neonatal mortality. Groups of 27 male and 27 female progeny of dams that received styrene were administered 300 mg/kg bw styrene in olive oil by gastric intubation once a week from weaning up to 120 weeks. Control groups of 12 male and 13 female mice received olive oil alone. The experiment was terminated at 120 weeks. There was no treatment-related effect on body weight or survival. At the time of observation of the first tumour, 12 male controls and 24 treated male progeny, 13 female controls and 24 treated progeny, and 10 control and 5 treated dams were still alive. There was no treatment-related difference in the incidences of tumours at any site in dams or progeny (Ponomarkov & Tomatis, 1978). [The Working Group noted the small numbers of animals.]

3.2.2 Rat

A group of 21 pregnant BDIV rats received a single administration of 1350 mg/kg bw styrene (purity, 99%) in olive oil by gastric intubation on day 17 of gestation. A control group of 10 pregnant rats received olive oil alone. There was a slight treatment-related increase in neonatal mortality. Groups of 73 male and 71 female progeny of dams that received styrene were administered 500 mg/kg bw styrene in olive oil by gastric intubation weekly from weaning up to 120 weeks. Control groups of 36 male and 39 female rats received olive oil alone. The experiment was terminated at 120 weeks. There was no treatment-related effect on body weight or survival. At the time of observation of the first tumour, 32 control and 54 treated male progeny and 35 control and 68 treated female progeny were still alive. Stomach tumours occurred in three female rats (adenoma, fibrosarcoma, carcinosarcoma) administered styrene and in one female rat (fibrosarcoma) in the control group. Non-neoplastic stomach lesions [morphology and incidence unspecified] were reported in rats administered styrene. There was no significant treatment-related increase in tumour incidence at any site (Ponomarkov & Tomatis, 1978).

3.3 Inhalation

Rat: Groups of 30 male and 30 female Sprague-Dawley rats, 13 weeks old, were exposed by inhalation in chambers to 25, 50, 100, 200 or 300 ppm styrene [105, 210, 420, 840, 1260 mg/m³] (purity, > 99%) for 4 h per day on five days a week for 52 weeks. The control groups comprised 60 male and 60 female rats. The study was terminated when the last animal died [duration unspecified]. No treatment-related effect on body weight or survival was reported [data not provided]. The combined incidence of benign and malignant mammary tumours was greater in treated female rats than in controls: 34/60 controls, 24/30 at 25 ppm, 21/30 at 50 ppm, 23/30 at 100 ppm, 24/30 at 200 ppm and 25/30 at 300 ppm [$p = 0.01$; Fisher's exact test; highest-dose animals compared with controls]. The incidence of malignant mammary tumours was significantly increased in treated females: 6/60 controls, 6/30 at 25 ppm, 4/30 at

50 ppm, 9/30 at 100 ppm, 12/30 at 200 ppm and 9/30 at 300 ppm [$p < 0.01$, Cochran-Armitage trend test]) (Maltoni *et al.*, 1982; Conti *et al.*, 1988). [The Working Group noted the incomplete reporting of the data and the high incidence of spontaneous mammary tumours in animals of this strain.]

3.4 Intraperitoneal administration

3.4.1 Mouse

In a screening assay based on increased multiplicity and incidence of lung tumours in a strain of mice highly susceptible to development of this neoplasm, a group of 25 female A/J mice, six to eight weeks old, received intraperitoneal injections of 20 μ mol styrene (purity, > 99%) in olive oil three times a week for a total of 20 injections. A vehicle control group of 25 mice received olive oil alone. The study was terminated 20 weeks after the last injection, and gross and microscopic examination was performed on all mice administered styrene; only lung tissue was examined from controls. There was no treatment-related increase in the incidence of lung tumours (3/25 versus 1/25 in controls). All 25 animals in a positive control group treated with 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK) developed lung tumours (Brunnemann *et al.*, 1992).

3.4.2 Rat

Groups of 40 male and 40 female Sprague-Dawley rats, 13 weeks old, received four intraperitoneal injections of 50 mg/animal styrene (purity, > 99%) in olive oil at two-month intervals. Control groups received injections of olive oil alone. The study was terminated when the last animal died [duration unspecified]. No treatment-related effect on body weight or survival was reported [data not provided]. There was no treatment-related increase in the incidence of benign and/or malignant tumours (Conti *et al.*, 1988). [The Working Group noted the incomplete reporting of data, the short duration of treatment and the low total dose.]

3.5 Subcutaneous administration

Rat: Groups of 40 male and 40 female Sprague-Dawley rats, 13 weeks old, received a single subcutaneous injection of 50 mg/animal styrene (purity, > 99%) in olive oil. Control groups of 40 male and 40 female rats received a subcutaneous injection of olive oil alone. The study was terminated when the last animal died [duration unspecified]. No treatment-related effect on body weight or survival was reported [data not provided]. The authors reported that there was no treatment-related increase in the incidence of benign and/or malignant tumours (Conti *et al.*, 1988). [The Working Group noted the incomplete reporting of data and the single low-dose treatment.]

4. Other Data Relevant for an Evaluation of Carcinogenicity and Its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

The pharmacokinetics and metabolism of styrene have been reviewed (WHO, 1983; Henschler, 1987; WHO, 1987; Guillemin & Berode, 1988; Bond, 1989; Beratergremium für Umweltrelevante Altstoffe, 1993).

4.1.1 Humans

(a) Absorption

In several studies, the pulmonary retention of styrene was 60–70% of the inhaled dose. In these studies, volunteers or workers were exposed to styrene for: over 1 h to 51.4, 216.1, 376 ppm [223, 936, 1629 mg/m³] and 2 h to 117 ppm [507 mg/m³] (Stewart *et al.*, 1968), 2 h to 70 ppm [303 mg/m³] (Wigaeus *et al.*, 1983, 1984; Löf *et al.*, 1986a), 2 h to 210 mg/m³ [48 ppm] at different workloads (Åstrand, 1986), 2 h and 5 h to 24 ppm [104 mg/m³] (Fiserova-Bergerova & Teisinger, 1965), 6 h to 80 ppm [347 mg/m³] (Ramsey *et al.*, 1980), 8 h to 22, 129, 235 ppm [95, 559, 1018 mg/m³] (Bardoděj & Bardodějová, 1970), 8 h to 32–85 mg/m³ [7.4–20 ppm] (Engström *et al.*, 1978a) and 8 h to 20, 40, 100, 200 mg/m³ [4.6, 9.2, 23.0, 46 ppm] (Wieczorek & Piotrowski, 1985).

Styrene in ambient air is absorbed through the skin at 2–5% of the dose absorbed in the respiratory tract (Riihimäki & Pfäffli, 1978; Wieczorek, 1985). Liquid styrene was found to penetrate the skin at a rate of 1 µg/(cm² × min). Contact of one hand (500 cm²) for 30 min with liquid styrene was estimated to be equivalent, in terms of absorption, to 4% of the dose retained in the body during exposure to 50 ppm [213 mg/m³] for 8 h (Berode *et al.*, 1985).

(b) Distribution

As the partition coefficients between air and different body tissues are 4100 for fat, 84–154 for other organs and 59 for blood, styrene was concluded to accumulate exclusively in fat tissue (Droz & Guillemin, 1983). Analysis of styrene in subcutaneous fat suggested accumulation and slow release (Wolff *et al.*, 1977; Engström *et al.*, 1978a,b). Its half-life was estimated from determinations in subcutaneous fat of six subjects exposed to 50 ppm (2 h), 15 and 20 ppm (8 h each) to be between two and five days (Engström *et al.*, 1978a,b). This finding led to the proposal that styrene levels in the body increase during a working week, and arguments for and against the hypothesis have been presented (see Bond, 1989). According to the physiologically based pharmacokinetic model of Perbellini *et al.* (1988) (described below), the styrene concentration in fat tissue increases during a working week because of high enrichment. In a study of workers exposed to 37 ppm [160 mg/m³] styrene, no evidence was found that it accumulates during a working week (Pekari *et al.*, 1993).

(c) Elimination in exhaled air and in urine

Only 0.7–4.4% of the amount of styrene absorbed was found to be exhaled unchanged (Stewart *et al.*, 1968; Fernández & Caperos, 1977; Caperos *et al.*, 1979; Guillemin & Bauer,

1979; Ramsey *et al.*, 1980). At the end of a 2-h exposure to 300 mg/m³ [69 ppm], the quotients arterial blood:alveolar air and arterial blood:inhaled air at steady state were found to be 62 and 7.5 (Wigaeus *et al.*, 1983). The large difference between the two values is due to rapid metabolic elimination of styrene. The higher value represents determination of the partition coefficient blood:air of styrene *in vivo* and is similar to the thermodynamic partition coefficient human blood:air determined *in vitro* at 37 °C, which was 61 (Wigaeus *et al.*, 1983), 59 (Droz & Guillemin, 1983) and 48 (Csanády *et al.*, 1994).

Unchanged styrene was also excreted in urine of workers exposed to a mean time-weighted styrene concentration of 87.9 mg/m³ [20.3 ppm]. The concentration in urine was about one-tenth that in blood (Gobba *et al.*, 1993).

(d) *Metabolic elimination*

In several studies, disappearance of styrene was measured in blood of workers and volunteers after exposure for 2–6 h to concentrations of 70 ppm [303 mg/m³] and 80 ppm [347 mg/m³] styrene for 1.5 h (Teramoto & Horiguchi, 1978), 4 h (Wigaeus *et al.*, 1983) and 41 h (Ramsey *et al.*, 1980) after exposure. For periods between 1.5 and 2 h, a fast elimination phase was observed, with half-lives of 0.58 h (Ramsey *et al.*, 1980), 0.67 h (Teramoto & Horiguchi, 1978) and 0.68 h (Wigaeus *et al.*, 1983). In the study of Ramsey *et al.* (1980), a second phase was distinguished, with a half-life of 13 h. A total blood clearance of 1.5–1.6 L/(kg×h) was calculated from the fast phase (Ramsey *et al.*, 1980; Wigaeus *et al.*, 1983). This value was almost identical to that for total blood flow through the liver, indicating that a high-affinity perfusion-limited pathway exists. Contributions from extrahepatic metabolism, uptake by adipose tissues and exhalation were considered to be small (Wigaeus *et al.*, 1983).

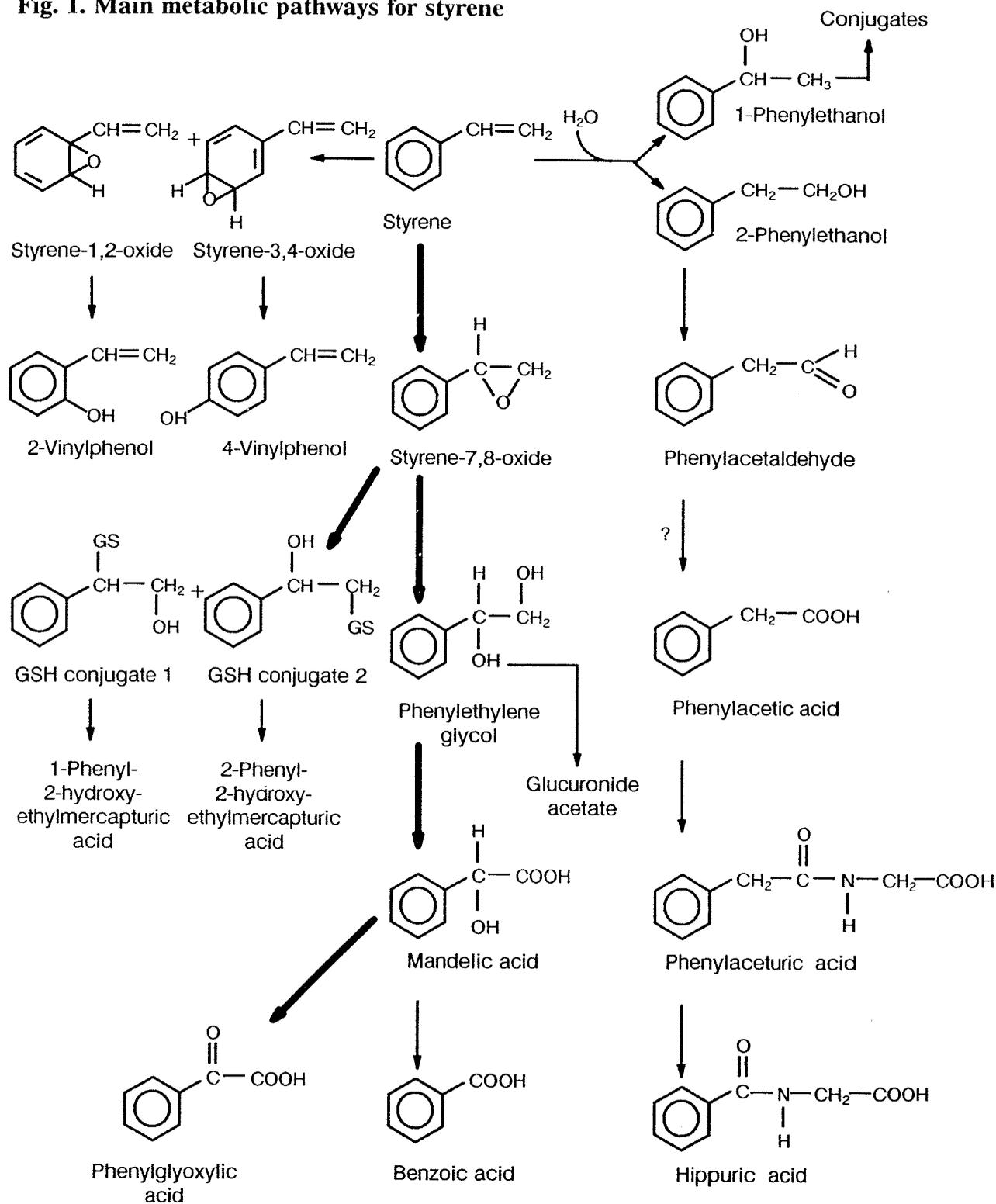
Experimental 2-h exposure to styrene at 296 mg/m³ [68 ppm] led to a significantly lower styrene level in the blood of occupationally pre-exposed subjects (14.7 µmol/L) than of those not previously exposed (21.2 µmol/L). The mean values for total clearance were 1.5 L/(kg×h) in the reference group and 2.0 L/(kg×h) in the pre-exposed group (Löf *et al.*, 1986a).

In workers, a linear correlation was found between atmospheric styrene concentrations up to about 150 ppm [650 mg/m³] and concentrations in venous blood (Bartolucci *et al.*, 1986; Brugnone *et al.*, 1993; Gobba *et al.*, 1993; Korn *et al.*, 1994). No linear correlation was found, however, between atmospheric and arterial blood concentrations of styrene in two volunteers exposed for 2 h to 26, 77, 201 and 386 ppm styrene [110, 328, 856 and 1672 mg/m³]. The relatively higher blood concentrations at the two higher doses seem to indicate saturability of styrene metabolism (Löf & Johanson, 1993).

(e) *Metabolites*

The metabolic pathways of styrene are shown in Figure 1. Styrene is oxidized to styrene-7,8-oxide by the cytochrome P450-mediated monooxygenase system. The responsible isozyme, first identified in human liver, was considered to be the ethanol-inducible CYP2E1 (Guengerich *et al.*, 1991); however, more recent evidence suggests that CYP2B6 is the main catalyst, followed by CYP2E1 and CYP1A2, and additional isozymes with lower activity for styrene may be involved (Nakajima *et al.*, 1993). Styrene has a high affinity for both CYP2B6

Fig. 1. Main metabolic pathways for styrene



Adapted from Ohtsuji & Ikeda (1971), Leibman (1975), Bardodej (1978), Vainio *et al.* (1984) and Watabe *et al.* (1978). Main pathways are indicated by thick arrows. GS, glutathione; GSH, reduced glutathione

and CYP2E1 isozymes (Michaelis-Menten constant [K_m] about 0.1 mmol/L), and other isozymes may be responsible for low-affinity catalysis.

In volunteers exposed for 2 h to about 300 mg/m³ [70 ppm] styrene, styrene-7,8-oxide was found in venous blood at a mean concentration of 0.05 µmol/L [6 µg/L] (Wigaeus *et al.*, 1983). Similar styrene-7,8-oxide concentrations, up to 0.04 µmol/L [4.8 µg/L] and 4.1 µg/L, were present in venous blood of workers exposed to styrene at concentrations up to 371 mg/m³ [86 ppm] (mean, 99 mg/m³ [23 ppm]) (Löf *et al.*, 1986b) and up to 73 ppm [311 mg/m³] (Korn *et al.*, 1994). Exposure to 20 ppm [87 mg/m³] styrene gave rise to a mean styrene-7,8-oxide concentration of 1 µg/L (Korn *et al.*, 1994). These concentrations are 5–20 times lower than the corresponding concentrations in the blood of styrene-exposed rodents (see below). Enzymatic hydrolysis of styrene-7,8-oxide yields phenylethylene glycol, which reached a blood concentration of 15–20% of the styrene concentration in blood of workers exposed for 2 h to 300 mg/m³ [69 ppm] styrene (Wigaeus *et al.*, 1983; Löf *et al.*, 1986a). Phenylethylene glycol (S enantiomer:R enantiomer, about 3) and 2-phenylethanol have been found in urine of styrene-exposed workers (Korn *et al.*, 1985, 1987). Phenylethylene glycol is further oxidized to mandelic and phenylglyoxylic acids, which are the main metabolites found in urine of people exposed to styrene, with 57–85% and 10–33% of an absorbed dose of styrene excreted as mandelic and phenylglyoxylic acids (Bardoděj & Bardodějová, 1970; Guillemin & Bauer, 1979). Mandelic acid was excreted in styrene-exposed workers as a racemic mixture with a 1.2-fold excess of the R enantiomer (Drummond *et al.*, 1989). In other studies, a 1.5-fold excess of the S enantiomer was found (Korn *et al.*, 1985, 1987).

Urinary excretion of mandelic acid and phenylglyoxylic acid is biphasic. After an 8-h exposure of workers to styrene at concentrations of 26–130 mg/m³ [6.1–30.5 ppm], both metabolites had a half-life of about 2.5 h for the first and 30 h for the second phase (Wieczorek & Piotrowski, 1988). These compounds are used in assessing occupational exposure to styrene. According to several studies, an exposure to 20 ppm [80 mg/m³] styrene corresponds, the following morning, to a combined mandelic acid and phenylglyoxylic acid level of about 2.9 mmol/L (Pekari *et al.*, 1993).

Alcohol inhibits urinary excretion of mandelic and phenylglyoxylic acids (Berode *et al.*, 1986; Černý *et al.*, 1990).

Conjugation of styrene-7,8-oxide with glutathione is of minor importance in humans, since urinary excretion of thioethers or of the mercapturic acid, *N*-acetyl-*S*-(2-phenyl-2-hydroxyethyl)cysteine, after exposure to styrene for 2 h at about 210 mg/m³ [48 ppm] amounted to only about 1% or less of the absorbed styrene (Aringer *et al.*, 1991; Norström *et al.*, 1992).

Small amounts of 4-vinylphenol (0.3% of mandelic acid) were found in urine of workers exposed to styrene at 130 ppm [563 mg/m³], indicating that ring oxidation of styrene leading to the formation of styrene-3,4-oxide is a minor metabolic pathway (Pfäffli *et al.*, 1981).

Several physiologically based pharmacokinetic models have been developed which are useful in predicting the behaviour of styrene in humans. The models of Droz and Guillemin (1983) and Perbellini *et al.* (1988), validated for experimental exposures to styrene at up to 100 ppm [433 mg/m³], simulate styrene concentrations in blood and alveolar air and urinary

excretion of mandelic acid and phenylglyoxylic acid during a working week. The models of Ramsey and Andersen (1984) and Löf and Johanson (1993) were validated with data on styrene in exhaled air and in arterial blood during and after experimental exposures to styrene at up to 376 and 386 ppm, respectively. Both models predict partially saturated metabolism at the highest exposure. Values for the K_m (0.36 mg/L in blood and 0.01 mmol/L [1 mg/L] in liver) and for the maximal rate of metabolism (V_{max}) (184 mg/h) were obtained in the first model by scaling from data obtained in rats. The V_{max} (2.9 mmol/h [300 mg/h]) was obtained in the second model by fitting to experimental data.

Csanády *et al.* (1994) developed a model that simulates the behaviour of both styrene and styrene-7,8-oxide. It was validated with data on styrene in exhaled air and venous blood after exposure to up to 376 ppm [1600 mg/m³] and with data on concentrations of styrene-7,8-oxide in blood of workers exposed to styrene at up to 72 ppm [305 mg/m³]. Interactions between inhaled styrene (20 and 50 ppm [85 and 213 mg/m³]) and 1,3-butadiene (5 and 15 ppm [11 and 33 mg/m³]) were predicted in a physiologically based pharmacokinetic model by extrapolation from data obtained in rats. No influence of 1,3-butadiene on styrene metabolism was seen, but styrene at 20 and 50 ppm reduced the metabolism of 1,3-butadiene at 15 ppm to 81 and 63%, respectively (Filser *et al.*, 1993a).

(f) Haemoglobin adducts

In a study of US fibreglass-reinforced plastics workers, passive personal measurements indicated individual exposures to styrene in air of 1–44 ppm [4.26–187.4 mg/m³] (arithmetic mean, 17.2 ppm [73.3 mg/m³]); extrapolation from urinary mandelic acid values gave an average air concentration of 15.2 ppm [64.8 mg/m³]. As measured by gas chromatography–mass spectrometry, the concentration of adducts of styrene-7,8-oxide to the N-terminal valine, *N*-(1-hydroxy-2-phenylethyl)valine, in haemoglobin was higher among the workers than among a control group of library workers; however, 43% of the exposed and none of the controls were smokers, and an eight-fold variation in the level of styrene-7,8-oxide–haemoglobin adducts was seen among the exposed workers. Furthermore, one exposed laminator showed an adduct level 5000-fold higher than others. Similar variation was seen among the controls. When trichotomized exposure categories were used on the basis of individuals' usual job assignment during the preceding four months, a dose–response relationship was seen, with a mean of 8.1 ± 0.8 pmol/g (including the highly deviant individual, 34.5 ± 11.6 pmol/g) for the high-exposure category, 4.7 ± 0.7 for the medium-low-exposure category and 2.2 ± 6.8 for the control group (Brenner *et al.*, 1991).

In a study of workers in Sweden using unsaturated polyester resin, styrene-induced adducts to the N-terminal valine of haemoglobin were measured by enrichment of adducted globin chains with ion-exchange chromatography and gas chromatography–mass spectrometry. Increased levels were seen in seven of 17 reinforced plastic workers (mean, 28 pmol/g globin) in relation to three out of 11 controls (mean, ≤ 13 pmol/g globin). A linear correlation was seen in a regression analysis between individual adduct levels and free styrene-7,8-oxide ($r = 0.7$) and styrene glycol ($r = 0.9$) in blood and with mandelic acid in urine ($r = 0.9$). Extrapolation from the concentrations of the metabolite in urine provides an estimate of about 75 ppm [319.5 mg/m³] styrene in workplace air (Christakopoulos *et al.*, 1993).

No carboxylic acid esters were detected in six workers exposed to styrene (Sepai *et al.*, 1993).

4.1.2 *Experimental systems*

(a) *Styrene*

The uptake and elimination of styrene were investigated in male Wistar rats during and after a 5-h steady-state exposure to styrene vapours at concentrations varying from 50 to 2000 ppm [217–8666 mg/m³] (Withey & Collins, 1979) and following intravenous administration of styrene at doses of 1.34, 4.01, 6.70 and 9.36 mg/kg bw (Withey & Collins, 1977). Styrene concentration–time courses were analysed in a two-compartment model. The rate coefficient for elimination from the central compartment was affected by dose, indicating saturation of metabolic elimination. The administered dose did not affect the apparent volume of distribution, which was about 10 times larger than the blood volume, indicating extensive distribution of styrene to the tissues. The concentration of styrene in perirenal fat was 10 times higher than that in any organ, indicating a great affinity of styrene for lipid depots.

Teramoto and Horiguchi (1978) investigated the absorption and distribution of styrene in rats [strain unspecified] after a 4-h exposure to styrene vapours at 500 and 1000 ppm [2166 and 4333 mg/m³]. A significant enrichment of styrene in adipose tissues was reported. In another experiment, the authors found no accumulation of styrene in the body after exposure to styrene at about 700 ppm [3033 mg/m³] for 4 h a day for five days.

The pharmacokinetics and distribution of styrene were investigated in male Sprague-Dawley rats after a 6-h exposure to styrene at concentrations of 80, 200, 600 and 1200 ppm [347, 867, 2600 and 5200 mg/m³] (Ramsey & Young, 1978). At each exposure level, the styrene concentration in blood increased rapidly during exposure and approached a maximal value at the end of exposure. The relationship between exposure concentration and blood concentration measured at the end of exposure was nonlinear, since a 15-fold increase in the exposure concentration resulted in a 63-fold increase in blood levels, indicating that metabolism of styrene became saturated. The measured styrene concentrations in blood and adipose tissue were used to develop a physiologically based pharmacokinetic model (Ramsey & Andersen, 1984).

The effects of the cytochrome P450 inhibitor, pyrazole, and of the inducer, phenobarbital, on the results of repeated exposures to styrene were investigated in male Fischer 344 rats (Andersen *et al.*, 1984) by evaluating measured blood styrene concentration–time courses in a physiologically based pharmacokinetic model. The metabolism of styrene was lower in rats pretreated with pyrazole (300 mg/kg 0.5 h before exposure) than in controls. After pretreatment with phenobarbital (80 mg/kg per day for four days before exposure), the V_{\max} was increased about six fold. Repeated exposures to styrene at 1000 ppm [4333 mg/m³] for 6 h/day for four days before exposure resulted in a two-fold increase in the value of V_{\max} .

Administration of single oral doses of 500 mg/kg bw styrene to untreated male Fischer 344 rats and to another group of rats 16 h after exposure by inhalation to 1000 ppm styrene for 6 h a day for four days did not significantly alter the area under the blood concentration–time curve (Mendrala *et al.*, 1993).

The uptake, distribution and elimination of styrene have been investigated in Sprague-Dawley rats and B6C3F1 mice in closed chambers after inhalation and after intraperitoneal and oral administration (Filser *et al.*, 1993a,b). In both species, the rate of metabolism of inhaled styrene was dependent on concentration. Deviations from linearity due to saturation of metabolic activation of styrene became apparent at concentrations above 200–300 ppm [867–1300 mg/m³] in both species (Ramsey & Andersen, 1984; Filser *et al.*, 1993b). Saturation of metabolism was reached at atmospheric concentrations of about 700 ppm [3033 mg/m³] in rats and 800 ppm [3466 mg/m³] in mice. In rats, pretreatment with diethyl-dithiocarbamate (200 mg/kg) reduced metabolism of styrene at an average body concentration of 0.34 $\mu\text{mol/kg}$ to only 2% of that in control animals; pretreatment was somewhat less effective in mice. The ability of styrene to influence its own metabolism during chronic exposure was investigated by exposing rats and mice for 6 h a day for five days to atmospheric concentrations of 150 and 500 ppm [650 and 2166 mg/m³]; no significant effect on the rate of metabolism of inhaled styrene was found. The experimental data were then used in a two-compartment model in order to calculate pharmacokinetic parameters. Clearance due to uptake, reflecting the transfer rate of styrene due to inhalation, was 63 ml/min for a 250-g rat and 12 ml/min for a 25-g mouse. These values represent 54% (rat) and 47% (mouse) of the alveolar ventilation (Arms & Travis, 1988). At steady state, the clearances of styrene uptake and of metabolism in relation to atmospheric concentrations below 300 ppm [1300 mg/m³] were almost equal. Consequently, less than 5% of the styrene reaching the body was exhaled unchanged in mice and rats exposed to styrene vapours at concentrations below that level. Maximal accumulation, determined as the thermodynamic partition coefficient whole body:air ($\text{ppm}_{\text{body}}/\text{ppm}_{\text{air}}$), was almost identical in the two species (about 420). These values were corroborated by estimates calculated on the basis of the oil:air and water:air partition coefficients. The bioaccumulation factor body:air was lower than the thermodynamic partition coefficient due to metabolic elimination. The lowest value for the bioaccumulation factor in rats, 2.7, was reached at a steady-state exposure concentration below 10 ppm [43 mg/m³]; in mice, it was 5.9, reached at below 20 ppm [87 mg/m³]. The V_{max} was estimated to be 224 $\mu\text{mol/h}$ per kg in rats and 625 $\mu\text{mol/h}$ per kg bw in mice (Table 13).

Dermal uptake was estimated to be 9.4% of total uptake (skin plus inhalation) in male Fischer 344 rats whose fur was closely clipped (McDougal *et al.*, 1990).

The tissue distribution of styrene and its metabolites was investigated in NMRI mice after intraperitoneal injection of 343 mg/kg bw [7-¹⁴C]-styrene (Löf *et al.*, 1983). Radioactivity was distributed rapidly in the tissues, but the results are difficult to evaluate in relation to pharmacokinetics.

The distribution and elimination of styrene was investigated in male CD2F1 mice after intraperitoneal injection of 200 mg/kg bw styrene (Pantarotto *et al.*, 1980). Absorption and elimination processes were described by first-order kinetics. Styrene was rapidly distributed among the tissues, and marked enrichment occurred in perirenal fat.

Pharmacokinetic interactions between styrene and 1,3-butadiene in Sprague-Dawley rats were investigated in a two-compartment model (Laib *et al.*, 1992) and in a physiological pharmacokinetic model (Filser *et al.*, 1993a). 1,3-Butadiene did not influence the metabolism of styrene, whereas styrene inhibited the metabolism of 1,3-butadiene. The

inhibition could be described by assuming a competitive mechanism at atmospheric styrene concentrations up to 100 ppm [433 mg/m³].

Table 13. Maximal rate of styrene metabolism in mice and rats

Species	Strain	V _{max} (μmol/h per kg)	Reference
Mouse	B6C3F1	625	Filser <i>et al.</i> (1993b)
	Unspecified	253	Ramsey & Anderson (1984)
	B6C3F1	600	Csanády <i>et al.</i> (1994)
Rat	Sprague-Dawley	224	Filser <i>et al.</i> (1993b)
	Sprague-Dawley	115	Ramsey & Anderson (1984) ^a
	Sprague-Dawley	224	Csanády <i>et al.</i> (1994) ^b
	Fischer 344	96	Andersen <i>et al.</i> (1984)

^aData set of Ramsey and Young (1978) reanalysed in a physiological pharmacokinetic model

^bData set of Filser *et al.* (1993b) reanalysed in a physiological pharmacokinetic model

Physiological pharmacokinetic models were developed to describe the disposition and metabolism of styrene in rat and man (Andersen *et al.*, 1984; Ramsey & Andersen, 1984; Paterson & Mackay, 1986) and in rat, mouse and man (Csanády *et al.*, 1994). In the last model, the metabolism of styrene was linked to styrene-7,8-oxide. The influence of alveolar ventilation and the blood:air partition coefficient for styrene on the pharmacokinetics of styrene and styrene-7,8-oxide were discussed.

(c) Metabolites

Monooxygenase-dependent epoxidation of styrene to styrene-7,8-oxide is the earliest step in the metabolism of styrene, as demonstrated *in vitro* in isolated perfused rat liver (Beije & Jenssen, 1982; Belvedere *et al.*, 1984), rat liver microsomes (Watabe *et al.*, 1978; Foureman *et al.*, 1989) and purified cytochrome P450 enzymes from rat liver (Foureman *et al.*, 1989). In all species examined (male and female Sprague-Dawley rats, CD1 mice, New Zealand rabbits and Dunkin Hartley guinea-pigs), monooxygenase-dependent formation and epoxide hydrolase-dependent hydration of styrene-7,8-oxide were more active in liver than in lungs, kidneys, spleen or heart (Cantoni *et al.*, 1978). Styrene monooxygenase and epoxide hydrolase activities have been detected in rabbit liver before birth (Romano *et al.*, 1985).

Intermittent exposure (6 h per day, five days per week, 11 weeks) of male Wistar rats to 300 ppm [1300 mg/m³] styrene enhanced the activities of drug hydroxylating (ethoxycoumarin *O*-deethylase, cytochrome P450) and conjugating (epoxide hydrolase) enzymes in liver and kidneys by up to two fold (Vainio *et al.*, 1979). A 24-h exposure of male Han/Wistar rats to 500 ppm [2166 mg/m³] styrene led to a 2.4-fold increase in styrene metabolism in liver microsomes, which was reported to be related to induction of cytochrome P450IIE1 (Elovaara *et al.*, 1991).

Styrene can also be oxidized in the presence of human erythrocytes *in vitro* by oxyhaemoglobin (Tursi *et al.*, 1983). Non-enzymatic epoxidation of styrene by haemoglobin and

myoglobin requires the presence of either molecular oxygen or hydrogen peroxide (Ortiz de Montellano & Catalano, 1985; Rao *et al.*, 1993). Styrene-7,8-oxide can also be formed experimentally in a number of co-oxidation reactions by peroxidase and other oxidants (Belvedere *et al.*, 1983; Ortiz de Montellano & Grab, 1986; Mickiewicz & Rzczycki, 1988).

Species-specific kinetics of the styrene and styrene-7,8-oxide metabolizing systems have been investigated under the same experimental conditions in hepatic microsomes obtained from male Fischer rats, male Sprague-Dawley rats, male B6C3F1 mice and humans (Mendrala *et al.*, 1993). When extrapolated to conditions *in vivo*, the K_m values for human, rat and mouse cytochrome P450-dependent monooxygenases were essentially similar, ranging from 9 $\mu\text{g/g}$ liver in humans to 4.3 $\mu\text{g/g}$ liver in mice not pretreated with styrene; the V_{max} values were relatively similar in rats and mice (41–62 mg/h/kg bw) but were much lower in the five human samples (3.2 mg/h per kg bw). The K_m values for epoxide hydrolase were low in humans (1.2 $\mu\text{g/g}$ liver), intermediate in rats (16–27 $\mu\text{g/g}$ liver) and high in mice (89 $\mu\text{g/g}$ liver); the V_{max} values ranged from 27 mg/h per kg bw in humans to 98 mg/h per kg bw in mice. Glutathione *S*-transferase activity towards styrene-7,8-oxide was apparently lower for humans (168 mg/h per kg bw) than for rodents (1280–2490 mg/h per kg bw).

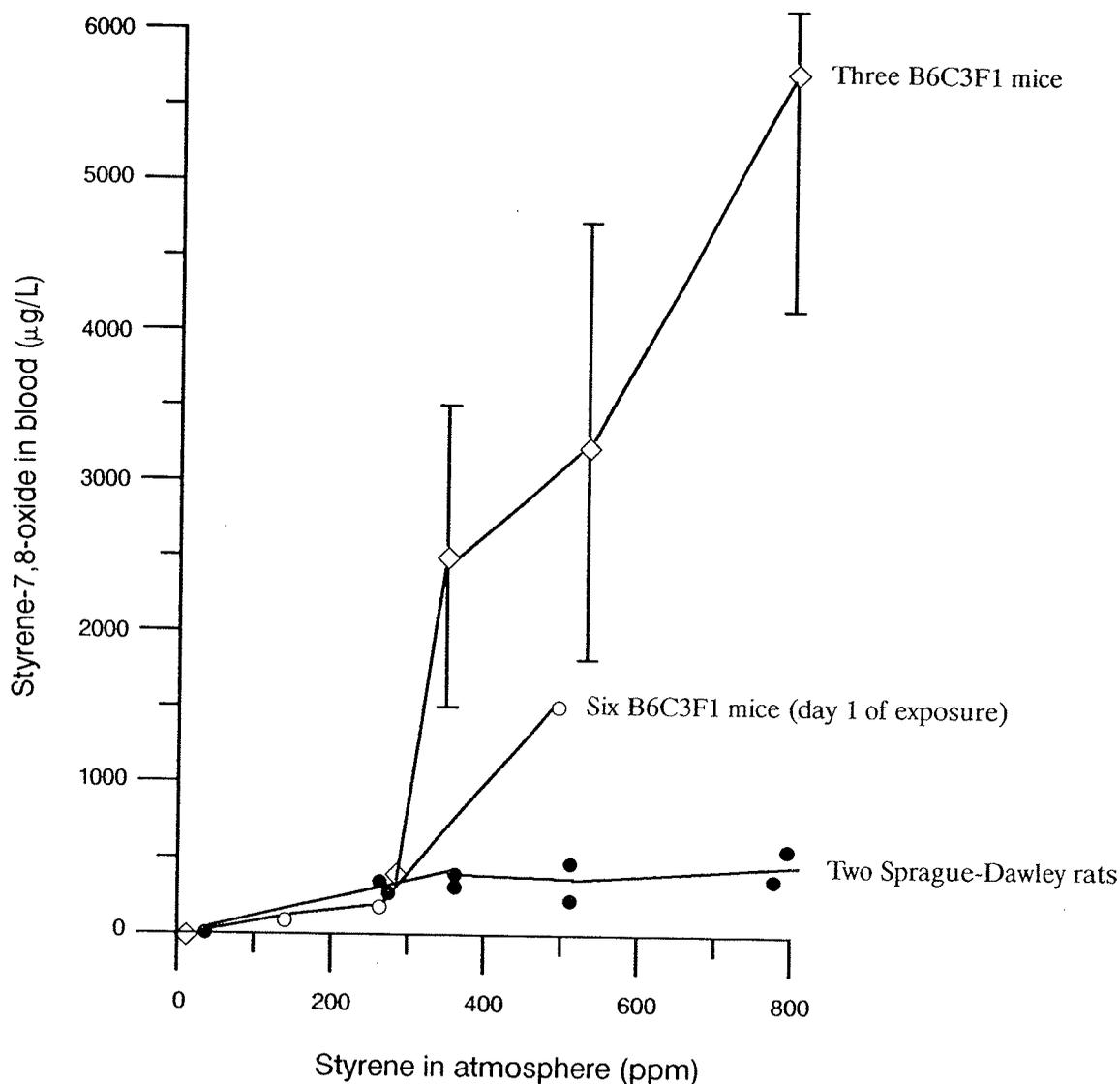
In a study reported in an abstract (Bogan *et al.*, 1993), kinetic parameters of oxidative styrene metabolism were investigated in hepatocytes of male Sprague-Dawley rats and male B6C3F1 mice. The apparent K_m values for the suspensions were (mean \pm SD): 25.5 \pm 7.8 $\mu\text{mol/L}$ for rats and 11.7 \pm 0.3 $\mu\text{mol/L}$ for mice. The V_{max} values were: 3.1 \pm 0.3 nmol/min per (5×10^6 viable cells) in rat hepatocytes and 11.4 \pm 0.7 nmol/min per (5×10^6 viable cells) in those of mice. These values were used to calculate maximal metabolic elimination rates *in vivo* on the basis of the amount of cells in the incubations, the cell densities in the livers of rats (143×10^6 per g) and mice (103×10^6 per g) and liver weights of 10 g for rats weighing 250 g and 1.4 g for mice weighing 25 g. The scaled values for one rat and one mouse *in vivo* were 53.2 \pm 5.2 $\mu\text{mol/h}$ and 19.4 \pm 1.2 $\mu\text{mol/h}$.

Styrene-7,8-oxide has been shown to be formed as a metabolite of styrene *in vivo* in male Fischer rats, male Sprague-Dawley rats, male NMRI mice, male and female B6C3F1 mice, male DBA/2 mice and male Swiss mice (Löf *et al.*, 1984; Kessler *et al.*, 1992; Mendrala *et al.*, 1993; Morgan *et al.*, 1993a). Styrene-7,8-oxide was determined in blood, liver, lungs, kidneys and subcutaneous fat of male NMRI mice 2 h after intraperitoneal administration of [$7\text{-}^{14}\text{C}$]-styrene (1.1–5.1 mmol/kg bw). The concentration in subcutaneous fat (up to about 100 nmol/g) was several times higher than that in other tissues (up to 5–20 nmol/g) (Löf *et al.*, 1984).

In Sprague-Dawley rats, the styrene-7,8-oxide concentration in blood reached a maximum of about 0.3 mg/L at atmospheric styrene concentrations above 360 ppm [1560 mg/m^3] (Kessler *et al.*, 1992). In the mouse strains investigated (DBA2, Swiss and B6C3F1), the styrene-7,8-oxide concentrations in blood increased greatly at styrene concentrations above 250 ppm [1083 mg/m^3] (Kessler *et al.*, 1992; Morgan *et al.*, 1993a,b), reaching a value of about 6 mg/L at 800 ppm [3466 mg/m^3], which was suggested to be due to glutathione depletion (Kessler *et al.*, 1992). This conjecture was confirmed in mice exposed for 6 h/day for three days to constant concentrations of styrene vapours in excess of 260 ppm [1127 mg/m^3] (Morgan *et al.*, 1993a,b). Physiologically based pharmacokinetic modelling of this effect suggests that glutathione transferase-mediated detoxification of styrene-7,8-oxide is

decreased (Csanády *et al.*, 1994). The amount of styrene-7,8-oxide in blood of humans exposed to styrene at concentrations below 100 ppm [433 mg/m^3] (Wigaeus *et al.*, 1983; Löf *et al.*, 1986b; Korn *et al.*, 1994) was 5–20 times lower than the corresponding values in rodents (Fig. 2).

Fig. 2. Concentrations of styrene-7,8-oxide in blood of male rodents in relation to concentrations of styrene in air



From Kesslet *et al.* (1992) and Morgan *et al.* (1993b)

A proportion of styrene-7,8-oxide is further metabolized by microsomal epoxide hydrolase to styrene glycol (Oesch *et al.*, 1971; Watabe *et al.*, 1978). A coupled mono-oxygenase-hydrolase multi-enzyme complex with direct substrate transfer was assumed by Oesch (1973) which would result in a first-pass effect for styrene-7,8-oxide formed from styrene within the endoplasmic reticulum.

After intraperitoneal administration of [7-¹⁴C]-styrene (1.1–5.1 mmol/kg bw) to male NMRI mice, styrene glycol was detected in all tissues examined (blood, liver, lungs, kidneys, brain, pancreas, subcutaneous fat), with the highest concentration in kidney. The concentrations increased linearly with dose and were proportional to the respective styrene-7,8-oxide concentrations (see above) in the tissues (Löf *et al.*, 1984). Further metabolism of styrene glycol yields mandelic acid, phenylglyoxylic acid and hippuric acid, which are the main metabolites excreted in the urine of Wistar rats after intraperitoneal treatment with styrene (Ohtsuji & Ikeda, 1971).

Rat microsomal monooxygenase is stereoselective, preferring the S to the R form (Fouremant *et al.*, 1989), and the metabolism of styrene glycol is stereoselective in rats *in vivo* (Drummond *et al.*, 1990). Racemic styrene glycol is excreted only as R-mandelic acid; S-styrene glycol is excreted principally as that enantiomer. In another study, the ratio of R-:S-mandelic acid was between 2 and 4, depending on the pretreatment of rats (Elovaara *et al.*, 1991).

A proportion of styrene-7,8-oxide is conjugated with glutathione, although direct conjugation of styrene without prior epoxide formation may also occur (Stock *et al.*, 1986). The glutathione conjugates with styrene-7,8-oxide are transformed into the mercapturic acids N-acetyl-S-(1-phenyl-2-hydroxyethyl)cysteine, N-acetyl-S-(2-phenyl-2-hydroxyethyl)-cysteine and N-acetyl-S-(phenylacetyl)cysteine and excreted in urine. About 10% of a single intraperitoneal dose of 250 mg/kg bw to female Wistar rats was excreted as mercapturic acids (Seutter-Berlage *et al.*, 1978). After 6-h exposures of male Sprague-Dawley rats to 25–200 ppm styrene [108–867 mg/m³], the amount of the two major mercapturic acids excreted during 24 h was about 74% of the excreted amount of mandelic acid and phenylglyoxylic acid at the highest exposure concentration (Truchon *et al.*, 1990).

Other putative metabolites identified in urine of styrene-exposed rats were 1-phenylethanol, 2-phenylethanol (Bakke & Scheline, 1970) and phenacetic acid (Delbressine *et al.*, 1980). The occurrence of small amounts of 4-vinylphenol (0.1% of styrene dose) in the urine of rats treated orally with 100 mg/kg bw styrene (Bakke & Scheline, 1970) suggested formation of the ring epoxide, styrene-3,4-oxide, as an intermediary metabolite of styrene (Pantarotto *et al.*, 1978).

Since glutathione conjugates are formed, the effect of styrene on glutathione levels was studied. Glutathione levels in the livers of male Wistar rats were significantly depleted, to 75% and 44%, following exposures (6 h per day, four days) to styrene at 200 and 400 ppm [867 and 1733 mg/m³], respectively. After exposure to 300 ppm styrene [1300 mg/m³] for 6 h per day, five days per week for 2–11 weeks, glutathione levels reached a minimum of 72% of the control level in lungs and 41% in liver after two weeks but returned to 110% in lungs and 80% in liver after six weeks (Vainio *et al.*, 1979). A 24-h exposure of male Han/Wistar rats to 500 ppm styrene [2166 mg/m³] resulted in glutathione levels of 84% of the control level in liver and 34% in lung; the concentration in kidneys was unaffected (Elovaara *et al.*, 1991). The hepatic glutathione content was reduced to about 50% of control levels for about 5 h following intraperitoneal injection of 300 mg/kg bw styrene to male Wistar rats (Katoh *et al.*, 1989).

(c) Protein adducts

Exposure to styrene results in binding to several amino acids in proteins *in vivo*. In rats administered 0.5, 1, 2 and 3 mmol styrene/kg bw by intraperitoneal injection, concentrations of the cysteine adduct of styrene-7,8-oxide in globin were measured after carbon-sulfur bond cleavage by the Raney nickel procedure (Ting *et al.*, 1990). A linear increase was seen in the amount of adduct produced with dose. Treatment with 1 mmol/kg styrene yielded 2.3 nmol/g globin. Alkylation was also determined following intraperitoneal administration of styrene to rats at 0, 0.5, 1 and 3 mmol/kg bw and of styrene-7,8-oxide at 0, 0.1, 0.3 and 1 mmol/kg bw (Rappaport *et al.*, 1993). The dose-response curves for alkylation of haemoglobin and albumin cysteine were linear and indicated that about 2% of the styrene dose was available as styrene-7,8-oxide in blood.

Byfält Nordqvist *et al.* (1985) administered [^{14}C]-styrene intraperitoneally to mice at doses of 0.12–4.9 mmol/kg bw. The valine adduct in haemoglobin, determined by a modified Edman procedure, constituted about 3% of total alkylation. Latriano *et al.* (1991) exposed rats for 6 h per day for five days to 1000 ppm styrene and found a 25-fold increase in valine adducts over that in controls.

4.2 Toxic effects

4.2.1 Humans

The odour threshold for styrene is $70\ \mu\text{g}/\text{m}^3$ [16 ppb] (WHO, 1987). It causes irritation of eyes, throat and respiratory tract at $84\ \text{mg}/\text{m}^3$ [19 ppm] (Lorimer *et al.*, 1976, 1978). Subjective health complaints were usually not seen in the glass-reinforced plastics industry with concentrations of styrene below $105\ \text{mg}/\text{m}^3$ [24 ppm] (Geuskens *et al.*, 1992).

Central and peripheral nervous system effects have been observed in styrene-exposed workers. Nerve conduction velocities were decreased (Lilis *et al.*, 1978; Rosén *et al.*, 1978; Cherry & Gautrin, 1990; Murata *et al.*, 1991), and electroencephalographic (Seppäläinen & Härkönen, 1976), dopaminergic (Mutti *et al.*, 1984a; Arfini *et al.*, 1987; Checkoway *et al.*, 1992), functional (Lindström *et al.*, 1976; Cherry *et al.*, 1980; Baker *et al.*, 1985; Gregersen, 1988) and psychiatric impairments (Flodin *et al.*, 1989) have been noted. Most effects have been seen at concentrations of about 100 ppm [$433\ \text{mg}/\text{m}^3$] styrene, although memory and neurobehavioural disturbances were seen at 10–30 ppm [$43\text{--}130\ \text{mg}/\text{m}^3$] and above (Flodin *et al.*, 1989; Letz *et al.*, 1990). Other studies have shown no evidence of neurotoxicity (Triebig *et al.*, 1989). The hearing threshold was unchanged in workers exposed to less than $150\ \text{mg}/\text{m}^3$ [35 ppm] (Muijser *et al.*, 1988; Möller *et al.*, 1990). In a mortality study of styrene-exposed workers, an increased number of deaths attributed to 'symptoms, senility and ill-defined conditions' was ascribed to a high local registration of these conditions in comparison with national statistics (Bond *et al.*, 1992).

The effects of styrene on the respiratory tract of workers exposed to concentrations above $100\ \text{mg}/\text{m}^3$ [433 ppm] include chronic bronchitis (Härkönen, 1977) and obstructive pulmonary changes (Chmielewski & Renke, 1976). Cases of styrene-induced asthma (Moscato *et al.*, 1987; Hayes *et al.*, 1991) and one of contact dermatitis (Sjöborg *et al.*, 1984) have also been reported.

Several studies reported signs of liver damage, as measured by liver enzyme activities in serum, but it was concluded in a review that no clear-cut trend towards altered liver function could be demonstrated (WHO, 1983). Elevated serum bile acid concentrations were observed in one study (Edling & Tagesson, 1984) but not in another (Härkönen *et al.*, 1984a). In one study, altered kidney function was indicated by increased urinary excretion of albumin in styrene-exposed workers (Askergren *et al.*, 1981).

Early studies on the effects of styrene on the haematopoietic and immune system did not consistently reveal changes (WHO, 1983). In a group of reinforced plastics workers exposed to 60 mg/m³ [14 ppm] styrene, an increased number of peripheral blood monocytes was noted (Hagmar *et al.*, 1989); in another group of workers, changes in lymphocyte sub-populations were observed (Mutti *et al.*, 1992).

4.2.2 Experimental systems

Acute exposure of animals to styrene causes irritation of the skin and respiratory tract and central nervous system effects. Liquid styrene is a skin irritant which, on direct contact, causes erythema. Long-term contact with styrene results in blistering of the skin and development of dermatitis, which is thought to result from defatting of the skin. Single exposures of rats and guinea-pigs to 1300 ppm [5633 mg/m³] styrene resulted in central nervous system effects, including weakness and unsteadiness. After exposure to 2500 ppm [10.8 g/m³] styrene for 10 h, both rats and guinea-pigs lost consciousness; exposure to 5000–10 000 ppm [21.7–43.3 g/m³] resulted in unconsciousness and death. The principal pathological findings in these animals were severe pulmonary irritation, congestion, oedema, haemorrhage and leukocytic infiltration (Bond, 1989).

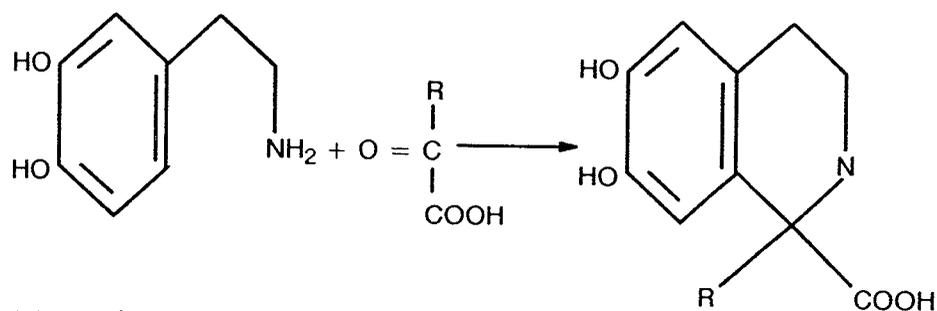
Ohashi *et al.* (1985) investigated the respiratory toxicity of styrene in rats. Epithelial changes occurred in the nose and trachea of animals exposed to 800 ppm [3466 mg/m³] styrene for 4 h per day for eight weeks. The changes included vacuolation of epithelial cells, nuclear pyknosis and exfoliation of epithelial cells. Changes in the nasal mucosa occurred at exposure levels of 30 ppm [130 mg/m³]. Morphological damage was more severe in the upper than in the lower respiratory tract.

Early studies on the neurotoxicity of styrene gave equivocal results (WHO, 1983), but a decrease in the activity of monoamine oxidase was seen in male rats after repeated oral doses of styrene (Zaprianov & Bainova, 1979). In subsequent studies in male rabbits, exposure to styrene caused a dose-dependent decrease in striatal and tuberoinfundibular dopamine content and an increase in homovanillic acid content, consistent with disturbance of the dopaminergic functions of the brain (Mutti *et al.*, 1984b). The levels of norepinephrine were unchanged, suggesting that the metabolites of styrene, phenyl glyoxylic acid and mandelic acid, condense with dopamine and deplete it through a direct chemical reaction of the α -keto acids (Fig. 3; Mutti *et al.*, 1988).

An increase in the prevalence of glial marker proteins was noted in Sprague-Dawley rats exposed to 320 ppm [1386 mg/m³] styrene for three months (Rosengren & Haglid, 1989), and was taken to be an indication of brain damage. Mild neurobehavioural disturbances were seen in rats exposed to 1400 ppm [6066 mg/m³] styrene for 18 weeks (Kulig, 1989) and in mice exposed to 425 ppm [1841 mg/m³] for two weeks (Teramoto *et al.*, 1988). Disturbances

of the auditory system were observed only with combined exposure to trichloroethylene (Rebert *et al.*, 1993).

Fig. 3. Possible molecular basis for dopamine condensation: 1,2,3,4-tetrahydroisoquinolines are formed non-enzymatically in the Pictet-Spengler reaction of the carbonylic group of an α -keto acid with the aminic group of dopamine.



Adapted from Mutti *et al.* (1988)

Exposure of B6C3F1 mice to 259 ppm [1122 mg/m³] styrene for 6 h a day for 14 days induced hepatic necrosis (Morgan *et al.*, 1993c). Hepatotoxicity in rats occurs concomitantly with depletion of glutathione and may be either a direct effect of styrene or mediated by lipid peroxidation (Srivastava *et al.*, 1983; Décarie & Chakrabarti, 1989; Katoh *et al.*, 1989).

Morphological changes have been observed in rat kidney (Chakrabarti *et al.*, 1987) and respiratory mucosa (Ohashi *et al.*, 1986) after exposure to styrene. Srivastava *et al.* (1989) showed that exposure of adult male Wistar rats to 400 mg/kg bw (but not 200 mg/kg bw) styrene daily by gavage for 60 days damaged seminiferous tubules, with reduced sperm count in the epididymis and changes in testicular enzymes, but had no effect on body or testicular weight. The same authors showed that young rats are more sensitive than older animals, with similar changes in sperm count and testicular enzymes when exposed to 200 mg/kg bw (but not 100 mg/kg bw) daily by gavage for the first 60 days of life (Srivastava *et al.*, 1992a). Only slight, transient effects were observed in male offspring of lactating dams exposed during lactation to 400 mg/kg bw styrene daily (Srivastava *et al.*, 1992b).

Effects in kidney and lung are associated with depletion of glutathione (Chakrabarti & Tuchweber, 1987; Elovaara *et al.*, 1990) and possibly with direct toxicity of glutathione conjugates on the kidney (Chakrabarti & Malick, 1991). At 210 mg/m³, styrene inhibited δ -aminolaevulinate dehydratase activity in rat erythrocytes and bone marrow (Fujita *et al.*, 1987).

Styrene suppressed the activity of mouse splenic T lymphocyte killer cells *in vitro* (Grayson & Gill, 1986). Male Swiss mice dosed orally with 20–50 mg/kg bw styrene daily for five days showed impairment of humoral and cell-mediated immunity (Dogra *et al.*, 1989); a similar dose regimen given for four weeks decreased the resistance of mice to viral, malarial and hookworm infections (Dogra *et al.*, 1992).

4.3 Reproductive and prenatal effects

4.3.1 Humans

The frequency of spontaneous abortions among women with definite or assumed exposure to styrene has been investigated in a number of studies. The majority do not indicate an increased risk in association with occupational exposure to styrene (Hemminki *et al.*, 1980; Härkönen & Holmberg, 1982; Lindbohm *et al.*, 1985; Ahlborg *et al.*, 1987; Taskinen *et al.*, 1989; Lindbohm *et al.*, 1990). A study in Canada (McDonald *et al.*, 1988) found an increased risk for spontaneous abortions (18 observed; SMR, 158; 90% CI, 102–235) among women employed in polystyrene manufacture. The expected figures were derived from the experience of 47 316 pregnant women who had worked for 30 h or more per week at the start of pregnancy. No styrene concentrations were given, and most of these women had had mixed exposures. Two studies in Finland found no increase in the frequency of spontaneous abortion or congenital malformation among the wives of men exposed occupationally to styrene (Taskinen *et al.*, 1989; Lindbohm *et al.*, 1991).

Exposure to styrene has been mentioned in some case reports of central nervous system defects in children born to mothers who were exposed to organic solvents during pregnancy (Holmberg, 1977, 1979). A cohort study showed no increased risk for congenital malformations among children of styrene-exposed women or of women married to styrene-exposed men (Härkönen *et al.*, 1984b).

A study on menstrual dysfunction among 174 styrene-exposed and 449 unexposed women from 36 US reinforced plastics companies showed no increase in risk associated with exposure to styrene (Lemasters *et al.*, 1985b). Children born to the 50 women with the highest exposure to styrene (mean, 82 ppm) had a 4% reduction in birth weight ($p = 0.08$); the women were also exposed to other, unidentified solvents (Lemasters *et al.*, 1989).

4.3.2 Experimental systems

Styrene has been shown to cross the placenta of rats. Sprague-Dawley rats exposed for 5 h on day 17 of gestation to 1000 (six rats) or 2000 (five rats) ppm styrene [4330 or 8660 mg/m³] had blood levels, respectively, of about 35 µg/g and 88 µg/g and fetal levels of 17 µg/g and 48 µg/g tissue (Withey & Karpinski, 1985). Transfer was also shown to occur on the 16th day of pregnancy in CD1,crj mice injected intravenously with 5 µCi (5 µl) [8-¹⁴C]styrene. By 6 h, the levels in the fetus were similar to those in maternal brain (Kishi *et al.*, 1989).

The reproductive toxicity of styrene has been reviewed by Jakobsen (1990) and very extensively by Brown (1991), who critically reviewed work published in 1960–90, including the extensive Russian and eastern European literature. The overall conclusion was that there is little indication that styrene exerts any specific developmental or reproductive toxicity.

Murray *et al.* (1978) carried out a standard teratological investigation in rats and rabbits using production-grade styrene (> 99.5% pure). Groups of 23–29 pregnant Sprague-Dawley rats were exposed (whole body) for 7 h per day to 0, 300 or 600 ppm styrene [0, 1299 or 2598 mg/m³] (with one control group for each exposure level) from days 6 to 15 of gestation, and the fetuses removed for examination on day 21. Groups of 24–32 rats were dosed by

gavage with 90 or 150 mg/kg bw styrene twice daily (total doses, 180 and 300 mg/kg bw per day) from days 6 to 15 of gestation and the fetuses examined on day 21. All treatments reduced weight gain in the dams during days 6–9, but there was no overall adverse effect on number or size of litters, resorptions, fetal weight or crown–rump length. The incidences of external, visceral or skeletal malformations were not increased, and variations were within historical control limits. Groups of 16–19 pregnant New Zealand white rabbits were exposed (whole body) for 7 h per day to 0, 300 or 600 ppm styrene (with one control group for each exposure level) from days 6 to 18 of gestation and the fetuses removed for examination on day 29. No toxicity and no effect on the body weight of the dams were observed, and no effects were seen on litter number or size, resorptions, fetal weight or crown–rump length. No malformed fetuses were found in any group and, other than a slightly increased incidence of unossified fifth sternbrae (within historical control range), no other variation was observed.

A three-generation reproduction study was carried out in rats by Beliles *et al.* (1985). Groups of Sprague-Dawley rats were exposed constantly to styrene in the drinking-water at concentrations of 0, 125 or 250 ppm [541 or 1082 mg/L] (averages measured, 112 and 221 ppm [485 and 957 mg/L]) for two years. After 90 days' treatment, groups of 10 males and 20 females and 15 and 30 controls were mated to produce an F1 generation. These pups were kept on the respective treatments and subsequently mated to produce two further generations (F2 and F3) of one litter each, all of which continued to be exposed to styrene in the drinking-water. No treatment-related change in fertility, litter size, pup viability, survival, sex ratio, body weight or bone-marrow cytogenetics was observed.

In a study of a variety of chemicals which are not known to be developmental toxins, given to Sprague-Dawley rats at lethal or near lethal doses in order to study the effects of severe maternal toxicity on fetal development, styrene (99% pure) was administered by gavage at a dose of 1147 mg/kg bw per day from days 6 to 15 of gestation. It produced a very marked reduction in maternal weight gain during the entire pregnancy, but in 13 animals that survived to day 20 there was no adverse effect on litter size, resorptions, fetal weight, malformations or variations, except for an increased proportion of fetuses with an enlarged renal pelvis (Chernoff *et al.*, 1990).

On the basis of the suggestion that toxic doses of chemicals that induce hepatic metallothionein in pregnant rats (and thus sequester zinc in their livers) can lead to developmental toxicity by inducing zinc deficiency in the embryos, Sprague-Dawley rats were dosed on day 11 of gestation with 300 mg/kg bw styrene orally. They showed no specific induction of metallothionein, no changes were seen in zinc levels in the fetuses and no developmental toxicity was seen in the eight litters examined (Daston *et al.*, 1991).

Zaidi *et al.* (1985) showed that exposure of albino rats [strain unspecified] to 200 mg/kg bw styrene by gavage during lactation increase the number of striatal dopamine receptors in pups at two and three weeks of age, resulting in some behavioural effects. No effects were seen if the rats were exposed only during gestation.

Groups of 12 Wistar rats were fed throughout gestation and lactation on a diet containing either a high (20% casein) or a low (8% casein) protein level. From day 6 of gestation onwards, half of the animals were given orally 100 mg/kg bw styrene daily. The low-protein diet slightly impaired postnatal growth and development of the pups, but those given styrene

as well were markedly affected, with effects on brain enzymes and receptor activity. Styrene had no adverse effect in the animals fed the high protein diet (Khanna *et al.*, 1991).

When Wistar rats were exposed to 50 or 300 ppm styrene [217 or 1299 mg/m³] by inhalation for 6 h per day (analysed levels, 60 and 293 ppm [260 and 1269 mg/m³]) on days 7–21 of gestation, no adverse effect was observed in the dams, but pup body weight on the day of delivery was depressed at both dose levels. Pup brain weight and protein content were not affected, but the levels of cerebral serotonin and 5-hydroxyindoleacetic acid were significantly reduced in the one-day-old pups exposed to the high-dose level (Kishi *et al.*, 1992).

Exposure of male (C3H/He × C57Bl/6J)F1 mice to styrene by inhalation (whole body) at 150 or 300 ppm [650 or 1299 mg/m³] for 6 h daily for five days, or to 175, 350 or 700 mg/kg bw intraperitoneally daily for five days did not increase the frequency of abnormal sperm heads at examination three and five weeks after the start of treatment (Salomaa *et al.*, 1985).

4.4 Genetic and related effects

4.4.1 Humans

Several reviews on the genetic toxicology of styrene include data on human genetic bio-monitoring (Barale, 1991; European Centre for Ecotoxicology and Toxicology of Chemicals, 1992; Norppa & Sorsa, 1993; Scott, 1993). Most data published since the re-evaluation of styrene (IARC, 1987b) relate to attempts to find adducts, DNA breakage or cytogenetic damage in association with occupational exposure to styrene.

(a) DNA adducts

Liu *et al.* (1988) measured adducts in one styrene-exposed worker by the ³²P-post-labelling technique; they reported a modification level of 8.6×10^{-7} nucleotides.

The numbers of DNA adducts were compared in lamination workers exposed to styrene at 200–400 mg/m³ in two plants and in agricultural workers, by a ³²P-postlabelling method (Vodička & Hemminki, 1993; Vodička *et al.*, 1993). *O*⁶-Guanine adducts were measured specifically using authentic standards. More than five times higher levels of *O*⁶-(2-hydroxy-1-phenylethyl)-2'-deoxyguanosine-3'-monophosphate were detected among the exposed workers than in controls. The level of adducts in one exposed group was 4.7 (SD ± 1.9) adducts per 10⁸ nucleotides, whereas it was 0.3 (SD ± 0.3) adducts per 10⁸ nucleotides in the controls. The mean adduct level was 7.3 (SD ± 4.9) in workers in the second plant and 1.1 (SD ± 1.3) in the control group.

(b) Alkali-labile sites/DNA single-strand breakage

Using the alkaline elution technique, Walles *et al.* (1993) demonstrated single-strand DNA breaks in the leukocytes of 17 men occupationally exposed to low concentrations of styrene in a plastics factory. [The Working Group noted that this method does not distinguish between alkali-labile sites and single-strand breaks in DNA.] The time-weighted average concentration of styrene in the breathing zone during an 8-h shift was 7.0 ppm [29.4 mg/m³] (range, 0.04–20 ppm [0.17–85.2 mg/m³]), and the arithmetic mean urinary mandelic acid concentration was 70 mg/g creatinine at the end of the shift. An exposure-dependent

increase in the frequency of single-strand breaks was observed at the end of the shift, but not before a shift or the following morning, suggesting repair of the damage. There was no effect of age, years of employment or use of wet snuff. Smoking influenced the frequency of single-strand breaks during a shift, but the increase due to working was significant in both smokers and nonsmokers. Linear regression analysis indicated that an 8-h exposure to 18 ppm [76 mg/m³] styrene, or the resulting urinary mandelic acid concentration of 240 mg/g creatinine, would result in a doubling of the normalized area above the DNA elution curve from the value for no styrene.

In a previous study by the same group, single-strand breakage at the end of a shift was increased in reinforced plastics industry workers manufacturing large plastic containers and exposed to much higher levels (estimated average, 300 mg/m³ on the basis of an average post-shift urinary mandelic acid concentration of 9.4 ± 6.4 mmol/L and a blood styrene glycol concentration of 2.5 ± 1.5 μ mol/L) than in the later study. The method used was the alkaline DNA unwinding technique. Pre-shift analyses were not done (Mäki-Paakkanen *et al.*, 1991).

(c) *Cytogenetic damage in lymphocytes*

Since the first published report of an association between exposure to styrene in the reinforced plastics industry and chromosomal damage in the lymphocytes of workers (Meretoja *et al.*, 1978a), studies have appeared reporting both the presence and absence of three cytogenetic end-points: chromosomal aberrations, sister chromatid exchange and micronuclei (Table 14). The conflicting nature of the results reported may be due to a number of factors. The early studies involved small numbers of individuals exposed to high levels, the highest exposures being those of workers in the reinforced plastics industry. In later studies, improved cytogenetic techniques were used, and two important confounding factors, age and smoking, were identified.

In the only study performed in the polystyrene manufacturing industry (Fleig & Thiess, 1978; Thiess & Fleig, 1978), the frequency of chromosomal aberrations in 12 employees with 2–39 years of exposure was not increased over that in controls matched for sex and age. The styrene concentrations in air were generally below 1 ppm [4.3 mg/m³], and the mandelic acid concentrations in urine were generally less than 50 mg/L. In the study of Fleig and Thiess (1978), an additional five workers in styrene monomer manufacture were included who had been employed for 14–25 years and had mandelic acid concentrations of less than 40 mg/L urine. No difference was seen in chromosomal aberration frequency as compared with that in matched controls. In contrast, the aberration frequency in 14 workers processing unsaturated polyester resins was greater than that in 20 controls. These workers had been exposed for 2–24 years (mean, 7.9 years) and had urinary mandelic acid concentrations of 102–> 1500 mg/L, indicating a much higher exposure than in the styrene monomer and polystyrene manufacturing industry.

Thiess *et al.* (1980) also found no significant increase in the frequency of chromosomal aberrations in 24 employees after 4–27 years of exposure to an average styrene monomer concentration of 6 ppm (1–11.5 ppm [25 (4.3–50) mg/m³]) in a laboratory or 58.1 ppm (0.7–178 ppm [250 (3–760) mg/m³]) in a pilot plant, when compared with a matched control group.

Table 14. Cytogenetic observations in lymphocytes from people occupationally exposed to styrene

No. exposed	No. of referents	Length of exposure (years)		Styrene in air (ppm)		Urinary mandelic acid ($\mu\text{g/g}$ creatinine)		Cytogenetic observation			Reference
		Range	Mean	Range	Mean	Range	Mean	CA	MN	SCE	
10	3	1-15	3.2	≤ 300		53-1646	570	+			Meretoja <i>et al.</i> (1978a)
16	6	1-15	6.3	≤ 300		23-3257	239	+		-	Meretoja <i>et al.</i> (1978a)
5	20	14-25		≤ 10		19-40 mg/L	30	-			Fleig & Thiess (1978)
12	20	3-39		0-47	2	< 5-100 mg/L	32	-			
14	20	2-24		50-300		102- > 1500 mg/L	593	(+)			
12	12	3-34		0-9		10-109 mg/L		-			Thiess & Fleig (1978)
6	6	0.5-10		14-192	39	225-2100	490	+			Högstedt <i>et al.</i> (1979)
24	24	4-27		0.7-170	58.1	0-320 mg/L		-			Thiess <i>et al.</i> (1980)
36	37	0.3-12		1-382	138			+			Andersson <i>et al.</i> (1980)
20	21	0.3-12		1-382	138					(+)	
16	13	0.6-9.3		1-211	70	90-4300 mg/L		-		-	Watanabe <i>et al.</i> (1981)
18	6	0.2-30		40-50		0-1041 mg/L	332	-		-	Watanabe <i>et al.</i> (1983)
38	20	1-23		1-36	13	9-316	65		+		Högstedt <i>et al.</i> (1983)
25 (22) ^a	22 (20)	1-22	9.4	7- > 96		45-1108 mg/L	458	+		(+)	Camurri <i>et al.</i> (1983)
43 (35) ^a	33 (28)	1-22		7- > 96		45-1440 mg/L	479	+			Camurri <i>et al.</i> (1984)
18	9			2-44	13.2			-		-	Hansteen <i>et al.</i> (1984)
15	13	1-26			24	< 152-304		-			Nordenson & Beckman (1984)
12	12	1-26			24	< 152-304			+		Nordenson & Beckman (1984)
36	19	1-11		1-236	36	35-972 $\mu\text{g/L}$	-			P	Pohlová & Srám (1985)
22	22	1-11		9-132		40-3000 $\mu\text{g/L}$	-				
21	21	1-25		8-63	24	0-1103 mg/L	243	-	-	-	Mäki-Paakkanen (1987)
32	32		18.8	27-55				+			Forni <i>et al.</i> (1988)
8	8		4.5	9-44				-			
11	11		10	28-140	61			-			Jablonická <i>et al.</i> (1988)
11	15	0.1-25.4	8.1	1-39	13	< 6-317 ^b	128 ^b	-			Hagmar <i>et al.</i> (1989)
20	22	0.1-25.4	8.1	1-39	13	< 6-317 ^b	128 ^b	-		-	

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Table 14 (contd)

No. exposed	No. of referents	Length of exposure (years)		Styrene in air (ppm)		Urinary mandelic acid ($\mu\text{g/g}$ creatinine)		Cytogenetic observation			Reference
		Range	Mean	Range	Mean	Range	Mean	CA	MN	SCE	
7	8 (smokers)		8.6	1.7-131	50		275			-	Kelsey <i>et al.</i> (1990)
13	12 (nonsmokers)		7.2	5.8-130	55		323			-	
11	11 (smokers)		6.4			< 152-3271 mg/L	1674	-	-	-	Mäki-Paakkanen <i>et al.</i> (1991)
6	6 (nonsmokers)		7.2			< 152-2526 mg/L	989	?	-	-	
17	17 (all)		6.7		70	< 152-3271 mg/L	1430	-	-	-	
10	9		2.7	1-44	11.2	96-2496	243		+	-	Brenner <i>et al.</i> (1991)
15	-				≤ 1.5						Yager <i>et al.</i> (1990, 1993)
16	-			0.2-55.3 ^c	2-25					+	
17	-				> 25						
50	54			5-182	43			-	-	-	Sorsa <i>et al.</i> (1991)
25	54			1-133	11			-	-	-	
7	7	1-18		5-24	12 ^d	46-345	186	-	-	-	Tomanin <i>et al.</i> (1992)
11	11	1.5-15		27-104	45 ^d	423-1325	725	+	-	-	

CA, chromosomal aberrations; MN, micronuclei; SCE, sister chromatid exchange; +, positive; (+), weakly positive; -, negative; ?, inconclusive; blank, not tested

^aNumbers in parentheses, samples for which data on both CA and SCE were available

^bMandelic acid + phenylglyoxylic acid (mmol/mmol creatinine)

^cLinear regression analysis

^dAssuming exposure to 50 ppm = 800 mg mandelic acid per g creatinine (American Conference of Governmental Hygienists, 1984)

Camurri *et al.* (1983) studied 25 workers at six sites (2–7 workers per site) in the reinforced plastics industry who were exposed to styrene at increasing concentrations ranging from 30 to > 400 mg/m³ (7–> 96 ppm) and 22 matched controls. They found a significant increase in the frequency of chromosomal aberrations (mainly of the chromatid type) in relation to exposure level. A significant linear regression was also reported for individual aberration frequencies in relation to the concentration of urinary mandelic acid plus phenylglyoxylic acid, which are indicators of recent exposure to styrene. The authors noted that other chemicals in the work environments may play a role in the process of chromosomal damage but concluded that styrene was probably the cause of the observed damage. [The Working Group noted that the frequencies of chromosomal aberrations in both exposed and control subjects were unusually high.] An increase in the frequency of sister chromatid exchange was also reported in workers exposed to the three highest styrene concentrations, but there was no dose–response relationship.

Andersson *et al.* (1980) measured chromosomal aberration and sister chromatid exchange frequencies in a group of Swedish boat builders who had worked with fibreglass reinforced plastics in 1973–78. The workers were divided into a low-dose group (137 mg/m³ × years; 22 workers for chromosomal aberrations, 14 for sister chromatid exchange) and a high-dose group (1204 mg/m³ × years; 16 workers for chromosomal aberrations, six for sister chromatid exchange) on the basis of cumulated exposure to styrene. There were 37 controls for the studies of chromosomal aberrations and 21 for studies of sister chromatid exchange. Both groups had small but significantly increased frequencies of chromosomal aberrations, so that there was no difference in aberration frequency between the groups. Although a correlation between frequency and accumulated exposure was reported for the low-dose group for 6–283 mg/m³ × years, the relationship was not substantiated in the high-dose group (710–1589 mg/m³ × years). A slight increase in the frequency of sister chromatid exchange was reported in the high-dose group only. [The Working Group did not consider that increase to be of biological significance.]

In a study by Forni *et al.* (1988), chromosomal aberrations were measured in 40 controls and 40 workers from two factories with high (factory A) and low (factory B) cumulative exposure-years, based on measurements of styrene in air; however, current exposures were higher in factory B than in factory A, as also documented by measurement of mandelic acid. In 32 workers in factory A, the frequency of chromosomal type aberrations was highly significantly increased over that in 32 matched controls (1.2 versus 0.3%). In workers in factory B, an increased frequency of chromatid aberrations (2.25 versus 1.0%) but not of the chromosomal type was recorded.

Mäki-Paakkanen *et al.* (1991) reported a correlation between total years of exposure to styrene and the percentage of cells with chromosomal aberrations in a group of 17 workers and 17 controls at a reinforced plastics plant where large plastic containers were made. Only nonsmokers had a small but significant increase, however, and only when gaps were included. [The Working Group did not consider the finding significant.] They found no increase in the frequencies of micronuclei or of sister chromatid exchange in workers exposed to styrene.

In a longitudinal study of 48 workers at reinforced plastic boat manufacturing facilities (Yager *et al.*, 1990, 1993), a clear relationship was established between the level of styrene exposure and the induction of sister chromatid exchange in smokers and nonsmokers

involved in the production of reinforced plastics. The concentrations of styrene in air exhaled by exposed individuals, measured seven times during one year, were plotted against the mean sister chromatid exchange frequencies, which were measured twice in the same year. A linear regression analysis showed a highly significant correlation between these two parameters. The authors reported that the relative contribution of each variable to regression of sister chromatid exchange frequency showed that smoking contributed about 62% and styrene exposure about 25% of the total variation. No increase in the frequency of micronuclei was observed in four samples taken during the year.

Tomanin *et al.* (1992) studied chromosomal aberration and micronucleus induction in workers in two factories where polyester resin was used. Seven workers were engaged at small fibreglass manufacturing plants and were exposed to styrene at 21–100 mg/m³ [5–24 ppm]; 10 workers in boat manufacturing plants had exposures of 112–435 mg/m³ [27–104 ppm]. The mean chromosomal aberration frequency in the latter group was significantly greater than that in matched controls, but that of the former group was not. [The Working Group noted that this result might indicate a relationship between styrene exposure and aberration frequency.] For individuals within the more heavily exposed group, however, there was no clear correlation between urinary mandelic acid and chromosomal aberration frequency. The authors stated that there was no correlation between aberration frequency and length of exposure. [The Working Group noted that other factors may be involved in the process of chromosomal damage besides exposure to styrene.] Micronucleus frequency was not significantly increased in exposed workers.

In three studies, induction of micronuclei was reported in lymphocytes of workers in the reinforced plastics industry. In only one of the studies (Brenner *et al.*, 1991) did the staining method used allow differentiation between subsequent cell divisions. The differences in the frequencies of micronuclei between the groups were highly significant. [The Working Group noted that there were some deficiencies in the matching of controls in this study.]

In a preliminary study of glycoporphin A variants, an increased frequency was found in styrene-exposed workers, but the results were confounded by differences in smoking habits and in age between the high and low exposure groups (Compton-Quintana *et al.*, 1993).

[The Working Group noted the following points: Exposure assessment is a general problem in studies of genotoxic effects. Measurements of styrene in the air or of styrene metabolites in urine or blood of individuals provide estimates of exposure that occurred on the day of sample collection or on the previous day.

Although the lifetime of lesions leading to cytogenetic changes is unknown, such changes may reflect recent exposure or exposure over many years. The situation is further complicated by the fact that no two occupational situations or workplaces are alike, and individual differences and seasonal variation are found in work load and intensity. Methodological differences in measurements of exposure in the various studies also make comparisons difficult. Time-weighted average concentrations of styrene in air do not take into account occasional high peak exposures, which may be important. The roles of individual genetic susceptibility to styrene and differences in its metabolism are also poorly understood. Consequently, it is difficult to establish a definitive relationship between styrene

exposure and chromosomal damage, although the methodological problems would tend to reduce the difference between exposed and unexposed workers.

Styrene is the most abundant compound in the air of plants in which reinforced plastics are used and would seem to be the likely cause of the observed chromosomal damage in workers; however, other factors may be involved. Styrene oxide, for example, may be present in the air of some work sites because of oxidation of styrene by various peroxides in lamination resins (Pfäffli & Säämänen, 1993). The possible role of the many other chemicals used in this branch of industry (see section 1) in inducing chromosomal damage in workers in the reinforced plastics industry has not been well characterized. Available data suggest that chromosomal aberrations occur more frequently in the lymphocytes of reinforced plastics laminators than do sister chromatid exchanges or micronuclei. Data obtained in animals exposed to styrene *in vivo*, however, suggest that DNA damage resulting from human exposure to styrene would produce primarily sister chromatid exchange rather than chromosomal aberrations.]

4.4.2 *Experimental systems*

(a) *DNA adducts*

A comprehensive review of DNA and protein binding by styrene and styrene oxide is available (Phillips & Farmer, 1994).

Byfält Nordqvist *et al.* (1985) reported that binding of ^{14}C -styrene to DNA in liver occurred in mice after intraperitoneal injection, and the adduct radiolabel co-chromatographed with 7-(hydroxyphenylethyl)guanine. In a subsequent study, ^3H -styrene was administered by inhalation to mice and rats for up to 9 h in a closed inhalation chamber, and DNA was isolated from liver and lungs (rats only) and purified to constant specific radioactivity. Radioactivity counts in the adduct fractions of nucleotides corresponded to very low binding levels: the covalent binding indices—($\mu\text{mol adduct/mol DNA nucleotide}$)/($\text{mmol chemical/kg bw}$)—in mouse liver were 0.05–0.18, and no binding was seen at the limit of detection of < 0.1 in rat liver. The index was 0.07 in the lungs of two of four female rats (Cantoreggi & Lutz, 1993).

(b) *Mutation and allied effects* (see also Table 15)

The SOS Chromotest in *Escherichia coli* strain PQ37 indicated negative results for DNA repair in one study and a positive result but no dose–response relationship in another.

Most studies have not demonstrated bacterial mutagenicity of styrene. This was a consistent finding in the absence of exogenous metabolic activation, but a few positive responses were reported in strains TA1535 and TA100 of *Salmonella typhimurium* in the presence of exogenous metabolic activation.

In a single study with *Saccharomyces cerevisiae*, styrene induced gene conversion, recombination and reverse mutation. Forward mutation was not induced in two studies with *Schizosaccharomyces pombe*.

Chromosomal aberrations were induced in one study in the plant *Allium cepa*. In a single study with *Drosophila melanogaster*, styrene induced sex-linked recessive lethal mutations but not aneuploidy.

Styrene induced DNA strand breaks in primary cultures of rat hepatocytes in one study. An exogenous metabolic activation system was required for mutation induction at the *hprt* locus of Chinese hamster V79 cells. Induction of sister chromatid exchange in Chinese hamster ovary cells required either exogenous metabolic activation or red blood cells, in separate studies. Sister chromatid exchange was induced in rat lymphocytes in a single study, and weak induction of chromosomal aberrations was seen in two studies with cultured Chinese hamster lung fibroblasts, one in the absence and one in the presence of exogenous metabolic activation.

Styrene did not induce transformation of C3H10T $\frac{1}{2}$ mouse cells *in vitro*, either alone or in a two-stage transformation assay with 3-methylcholanthrene.

Styrene induced sister chromatid exchange and micronuclei in human lymphocytes in whole blood cultures. Styrene at 2.0 mmol/L induced a 4.9-fold increase in sister chromatid exchange frequency in whole blood cultures but only a weak, 1.3-fold increase in isolated human lymphocytes (Norppa & Järventaus, 1992). Styrene also induced chromosomal aberrations in a dose-dependent manner in human whole blood cultures, but the response was weaker in isolated lymphocytes.

In mice *in vivo*, DNA strand breaks were induced in a single study and sister chromatid exchange in several; chromosomal aberrations were induced in only one of seven studies and micronuclei in two of six.

A positive dose-response relationship was seen for sister chromatid exchange frequency in peripheral blood and spleen lymphocytes and lung cells of B6C3F1 female mice exposed to concentrations of 125, 250 or 500 ppm [532.5, 1065 or 2130 mg/m³] styrene by inhalation for 6 h a day for 14 days. Analysis of chromosomal breakage in splenocytes and lung cells and of micronuclei in blood erythrocytes and splenocytes showed no exposure-related response (Kligerman *et al.*, 1992).

In a comparative study of Porton rats and LACA Swiss mice, sister chromatid exchanges in splenocytes and micronuclei in bone-marrow cells were analysed following single intraperitoneal injections of 150–3000 mg/kg bw styrene, and sperm morphology was studied after five daily injections of 50–2000 mg/kg. Styrene produced weak but significant responses at all of three end-points in mice and in sperm morphology and sister chromatid exchange in rats (Simula & Priestly, 1992).

Preston and Abernathy (1993) exposed male rats to styrene at concentrations up to 1000 ppm [4260 mg/m³] for 6 h per day for four weeks and found no increase in the frequencies of either chromosomal aberrations or sister chromatid exchange in peripheral lymphocytes. In a positive control group exposed to ethylene oxide at 150 ppm [270 mg/m³], however, the frequency of sister chromatid exchange was increased but that of chromosomal aberrations was not.

Kligerman *et al.* (1993) exposed mice and rats to styrene by inhalation at concentrations of up to 500 ppm [2130 mg/m³] for 6 h/day for 14 consecutive days. Small but significant concentration-related increases in the frequencies of sister chromatid exchange were noted in both mice and rats. There was no significant increase in DNA strand breakage in rats or in the frequencies of chromosomal aberrations or micronuclei in either rats or mice.

Table 15. Genetic and related effects of styrene

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ECB, <i>Escherichia coli</i> , SOS repair	?	0	100.0000	Głońska & Dziadziuszko (1986)
ECB, <i>Escherichia coli</i> , SOS repair	-	0	10000.0000	Brams <i>et al.</i> (1987)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	(+)	(+)	52.0000	Vainio <i>et al.</i> (1976)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	0.0000	de Meester <i>et al.</i> (1977)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	500.0000	Stoltz & Withey (1977)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	0	-	250.0000	Watabe <i>et al.</i> (1978)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	104.0000	Busk (1979)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	250.0000	De Flora (1979)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	312.0000	Florin <i>et al.</i> (1980)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	+	1000.0000 ^c	de Meester <i>et al.</i> (1981)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	500.0000	Brams <i>et al.</i> (1987)
SA3, <i>Salmonella typhimurium</i> TA1530, reverse mutation	+	+	0.0200 ^c	de Meester <i>et al.</i> (1981)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	+	0.5000	Vainio <i>et al.</i> (1976)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	+	52.0000	de Meester <i>et al.</i> (1977)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	500.0000	Stoltz & Withey (1977)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	0	-	250.0000	Watabe <i>et al.</i> (1978)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	104.0000	Busk (1979)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	250.0000	De Flora (1979)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	312.0000	Florin <i>et al.</i> (1980)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	0	+	521.0000	Poncelet <i>et al.</i> (1980)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	+	1 000.0000 ^c	de Meester <i>et al.</i> (1981)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	52.0000	Vainio <i>et al.</i> (1976)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	0.0000	de Meester <i>et al.</i> (1977)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	500.0000	Stoltz & Withey (1977)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	0	-	250.0000	Watabe <i>et al.</i> (1978)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	104.0000	Busk (1979)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	312.0000	Florin <i>et al.</i> (1980)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	1 000.0000 ^c	de Meester <i>et al.</i> (1981)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	52.0000	Vainio <i>et al.</i> (1976)

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Table 15 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	0.0000	de Meester <i>et al.</i> (1977)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	500.0000	Stoltz & Withey (1977)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	0	-	250.0000	Watabe <i>et al.</i> (1978)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	104.0000	Busk (1979)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	250.0000	De Flora (1979)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	1 000.0000 ^c	de Meester <i>et al.</i> (1981)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	500.0000	Brams <i>et al.</i> (1987)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	52.0000	Vainio <i>et al.</i> (1976)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	0.0000	de Meester <i>et al.</i> (1977)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	500.0000	Stoltz & Withey (1977)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	0	-	250.0000	Watabe <i>et al.</i> (1978)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	104.0000	Busk (1979)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	250.0000	De Flora (1979)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	312.0000	Florin <i>et al.</i> (1980)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	1 000.0000 ^c	de Meester <i>et al.</i> (1981)
SCG, <i>Saccharomyces cerevisiae</i> , gene conversion	+	0	104.0000	Del Carratore <i>et al.</i> (1983)
SCH, <i>Saccharomyces cerevisiae</i> , homozygosis	+	0	104.0000	Del Carratore <i>et al.</i> (1983)
SCR, <i>Saccharomyces cerevisiae</i> , reverse mutation	+	0	104.0000	Del Carratore <i>et al.</i> (1983)
SZF, <i>Schizosaccharomyces pombe</i> , forward mutation	-	-	10 400.0000	Loprieno <i>et al.</i> (1976)
SZF, <i>Schizosaccharomyces pombe</i> , forward mutation	0	-	2 080.0000	Bauer <i>et al.</i> (1980)
ACC, <i>Allium cepa</i> , chromosomal aberrations	+	0	90.0000	Linnainmaa <i>et al.</i> (1978a,b)
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutations	+	0	182.0000	Donner <i>et al.</i> (1979)
DMN, <i>Drosophila melanogaster</i> , aneuploidy	-	0	500.0000	Penttilä <i>et al.</i> (1980)
DIA, DNA strand breaks, rat primary hepatocytes <i>in vitro</i>	+	0	312.0000	Sina <i>et al.</i> (1983)
G9H, Gene mutation, Chinese hamster lung V79 cells, <i>hprt</i> locus	-	0	1 771.0000	Loprieno <i>et al.</i> (1976)
G9H, Gene mutation, Chinese hamster lung V79 cells, <i>hprt</i> locus	-	+	6 250.0000	Beije & Jenssen (1982)
SIC, Sister chromatid exchange, Chinese hamster ovary cells <i>in vitro</i>	-	+	455.0000	de Raat (1978)
SIC, Sister chromatid exchange, Chinese hamster ovary cells <i>in vitro</i>	-	+ ^d	830.0000	Norppa <i>et al.</i> (1985)
SIR, Sister chromatid exchange, rat lymphocytes <i>in vitro</i>	+	0	50.0000	Norppa <i>et al.</i> (1985)
CIC, Chromosomal aberrations, Chinese hamster lung cells <i>in vitro</i>	-	(+)	250.0000	Matsuoka <i>et al.</i> (1979)

Table 15 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CIC, Chromosomal aberrations, Chinese hamster lung cells <i>in vitro</i>	(+)	0	100.0000	Ishidate & Yoshikawa (1980)
TCM, Cell transformation, C3H10T1/2 mouse cells	-	0	10.0000	Male <i>et al.</i> (1985)
SHL, Sister chromatid exchange, human whole blood lymphocytes <i>in vitro</i>	+	0	104.0000	Norppa <i>et al.</i> (1980a)
SHL, Sister chromatid exchange, human whole blood lymphocytes <i>in vitro</i>	+	0	104.0000	Norppa & Vainio (1983)
SHL, Sister chromatid exchange, human whole blood lymphocytes <i>in vitro</i>	+ ^e	0	50.0000	Norppa <i>et al.</i> (1983)
SHL, Sister chromatid exchange, human whole blood lymphocytes <i>in vitro</i>	+	0	1.0000	Chakrabarti <i>et al.</i> (1993)
MIH, Micronucleus formation, human whole blood lymphocytes <i>in vitro</i>	+	0	270.0000	Linnainmaa <i>et al.</i> (1978a)
CHL, Chromosomal aberrations, human whole blood lymphocytes <i>in vitro</i>	+	0	270.0000	Linnainmaa <i>et al.</i> (1978a)
CHL, Chromosomal aberrations, human lymphocytes <i>in vitro</i>	+	0	50.0000	Pohlova <i>et al.</i> (1985)
CHL, Chromosomal aberrations, human whole blood lymphocytes <i>in vitro</i>	+	0	208.0000	Jantunen <i>et al.</i> (1986)
HMM, Host-mediated assay, <i>Saccharomyces cerevisiae</i> gene conversion in mouse	+		1000.0000	Loprieno <i>et al.</i> (1976)
DVA, DNA strand breaks, mouse, various organs <i>in vivo</i>	+		170.0000 × 1 ip	Wallis & Orsén (1983)
DVA, Single-strand DNA breakage, rat peripheral lymphocytes <i>in vivo</i>	-		450.0000 inhal. 6 h/d × 14	Kligerman <i>et al.</i> (1993)
SVA, Sister chromatid exchange, mouse bone-marrow and liver cells <i>in vivo</i>	+		850.0000 inhal. 6 h/d × 4	Conner <i>et al.</i> (1979)
SVA, Sister chromatid exchange, mouse bone-marrow, liver and alveolar macrophages <i>in vivo</i>	+		580.0000 inhal. 6 h/d × 4	Conner <i>et al.</i> (1980)
SVA, Sister chromatid exchange, mouse bone-marrow cells <i>in vivo</i>	+		500.0000 × 1 ip	Sharief <i>et al.</i> (1986)
SVA, Sister chromatid exchange, mouse lymphocytes <i>in vivo</i>	+		450.0000 inhal. 6 h/d × 14	Kligerman <i>et al.</i> (1992)
SVA, Sister chromatid exchange, mouse lung cells <i>in vivo</i>	+		450.0000 inhal. 6 h/d × 14	Kligerman <i>et al.</i> (1992)
SVA, Sister chromatid exchange, mouse splenocytes <i>in vivo</i>	(+)		450.0000 × 1 ip	Simula & Priestly (1992)
SVA, Sister chromatid exchange, rat splenocytes <i>in vivo</i>	+		750.0000 × 1 ip	Simula & Priestly (1992)
SVA, Sister chromatid exchange, rat peripheral lymphocytes <i>in vivo</i>	+		225.0000 inhal. 6 h/d × 14	Kligerman <i>et al.</i> (1993)
MVM, Micronucleus formation, mouse bone-marrow cells <i>in vivo</i>	+		250.0000 × 1 ip	Norppa (1981)
MVM, Micronucleus formation, mouse splenocytes <i>in vivo</i>	-		900.0000 inhal. 6 h/d × 14	Kligerman <i>et al.</i> (1992)
MVM, Micronucleus formation, mouse erythrocytes <i>in vivo</i>	-		900.0000 inhal. 6 h/d × 14	Kligerman <i>et al.</i> (1992)
MVM, Micronucleus formation, mouse bone-marrow cells <i>in vivo</i>	(+)		600.0000 × 1 ip	Simula & Priestly (1992)
MVR, Micronucleus formation, rat bone-marrow cells <i>in vivo</i>	-		3000.0000 × 1 ip	Simula & Priestly (1992)
MVR, Micronucleus formation, rat peripheral lymphocytes <i>in vivo</i>	-		450.0000 inhal. 6 h/d × 14	Kligerman <i>et al.</i> (1993)

Table 15 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVC, Micronucleus formation, Chinese hamster bone-marrow cells <i>in vivo</i>	-		1000.0000 × 1 ip	Penttilä <i>et al.</i> (1980)
CBA, Chromosomal aberrations, rat bone-marrow cells <i>in vivo</i>	+		270.0000 inhal. 6 h/d; 5 d/wk; 9 wk	Meretoja <i>et al.</i> (1978b)
CBA, Chromosomal aberrations, Chinese hamster bone-marrow cells <i>in vivo</i>	-		225.0000 inhal. 6 h/d; 5 d/wk; 3 wk	Norppa <i>et al.</i> (1980b)
CBA, Chromosomal aberrations, mouse bone-marrow cells <i>in vivo</i>	-		500.0000 × 4 po	Sbrana <i>et al.</i> (1983)
CBA, Chromosomal aberrations, rat bone-marrow cells <i>in vivo</i>	-		900.0000 inh. 6 h/d; 5 d/wk; 1 yr	Sinha <i>et al.</i> (1983)
CBA, Chromosomal aberrations, mouse bone-marrow cells <i>in vivo</i>	-		750.0000 × 1 ip	Sharief <i>et al.</i> (1986)
CVA, Chromosomal aberrations, mouse splenocytes <i>in vivo</i>	-		900.0000 inhal. 6 h/d × 14	Kligerman <i>et al.</i> (1992)
CVA, Chromosomal aberrations, mouse lung cells <i>in vivo</i>	-		900.0000 inhal. 6 h/d × 14	Kligerman <i>et al.</i> (1992)
CVA, Chromosomal aberrations, rats peripheral lymphocytes <i>in vivo</i>	-		450.0000 inhal. 6 h/d × 14	Kligerman <i>et al.</i> (1993)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	-		0.0000	Meretoja <i>et al.</i> (1978a)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	(+)		80.0000	Andersson <i>et al.</i> (1980)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	-		40.0000	Watanabe <i>et al.</i> (1981)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	(+)		35.0000	Camurri <i>et al.</i> (1983)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	-		29.0000	Watanabe <i>et al.</i> (1983)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	-		7.5000	Hansteen <i>et al.</i> (1984)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	-		14.0000	Mäki-Paakanen (1987)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	-		31.0000	Kelsey <i>et al.</i> (1990)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	+		9.0000	Yager <i>et al.</i> (1990)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	-		6.0000	Brenner <i>et al.</i> (1991)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	-		41.0000 ^f	Mäki-Paakanen <i>et al.</i> (1991)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	-		2.5000	Sorsa <i>et al.</i> (1991)
MVH, Micronucleus formation, human lymphocytes <i>in vivo</i>	+		7.3000	Högstedt <i>et al.</i> (1983)
MVH, Micronucleus formation, human lymphocytes <i>in vivo</i>	+		14.0000	Nordenson & Beckman (1984)
MVH, Micronucleus formation, human lymphocytes <i>in vivo</i>	-		14.0000	Mäki-Paakanen (1987)
MVH, Micronucleus formation, human lymphocytes <i>in vivo</i>	-		7.5000	Hagmar <i>et al.</i> (1989)
MVH, Micronucleus formation, human lymphocytes <i>in vivo</i>	+		6.0000	Brenner <i>et al.</i> (1991)
MVH, Micronucleus formation, human lymphocytes <i>in vivo</i>	-		41.0000 ^f	Mäki-Paakanen <i>et al.</i> (1991)

Table 15 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVH, Micronucleus formation, human lymphocytes <i>in vivo</i>	-		25.0000	Sorsa <i>et al.</i> (1991)
MVH, Micronucleus formation, human lymphocytes <i>in vivo</i>	-		26.0000 ^f	Tomanin <i>et al.</i> (1992)
MVH, Micronucleus formation, human lymphocytes <i>in vivo</i>	-		9.0000	Yager <i>et al.</i> (1993)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-		0.0000	Fleig & Thiess (1978)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	+		0.0000	Meretoja <i>et al.</i> (1978a)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-		0.5500	Theiss & Fleig (1978)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	+		22.0000	Högstedt <i>et al.</i> (1979)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	+		19.0000	Andersson <i>et al.</i> (1980)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-		33.0000	Thiess <i>et al.</i> (1980)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-		40.0000	Watanabe <i>et al.</i> (1981)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	+		7.0000	Camurri <i>et al.</i> (1983)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-		29.0000	Watanabe <i>et al.</i> (1983)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-		7.5000	Hansteen <i>et al.</i> (1984)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-		14.0000	Nordenson & Beckman (1984)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-		22.0000	Pohlova & Srám (1985)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-		14.0000	Mäki-Paakanen (1987)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	+		0.0000	Forni <i>et al.</i> (1988)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-		36.0000	Jablonická <i>et al.</i> (1988)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-		7.5000	Hagmar <i>et al.</i> (1989)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-		41.0000 ^f	Mäki-Paakanen <i>et al.</i> (1991)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-		25.0000	Sorsa <i>et al.</i> (1991)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	+		26.0000 ^f	Tomanin <i>et al.</i> (1992)
DVH, DNA single-strand breaks, human lymphocytes <i>in vivo</i>	+		6.0000	Brenner <i>et al.</i> (1991)
DVH, DNA single-strand breaks, human lymphocytes <i>in vivo</i>	+		41.0000 ^f	Mäki-Paakanen <i>et al.</i> (1991)
DVH, DNA single-strand breaks, human lymphocytes <i>in vivo</i>	+		10.0000	Wallis <i>et al.</i> (1993)
BVD, Binding (covalent) to DNA, mouse liver <i>in vivo</i>	+		114.0000 × 1 ip	Byfält Nordqvist <i>et al.</i> (1985)
BVD, Binding (covalent) to DNA, mouse liver <i>in vivo</i>	(+)		110.0000 inhal. 9 h	Cantoreggi & Lutz (1993)
BVD, Binding (covalent) to DNA, rat liver and lung <i>in vivo</i>	?		39.0000 inhal. 6 h	Cantoreggi & Lutz (1993)
SPM, Sperm morphology, mouse <i>in vivo</i>	(+)		200.0000 × 5 ip	Simula & Priestly (1992)
SPR, Sperm morphology, rat <i>in vivo</i>	(+)		1000.0000 × 5 ip	Simula & Priestly (1992)

Table 15 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Protein binding</i>				
BVP, Binding (covalent) to haemoglobin, mouse <i>in vivo</i>	+		114.0000 × 1 ip	Byfält Nordqvist <i>et al.</i> (1985)
BHP, Binding (covalent) to haemoglobin, humans <i>in vivo</i>	(+)		6.0000 inhal.	Brenner <i>et al.</i> (1991)
BHP, Binding (covalent) to haemoglobin, humans <i>in vivo</i>	(+)		45000.0000 ^f inhal.	Christakopoulos <i>et al.</i> (1993)

^a+, positive; (+), weakly positive; -, negative; 0, not tested; ?, inconclusive (variable response within several experiments within an adequate study)

^bIn-vitro tests, µg/ml; in-vivo tests, mg/kg bw

^cAtmospheric concentration (µg/ml)

^dActivation by erythrocytes

^ePurified lymphocytes showed weaker response: LED, 208 µg/ml

^fBased on urinary mandelic acid concentration

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Styrene has been produced since the 1920s by catalytic dehydrogenation of ethylbenzene. It is one of the most important monomers, worldwide, and finds major use in the production of polystyrene, acrylonitrile–butadiene–styrene resins, styrene–butadiene rubbers and latexes, and unsaturated polystyrene resins. Occupational exposure levels, measured both by air measurements and biological monitoring, have been highest in the manufacture of fibre glass-reinforced polyester products and lower in the production of styrene, polystyrene and styrene-based plastics and rubbers.

5.2 Human carcinogenicity data

Epidemiological studies of styrene have been done in three types of industry: production of glass-reinforced plastic products, production of styrene monomer and styrene polymerization and production of styrene–butadiene rubber. The malignancies observed in excess most frequently are of the lymphatic and haematopoietic system.

In a European multinational study of over 40 000 workers in the glass-reinforced plastics industry, no overall excess of deaths from lymphatic and haematopoietic cancers was observed in comparison with national controls. Within the cohort, the risks for these cancers were significantly related to average intensity of exposure and to years since first exposure but were not related to cumulative exposure.

A study of cancer incidence in the reinforced plastics industry in Denmark involved 12 800 male workers who had been included within the European multinational mortality study and a further 24 000 workers with lower probability of exposure to styrene. A non-significant overall increase in risk was seen for lymphatic and haematopoietic cancer. The increase was concentrated mainly in those workers not previously included in the international cohort, in short-term workers with at least 10 years since first employment and in those employed before 1970.

In a large study of the reinforced plastics industry in the USA, no overall increase in risk for lymphatic and haematopoietic cancer was seen, although a nonsignificant increase was found among workers with the highest exposure.

A study of chemical workers in the production of styrene and styrene derivatives in the USA found a nonsignificant association between exposure to styrene and lymphatic and haematopoietic cancers. A smaller study from the United Kingdom also found a nonsignificant association with cancers at this site but lacked detailed information on exposure.

A large cohort study of the styrene–butadiene rubber industry showed increased risks for lymphatic and haematopoietic malignancies, but a nested case–control analysis that evaluated exposure to both styrene and butadiene found no relationship with exposure to styrene. Two additional studies showed increased risks for lymphatic and haematopoietic cancers but provided little information on exposure to styrene.

Exposures to styrene are highest in the reinforced plastics industry, where less opportunity for confounding occurs than in the other industries studied. The two largest, most

informative, but partly overlapping, studies of reinforced plastics manufacturers have certain features that are suggestive of a cancer hazard insofar as, in one, risk increased with average intensity of exposure and time since first exposure, and in the other risk was greatest in men employed at times when the highest exposures occurred. More importantly, however, they do not indicate an increase in risk with increasing cumulative exposure to styrene (as the excesses occurred mainly in short-term employees), and there is no overall increase in risk for lymphatic and haematopoietic cancer in studies of the reinforced plastics industry.

5.3 Animal carcinogenicity data

Styrene was tested for carcinogenicity in mice and rats by oral administration and in rats by inhalation exposure. Administration of styrene by gastric intubation resulted in a small increase in the incidence of pulmonary tumours in male mice and of hepatocellular adenomas in females and no increase in tumour incidence in rats. Prenatal exposure followed by postnatal gastric intubation of styrene resulted in a significant increase in the occurrence of pulmonary tumours in male and female mice of one strain and no increase in tumour incidence in rats. Exposure of rats to styrene by inhalation in one study was associated with an increase in the incidence of mammary tumours in females; however, because of limitations in the reporting of the data, the results of the study were considered to be inconclusive. Two studies by gastric intubation of a styrene/ β -nitrostyrene mixture in mice and rats were of limited value for the evaluation.

5.4 Other relevant data

Styrene is absorbed by inhalation and dermal transfer in both man and rat. In man, 60–70% of inhaled styrene is absorbed. It is rapidly distributed throughout the body in treated rats. A large percentage of absorbed styrene is excreted as urinary mandelic and phenylglyoxylic acids, glutathione conjugates forming a minor fraction of the metabolites. Saturation of metabolic activation of styrene becomes apparent at concentrations above 200–300 ppm (850–1280 mg/m³) in rats and mice, and above 100–200 (430–850 mg/m³) ppm in humans. The dominant first metabolite is styrene-7,8-oxide, the formation of which appears to be catalysed in man principally by the cytochrome P450 isoenzyme CYP2B6 but also by CYP2E1 and CYP1A2. Isolated erythrocytes are also capable of nonenzymatic conversion of styrene to styrene-7,8-oxide. The amounts of styrene-7,8-oxide present in the blood of rats and mice exposed to styrene at concentrations below 100 ppm (430 mg/m³) were about 5–20 fold greater than those in similarly exposed humans.

Exposure to styrene leads to the formation of both protein and DNA adducts in man, rat and mouse. The levels of the N-terminal valine adduct of haemoglobin, *N*-(1-hydroxy-2-phenylethyl)valine, have been found to be four times higher in styrene-exposed workers than in controls, and the levels of the DNA adduct, *O*⁶-(2-hydroxy-1-phenylethyl)-2'-deoxyguanosine-3'-monophosphate, have been found to be about five times higher than in controls.

Central and peripheral neurotoxicity have been described in workers, rats and rabbits exposed to styrene, but the mechanism has not been established.

No clear association was seen in a number of studies between occupational exposure of either mothers or fathers to styrene and the frequency of spontaneous abortions or congenital malformations. In rats and rabbits exposed to styrene at doses up to those that induce maternal toxicity, no adverse reproductive effect has been observed. Damage to seminiferous tubules and decreased sperm counts have been observed in male rats.

Some 25 studies on chromosomal aberrations, micronuclei and sister chromatid exchange have been performed in workers exposed to styrene in various countries and different industries. These have provided variable results with regard to the association between exposure to styrene and chromosomal damage. While clear dose-response relationships were not observed, those studies that showed effects were conducted in the reinforced plastics industry, where exposure to styrene is high; only one study was available on the styrene monomer and polystyrene manufacturing industries. Chromosomal aberrations were observed in 9 of 22, sister chromatid exchange in 3 of 12 and micronuclei in 3 of 11 studies.

The frequency of single-strand DNA breakage/alkali-labile sites was increased in workers exposed to styrene at less than 20 ppm (85 mg/m³).

Chromosomal aberrations have not been seen in most studies in rodents, while several studies indicate weak induction of sister chromatid exchange in various tissues of rats and mice. Contradictory results have been obtained with regard to the induction of micronuclei in mice.

Significant increases have been observed consistently in the frequency of sister chromatid exchange and chromosomal aberrations in human lymphocytes *in vitro*. Most studies did not show mutation in bacteria, although mutation was seen in some studies in the presence of an exogenous metabolic activation system.

5.5. Evaluation¹

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

There is *limited evidence* in experimental animals for the carcinogenicity of styrene.

In making the overall evaluation, the Working Group took into consideration the following supporting evidence: Styrene is metabolized to styrene-7,8-oxide, which binds covalently to DNA and shows activity in various in-vitro and in-vivo assays for genetic effects. The genetic and related effects of styrene are therefore associated with its oxidation, which also occurs, e.g. in human whole blood cultures, where styrene induces dose-related responses of chromosomal damage at low concentrations. Styrene-7,8-oxide is detected in blood of workers exposed to styrene. Adducts in haemoglobin and DNA, DNA single-strand breaks/alkali-labile sites, as well as significant increases in the frequency of chromosomal damage have been found in workers exposed to styrene in the reinforced plastics industry. Positive results are associated with higher overall styrene levels and negative results with decreasing exposures to styrene. Although in human studies the role of other contaminants

¹For definition of the italicized terms, see Preamble, pp. 27-30.

cannot be excluded, their occurrence is variable and their concentrations are very low in comparison with that of styrene.

Overall evaluation

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

6. References

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STYRENE-7,8-OXIDE

This substance was considered by previous Working Groups, in February 1976 (IARC, 1976), February 1978 (IARC, 1979) and June 1984 (IARC, 1985). 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.: 96-09-3

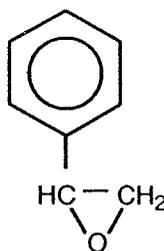
Replaced CAS Reg. No.: 62497-63-6

Chem. Abstr. Name: Phenyloxirane

IUPAC Systematic Name: (Epoxyethyl)benzene

Synonyms: 1,2-Epoxyethylbenzene; 1,2-epoxy-1-phenylethane; epoxystyrene; α,β -epoxystyrene; phenethylene oxide; 1-phenyl-1,2-epoxyethane; phenylethylene oxide; 2-phenyloxirane; styrene epoxide; styrene oxide; styryl oxide

1.1.2 Structural and molecular formulae and relative molecular mass



C_8H_8O

Relative molecular mass: 120.15

1.1.3 Chemical and physical properties of the pure substance

From Union Carbide Corp. (1984) and Rhone-Poulenc Chimie (1985), unless otherwise specified.

(a) *Description:* Colourless liquid

(b) *Boiling-point:* 194.1 °C

(c) *Freezing-point:* -36.7 °C

- (d) *Density*: 1.050–1.054 at 20 °C/4 °C
- (e) *Spectroscopy data*: Infrared, ultraviolet [2303], nuclear magnetic resonance and mass spectral data have been reported (Sadtler Research Laboratories, 1991; US National Library of Medicine, 1993a).
- (f) *Solubility*: Slightly soluble in water (3 g/L at 25 °C); soluble in acetone, benzene, carbon tetrachloride, heptane and methanol
- (g) *Volatility*: Vapour pressure, < 1 mm Hg [133 Pa] at 20 °C
- (h) *Stability*: Flash-point, 80–82 °C (open cup); polymerizes exothermically and reacts violently with water in the presence of catalysts (acids, bases, certain salts)
- (i) *Octanol–water partition coefficient (P)*: log P, 1.61 (Sangster, 1989)
- (j) *Conversion factor*: $\text{mg/m}^3 = 4.91 \times \text{ppm}^a$

1.1.4 Technical products and impurities

Styrene-7,8-oxide exists in two optical isomers (see section 4), and the commercial product is a racemic mixture. Typical product specifications are for 99% minimal purity and 0.1–0.2% maximal water content (Union Carbide Corp., 1984; Rhone-Poulenc Chimie, 1985).

1.1.5 Analysis

Styrene-7,8-oxide can be determined in air by gas chromatography with mass spectrometry or flame ionization detection. The sample is collected on solid sorbent and desorbed thermally or with ethyl acetate (Pellizzari *et al.*, 1976; Taylor, 1979; Stampfer & Hermes, 1981). Detection limits as low as 2 ng/m³ have been reported (Krost *et al.*, 1982).

1.2 Production and use

1.2.1 Production

Styrene-7,8-oxide is produced commercially by the reaction of styrene with chlorine and water to form styrene chlorohydrin, followed by cyclization with aqueous base to produce styrene-7,8-oxide. It is also prepared by epoxidation of styrene with peroxyacetic acid (US National Library of Medicine, 1993a).

Information available in 1991 indicated that styrene-7,8-oxide was produced by three companies in Japan and one in the USA (Chemical Information Services Ltd, 1991).

1.2.2 Use

Styrene-7,8-oxide is used as a chemical intermediate in several processes. Hydrogenation yields 2-phenylethanol, which is also known as 'oil of roses', a widely used perfume base. Esters useful in fragrance applications can be made by reacting styrene-7,8-oxide with

^aCalculated from: $\text{mg/m}^3 = (\text{relative molecular mass}/24.45) \times \text{ppm}$, assuming normal temperature (25 °C) and pressure (101.3 kPa)

carboxylic acids. Reaction of styrene-7,8-oxide with ethanolamine yields an intermediate used in the synthesis of tetramisole, a commercial anthelmintic. The low viscosity of styrene-7,8-oxide and its reactivity have led to its use as a reactive diluent for epoxy resins. It is also reported to be used in cross-linked polyesters and polyurethanes. It is added in small quantities as a reactive acid scavenger to improve the stability of hydraulic fluids, chlorinated cleaning compositions, petroleum distillates, dielectric fluids and acid-sensitive polymers and copolymers. Styrene-7,8-oxide can be homopolymerized to poly(styrene glycols) and copolymerized with other epoxides. It is also used in adhesive formulations, as a polypropylene catalyst deactivator, to make graft copolymers of cotton, silk and wool, as a lubricant for acetal polymers and in sealant formulations based on silylated polyurethanes (Union Carbide Corp., 1984).

1.3 Occurrence

1.3.1 *Natural occurrence*

Styrene-7,8-oxide is not known to occur as a natural product.

1.3.2 *Occupational exposure*

The National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health between 1981 and 1983 indicated that 450 employees were potentially exposed to styrene-7,8-oxide in the USA (US National Institute for Occupational Safety and Health, 1993). Of this number 59% were estimated to be exposed to styrene-7,8-oxide and 41% to materials containing styrene-7,8-oxide. The estimate is based on a survey of US companies and did not involve measurements of actual exposures.

Occupational exposure to styrene-7,8-oxide may occur because of its formation from styrene in industries where polyester resins with styrene are used when peroxides are added to the resin. In Finnish factories for producing boats, car parts and building materials from polyester-based reinforced plastics, the average styrene-7,8-oxide levels in personal air samples were found to be 0.04 ppm [0.20 mg/m³] for hand lay-up and 0.12 ppm [0.59 mg/m³] for spray application; the corresponding styrene levels were 133 and 130 ppm [567 and 554 mg/m³] (Pfäffli *et al.*, 1979). In a Norwegian factory where similar processes were used, styrene-7,8-oxide levels ranged from < 0.003 to 0.12 ppm [< 0.015–0.59 mg/m³] and concurrent styrene levels from 17 to 289 ppm [72–1230 mg/m³] (Fjeldstad *et al.*, 1979). Similarly, in a boat manufacturing company in the USA, the mean styrene-7,8-oxide level was 0.14 mg/m³ for the 19 workers most heavily exposed to styrene (mean, 64 mg/m³) (Rappaport *et al.*, 1991). Data obtained in 32 Finnish plants allow the rough calculation of a ratio of styrene-7,8-oxide to styrene of 1:1000 (Säämänen *et al.*, 1993).

Acetophenone and benzaldehyde, oxidized products of styrene-7,8-oxide, were quantified in personal samples at mean levels of 0.47 and 0.48 ppm [2.3 and 2.4 mg/m³], respectively, during spray application in Finland (Pfäffli *et al.*, 1979).

1.3.3 *Water and sediments*

In a comprehensive survey of 4000 samples of wastewater taken from a broad range of industrial and publicly owned treatment works in the USA, styrene-7,8-oxide was identified

in one discharge from rubber processing at a level of 46.2 ppb [$\mu\text{g/L}$] (US National Library of Medicine, 1993a).

1.3.4 *Other*

Annual total air emissions of styrene-7,8-oxide in the USA, reported to the US Environmental Protection Agency by industrial facilities, were 464 kg in 1987 from two locations, 1050 kg in 1988 from six locations, 918 kg in 1989 from five locations, 1099 kg in 1990 from five locations and 760 kg in 1991 from five locations. Total releases to ambient water in 1987 were estimated to result in 353 kg (US National Library of Medicine, 1993b).

1.4 Regulations and guidelines

No regulations or guidelines have been established for occupational exposure to styrene-7,8-oxide (American Conference of Governmental Industrial Hygienists, 1993; ILO, 1993; UNEP, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 52 male and 52 female B6C3F1 mice, seven weeks old, were administered 0 (control), 375 or 750 mg/kg bw styrene-7,8-oxide (purity, 96.6%; two of the three impurities were unspecified amounts of benzaldehyde and benzene) in corn oil by gastric intubation daily three times a week for 104 weeks. Three to four weeks after the last dose, all surviving animals were killed. There was a marked reduction in the survival of high-dose male and female mice, and body weights were reduced in both groups. Treatment resulted in a significant ($p < 0.001$) increase in the incidence of squamous-cell carcinoma of the forestomach in males at both dose levels (control, 0/51; low-dose, 16/51; high-dose, 15/52) and in females at the low dose (control, 0/51; low-dose, 10/50; high-dose, 3/51), and a significant increase in the incidence of squamous-cell papillomas at both dose levels in males (control, 2/51; low-dose, 22/51; high-dose, 8/52) and females (control, 0/51; low-dose, 14/50; high-dose, 17/51). The incidences of squamous-cell papillomas and carcinomas (combined) were: males—control, 2/51; low-dose, 37/51; high-dose, 21/52; and females—control, 0/51; low-dose, 24/50; high-dose, 20/51 ($p < 0.001$). Low-dose males had a significant increase in the incidence of hepatocellular tumours: 12/51 in controls; 28/52 in the low-dose group ($p < 0.001$; Fisher's exact test) (Lijinsky, 1986).

3.1.2 Rat

Groups of 40 male and 40 female Sprague-Dawley rats, 13 weeks old, were administered 0 (control), 50 or 250 mg/kg bw styrene-7,8-oxide [purity unspecified] in olive oil by gastric intubation daily on four to five days per week for 52 weeks. Rats were kept until they died; the last death occurred 156 weeks after initial dosing. There was no effect of treatment on survival or body weight. Treatment resulted in a dose-dependent increase in the incidence of squamous-cell carcinoma of the forestomach in males (control, 0/40; low-dose, 11/40; high-dose, 30/40) and females (control, 0/40; low-dose, 8/40; high-dose, 33/40). The incidences of squamous-cell papilloma/acanthoma were: males—control, 0/40; low-dose, 3/40; high-dose, 9/40; and females—control, 0/40; low-dose, 3/40; high-dose, 5/40. The incidences of acanthosis and dysplasia of the forestomach epithelium were treatment-related. No increase in the incidence of tumours at other sites was found (Maltoni *et al.*, 1979; Conti *et al.*, 1988).

Groups of 52 male and 52 female Fischer 344/N rats, nine weeks old, were administered 0 (control), 275 or 550 mg/kg styrene-7,8-oxide (purity, 96.6%; two of the three impurities were unspecified amounts of benzaldehyde and benzene) in corn oil by gastric intubation daily three times a week for 104 weeks. The experiment was terminated at 107–108 weeks. Body weights and survival were reduced in high-dose males and females. Treatment resulted in a significant increase in the incidence of squamous-cell carcinoma of the forestomach in males (control, 0/52; low-dose, 35/52; high-dose, 43/51) and females (control, 0/52; low-dose, 21/52; high-dose, 24/52); the incidence of squamous-cell papilloma was also increased (males: 1/52, 23/52, 18/51; females: 0/52, 21/52, 24/52) in treated rats. The incidences of combined squamous-cell papilloma and carcinoma were: males—control, 1/52; low-dose, 50/52; high-dose, 50/51; and females—control, 0/52; low-dose, 46/52; high-dose, 50/52. No increase in the incidence of tumours at other sites was found (Lijinsky, 1986).

3.2 Prenatal exposure followed by postnatal oral administration

Rat: A group of 14 pregnant female BDIV inbred rats [age unspecified] was administered 200 mg/kg bw styrene-7,8-oxide (purity, 97%) in olive oil by gastric intubation on day 17 of gestation. Their offspring (62 females and 43 males) received 96 doses of styrene-7,8-oxide (100–150 mg/kg bw) in olive oil by gastric intubation once a week beginning at four weeks of age. The study was terminated at 120 weeks to give estimated total doses of 2.5 g for females and 5.0 g for males. Control groups of 49 male and 55 female rats with no prenatal exposure received olive oil alone. At the time of appearance of the first tumour, 60 female and 42 male progeny that had been treated with styrene-7,8-oxide were still alive. The incidences of forestomach tumours in control and treated groups were: papilloma—males, 0/49 versus 7/42 ($p < 0.003$); females, 2/55 versus 2/60 ($p > 0.05$); carcinoma *in situ*—males, 0/49 versus 4/42 ($p < 0.04$); females, 0/55 versus 6/60 ($p < 0.02$); carcinoma—males, 0/49 versus 10/42 ($p < 0.0002$); females, 1/55 versus 16/60 ($p < 0.0001$). Hyperplasia, dysplasia and hyperkeratosis of the forestomach were also reported in treated rats. There was no difference between treated and control groups in the incidence of tumours at other sites (Ponomarev *et al.*, 1984).

3.3 Skin application

Mouse: A group of 40 C3H mice [sex unspecified], 13 weeks old, received three weekly applications of a 5% solution of styrene-7,8-oxide in acetone [volume unspecified] on the clipped dorsal skin for life. No skin tumour was observed in the 17 mice that survived to 24 months. Another group of 40 C3H mice was similarly treated with a 10% solution of styrene-7,8-oxide in acetone: 18 mice survived to 12 months, and only two mice survived to 17 months. No skin tumour was observed (Weil *et al.*, 1963). [The Working Group noted the incomplete reporting of the study.]

A group of 30 male Swiss ICR/Ha mice, eight weeks old, received three weekly applications of 100 mg of a 10% solution of styrene-7,8-oxide in benzene on clipped dorsal skin for life. The median survival time was 431 days. Three mice developed skin tumours, one of which was a squamous-cell carcinoma. Of 150 benzene-painted controls, 11 developed skin tumours, one of which was a squamous-cell carcinoma (Van Duuren *et al.*, 1963). [The Working Group noted the potential carcinogenicity of the vehicle.]

4. Other Data Relevant for an Evaluation of Carcinogenicity and Its Mechanisms

4.1 Absorption, distribution, excretion and metabolism

For a review of the metabolism and pharmacokinetics of styrene-7,8-oxide see the monograph on styrene.

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

(a) Styrene-7,8-oxide

In mice that received an intraperitoneal injection of styrene, the maximal concentrations of styrene-7,8-oxide were higher in subcutaneous adipose tissue than in the other tissues studied 1–5 h after injection (Nordquist *et al.*, 1983; Löf *et al.*, 1984).

The absorption and elimination of styrene-7,8-oxide were investigated in CD2F1 mice after a single intraperitoneal injection of 200 mg/kg bw in corn oil (Bidoli *et al.*, 1980). Styrene-7,8-oxide was rapidly absorbed, reaching a peak concentration in blood of 40 ± 7 $\mu\text{g/ml}$ at 7 min, after which it rapidly disappeared; at 60 min, it was no longer detectable. The area under the curve for the time course of the blood concentration of styrene-7,8-oxide was $329 \text{ min} \times \mu\text{g/g}$.

The pharmacokinetics of styrene-7,8-oxide in male Fischer 344 rats was studied after oral administration of 275 and 550 mg/kg bw (Langvardt & Nolan, 1991). Wide variation was seen in the measured blood concentrations, which ranged from 0.27 to 8.84 $\mu\text{g/ml}$ in animals given the low dose and from 2.1 to 32.4 $\mu\text{g/ml}$ in those given the high dose. The areas under

the curve for the time course of the blood concentration of styrene-7,8-oxide were 47 and 286 min \times μ g/g.

The uptake, distribution and elimination of styrene-7,8-oxide were investigated in Sprague-Dawley rats and B6C3F1 mice after intraperitoneal and oral administration of 200 mg/kg bw. Styrene-7,8-oxide was rapidly absorbed, reaching a peak concentration within 15 min. The blood concentrations varied widely between animals after oral administration. The areas under the curve for the time course of the blood concentration of styrene-7,8-oxide after intraperitoneal and oral administration were 18 and 0.76 h \times μ g/ml in rats and 12 and 0.01 h \times μ g/ml in mice, respectively. The significantly reduced bioavailability of styrene-7,8-oxide after oral administration was due to hydrolysis in the acidic environment of the stomach (Kessler *et al.*, 1992), as indicated by the finding of acid-catalysed hydrolysis of styrene-7,8-oxide *in vitro* (Ross *et al.*, 1982).

A physiological pharmacokinetic model was developed to describe the disposition and metabolism of styrene and styrene-7,8-oxide in mouse, rat and man (Csanády *et al.*, 1994) after inhalation or intravenous, oral or intraperitoneal administration of styrene, and after intravenous, oral or intraperitoneal administration of styrene-7,8-oxide. The model includes oxidation of styrene to styrene-7,8-oxide, the intracellular first-pass hydrolysis of styrene-7,8-oxide catalysed by epoxide hydrolase and the conjugation of styrene-7,8-oxide with glutathione. Conjugation is described by an ordered sequential 'ping-pong' mechanism between glutathione, styrene-7,8-oxide and glutathione *S*-transferase. The model was validated with data sets from a number of laboratories on the pharmacokinetics of styrene and styrene-7,8-oxide in rodents and man. The effects of alveolar ventilation and the blood:air partition coefficient of styrene on the pharmacokinetics of styrene and styrene-7,8-oxide were investigated by sensitivity analysis. The sensitivity coefficients calculated for steady-state exposure to styrene at 500 ppm [2130 mg/m³] indicated that small changes in the balance of production and elimination could cause drastic changes in the body burden of styrene-7,8-oxide in mice but not in rats or humans. These findings might explain the greater mortality among mice exposed to 250 and 500 ppm [1065 and 2130 mg/m³] styrene (Morgan *et al.*, 1993).

Styrene-7,8-oxide is the metabolite of styrene that is catalysed by the cytochrome P450 monooxygenase system and non-enzymatically by oxyhaemoglobin (Belvedere *et al.*, 1983). Further metabolic reactions are catalysed by epoxide hydrolase and glutathione *S*-transferase. When human cytosolic and microsomal epoxide hydrolases were assayed with styrene-7,8-oxide, the microsomal activity was greater than the cytosolic activity (Schlady *et al.*, 1988). Human liver glutathione *S*-transferase cytosolic fractions occur in two forms, μ and α , of which the μ form was more active with styrene-7,8-oxide, with a K_m of 4.9 mmol/L and a V_{max} of 22 nmol/mg per min (Pacifici *et al.*, 1987). About one-half of individuals in many Caucasian populations lack this enzyme (Warholm *et al.*, 1981). Glutathione *S*-transferase and epoxide hydrolase activities were detected in many fetal tissues (Pacifici & Rane, 1982), and the α and π forms of glutathione *S*-transferase are present in fetal liver (Pacifici *et al.*, 1988).

The enzymes that metabolize styrene-7,8-oxide are stereoselective, in that the *S* enantiomer is favoured over the *R* in subsequent hydrolysis by epoxide hydrolase (Watabe *et al.*, 1981). In contrast, glutathione *S*-transferase, including the μ form, favours the *R* isomer

(Hiratsuka *et al.*, 1989). The R forms were substituted to C7 and the S forms to C8 (Dostal *et al.*, 1986).

Isolated, perfused rat liver rapidly metabolized styrene-7,8-oxide to styrene glycol, mandelic acid and glutathione conjugates (Ryan & Bend, 1977; Steele *et al.*, 1981). Microsomal conjugation of styrene-7,8-oxide with glutathione yielded about 60% *S*-(1-phenyl-2-hydroxyethyl)glutathione and 40% *S*-(2-phenyl-2-hydroxyethyl)glutathione (Pachecka *et al.*, 1979). (See the monograph on styrene for further description of styrene-7,8-oxide metabolism.)

The main route of excretion of styrene-7,8-oxide metabolites in animals is via the kidney: in rabbits, about 80% of a single oral dose was excreted in the urine (James & White, 1967). Acidic urinary metabolites of styrene-7,8-oxide derived from glutathione conjugates are species dependent: in rats, the only products detected are mercapturic acids; in guinea-pigs, the major bivalent sulfur acids are the corresponding mercaptoacetic acids, together with mercaptolactic and mercaptopyruvic and mercapturic acids. 3,4-Dihydroxy-3,4-dihydro-1-vinylbenzene has been reported as a urinary metabolite of both styrene and styrene-7,8-oxide in rats and guinea-pigs (Nakatsu *et al.*, 1983).

(b) Protein adducts

In vitro, styrene-7,8-oxide bound to histidine in human haemoglobin (Kaur *et al.*, 1989) but predominantly to cysteine in human plasma proteins (Hemminki, 1986). It bound to polyamino acids in the order: polycysteine >> polyhistidine > polylysine > polyserine (Hemminki, 1983). Cysteine alkylation was determined following intraperitoneal administration of styrene-7,8-oxide to rats (Rappaport *et al.*, 1993).

Covalent binding to plasma proteins and haemoglobin were determined in male mice [strain unspecified] after intraperitoneal administration of [7-¹⁴C]styrene and [7-³H]-styrene-7,8-oxide. A dose-dependent increase in alkylated plasma proteins was seen 5 h after injection of 0.12–4.9 mmol/kg bw styrene or 2 h after injection of 0.12–2.4 mmol/kg bw styrene or 0.037–1.1 mmol/kg bw styrene-7,8-oxide. The plasma-protein binding ratio of styrene-7,8-oxide to styrene increased with dose, a result that is consistent with the saturable metabolism of styrene. In contrast, binding to haemoglobin 2 h after injection of 1.1–4.9 mmol/kg bw styrene was proportionally higher at high doses. One explanation given by the authors is an increased importance of metabolic activation of styrene by erythrocytes when a higher proportion of styrene escapes the hepatic metabolizing enzymes. Following administration of 0.037–1.1 mmol/kg bw styrene-7,8-oxide, proportionally greater binding to plasma proteins was observed at the highest dose (Byfält Nordqvist *et al.*, 1985).

In mice treated intraperitoneally with styrene-7,8-oxide at 50–250 mg/kg bw, a disproportionate increase in binding was seen at higher dose levels. A lesser but similar effect was seen in rats, which showed an about three-fold lower adduct level at equivalent doses of styrene-7,8-oxide. In mice administered styrene at the same doses, about 5% of styrene was available as the oxide (Osterman Golkar, 1992).

Female Wistar rats treated intraperitoneally with styrene-7,8-oxide at 83–833 µmol/kg bw had haemoglobin carboxylic acid esters of styrene-7,8-oxide, the level of which increased with dose (disproportionately at higher doses). The lowest dose (83 µmol/kg bw) resulted in

an adduct level of 16.7 pmol/g globin, and 833 $\mu\text{mol/kg}$ bw yielded 724 pmol/g globin (Sepai *et al.*, 1993).

A similar study on covalent binding of styrene and styrene-7,8-oxide to albumin and haemoglobin was performed in Sprague-Dawley rats. Linear relationships were observed between adduct levels and intraperitoneal doses of 0.5–3 mmol/kg bw styrene and 0.1–1 mmol/kg bw styrene-7,8-oxide. Comparison of the slopes revealed a much greater production of protein adducts following administration of styrene-7,8-oxide, the slope derived for styrene being only 2% of that for styrene-7,8-oxide (Rappaport *et al.*, 1993).

4.1.3 Comparison of humans and animals

Pieces of human liver from five accident victims selected for organ transplantation were obtained through the Nashville (USA) regional organ procurement agency. No information was available on the donors, other than that the livers were free of debilitating diseases, such as human immunodeficiency viral infection and hepatitis A and B. The activities of cytochrome P450 monooxygenase and microsomal and cytosolic forms of epoxide hydrolase and glutathione *S*-transferase were then compared in the livers of humans, Fischer 344 and Sprague-Dawley rats and B6C3F1 mice (Mendrala *et al.*, 1993). The affinities of the monooxygenases (inverse K_m values) were essentially similar: 0.09 mmol in humans and 0.05 mmol in mice not pretreated with styrene. The V_{max} values were similar in rats and mice (9.3–13 nmol/mg protein per min) but were lower in the five human samples (2.1 nmol/mg per min). The K_m values for epoxide hydrolase were low in humans (0.01 mmol), intermediate in rats (0.13–0.23 mmol) and high in mice (0.74 mmol); the V_{max} values did not differ between the species. Humans apparently had the lowest glutathione *S*-transferase activity towards styrene-7,8-oxide.

4.2 Toxic effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

Human plasma α_1 -proteinase inhibitor was inactivated *in vitro* by styrene-7,8-oxide (Ansari *et al.*, 1988a). Administration of styrene-7,8-oxide together with acrolein or pyruvic aldehyde caused greater inhibition than any compound alone (Ansari *et al.*, 1988b). Styrene-7,8-oxide inhibited the activity of glutathione *S*-transferase π isolated from human erythrocytes to about one-half at a concentration of 2 nmol (Ansari *et al.*, 1987).

Styrene-7,8-oxide causes corneal injury in rabbits (Weil *et al.*, 1963); even dilutions as low as 1% cause eye irritation (Hine & Rowe, 1980). Intradermal injections sensitized the skin of guinea-pigs (Weil *et al.*, 1963)

One intraperitoneal dose of 375 mg/kg bw styrene-7,8-oxide decreased the rat-liver mixed-function oxidase activity for certain substrates and in total cytochrome P450 content (Parkki *et al.*, 1976). Styrene-7,8-oxide decreased the glutathione content of rat liver *in vivo* at doses of 50 and 200 mg/kg bw (Marniemi *et al.*, 1977).

Styrene-7,8-oxide administered intraperitoneally to inbred male albino rats at doses of 25 and 50 mg/kg bw increased the levels of noradrenaline in the cerebral cortex, increased the activity of 5-hydroxytryptamine and decreased the activity of monoamine oxidase in several regions of the brain (Husain *et al.*, 1985). In similar experiments, styrene-7,8-oxide treatment increased the total number of dopamine receptors (Zaidi *et al.*, 1985). Styrene-7,8-oxide administered as single intraperitoneal doses of 100–400 mg/kg bw to Sprague-Dawley rats decreased the level of glutathione in the brain (Trenga *et al.*, 1991). The effect was potentiated by arylamide, which caused necrosis of cerebellar granule cells and some small neurones of the cerebral cortex (Beiswanger *et al.*, 1993).

Cell proliferation (as measured by the proportion of nuclei labelled with 5-bromo-2'-deoxyuridine delivered from a subcutaneously implanted osmotic pump during the last 24 h of the experiment) was increased in three regions of the forestomach of male Fischer 344 rats after gavage administration of styrene-7,8-oxide three times per week for four weeks. The doses used were 0, 137, 275 and 550 mg/kg bw. Only marginal morphological changes were observed occasionally (Cantoreggi *et al.*, 1993).

4.3 Reproductive and prenatal effects

4.3.1 Humans

No data were available to the Working Group.

4.3.2 Experimental systems

Only one study of the reproductive toxicity of styrene-7,8-oxide in mammals has been published (Sikov *et al.* 1986). Six groups of at least 31 Wistar rats were exposed by inhalation (whole body) to 100 ppm [490 mg/m³] or 300 ppm [1470 mg/m³] styrene-7,8-oxide (purity, 99%) vapour for 7 h per day either during a three-week (five days/week) pregestational period, during a three-week (five days/week) pregestational period and through days 1–19 of gestation, or on gestational days 1–19 only. A control group was exposed to air during the whole period. Fetuses were examined on day 21. There was extensive mortality among rats that received prolonged exposure to 100 ppm; exposure to 300 ppm was discontinued after one day because of mortality. Maternal weight gain was reduced in all groups receiving 100 ppm. Exposure only prior to mating had no effect on mating or fertility. Gestational exposure decreased the number of animals pregnant at term by increasing preimplantation loss of embryos; fetal weights and lengths were reduced, and the incidences of retarded ossification of the sternbrae and occipital bones were increased. In the same study, groups of 23–24 New Zealand white rabbits were exposed by inhalation to 0, 15 or 50 ppm [74 or 245 mg/m³] (measured concentrations, 14.6 and 51 ppm) styrene-7,8-oxide (purity, 99%) vapour for 7 h per day on days 1–24 of gestation. Fetuses were examined on day 30. Maternal toxicity was observed at the highest dose only, resulting in increased mortality (19/24 *versus* 1/23 in controls and 4/24 at 15 ppm) and decreased food consumption and weight gain. There was no effect on the proportion pregnant at term, i.e. there was no marked preimplantation loss, but there was an increase in postimplantation loss, with 0.25, 0.93 and 1.5 resorptions per litter in the control, low- and high-dose groups, respectively. There was no effect on fetal

weight, and no increase in the incidence of malformations was observed in either rats or rabbits.

4.4 Genetic and related effects

4.4.1 Humans

No published data on the effects of exposure of humans to styrene-7,8-oxide alone were available to the Working Group.

4.4.2 Experimental systems (see also Table 1 and Appendices 1 and 2)

(a) DNA adducts

A comprehensive review of DNA adduct formation with styrene-7,8-oxide is available (Phillips & Farmer, 1994).

The relative yields of alkylated deoxynucleosides in DNA in aqueous buffer were deoxyguanosine > deoxycytidine > deoxyadenosine > thymidine, the dominant product being 7-alkylguanine (Savela *et al.*, 1986). When radioactive styrene-7,8-oxide was reacted with double- and single-stranded DNA, the latter produced more adducts, the majority (54%) of which were 7-guanine adducts, representing similar proportions of α and β isomers (Vodička & Hemminki, 1988a). Depurination of 7-alkyldeoxyguanosine derivatives of styrene-7,8-oxide occurred at the same rate as for 7-methyldeoxyguanosine, while depurination of 7-alkylguanine was 15 times slower in single-stranded DNA and 55 times slower in double-stranded DNA (Vodička & Hemminki, 1988b).

7-Alkylguanine adducts of styrene-7,8-oxide were demonstrated in five organs of mice after intraperitoneal injection of styrene-7,8-oxide (Byfält Nordqvist *et al.*, 1985).

Using ^{32}P -postlabelling methods (Liu *et al.*, 1988) with mammalian cells in culture, Pongracz *et al.* (1989) detected six adducts and identified two isomers of O^6 -modified deoxyguanosines, O^6 -(2-hydroxy-2-phenylethyl)-2'-deoxyguanosine-3',5'-bisphosphate and O^6 -(2-hydroxy-1-phenylethyl)-2'-deoxyguanosine-3',5'-bisphosphate. Hemminki *et al.* (1990) studied the stability of the deoxyguanosine 3'-monophosphate 7-alkylation products for postlabelling, but considerable lability of the 7-guanine adducts was observed. Further ^{32}P -postlabelling was performed using N^7 , N^2 and O^6 adducts of styrene-7,8-oxide (Vodička & Hemminki, 1991). No phosphorylation products of N^7 adducts were seen, while one of the two diastereomeric N^2 adducts was labelled with 20% efficiency, two of the three O^6 adducts with 5% efficiency and the third with 10% labelling efficiency, suggesting stereoselectivity of the kinase reaction.

Pongracz *et al.* (1992) detected six adducts of styrene-7,8-oxide in calf thymus DNA by ^{32}P -postlabelling, the N^2 -guanosine derivatives being the major products. Combination of mass spectrometry with the postlabelling assay allowed identification of three new hydrophobic bis-substituted adducts representing N^1 , N^2 and N^2 , O^6 modifications (Kaur *et al.*, 1993). These modifications are unlikely to occur *in vivo* (Phillips & Farmer, 1994).

Radiolabelled [^3H]styrene-7,8-oxide was used to search for adducts in different parts of the gastrointestinal tract and liver of rats and mice *in vivo*. Covalent binding of

styrene-7,8-oxide occurred below the limit of detection in all tissues (Cantoreggi & Lutz, 1992). [The Working Group calculated that the maximal possible covalent binding index—(pmol adduct/mol DNA nucleotide)/(mmol chemical/kg bw)—was < 0.6 for mouse liver DNA 2 h after intraperitoneal injection.] In further studies with higher concentrations of styrene-7,8-oxide, binding to DNA in rat forestomach was detected, the covalent binding index being 1.0 (Lutz *et al.*, 1993).

(b) *Mutation and allied effects*

Styrene-7,8-oxide induced SOS repair and reverse mutations in *Salmonella typhimurium* and *Escherichia coli*. Forward mutations were also induced in *S. typhimurium* and *Klebsiella pneumoniae*. The R enantiomer of styrene-7,8-oxide was slightly more mutagenic in *S. typhimurium* TA100 than the respective S enantiomer (Seiler, 1990; Sinsheimer *et al.*, 1993). The sensitivity of *S. typhimurium* TA100 mutants to DL-1,2,4-triazole-3-alanine indicated that > 95% of the mutants were *his* locus revertants, the remainder being suppressors (Einistö *et al.*, 1993).

Forward mutations and gene conversion were induced in yeasts, both *in vitro* and in the mouse host peritoneal assay. Chromosomal aberrations and micronuclei were induced in the plant, *Allium cepa*.

Sex-linked recessive lethal mutations were induced by styrene-7,8-oxide in *Drosophila melanogaster* in a single study.

In cultured mammalian cells, styrene-7,8-oxide induced DNA single-strand breaks (but not double-strand breaks or cross-links), mutations at the *hprt* and *tk* loci, sister chromatid exchange, micronuclei and chromosomal aberrations. Styrene-7,8-oxide tested in human cells in culture induced sister chromatid exchange, micronuclei and chromosomal aberrations. All of the reports of significant increases in the frequencies of sister chromatid exchange and chromosomal aberrations relate to cultures of lymphocytes.

Styrene-7,8-oxide did not induce morphological transformation of C3H/10T $\frac{1}{2}$ Cl8 cells but enhanced the transforming activity of 3-methylcholanthrene in a two-stage transformation assay.

Responses to styrene-7,8-oxide *in vivo* are more variable. [The Working Group noted that the purity of the test compound was frequently stated in the publications but that the styrene-7,8-oxide used was probably a mixture of optical isomers, except when the R and S enantiomers were specified. Exposure was usually by inhalation or intraperitoneal injection (see Table 1).] DNA strand breaks were induced in a single study. Sister chromatid exchange was induced in mouse bone-marrow cells in one study with the S enantiomer of styrene-7,8-oxide but not with the R enantiomer. In another study, small increases in sister chromatid exchange frequencies were seen in liver cells and alveolar macrophages but not in bone-marrow cells in mice. A negative response was also obtained in bone-marrow cells of Chinese hamsters. Micronuclei were not induced by styrene-7,8-oxide in mouse or Chinese hamster bone marrow. Chromosomal aberrations were induced in mouse bone-marrow cells in one study with the S enantiomer of styrene-7,8-oxide but not with the R enantiomer. Conflicting results were obtained in two other studies with mice, since a significant increase in the frequency of bone-marrow cell chromosomal aberrations was reported in one study at a five-fold lower dose level than was used in another study in which no significant response

Table 1. Genetic and related effects of styrene-7,8-oxide

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
PRB, <i>Salmonella typhimurium umu</i> , SOS induction	+	0	0.0700	Nakamura <i>et al.</i> (1987)
ECB, <i>Escherichia coli</i> PQ37, SOS induction	+	0	100.0000	Głosńska & Dziadziuszko (1986)
ECB, <i>Escherichia coli</i> PQ37, SOS induction	-	-	12000.0000	Brams <i>et al.</i> (1987)
ECB, <i>Escherichia coli</i> PQ37, SOS induction	+	0	36.0000	von der Hude <i>et al.</i> (1990)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation (spot test)	+	0	200.0000	Milvy & Garro (1976)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	0.6000	Vainio <i>et al.</i> (1976)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	60.0000	de Meester <i>et al.</i> (1977)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	146.0000	Sugiura <i>et al.</i> (1978a)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	250.0000	Wade <i>et al.</i> (1978)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	250.0000	Watabe <i>et al.</i> (1978)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	600.0000	Watabe <i>et al.</i> (1980)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	120.0000	Busk (1979)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	125.0000	El-Tantawy & Hammock (1980)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	240.0000	Yoshikawa <i>et al.</i> (1980)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	0.0000	De Flora (1981)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	768.0000	de Meester <i>et al.</i> (1981)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	144.0000	Sugiura & Goto (1981)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	120.0000	Turchi <i>et al.</i> (1981)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	48.0000	Pagano <i>et al.</i> (1982)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	60.0000	Glatt <i>et al.</i> (1983)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	300.0000	Brams <i>et al.</i> (1987)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+ ^c	+	500.0000	Hughes <i>et al.</i> (1987)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	0.0000	Claxton <i>et al.</i> (1991)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	60.0000	Einistö <i>et al.</i> (1993)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	120.0000	Sinsheimer <i>et al.</i> (1993)

Table 1 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA3, <i>Salmonella typhimurium</i> TA1530, reverse mutation	+	+	768.0000	de Meester <i>et al.</i> (1981)
SA4, <i>Salmonella typhimurium</i> TA104, reverse mutation	+	0	120.0000	Einistö <i>et al.</i> (1993)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation (spot test)	+	0	5000.0000	Milvy & Garro (1976)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	+	0.6000	Vainio <i>et al.</i> (1976)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	+	24.0000	de Meester <i>et al.</i> (1977)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	+	125.0000	Stoltz & Withey (1977)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	+	60.0000	Loprieno <i>et al.</i> (1978)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	(+)	0	250.0000	Wade <i>et al.</i> (1978)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	0	50.0000	Watabe <i>et al.</i> (1978)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	+	60.0000	Busk (1979)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	0	60.0000	El-Tantawy & Hammock (1980)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	+	0.0000	De Flora (1981)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	+	768.0000	de Meester <i>et al.</i> (1981)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation (spot test)	-	0	5000.0000	Milvy & Garro (1976)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	600.0000	Vainio <i>et al.</i> (1976)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	6000.0000	de Meester <i>et al.</i> (1977)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	0	0.0000	Wade <i>et al.</i> (1978)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	(+)	0	250.0000	Watabe <i>et al.</i> (1978)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	0	500.0000	El-Tantawy & Hammock (1980)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	0.0000	De Flora (1981)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	1150.0000	de Meester <i>et al.</i> (1981)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation (spot test)	-	0	5000.0000	Milvy & Garro (1976)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	+	6.0000	Vainio <i>et al.</i> (1976)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	6000.0000	de Meester <i>et al.</i> (1977)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	0	250.0000	Watabe <i>et al.</i> (1978)

Table 1 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	0.0000	De Flora (1981)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	1150.0000	de Meester <i>et al.</i> (1981)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation (spot test)	-	0	5000.0000	Milvy & Garro (1976)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	600.0000	Vainio <i>et al.</i> (1976)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	6000.0000	de Meester <i>et al.</i> (1977)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	0	0.0000	Wade <i>et al.</i> (1978)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	0	250.0000	Watabe <i>et al.</i> (1978)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	250.0000	Ueno <i>et al.</i> (1978)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	0	500.0000	El-Tantawy & Hammock (1980)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	0.0000	De Flora (1981)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	1150.0000	de Meester <i>et al.</i> (1981)
SAS, <i>Salmonella typhimurium</i> TA97, reverse mutation	+	0	300.0000	Brams <i>et al.</i> (1987)
SAS, <i>Salmonella typhimurium</i> TA4001, reverse mutation	+	0	240.0000	Einistö <i>et al.</i> (1993)
SAS, <i>Salmonella typhimurium</i> TA4006, reverse mutation	(+)	0	960.0000	Einistö <i>et al.</i> (1993)
ECW, <i>Escherichia coli</i> WP2 <i>uvrA</i> , reverse mutation	+	0	720.0000	Sugiura <i>et al.</i> (1978b)
ECW, <i>Escherichia coli</i> WP2 <i>uvrA</i> , reverse mutation	+	0	480.0000	Sugiura & Goto (1981)
KPF, <i>Klebsiella pneumoniae</i> , forward mutation	+	0	120.0000	Voogd <i>et al.</i> (1981)
SCG, <i>Saccharomyces cerevisiae</i> , gene conversion	+	0	1200.0000	Loprieno <i>et al.</i> (1976)
SZF, <i>Schizosaccharomyces pombe</i> , forward mutation	+	0	600.0000	Loprieno <i>et al.</i> (1976)
ACC, <i>Allium cepa</i> , chromosomal aberrations and micronuclei	+	0	500.0000	Linnainmaa <i>et al.</i> (1978a,b)
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutations	+	0	1.0000 inhal.	Donner <i>et al.</i> (1979)
DIA, DNA strand breaks, rat hepatocytes <i>in vitro</i>	+	0	36.0000	Sina <i>et al.</i> (1983)
DIA, DNA strand breaks, Pc12 cells <i>in vitro</i>	+	0	3.6000	Dypbukt <i>et al.</i> (1992)
G9H, Gene mutation, Chinese hamster lung V79 cells, <i>hprt</i> locus	+	0	1020.0000	Loprieno <i>et al.</i> (1976)
G9H, Gene mutation, Chinese hamster lung V79 cells, <i>hprt</i> locus	+	0	1020.0000	Bonatti <i>et al.</i> (1978)

Table 1 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
G9H, Gene mutation, Chinese hamster lung V79 cells, <i>hprt</i> locus	+	0	504.0000	Loprieno <i>et al.</i> (1978)
G9H, Gene mutation, Chinese hamster lung V79 cells, <i>hprt</i> locus	+	-	240.0000	Beije & Jenssen (1982)
G9H, Gene mutation, Chinese hamster lung V79 cells, <i>hprt</i> locus	(+)	0	100.0000	Nishi <i>et al.</i> (1984)
G5T, Gene mutation, mouse lymphoma L5178Y cells, <i>tk</i> locus	+	-	13.8000	Amacher & Turner (1982)
SIC, Sister chromatid exchange, Chinese hamster ovary cells <i>in vitro</i>	+	+	50.0000	de Raat (1978)
SIC, Sister chromatid exchange, Chinese hamster V79 cells <i>in vitro</i>	+	0	20.0000	Nishi <i>et al.</i> (1984)
SIC, Sister chromatid exchange, Chinese hamster V79 cells <i>in vitro</i>	+	0	15.0000	von der Hude <i>et al.</i> (1991)
MIA, Micronucleus formation, Chinese hamster V79 cells <i>in vitro</i>	+	0	90.0000	Turchi <i>et al.</i> (1981)
CIC, Chromosomal aberrations, Chinese hamster V79 cells <i>in vitro</i>	+	0	90.0000	Turchi <i>et al.</i> (1981)
TCM, Cell transformation, C3H10T1/2 mouse cells <i>in vitro</i>	- ^d	0	1.2000	Male <i>et al.</i> (1985)
SHL, Sister chromatid exchange, human lymphocytes <i>in vitro</i>	+	0	8.4000	Norppa <i>et al.</i> (1981)
SHL, Sister chromatid exchange, human lymphocytes <i>in vitro</i>	+	0	1.0000	Pohlova <i>et al.</i> (1985)
MIH, Micronucleus formation, human cells <i>in vitro</i>	+	0	80.0000	Linainmaa <i>et al.</i> (1978a,b)
CHL, Chromosomal aberrations, human lymphocytes <i>in vitro</i>	+	0	60.0000	Fabry <i>et al.</i> (1978)
CHL, Chromosomal aberrations, human lymphocytes <i>in vitro</i>	+	0	80.0000	Linnainmaa <i>et al.</i> (1978a,b)
CHL, Chromosomal aberrations, human lymphocytes <i>in vitro</i>	+	0	24.0000	Norppa <i>et al.</i> (1981)
CHL, Chromosomal aberrations, human lymphocytes <i>in vitro</i>	+	0	3.0000	Pohlova <i>et al.</i> (1985)
HMM, Host-mediated assay, <i>Saccharomyces cerevisiae</i> in mice	(+)		100×1, gavage	Loprieno <i>et al.</i> (1976)
HMM, Host-mediated assay, <i>Schizosaccharomyces pombe</i> in mice	(+)		100×1, gavage	Loprieno <i>et al.</i> (1976)
DVA, DNA strand breaks, mouse tissue <i>in vivo</i>	+		600×1, ip	Wallis & Orsen (1983)
SVA, Sister chromatid exchange, Chinese hamster bone-marrow cells <i>in vivo</i>	-		86 inhal.×2	Norppa <i>et al.</i> (1979)
SVA, Sister chromatid exchange, Chinese hamster bone-marrow cells <i>in vivo</i>	-		500×1 ip	Norppa <i>et al.</i> (1979)
SVA, Sister chromatid exchange, mouse liver cells <i>in vivo</i>	(+)		72 inhal. 5 h×1	Conner <i>et al.</i> (1982)
SVA, Sister chromatid exchange, mouse alveolar macrophages <i>in vivo</i>	(+)		72 inhal. 5 h×1	Conner <i>et al.</i> (1982)
SVA, Sister chromatid exchange, mouse bone-marrow cells <i>in vivo</i>	-		72 inhal. 5 h×1	Conner <i>et al.</i> (1982)

Table 1 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SVA, Sister chromatid exchange, mouse bone-marrow cells <i>in vivo</i>	+ ^e		100×1 ip	Sinsheimer <i>et al.</i> (1993)
MVM, Micronucleus formation, BALB/c mouse bone-marrow cells <i>in vivo</i>	-		250×1 ip	Fabry <i>et al.</i> (1978)
MVC, Micronucleus formation, Chinese hamster bone-marrow cells <i>in vivo</i>	-		250×1 ip	Pentillä <i>et al.</i> (1980)
CBA, Chromosomal aberrations, BALB/c mouse bone-marrow cells <i>in vivo</i>	-		250×1 ip	Fabry <i>et al.</i> (1978)
CBA, Chromosomal aberrations, male CD-1 mouse bone-marrow cells <i>in vivo</i>	+		50×1, gavage	Loprieno <i>et al.</i> (1978)
CBA, Chromosomal aberrations, male Chinese hamster bone-marrow cells <i>in vivo</i>	-		86 inhal.×2	Norppa <i>et al.</i> (1979)
CBA, Chromosomal aberrations, Chinese hamster bone-marrow cells <i>in vivo</i>	-		500×1 ip	Norppa <i>et al.</i> (1979)
CBA, Chromosomal aberrations, mouse bone-marrow cells <i>in vivo</i>	+ ^e		100×1 ip	Sinsheimer <i>et al.</i> (1993)
DLM, Dominant lethal mutation, male mice <i>in vivo</i>	-		250×1 ip	Fabry <i>et al.</i> (1978)
BVD, Binding (covalent) to DNA, male CD rat stomach, liver <i>in vivo</i>	-		240×1 po	Cantoreggi & Lutz (1992)
BVD, Binding (covalent) to DNA, male B6C3F1 mouse liver <i>in vivo</i>	-		165×1 ip	Cantoreggi & Lutz (1992)
BVD, Binding (covalent) to DNA, male CD rat forestomach <i>in vivo</i>	(+)		1.3×1 po	Lutz <i>et al.</i> (1993)

^a+, positive; (+), weakly positive; -, negative; 0, not tested; ?, inconclusive (variable responses in several experiments within an adequate study)

^bIn-vitro tests, µg/ml; in-vivo tests, mg/kg bw

^cIncubated in Tedlar bags

^dPositive in a two-stage assay

^eS isomer only

occurred. No chromosomal aberrations were reported in a single study on Chinese hamster bone marrow.

No dominant lethal effect was observed in male mice.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Styrene-7,8-oxide is produced by cyclization of styrene chlorohydrin and by epoxidation of styrene with peroxyacetic acid. It is used mainly in the preparation of fragrances and as a reactive diluent in epoxy resin formulations. Few data are available on levels of occupational exposure to styrene-7,8-oxide. It has been detected in association with styrene, but at much lower levels, in industries where unsaturated polyester resins are used.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Styrene-7,8-oxide was tested for carcinogenicity in one experiment in mice and in two experiments in rats by oral gavage. It produced benign and malignant tumours of the forestomach in animals of each species and sex and induced hepatocellular tumours in male mice. It was also tested in one strain of rats by prenatal exposure followed by postnatal gastric intubation, producing benign and malignant tumours of the forestomach.

5.4 Other relevant data

Styrene-7,8-oxide is absorbed by rabbits and rats following its oral administration. In mice, the highest tissue concentrations are found in kidney, adipose tissue and blood. Styrene-7,8-oxide is hydrolysed rapidly in the acid environment of the stomach. Almost all of an administered dose of styrene-7,8-oxide is excreted in the urine of experimental animals. Styrene-7,8-oxide can be metabolized by epoxide hydrolase to the glycol or by glutathione *S*-transferase to glutathione conjugates. A small amount may be reduced to styrene. Styrene glycol is further metabolized to mandelic, phenyl glyoxylic and hippuric acids.

Styrene-7,8-oxide bound to histidine in haemoglobin and to cysteine in plasma proteins *in vitro*. Low levels of covalent binding to DNA were observed in the stomachs of orally dosed rats. In rat brain, it can decrease the activity of some neurotransmitters and monoamine oxidase, and it increases the availability of dopamine receptors. Glutathione *S*-transferase from human erythrocytes was inhibited by low concentrations of styrene-7,8-oxide.

No teratogenic effect was observed in rats or rabbits treated with doses of styrene-7,8-oxide up to the lethal level.

No data were available on the genetic and related effects of styrene-7,8-oxide in humans.

Both positive and negative results have been obtained with styrene-7,8-oxide for a variety of genetic end-points *in vivo*. Chromosomal aberrations and sister chromatid exchange were induced in mouse bone marrow only after treatment with the S enantiomer and not with the R enantiomer. DNA damage, mutations and chromosomal aberrations have been observed consistently in mammalian and nonmammalian systems *in vitro*.

5.5 Evaluation¹

There is *inadequate evidence* in humans for the carcinogenicity of styrene-7,8-oxide.

There is *sufficient evidence* in experimental animals for the carcinogenicity of styrene-7,8-oxide.

In making the overall evaluation, the Working Group took into consideration the following supporting evidence. Styrene-7,8-oxide:

- (i) forms covalent adducts with DNA in humans, rats and mice;
- (ii) induces gene mutation in bacteria and rodent cells *in vitro*;
- (iii) induces chromosomal aberrations, micronuclei and sister chromatid exchange in human cells *in vitro*; and
- (iv) induces chromosomal aberrations and sister chromatid exchange in mice *in vivo*.

Overall evaluation

Styrene-7,8-oxide is *probably carcinogenic to humans (Group 2A)*.

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¹For definition of the italicized terms, see Preamble, pp. 27-30.

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4-VINYLCYCLOHEXENE

This substance was considered by previous Working Groups, in February 1976 (IARC, 1976) and in June 1985 (IARC, 1986). 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

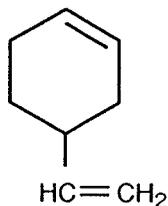
Chem. Abstr. Serv. Reg. No.: 100-40-3

Chem. Abstr. Name: 4-Ethenylcyclohexene

IUPAC Systematic Name: 4-Vinylcyclohexene

Synonyms: 1-Vinyl-3-cyclohexene; 4-vinyl-1-cyclohexene

1.1.2 Structural and molecular formulae and relative molecular mass



C₈H₁₂

Relative molecular mass: 108.18

1.1.3 Chemical and physical properties of the pure substance

- Description:* Colourless liquid (US National Toxicology Program, 1986)
- Boiling-point:* 128.9 °C (Lide, 1991)
- Freezing-point:* -108.9 °C (Lewis, 1993)
- Density:* 0.8299 at 20 °C/4 °C (Lide, 1991)
- Spectroscopy data:* Infrared [6321], ultraviolet, nuclear magnetic resonance and mass spectral data have been reported (US National Toxicology Program, 1986; Sadtler Research Laboratories, 1991; US National Library of Medicine, 1993).
- Solubility:* Soluble in benzene, diethyl ether and petroleum ether (Lide, 1991); slightly soluble in water (50 mg/L) (DuPont Chemicals, undated)

- (g) *Volatility*: Vapour pressure, 15 mm Hg [2 kPa] at 25 °C (DuPont Chemicals, 1992); relative vapour density (air = 1), 3.76 (US National Library of Medicine, 1993)
- (h) *Stability*: Flash-point, 21.2 °C (open cup); temperatures above 26.6 °C and prolonged exposure to oxygen lead to discolouration and gum formation (Lewis, 1993)
- (i) *Conversion factor*: $\text{mg/m}^3 = 4.42 \times \text{ppm}^a$

1.1.4 Technical products and impurities

4-Vinylcyclohexene is available as a commercial product with the following specifications: purity, 97 wt% min.; 1,5-cyclooctadiene, 3 wt% max.; water, 200 ppm max.; and *tert*-butylcatechol (inhibitor), 25–200 ppm (DuPont Chemicals, undated). It is also available in research quantities at a purity of 97–99%, inhibited with 50 ppm *para-tert*-butylcatechol (Aldrich Chemical Co., 1992). Trace quantities of 1,5,9-cyclododecatriene and 1,2-divinylcyclobutane have been detected in commercial 4-vinylcyclohexene (Miller, 1978). The commercial product, a dimer of 1,3-butadiene, is not considered to contain significant quantities of 1,3-butadiene.

1.1.5 Analysis

4-Vinylcyclohexene can be monitored in workplace air by gas chromatography with flame ionization detection at levels down to 1 ppm (4.4 mg/m^3) (Bianchi & Muccioli, 1981). Low levels were detected by collecting large volumes of air (120 L) on multiple charcoal tubes, desorbing with trichlorofluoromethane and analysing by gas chromatography–mass spectrometry (Cocheo *et al.*, 1983).

4-Vinylcyclohexene has been determined by gas chromatography–mass spectrometry in polymer products after extraction with dimethylformamide and in food after extraction with hexane (Tan *et al.*, 1989).

1.2 Production and use

1.2.1 Production

4-Vinylcyclohexene is isolated as a by-product of other production processes involving 1,3-butadiene (see IARC, 1992), including the production of vinylbornene, dodecane-dioic acid and 1,3-butadiene itself. The 4-vinylcyclohexene isolated in these processes may be sold or converted to the diepoxide, or may be recycled for further use within the production facility (Chemical Manufacturers Association, 1991).

4-Vinylcyclohexene is prepared commercially by catalytic dimerization of 1,3-butadiene at 110–425 °C and 1.3–100 MPa. The catalysts used are typically silicon carbide and copper or chromium salts (Kirshenbaum, 1978).

No data were available to the Working Group on production volume.

^aCalculated from: $\text{mg/m}^3 = (\text{relative molecular mass}/24.45) \times \text{ppm}$, assuming normal temperature (25 °C) and pressure (101.3 kPa)

1.2.2 Use

4-Vinylcyclohexene has been used as an intermediate for producing flame retardants, flavours and fragrances; in the manufacture of polyolefins; as a solvent; and in the manufacture of special chemicals such as the diepoxide (see monograph, p. 361) (DuPont Chemicals, undated).

1.3 Occurrence

1.3.1 Natural occurrence

4-Vinylcyclohexene is not known to occur as a natural product.

1.3.2 Occupational exposure

Industrial settings in which workers are potentially exposed include the production of 1,3-butadiene, 4-vinylcyclohexene and 4-vinylcyclohexene diepoxide; production of butadiene-based rubber; rubber vulcanization in the manufacture of shoe soles, tyres and other rubber products; extrusion of electrical cable insulation; flame retardant manufacture; insecticide manufacture; and plasticizer manufacture (Chemical Manufacturers Association, 1991; US National Library of Medicine, 1993). The use of closed vessels in the manufacture and use of 4-vinylcyclohexene in chemical processes limits potential exposure, except for accidental spills and leaks.

Short-term (30-min) area samples taken in three factories of the Italian rubber manufacturing industry contained 0.03–0.21 mg/m³ in the vulcanization area of a shoe-sole factory, 0–0.003 mg/m³ in the extrusion area of a tyre retreading factory and 0–0.01 mg/m³ in the extrusion area of an electrical cable insulation plant (Cocheo *et al.*, 1983). In the USA, the air concentrations of 4-vinylcyclohexene in a tyre curing room ranged from 54.4 to 97.7 ppb (0.24–0.43 mg/m³) (Rappaport & Fraser, 1977).

Full-shift time-weighted average air concentrations of 4-vinylcyclohexene measured in various US industrial sectors are summarized in Table 1. The highest levels were found during vinylnorbornene production (< 0.04–5.3 mg/m³) for supervisors, process operators and maintenance workers and during styrene–butadiene/polybutadiene rubber production (< 0.01–5.3 mg/m³) for polymerization process operators, maintenance workers and supervisors.

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (1991, 1992, 1993a,b and Anon., 1991) proposed an 8-h time-weighted average threshold limit value for 4-vinylcyclohexene of 0.4 mg/m³ in 1990 and established that level in 1992. They also classified 4-vinylcyclohexene as a suspected human carcinogen (A2).

2. Studies of Cancer in Humans

No data were available to the Working Group.

Table 1. Air concentrations of 4-vinylcyclohexene in personal samples taken in various industries in the USA

Industry	Operation/process	No. of samples	Air concentration (range; mg/m ³)
Vinylbornene production	Process operator	59	[< 0.04–5.3]
	Waste treatment	21	[< 0.04–0.58]
	Supervisor	2	[4.4]
	Maintenance	12	[0.18–4.9]
Epoxidation	Process operator	10	[0.36–0.4]
	Laboratory technician	5	[0.35]
	Maintenance	4	[≤ 0.04]
Butadiene production	Various	110	[< 0.18]
Styrene-butadiene and poly-butadiene rubber production	Polymer operator	110	< 0.01–5.1
	Recovery operator	49	< 0.01–0.7
	Finishing operator	41	< 0.01–0.4
	Laboratory technician	75	< 0.01–1.9
	Maintenance	89	< 0.01–3.0
	Waste treatment	16	< 0.01–0.6
	Supervisor	21	< 0.01–5.3
	Tank farm generator	10	< 0.01–0.09
Tyre manufacture	Tyre building	12	< 0.01–0.07
	Tyre curing	12	< 0.01–0.07

From Chemical Manufacturers Association (1991); year of measurement unspecified

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 Mouse

Groups of 50 male and 50 female B6C3F1 mice, eight weeks old, were administered 0 (control), 200 or 400 mg/kg bw 4-vinylcyclohexene (purity, > 98%; impurities in two lots of test chemical included 0.01% butylated hydroxytoluene in one and 0.005% *tert*-butylcatechol in the other, which had been added as inhibitors of peroxide formation) in corn oil by gastric intubation on five days per week for 103 weeks. Body weights and survival were decreased in both males (control, 37/50; low-dose, 39/50; high-dose, 7/50) and females (40/50, 39/50, 17/50, respectively) in the high-dose groups. Female mice showed a significant treatment-related increase in the incidence of granulosa-cell tumours of the ovary (control, 1/49; low-dose, 10/48; high-dose 13/47) and of mixed tumours composed of epithelial and granulosa cells (0/49, 25/48, 11/47, respectively) of the ovary ($p < 0.001$; incidental tumour trend test). The incidences of granulosa-cell hyperplasia and tubular-cell hyperplasia of the ovary were also increased in treated females. There was a significantly ($p = 0.027$; incidental tumour trend test) increased incidence of adrenal subcapsular adenoma in treated female mice (control, 0/50; low-dose, 3/49; high-dose, 4/48). After adjustment for mortality, the incidences of lymphoma (control, 4/50; low-dose, 7/50; high-dose, 5/50; $p = 0.01$; incidental

tumour trend test; $p = 0.001$ incidental pair-wise test for high dose *versus* control) and of alveolar-bronchiolar adenoma or carcinoma (control, 4/49; low-dose, 11/50; high-dose, 4/50; $p = 0.047$; incidental tumour trend test) were slightly increased in treated males (US National Toxicology Program, 1986; Collins *et al.*, 1987).

3.1.2 Rat

Groups of 50 male and 50 female Fischer 344/N rats, seven weeks old, were administered 0 (control), 200 or 400 mg/kg bw 4-vinylcyclohexene (purity, as above) in corn oil by gastric intubation on five days per week for 103 weeks. Body weights were reduced in males given the high dose, and survival was reduced in treated males (control, 33/50; low-dose, 13/50; high-dose, 5/50) and females (40/50, 28/50, 13/50, respectively). The incidence of squamous-cell papilloma or carcinoma of the skin was increased in high-dose males (control, 0/50; low-dose, 1/50; high-dose, 4/50; $p = 0.001$, life table pair-wise comparison of high-dose with controls). The incidence of clitoral gland adenoma or squamous-cell carcinoma was increased in low-dose females (control, 1/50; low-dose, 5/50; high-dose, 0/49; $p = 0.04$, incidental tumour test, pairwise comparison of low-dose with controls) (US National Toxicology Program, 1986; Collins *et al.*, 1987). [The Working Group noted the poor survival.]

3.2 Skin application

Mouse: A group of 30 male Swiss ICR/HA mice, eight weeks old, received skin applications of 45 mg 4-vinylcyclohexene (purified by removing autooxidation products with ferrous sulfate) in 0.1 ml of a 50% solution of benzene three times per week for life (approximately 100 mg of solution per application). The median survival time was 375 days. Five squamous-cell papillomas and one squamous-cell carcinoma of the skin occurred, while the incidence of skin tumours in a control group of 150 mice treated with benzene was 10 papillomas and one carcinoma [$p = 0.04$, Fisher's exact test] (Van Duuren *et al.*, 1963). [The Working Group noted the carcinogenic potential of the vehicle.]

A group of 30 male Swiss ICR/HA mice [age unspecified] received skin applications of 9 mg 4-vinylcyclohexene [purity not specified but stated to be 'oxygen-free'] in 0.1 ml of a 50% solution of benzene, three times a week for life. The median survival was 565 days; no skin tumour occurred (Van Duuren, 1965). [The Working Group noted the low dose used.]

3.3 Carcinogenicity of metabolites

See the monograph on 4-vinylcyclohexene diepoxide.

4. Other Data Relevant for 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 single dose of 400 mg/kg bw ^{14}C -4-vinylcyclohexene given by oral gavage in corn oil to fasted female B6C3F1 mice and Fischer 344 rats resulted in peak blood levels of about 100 nmol/ml between 1 and 2 h after administration. Mice eliminated 95% of the radioactivity within 24 h, whereas rats required 48 h. The main routes of excretion of the radioactivity were the urine (50–60%) and expired air (30–40%). The concentration of 4-vinylcyclohexene was highest in adipose tissue (about 5 $\mu\text{mol/g}$), which was about 10 times higher than that in tissues such as liver, skin and ovary (Smith *et al.*, 1990a).

4-Vinylcyclohexene is metabolized mainly to 4-vinyl-1,2-epoxycyclohexane. A peak blood level of 41 nmol/ml of the epoxide was detected 1–2 h after intraperitoneal injection of 800 mg/kg 4-vinylcyclohexene in female mice. In female Fischer 344 rats, the epoxide blood level was never above the limit of detection of 2.5 nmol/ml. 4-Epoxyethylcyclohexene was not detected in the blood of either species at the same limit of detection (Smith *et al.*, 1990a).

Microsomal mixed-function oxidases from the livers of Wistar rats and Swiss mice metabolize 4-vinylcyclohexene (1; see Fig. 1) to 4-vinyl-1,2-epoxycyclohexane (2), 4-epoxyethylcyclohexene (3) and traces of 4-epoxyethyl-1,2-epoxycyclohexane (4-vinylcyclohexene diepoxide) (4) (see monograph, p. 361). These epoxides are further hydrolysed by epoxide hydrolase to the corresponding diols: 4-vinylcyclohexane-1,2-diol (5), 4-dihydroxyethylcyclohexene (6) and possibly 4-epoxyethylcyclohexane-1,2-diol (7). The last two metabolites may be further metabolized to 4-dihydroxyethyl-1,2-epoxycyclohexane (8) and the tetrol 4-dihydroxyethylcyclohexane-1,2-diol (Gervasi *et al.*, 1981; Watabe *et al.*, 1981).

4.1.3 *Comparison of humans and animals*

Liver microsomes from B6C3F1 mice formed 4-vinyl-1,2-epoxycyclohexane 13 times faster than microsomes from humans and six times faster than those from Fischer 344 rats. The rate of 4-vinylcyclohexene epoxidation by hepatic microsomes derived from men and women was similar. The rate of formation of 4-epoxyethylcyclohexene was about six times lower than that of 4-vinyl-1,2-epoxycyclohexane in humans (Smith & Sipes, 1991).

4.2 Toxic effects

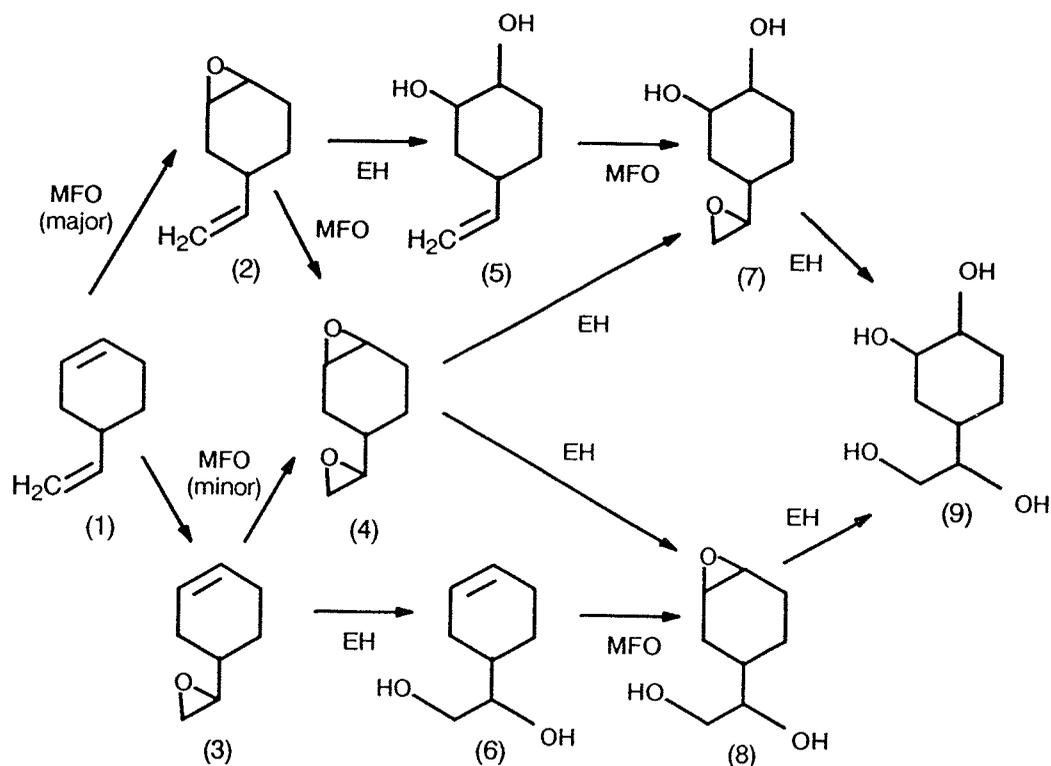
4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

B6C3F1 mice were administered 0, 75, 150, 300, 600 or 1200 mg/kg bw 4-vinylcyclohexene and Fischer 344 rats 0, 50, 100, 200, 400 or 800 mg/kg bw, daily by oral gavage in corn oil on five days per week for 13 weeks. Extensive mortality was observed in the mice treated with 1200 mg/kg, and the final body weights of male rats administered ≥ 400 mg/kg, of female rats administered 800 mg/kg and of female mice receiving 600 mg/kg were reduced. Compound-related histopathological effects included hyaline droplet degeneration of the proximal convoluted tubules of the kidney in treated male rats and a reduction in the number

Fig. 1. Possible pathways for the metabolism of 4-vinylcyclohexene by hepatic microsomes



From Gervasi *et al.* (1981); EH, epoxide hydrolase; MFO, mixed-function oxidases
 (1) 4-Vinylcyclohexene; (2) 4-vinyl-1,2-epoxycyclohexane; (3) 4-epoxyethylcyclohexene;
 (4) 4-epoxyethyl-1,2-epoxycyclohexane (4-vinylcyclohexene diepoxide); (5) 4-vinylcyclohexane-1,2-diol; (6) 4-dihydroxyethylcyclohexene; (7) 4-epoxyethylcyclohexane-1,2-diol; (8) 4-dihydroxyethyl-1,2-epoxycyclohexane; (9) 4-dihydroxyethylcyclohexane-1,2-diol

of primary follicles and mature graafian follicles in the ovaries of female mice receiving 1200 mg/kg. No compound-related gross or histopathological effect was evident in treated female rats or in male mice in the 13-week study (Collins & Manus, 1987).

4-Vinylcyclohexene and its epoxides were compared with respect to the dose required to reduce the number of oocytes to 50% that of controls (ED_{50}). After intraperitoneal injection to female B6C3F1 mice, the ED_{50} doses were 2.7 mmol/kg bw for 4-vinylcyclohexene, 0.5 mmol/kg bw for 4-vinyl-1,2-epoxycyclohexane, 0.7 mmol/kg bw for 4-epoxyethylcyclohexene and 0.2 mmol/kg bw for 4-vinylcyclohexene diepoxide. In female Fischer 344 rats, the ED_{50} dose was > 7.4 mmol/kg bw for 4-vinylcyclohexene, 1.4 mmol/kg bw for the 1,2-epoxide and 0.4 mmol/kg bw for the diepoxide (Smith *et al.*, 1990b).

4-Vinylcyclohexene was administered on five days a week by oral gavage to Fischer 344 rats and B6C3F1 mice in a 90-day toxicity study and in a two-year carcinogenicity study at 200 and 400 mg/kg bw (US National Toxicology Program, 1986). In the 90-day study, ovarian atrophy was seen in female mice but no significant microscopic lesions were seen in rats. After two years of exposure, 4-vinylcyclohexene produced ovarian hyperplasia and neoplasia

in mice but not in rats. The findings were interpreted as indicating a relationship between previous ovarian toxicity and subsequent ovarian neoplasia (Maronpot, 1987).

As reported in an abstract, DeMerrell *et al.* (1992) treated groups of eight male B6C3F1 mice with 0 or 800 mg/kg bw 4-vinylcyclohexene intraperitoneally daily for 30 days and killed them one day later. There was no effect on testicular weight or histological appearance or on plasma follicle-stimulating hormone level. Similarly, the reduction in small and growing follicles induced by administration of 650 mg/kg bw [6.0 mmol/kg] 4-vinylcyclohexene per day intraperitoneally for 30 days to groups of 15 B6C3F1 mice was not accompanied by any change in plasma follicle-stimulating hormone levels (Hooser *et al.*, 1993).

4.3 Reproductive and prenatal effects

4.3.1 Humans

No data were available to the Working Group.

4.3.2 Experimental systems

Grizzle *et al.* (1994) treated Swiss CD-1 mice with 4-vinylcyclohexene in corn oil at 0 (40 males and 40 females), 100, 250 or 500 mg/kg bw (20 males and 20 females in each treatment group) orally by gavage daily in a continuous breeding study for 14 weeks of cohabitation. No effect was observed on number of litters, pups per litter or percentage born alive. The progeny of the control and 500-mg/kg groups were then mated and their respective treatments continued without a break. No adverse effects of treatment on reproductive performance were observed. In the F1 males treated with 500 mg/kg bw, testicular spermatid count was decreased by 17%, with no effect on epididymal sperm number or testicular weight. In the treated F1 females, there were significantly reduced numbers of primordial (33%), growing (55%) and antral (33%) oocytes. Ovarian weight and oestrus cycles were unaffected.

4.4 Genetic and related effects (see also Table 2 and Appendices 1 and 2)

4.4.1 Humans

No data were available to the Working Group.

4.4.2 Experimental systems

4-Vinylcyclohexene was not mutagenic to *Salmonella typhimurium*.

4-Vinylcyclohexene diepoxide and its metabolites are described in a separate monograph. The two mono-epoxides, 4-vinyl-1,2-epoxycyclohexane and 4-epoxyethylcyclohexene were not mutagenic to *S. typhimurium* in the absence of an exogenous metabolic system. 4-Vinyl-1,2-epoxycyclohexane was also not mutagenic to cultured Chinese hamster lung V79 cells at the *hprt* locus, but did cause micronuclei and aberrant anaphases in these cells.

Table 2. Genetic and related effects of 4-vinylcyclohexene and its metabolites

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	500.0000	Zeiger <i>et al.</i> (1987)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	500.0000	Zeiger <i>et al.</i> (1987)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	500.0000	Zeiger <i>et al.</i> (1987)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	500.0000	Zeiger <i>et al.</i> (1987)
4-Vinyl-1,2-epoxycyclohexane				
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	0	620.0000	Watabe <i>et al.</i> (1980)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	0	400.0000 ^c	Turchi <i>et al.</i> (1981)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	0	1200.0000	Watabe <i>et al.</i> (1980)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	0	1200.0000	Watabe <i>et al.</i> (1980)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	0	1200.0000	Watabe <i>et al.</i> (1980)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	0	1200.0000	Watabe <i>et al.</i> (1980)
G9H, Gene mutation, Chinese hamster lung V79 cells, <i>hprt</i> locus	-	0	2500.0000	Turchi <i>et al.</i> (1981)
MIA, Micronucleus formation ^d , Chinese hamster lung V79 cells	+	0	300.0000	Turchi <i>et al.</i> (1981)
4-Epoxyethylcyclohexene				
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	0	620.0000	Watabe <i>et al.</i> (1980)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	0	1200.0000	Watabe <i>et al.</i> (1980)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	0	1200.0000	Watabe <i>et al.</i> (1980)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	0	1200.0000	Watabe <i>et al.</i> (1980)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	0	1200.0000	Watabe <i>et al.</i> (1980)

^a +, positive; (+), weakly positive; -, negative; 0, not tested; ?, inconclusive (variable responses in several experiments within an adequate study)

^bIn-vitro tests, µg/ml; in-vivo tests, mg/kg bw

^cOvernight incubation with bacteria before plating, 0.1 ml sample, but also negative in standard test

^dAberrant anaphases were observed at the same dose.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

4-Vinylcyclohexene is produced by catalytic dimerization of 1,3-butadiene. 4-Vinylcyclohexene has been used as a chemical intermediate for production of flame retardants, flavours and fragrances, in the manufacture of polyolefins, as a solvent and in the manufacture of its diepoxide. Low levels of occupational exposure have been measured during the production and use of 1,3-butadiene.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

4-Vinylcyclohexene was tested for carcinogenicity in one experiment in mice and in one experiment in rats by gastric intubation and in two skin application studies in mice. Administration of 4-vinylcyclohexene by gastric intubation produced granulosa-cell and mixed tumours of the ovary and adrenal subcapsular tumours in female mice. In male mice, there was an increase in the incidence of lymphoma and of lung tumours. Following gastric intubation in rats, increased incidences of squamous-cell tumours of the skin in males and of clitoral gland tumours in females were observed. The studies by skin application were inadequate for evaluation.

5.4 Other relevant data

4-Vinylcyclohexene is distributed mainly to adipose tissue in rodents. The ethylene carbons are eliminated mainly in urine and expired air. Metabolism primarily involves oxidation to 4-vinylcyclohexane-1,2-epoxide, which is formed 13 times faster by liver microsomes from mice and twice as fast by those from rats than by human microsomes. 4-Vinyl-1,2-epoxycyclohexane, 4-epoxyethylcyclohexene and, particularly, the diepoxide are more toxic to mouse oocytes than 4-vinylcyclohexene itself. Treatment with 4-vinylcyclohexene decreased the number of oocytes in mice but not in rats. The difference seemed to be due to the reduced ability of the rat to metabolize 4-vinylcyclohexene to epoxides.

No data were available on the genetic and related effects of 4-vinylcyclohexene in humans.

4-Vinylcyclohexene and its mono-epoxide metabolites were not mutagenic to *Salmonella typhimurium*. 4-Vinyl-1,2-epoxycyclohexane induced micronuclei but not *hprt* mutations in cultured Chinese hamster cells.

5.5 Evaluation¹

There is *inadequate evidence* in humans for the carcinogenicity of 4-vinylcyclohexene. There is *sufficient evidence* in experimental animals for the carcinogenicity of 4-vinylcyclohexene.

Overall evaluation

4-Vinylcyclohexene is *possibly carcinogenic to humans (Group 2B)*.

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¹For definition of the italicized terms, see Preamble, pp. 27-30.

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4-VINYLCYCLOHEXENE DIEPOXIDE

This substance was considered by a previous Working Group, in February 1976 (IARC, 1976), under the name 1-epoxyethyl-3,4-epoxycyclohexane. 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.: 106-87-6

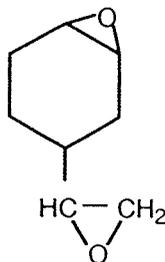
Replaced CAS Reg. No.: 25550-49-6

Chem. Abstr. Name: 3-Oxiranyl-7-oxabicyclo[4.1.0]heptane

IUPAC Systematic Name: 3-(Epoxyethyl)-7-oxabicyclo[4.1.0]heptane

Synonyms: 1,2-Epoxy-4-(epoxyethyl)cyclohexane; 1-(epoxyethyl)-3,4-epoxycyclohexane; 3-(1,2-epoxyethyl)-7-oxabicyclo[4.1.0]heptane; vinylcyclohexene diepoxide; 4-vinyl-1-cyclohexene diepoxide; 4-vinyl-1,2-cyclohexene diepoxide; 4-vinylcyclohexene dioxide; 1-vinyl-3-cyclohexene dioxide; 4-vinyl-1-cyclohexene dioxide

1.1.2 Structural and molecular formulae and relative molecular mass



$C_8H_{12}O_2$

Relative molecular mass: 140.18

1.1.3 Chemical and physical properties of the pure substance

(a) *Description:* Colourless liquid (American Conference of Governmental Industrial Hygienists, 1986)

(b) *Boiling-point:* 227 °C (Lide, 1991)

- (c) *Melting-point*: -108.9 °C (freezing-point) (American Conference of Governmental Industrial Hygienists, 1986)
- (d) *Density*: 1.0986 at 20 °C/20 °C (Lide, 1991)
- (e) *Spectroscopy data*: Infrared, ultraviolet and nuclear magnetic resonance spectral data have been reported (Weast & Astle, 1985; US National Toxicology Program, 1989).
- (f) *Solubility*: Soluble in water (Lide, 1991)
- (g) *Volatility*: 0.1 mm Hg [13 Pa] at 20 °C (American Conference of Governmental Industrial Hygienists, 1986)
- (h) *Conversion factor*: $\text{mg/m}^3 = 5.73 \times \text{ppm}^a$

1.1.4 *Technical products and impurities*

No data were available to the Working Group.

1.1.5 *Analysis*

No data were available to the Working Group.

1.2 **Production and use**

1.2.1 *Production*

4-Vinylcyclohexene diepoxide is manufactured by epoxidation of 4-vinylcyclohexene with peroxyacetic acid in an inert solvent (Wallace, 1964). Production of 4-vinylcyclohexene diepoxide provides a commercial outlet for 4-vinylcyclohexene (see monograph, p. 347) recovered as a by-product during production of vinylnorbornene (Chemical Manufacturers Association, 1991).

1.2.2 *Use*

4-Vinylcyclohexene diepoxide is used as a reactive diluent for other diepoxides and for epoxy resins derived from bisphenol A and epichlorohydrin (Union Carbide Corp., 1964). One of the applications is in embedding biological tissues in epoxy polymer for electron microscopy (Fluka Chemika-BioChemika, 1993).

1.3 **Occurrence**

1.3.1 *Natural occurrence*

4-Vinylcyclohexene diepoxide is not known to occur as a natural product.

1.3.2 *Occupational exposure*

Little information exists on the number of workers who may be exposed to 4-vinylcyclohexene diepoxide. The National Occupational Exposure Survey conducted by the National

^aCalculated from: $\text{mg/m}^3 = (\text{molecular weight}/24.45) \times \text{ppm}$, assuming normal temperature (25 °C) and pressure (101.3 kPa)

Institute for Occupational Safety and Health between 1981 and 1983 indicated that 6200 US employees were potentially exposed to a product containing 4-vinylcyclohexene diepoxide (US National Institute for Occupational Safety and Health, 1993). The estimate is based on a survey of US companies and did not involve measurements of actual exposures. Industry sectors in which potential exposure was identified include electrical lighting and wiring equipment manufacture, use, manufacture and maintenance of aircraft, missiles and space vehicles, and manufacture of measurement and control devices. Exposures in these sectors probably reflect the use of epoxy resins and glues.

There are no published data on exposure to 4-vinylcyclohexene diepoxide. Exposures may occur during manufacture of 4-vinylcyclohexene diepoxide from 4-vinylcyclohexene or in production and use of epoxy-based polyglycols and resins. Laboratory workers may be exposed during preparation of epoxy resin tissue embedding agents for electron microscopy (Ringo *et al.*, 1982).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for 4-vinylcyclohexene diepoxide in 12 countries are presented in Table 1.

Table 1. Occupational exposure limits and guidelines for 4-vinylcyclohexene diepoxide

Country	Year	Concentration (mg/m ³)	Interpretation
Argentina	1991	60	TWA; potential carcinogen; skin
Australia	1983	40	TWA; suspected human carcinogen; skin
Belgium	1984	60	TWA; probable human carcinogen; skin
Canada	1986	60	TWA
Denmark	1988	60	TWA
Finland	1989	60 120	TWA; suspected human carcinogen STEL
Germany	1993	None	Animal carcinogen
Mexico	1984	60	TWA
Netherlands	1986	60	TWA
Switzerland	1987	60	TWA; potential carcinogen
United Kingdom	1992	60	TWA; maximum exposure limit; under review
USA			
ACGIH	1994	57	TWA; suspected human carcinogen; skin
NIOSH	1992	60	Potential carcinogen

From Arbejdstilsynet (1988); US National Institute for Occupational Safety and health (1992); American Conference of Governmental Industrial Hygienists (1993); Deutsche Forschungsgemeinschaft (1993); ILO (1993); Työministeriö (1993); UNEP (1993)

TWA, time-weighted average; STEL, short-term exposure limit; skin, absorption through the skin may be a significant source of exposure.

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3.1 Skin application

3.1.1 Mouse

A group of 20 male albino mice [strain and age unspecified] received a daily skin application of 16 mg of a commercial sample (contaminated with water-insoluble material) of 4-vinylcyclohexene diepoxide in acetone on five days a week for 12 months. The last mouse died 21 months after the start of the treatment. Skin tumours occurred in 11/20 mice; nine were reported to be squamous-cell carcinomas and/or sarcomas (Hendry *et al.*, 1951). [The Working Group noted that no data on controls were provided.]

A group of 30 male Swiss ICR/Ha mice, eight weeks old, received skin applications of a 10% solution of 4-vinylcyclohexene diepoxide [purity unspecified] in benzene three times per week for life. The mean survival for the group was 326 days. Skin tumours occurred in 14 mice; nine were squamous-cell carcinomas. In a group of 150 controls painted with benzene, skin tumours occurred in 11 mice; one was a squamous-cell carcinoma. Of 207 untreated mice, 13 had skin tumours, one of which was a squamous-cell carcinoma [$p < 0.001$] (Van Duuren *et al.*, 1963). [The Working Group noted the carcinogenic potential of the vehicle.]

A group of 30–40 [exact number unspecified] C3H mice [sex unspecified], 13 weeks old, received skin applications of a 10% solution of 4-vinylcyclohexene diepoxide [purity unspecified] in acetone three times per week for 21 months. Of 18 mice that survived for 12 or more months, three developed skin tumours; one tumour was malignant (Weil *et al.*, 1963). [The Working Group noted the limited reporting and that no data on controls were provided.]

Groups of 60 male and 60 female B6C3F1 mice, eight to nine weeks old, each received daily skin applications of 0 (control), 2.5, 5 or 10 mg 4-vinylcyclohexene diepoxide (purity, approximately 97%) in 0.1 ml acetone on five days per week for 103 weeks. High-dose females were treated for only 85 weeks because of toxicity, and all high-dose male mice had died by week 83. At 15 months, 10 mice from each group were killed and examined; all other survivors were killed at 113 weeks of age, except for 12 high-dose females which were killed at 93 weeks. Survival at the end of the experiment was 38/50, 35/50, 4/50 and 30/50, 31/50, 15/50 for the control, low- and mid-dose groups of males and females, respectively. There was a significant increase in the incidence of squamous-cell carcinoma of the skin in males (control, 0/50; low-dose, 14/50; mid-dose, 39/50; high-dose, 42/50) and females (0/50, 6/50, 37/50, 41/50, respectively) ($p < 0.001$; logistic regression trend tests). Treatment-related non-neoplastic lesions of the skin in mice of each sex included necrotizing inflammation, hyperkeratosis and acanthosis. In female mice, there were significant increases in the incidences of granulosa-cell tumours (control, 0/50; low-dose, 0/49; mid-dose, 7/49;

high-dose, 12/50) and benign mixed tumours (0/50, 0/49, 11/49, 6/50, respectively) of the ovary ($p < 0.001$, logistic regression trend tests). Non-neoplastic lesions in the ovary included a treatment-related increase in follicular atrophy and tubular hyperplasia. The incidence of alveolar-bronchiolar adenoma or carcinoma of the lung was significantly increased in mid-dose females (control, 4/50; low-dose, 9/50; mid-dose, 11/50; high-dose, 7/50; $p = 0.032$, logistic regression test) (US National Toxicology Program, 1989; Chhabra *et al.*, 1990a).

3.1.2 Rat

Groups of 60 male and 60 female Fischer 344/N rats, seven to eight weeks old, received daily skin applications of 0 (control), 15 or 30 mg/animal 4-vinylcyclohexene diepoxide (purity, approximately 97%) in 0.3 ml acetone on five days per week for 105 weeks. At 15 months, 10 rats from each group were killed; all other survivors were killed at 114 weeks of age. Body weights were reduced (9–14%) in the high-dose male and female rats in comparison with controls. Survival was reduced in all groups of males (control, 7/50; low-dose, 8/50; high-dose, 4/50) and was significantly reduced ($p = 0.005$; life-table test) in high-dose females (27/50, 23/50, 15/50, respectively). There was a significant increase ($p < 0.001$; logistic regression trend test) in the incidence of squamous-cell carcinoma of the skin in treated males (control, 0/50; low-dose, 33/50; high-dose, 36/50) and females (0/50, 16/50, 34/50, respectively). The incidence of squamous-cell papillomas was increased in male rats (control, 0/50; low-dose, 3/50; high-dose, 6/50; $p = 0.015$, logistic regression, pair-wise comparison of high-dose to controls); that of basal-cell adenoma or basal-cell carcinoma was increased in males (0/50, 1/50, 6/50, respectively; $p = 0.011$, logistic regression pair-wise comparison); and that of basal-cell carcinoma was increased in females (0/50, 3/50, 4/50, respectively; $p = 0.015$, logistic regression trend test). Treatment-related non-neoplastic lesions of the skin in rats of each sex included sebaceous gland hypertrophy and acanthosis (US National Toxicology Program, 1989; Chhabra *et al.*, 1990a).

4. Other Data Relevant for 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 4-vinylcyclohexene diepoxide includes hydrolysis of the epoxide groups to the respective glycols or conjugation with glutathione. Enzymatic formation of 4-epoxyethylcyclohexane-1,2-diol, 4-dihydroxyethyl-1,2-epoxycyclohexane and 4-dihydroxyethylcyclohexane-1,2-diol by epoxide hydrolase in rabbit liver microsomes showed V_{\max} values of 4.7, 10.2 and 1.1 nmol/mg protein per min, respectively. The enzymatic rates of

hydrolysis of 4-epoxyethylcyclohexane-1,2-diol and 4-dihydroxyethyl-1,2-epoxycyclohexane to the tetrol had V_{\max} values of 10.0 and 7.6 nmol/mg protein per min, respectively. Non-enzymatic hydrolysis was less important (Watabe & Sawahata, 1976).

Elimination of 4-vinylcyclohexene diepoxide occurred mainly via the urine. The tetrol was the major metabolite in rat urine, whereas the major metabolites in mouse urine appear to be polar conjugates (Salyers *et al.*, 1993).

4.2 Toxic effects

4.2.1 Humans

A case report described allergic contact dermatitis in a female electron microscopist. Sensitization was detected three months after she started using 4-vinylcyclohexene diepoxide in the laboratory. Disposable latex and polyvinyl chloride gloves were found to be permeable to 4-vinylcyclohexene diepoxide, so that dermatitis was elicited after sensitization (Dannaker, 1988).

4.2.2 Experimental systems

4-Vinylcyclohexene diepoxide exerts both local and systemic toxicity. Fourteen-day dermal exposure of male B6C3F1 mice to 10 mg/mouse per day resulted in epidermal hyperplasia and hyperkeratosis. At 20 mg/mouse per day, 80% died. Fischer 344 rats tolerated five times higher concentrations of the compound per skin surface area (US National Toxicology Program, 1989).

Groups of 10 female and 10 male Fischer 344 rats were treated with skin applications of 0.1 ml 4-vinylcyclohexene diepoxide in acetone (3.75–60 mg/rat per day) on five days per week for 13 weeks. As part of the same study, groups of 10 male and 10 female B6C3F1 mice were treated similarly but with doses of 0.625–10 mg/mouse per day. Rats given the highest dose had reduced body weight gain and compound dose-related redness and ulceration at the application site, diffuse sebaceous gland hyperplasia, and acanthosis and hyperkeratosis of the stratified squamous epithelium. Thymus weights were reduced in male rats. Mice showed acanthosis of the stratified squamous epithelium at the application site. Liver and kidney weights were increased in both male and female mice. In a 13-week gavage study with doses of 62.5–1000 mg/kg bw on five days per week, mice showed forestomach hyperplasia and hyperkeratosis; ovarian atrophy was seen in females and degeneration of the germinal epithelium in testis of males at dose levels of ≥ 250 mg/kg. In rats, hyperplasia and hyperkeratosis of the forestomach were recorded at ≥ 125 mg/kg, and renal tubular-cell degeneration and/or regeneration was recorded at ≥ 500 mg/kg (Chhabra *et al.*, 1990b).

Liver and kidney (weight increase), thymus (weight decrease) and bone marrow (hypoplasia) were additional target tissues for the toxicity of 4-vinylcyclohexene diepoxide. Most of the effects showed a strongly nonlinear dose-response relationship. In a comparative study of the ovarian toxicity of 4-vinylcyclohexene diepoxide and some related compounds over 30 days, doses required to reduce the small oocyte counts were 0.2 and 0.4 mmol/kg per day for B6C3F1 mice and Fischer 344 rats, respectively. These values are much lower and closer for the two species than the corresponding values for 4-vinylcyclohexene, which were 2.7 and > 7.4 (highest dose given) mmol/kg per day (Smith *et al.*, 1990).

4.3 Reproductive and prenatal effects

No data were available to the Working Group.

4.4 Genetic and related effects (see also Table 2 and Appendices 1 and 2)

4.4.1 Humans

No data were available to the Working Group.

4.4.2 Experimental systems

4-Vinylcyclohexene diepoxide was mutagenic to *Salmonella typhimurium* and to *Saccharomyces cerevisiae*. It also caused gene conversion and mitotic crossing-over in *S. cerevisiae*.

Micronuclei were induced by the compound in cells of two plant species, *Allium cepa* and *Vicia faba*.

4-Vinylcyclohexene diepoxide induced mutations at both the *hprt* and *tk* loci in cultured mammalian cells. In rodent cell lines, it induced sister chromatid exchange and chromosomal aberrations; micronuclei were not induced by a single high dose.

4-Epoxyethylcyclohexane-1,2-diol, tested in a single study, was not mutagenic to *Salmonella typhimurium*. In the same study, micronuclei but not mutations were induced at a single dose in cultured mammalian cells.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

4-Vinylcyclohexene diepoxide is produced by epoxidation of 4-vinylcyclohexene with peroxyacetic acid. It is used as a reactive diluent for other diepoxides and for epoxy resins. No data are available on levels of occupational exposure to 4-vinylcyclohexene diepoxide.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

4-Vinylcyclohexene diepoxide was tested for carcinogenicity by skin application in three studies in mice and in one study in rats. Skin application of 4-vinylcyclohexene diepoxide produced benign and malignant skin tumours in all studies in mice and in the study in rats. In one study in mice, it also increased the incidences of ovarian and lung tumours in females.

5.4 Other relevant data

4-Vinylcyclohexene diepoxide can be absorbed through the skin of rodents. Higher concentrations tend to be found in the ovary rather than in other organs, and virtually all

Table 2. Genetic and related effects of 4-vinylcyclohexene diepoxide

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	550.0000	Murray & Cummins (1979)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation (spot test)	+	0	5000.0000	Wade <i>et al.</i> (1979)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	(+)	0	125.0000	El-Tantawy & Hammock (1980)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	700.0000	Watabe <i>et al.</i> (1980)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	1050.0000	Frantz & Sinsheimer (1981)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	140 ^c	Turchi <i>et al.</i> (1981)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	50.0000	Mortelmans <i>et al.</i> (1986)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	0	0.0000	Ringo <i>et al.</i> (1982)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	0	125.0000	El-Tantawy & Hammock (1980)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	0	1050.0000	Frantz & Sinsheimer (1981)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	+	170.0000	Mortelmans <i>et al.</i> (1986)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	0	500.0000	El-Tantawy & Hammock (1980)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	?	(+)	1700.0000	Mortelmans <i>et al.</i> (1986)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation (spot test)	+	0	5000.0000	Wade <i>et al.</i> (1979)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	0	500.0000	El-Tantawy & Hammock (1980)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	+	+	500.0000	Mortelmans <i>et al.</i> (1986)
SCG, <i>Saccharomyces cerevisiae</i> D4, gene conversion	+	0	3500.0000	Bronzetti <i>et al.</i> (1980)
SCH, <i>Saccharomyces cerevisiae</i> D7, mitotic crossing-over	+	0	3500.0000	Bronzetti <i>et al.</i> (1980)
SCR, <i>Saccharomyces cerevisiae</i> D7, reverse mutation	+	0	3500.0000	Bronzetti <i>et al.</i> (1980)
PLI, <i>Allium cepa</i> , micronucleus formation	+	0	700.0000	Ronchi <i>et al.</i> (1986)
PLI, <i>Vicia faba</i> , micronucleus formation	+	0	1400.0000	Ronchi <i>et al.</i> (1986)
G9H, Gene mutation, Chinese hamster V79 lung cells, <i>hprt</i> locus	+	0	140.0000	Gervasi <i>et al.</i> (1981)
G9H, Gene mutation, Chinese hamster V79 lung cells, <i>hprt</i> locus	+	0	700.0000	Turchi <i>et al.</i> (1981)
G5T, Gene mutation, mouse lymphoma L5178 cells, <i>tk</i> locus	+	0	25.0000	McGregor <i>et al.</i> (1988)
SIC, Sister chromatid exchange, Chinese hamster ovary cells	+	+	3.7300	US National Toxicology Program (1989)
MIA, Micronucleus formation, Chinese hamster V79 lung cells	-	0	280.0000	Turchi <i>et al.</i> (1981)

Table 2 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CIC, Chromosomal aberrations, Chinese hamster ovary cells	+	+	37.8000	US National Toxicology Program (1989)
4-Epoxyethyl cyclohexane-1,2-diol				
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	0	1600.0000 ^c	Turchi <i>et al.</i> (1981)
G9H, Gene mutation, Chinese hamster lung V79 cells, <i>hprt</i> locus	-	0	3200.0000	Turchi <i>et al.</i> (1981)
MIA, Micronucleus formation, Chinese hamster V79 lung cells	+	0	320.0000	Turchi <i>et al.</i> (1981)

^a+, positive; (+), weakly positive; -, negative; 0, not tested; ?, inconclusive (variable responses in several experiments within an adequate study)

^bIn-vitro tests, µg/ml; in-vivo tests, mg/kg bw

^cOvernight incubation with the bacteria before plating, 0.1 ml/sample; negative in standard plate test

elimination occurs via the urine. Its metabolism involves hydration to a mixture of glycols and conjugation with glutathione.

4-Vinylcyclohexene diepoxide is locally toxic and, when given orally, causes ovarian degeneration in both mice and rats and testicular degeneration in mice, as well as lesser effects in other organs.

No data were available on the genetic and related effects of 4-vinylcyclohexene diepoxide in humans.

4-Vinylcyclohexene diepoxide induced gene mutation, sister chromatid exchange and chromosomal aberrations but not micronuclei in mammalian cells *in vitro*. It was mutagenic in bacteria and caused gene conversion and mitotic crossing-over in *Saccharomyces cerevisiae*.

A metabolite of 4-vinylcyclohexene diepoxide, 4-epoxyethylcyclohexane-1,2-diol, was not mutagenic to *Salmonella typhimurium*.

5.5 Evaluation¹

There is *inadequate evidence* in humans for the carcinogenicity of 4-vinylcyclohexene diepoxide.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 4-vinylcyclohexene diepoxide.

Overall evaluation

4-Vinylcyclohexene diepoxide *is possibly carcinogenic to humans (Group 2B)*.

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¹For definition of the italicized terms, see Preamble, pp. 27-30.

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VINYL TOLUENE

1. Exposure Data

1.1 Chemical and physical data

Commercial vinyl toluene is a mixture of *meta* and *para* isomers with small amounts of *ortho* isomer. Chemical and physical data are given for the individual isomers and for the commercial mixture, when available.

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 25013-15-4

Replaced CAS Reg. No.: 1321-45-5

Chem. Abstr. Name: Ethenylmethylbenzene

IUPAC Systematic Name: *ar*-Methylstyrene

Synonyms: Methylstyrene; methylvinylbenzene; tolylethylene

Chem. Abstr. Serv. Reg. No.: 611-15-4

Chem. Abstr. Name: 1-Ethenyl-2-methylbenzene

IUPAC Systematic Name: *ortho*-Methylstyrene

Synonyms: 2-Ethenylmethylbenzene; 2-methylstyrene; 1-methyl-2-vinylbenzene; 2-vinyltoluene; *ortho*-vinyltoluene

Chem. Abstr. Serv. Reg. No.: 100-80-1

Chem. Abstr. Name: 1-Ethenyl-3-methylbenzene

IUPAC Systematic Name: *meta*-Methylstyrene

Synonyms: 3-Ethenylmethylbenzene; 3-methylstyrene; 1-methyl-3-vinylbenzene; 3-vinyltoluene; *meta*-vinyltoluene

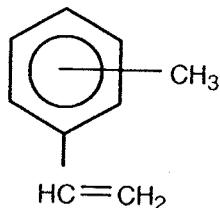
Chem. Abstr. Serv. Reg. No.: 622-97-9

Chem. Abstr. Name: 1-Ethenyl-4-methylbenzene

IUPAC Systematic Name: *para*-Methylstyrene

Synonyms: 4-Ethenylmethylbenzene; 4-methylstyrene; 1-methyl-4-vinylbenzene; 1-*para*-tolylethene; 4-vinyltoluene; *para*-vinyltoluene

1.1.2 Structural and molecular formulae and relative molecular mass



C_9H_{10}

Relative molecular mass: 118.18

1.1.3 Chemical and physical properties of the pure substance

- (a) *Description*: Colourless liquid (Dow Chemical Co., 1991), with a strong disagreeable odour (US National Library of Medicine, 1993)
- (b) *Boiling-point*: 167.7 °C (commercial mixture, *meta* and *para*); 169.8 °C (*ortho*); 171.6 °C (*meta*); 172.8 °C (*para*) (Eller, 1984)
- (c) *Freezing-point*: -77 °C (commercial mixture) (Deltech Corp., 1992)
- (d) *Density*: 0.898 g/ml at 20 °C (commercial mixture, *meta* and *para*); 0.904 g/ml at 20 °C (*ortho*); 0.911 g/ml at 20 °C (*meta* and *para*) (Eller, 1984)
- (e) *Spectroscopy data*: Infrared [D1711 (mixed isomers), 15063 (*meta*), 21205 (*para*)], ultraviolet [4418 (*meta*), 8303 (*para*)], nuclear magnetic resonance and mass [450 (*meta*), 185 (*para*)] spectral data have been reported (US National Toxicology Program, 1990; Sadtler Research Laboratories, 1991; US National Library of Medicine, 1993)
- (f) *Solubility*: Insoluble in water (89 mg/L) (commercial mixture) (Dow Chemical Co., 1991); completely soluble in acetone, benzene, carbon tetrachloride, diethyl ether, ethanol and *n*-heptane (US National Library of Medicine, 1993)
- (g) *Volatility*: Vapour pressure, 1.6 mm Hg [220 Pa] at 25 °C (commercial mixture, *meta* and *para*); 1.8 mm Hg [240 Pa] at 25 °C (*ortho* and *para*); 1.9 mm Hg [260 Pa] at 25 °C (*meta*) (Eller, 1984); 1.1 mm Hg [147 Pa] at 20 °C; relative vapour density (air = 1), 4.08 (commercial mixture) (Dow Chemical Co., 1991)
- (h) *Stability*: Lower explosive limit, 1.1%; polymerizes slowly at room temperature (Deltech Corp., 1991, 1992)
- (i) *Conversion factor*: $\text{mg/m}^3 = 4.83 \times \text{ppm}^a$

1.1.4 Technical products and impurities

Commercial vinyl toluene is primarily a mixture of *meta*- and *para*-vinyl toluene, usually with 56–60% *meta*, 40–45% *para* and 1% *ortho*. It is available as a commercial product with the following characteristics: purity, 99.2 wt% min.; polymer, 25 ppm max.; and *para-tert*-butylcatechol (inhibitor), 10–15 ppm or 45–55 ppm (Dow Chemical Co., 1988, 1989; Deltech Corp., 1991; Dow Chemical Co., 1991; Deltech Corp., 1992).

1.1.5 Analysis

Vinyl toluene is determined in workplace air by packed capillary column gas chromatography with a flame ionization detector. The sample is adsorbed on charcoal and desorbed with carbon disulfide. This method (NIOSH Method 1501) has an estimated limit of detection of 0.001–0.01 mg per sample (Eller, 1984).

^aCalculated from: $\text{mg/m}^3 = (\text{molecular weight}/24.45) \times \text{ppm}$, assuming normal temperature (25 °C) and pressure (101.3 kPa)

1.2 Production and use

1.2.1 Production

Vinyl toluene has been produced commercially in the USA since the late 1940s by the dehydrogenation of *meta*- and *para*-ethyl toluene with zinc oxide catalyst and by catalytic reforming (Kniel *et al.*, 1980; US National Toxicology Program, 1990). In 1992, vinyl toluene (mixed isomers) was produced or distributed in laboratory and larger quantities by seven companies in the USA and one company each in France, Japan, Switzerland and the United Kingdom (Directories Publishing Co., 1994).

1.2.2 Use

Vinyl toluene is a reactive monomer which polymerizes to form a clear, colourless polymer. Like styrene, vinyl toluene can be polymerized by any of the conventional methods of initiation and in the presence of inert materials such as fillers, dyes, solvents, resins, rubbers and plasticizers. Copolymers of vinyl toluene and other monomers such as acrylate, acrylonitrile, 1,3-butadiene, divinylbenzene, methacrylate and maleic anhydride are used in products with widely different physical properties (Dow Chemical Co., 1989; Deltech Corp., 1992).

Vinyl toluene is used in the coatings industry as a modifier for drying oils and oil-modified alkyds. It is also used as a replacement for styrene in unsaturated polyester resins where high-temperature cures and little shrinkage are desired. When used in this way, vinyl toluene contributes a lower vapour pressure at a given temperature and a higher flash-point (Deltech Corp., 1992). As a copolymer with styrene, it is used to increase the operating temperature range of paints, coatings and varnishes (Lewis *et al.*, 1983).

1.3 Occurrence

Vinyl toluene isomers may be released to the environment in wastewater and in atmospheric emissions resulting from its manufacture or use in resin production and plastics (Liepins *et al.*, 1977; Perry *et al.*, 1979; Sandmeyer, 1981). Vinyl toluene isomers may also be released to the environment in engine exhaust (Fleming, 1970; US National Library of Medicine, 1993), wood smoke (Kleindienst *et al.*, 1986) and emissions from the combustion of polyethylene and polystyrene polymers (Hawley-Fedder *et al.*, 1984a,b). Exhaust gas from motor boat engines has been found to pollute waterways with vinyl toluene isomers (Jüttner, 1988). *ortho*-Vinyl toluene has been identified as a biodegradation product of *ortho*-ethyl toluene (Kappeler & Wuhrmann, 1978).

1.3.1 Natural occurrence

ortho-Vinyl toluene has been identified in the essential oil of *Distichlis spicata*, a marsh grass found in Mississippi salt marshes in the USA (Mody *et al.*, 1975).

1.3.2 Occupational exposure

The National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health between 1981 and 1983 indicated that 25 400 employees

were potentially exposed to vinyl toluene in the USA (US National Institute for Occupational Safety and Health, 1993). Of this number 1% were estimated to be exposed to vinyl toluene and 99% to materials containing vinyl toluene. The estimate is based on a survey of US companies and did not involve measurements of actual exposures.

Few data have been published on levels of vinyl toluene vapour in the workplace. It was below detection levels in personal and area samples taken at a plant where polyester resin spray-up, lay-up and moulding processes were used. Vinyl toluene was included in the analysis because of the presence of empty containers indicating its use in the past (Rosensteel, 1979). Similarly, vinyl toluene was not detected (< 0.05 ppm [< 0.24 mg/m³]) in seven full-shift area samples taken in a mine where roof bolting was done with a resin containing vinyl toluene as one of the main ingredients (Cornwell & Stark, 1987).

1.3.3 Air

ortho-Vinyl toluene was identified in indoor air of houses in Washington DC and Chicago, IL, USA (Jarke *et al.*, 1981). Trace levels of vinyl toluene [isomer unspecified] were detected in air samples collected in Nitro, WV, USA (Erickson & Pellizzari, 1978).

Vinyl toluene has been detected in exhaust emissions from spark-ignition engines (*ortho*, *meta* and *para* isomers) (Fleming, 1970; US National Library of Medicine, 1993), wood smoke (*meta* isomer) (Kleindienst *et al.*, 1986), emissions from the incineration of polyethylene and polystyrene polymers (*ortho*, *meta* and *para* isomers) (Hawley-Fedder *et al.*, 1984a,b) and volatile emissions from polychloroprene-based building materials (*ortho* isomer) (Kiselev *et al.*, 1983).

1.3.4 Water

Vinyl toluene [isomer unspecified] was identified in water samples collected from the River Lee in the United Kingdom (Waggott, 1981), and *ortho*, *meta* and *para* isomers were identified in water samples collected in August 1984 from Lake Constance (Germany) after a period of heavy boat traffic. The concentration of *ortho*-vinyl toluene in water samples collected throughout the day varied between 3 and 72 mg/L. Emissions from motor boat engines were identified as the primary source of the volatile organic compounds found in the water (Jüttner, 1988).

ortho-Vinyl toluene was tentatively identified in concentrates of effluents from wastewater treatment plants in Lake Tahoe, Pomona and Orange County, CA, USA (Lucas, 1984). Vinyl toluene [isomer unspecified] was detected in effluents from six of 63 US industrial plants at concentrations ranging from < 10 to > 100 µg/L (Perry *et al.*, 1979).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for vinyl toluene in 18 countries are presented in Table 1.

The US Food and Drug Administration (1993) established regulations for the use of monomers, polymers, copolymers and homopolymers of vinyl toluene (methylstyrene) in products in contact with food, including food packaging adhesives (21 CFR 175.105),

Table 1. Occupational exposure limits and guidelines for vinyl toluene (all isomers, unless otherwise noted)

Country	Year	Concentration (mg/m ³)	Interpretation
Argentina	1991	240	TWA
		485	STEL (15 min)
Australia	1983	240	TWA
		485	STEL
Austria	1982	480	TWA
Belgium	1984	240	TWA
		485	STEL
Canada	1986	240	TWA
		485	STEL
Denmark	1988	120 ^a	TWA
Finland	1993	240	TWA
		480	STEL
France	1993	240	TWA
Germany	1993	480	TWA (MAK); substance with intense odour ^b
Indonesia	1978	480	TWA
Mexico	1984	240	TWA
		485	STEL
Netherlands	1986	240	TWA
Romania	1975	300	Average
		400	Maximum
Sweden	1991	120	TWA; skin
		350	STEL (15 min)
Switzerland	1987	240	TWA
United Kingdom	1992	480	TWA
		720	STEL (10 min)
USA			
ACGIH	1994	242	TWA
		483	STEL
OSHA	1992	480	TWA
NIOSH (REL)	1990	480	TWA
Venezuela	1978	480	TWA
		720	Ceiling

From Cook (1987); Arbejdstilsynet (1988); ILO (1991); US National Institute for Occupational Safety and Health (NIOSH) (1992); US Occupational Safety and Health Administration (OSHA) (1992); American Conference of Governmental Industrial Hygienists (ACGIH) (1993); Deutsche Forschungsgemeinschaft (1993); Institut National de Recherche et de Sécurité (1993); Työministeriö (1993); UNEP (1993)

TWA, time-weighted average; STEL, short-term exposure limit; TLV, threshold limit value; PEL, permissible exposure level; REL, recommended exposure level; MAK, maximale arbeitsplatzkonzentration (maximal workplace concentration); skin, absorption through the skin may be a significant source of exposure

^aIncludes mixed isomers and each isomer

^bConcentration that should never be exceeded is twice the MAK for 10 min, four times per shift

resinous and polymeric coatings for polyolefin films (21 CFR 175.300, 175.320), paper and paperboard in contact with dry food (21 CFR 176.180), cellophane (21 CFR 177.1200), poly(*para*-vinyltoluene) and rubber-modified poly(*para*-vinyltoluene) (21 CFR 176.1635), rubber articles intended for repeated use (21 CFR 177.2600) and a component of polyolefin film (21 CFR 178.3610).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 60 male and 60 female Swiss mice, six weeks old, were administered 0 (control), 10, 50 or 250 mg/kg bw vinyl toluene (purity, > 99%; 96.8% *para* isomer and 3% *meta* isomer) by gastric intubation in olive oil once a day on five days a week for 78 weeks. The study was terminated at 83 weeks, when the survival rate was reduced to less than 50% in at least one group. There was no treatment-related effect on survival of female mice or on body weight in mice of either sex; survival of male mice was reduced in treated groups, but the authors concluded that both the chemical and amyloidosis were causal factors in the increased mortality [survival data not provided]. There was no significant treatment-related increase in either the percentage of mice with malignant tumours or with benign and malignant tumours combined or in the number of malignant tumours per mouse (Conti *et al.*, 1988).

3.1.2 *Rat*

Groups of 60 or 90 male and 60 or 90 female Sprague-Dawley rats, six weeks old, were administered 10, 50, 250 or 500 mg/kg bw vinyl toluene (purity, > 99%; 96.8% *para* isomer and 3% *meta* isomer) by gastric intubation in olive oil once a day on five days a week for 108 weeks. Control groups of 60 male and 60 female rats received olive oil alone. Five rats from the 500-mg/kg group were killed at 54 and 107 weeks. The study was terminated at 123 weeks when the survival rate was reduced to less than 50% in at least one group. Survival of male rats receiving 250 and 500 mg/kg bw was reduced [exact data not provided]. There was no treatment-related effect on survival in female rats or on body weights of male or female rats and no treatment-related increase in either the percentage of rats with malignant tumours or with benign and malignant tumours combined nor in the number of malignant tumours per rat (Conti *et al.*, 1988).

3.2 Inhalation

3.2.1 *Mouse*

Groups of 50 male and 50 female B6C3F1 mice, eight to nine weeks old, were exposed by whole-body inhalation to 0 (control), 10 or 25 ppm [48.2 or 120.5 mg/m³] vinyl toluene

(approximately 99% pure; 65–71% *meta* and 32–35% *para* isomers) for 6 h per day on five days a week for 103 weeks and were sacrificed one week following the last exposure. Complete gross and microscopic examination was performed on all high-dose and control mice at terminal sacrifice and on all mice that died or were sacrificed when moribund prior to the end of the study. Mice in the mid-dose groups were examined microscopically for gross lesions and for alterations in lung and nasal passages. There was a dose-related decrease in body weights in animals of each sex. Survival at termination of the experiment was 33/50, 40/50 and 41/50 for male mice and 36/50, 37/50 and 34/50 for female mice in the control, low- and high-dose groups, respectively. There was no treatment-related increase in the incidence of any tumour in male or female mice. There were significant (logistic regression test) treatment-related decreases in the incidences of lymphoma (7/50, 2/50, 0/50; $p = 0.006$) and alveolar–bronchiolar tumours (12/50, 5/50, 2/50; $p = 0.003$) in male mice and of liver tumours (9/50, 5/50, 2/50; $p = 0.021$) in female mice. Hyperplasia of the respiratory epithelium and inflammation of the nasal mucosa and lung were observed in all treated groups (US National Toxicology Program, 1990).

3.2.2 Rat

Groups of 49 or 50 male and 50 female Fischer 334/N rats, 9–10 weeks old, were exposed by whole-body inhalation to 0 (control), 100 or 300 ppm [482 or 1447 mg/m³] vinyl toluene (purity, approximately 99%; 65–71% *meta* and 32–35% *para* isomers) for 6 h per day on five days a week for 103 weeks and sacrificed one week after the last exposure. Body weights of high-dose males and low-dose females were decreased. Survival at termination of the experiment was 19/49, 17/50 and 19/50 for males and 31/50, 28/50 and 26/50 for female rats in the control, low- and high-dose groups, respectively. There was no treatment-related change in the incidence of any type of tumour in male or female rats. A dose-related increase in the incidence of hyperplasia of the respiratory epithelium of the nasal passages was observed in animals of each sex (US National Toxicology Program, 1990).

4. Other Data Relevant for 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

para-Vinyl toluene was oxidized to the side-chain diol by the cytochrome P450-mediated system in rat liver microsomes *in vitro* at a rate similar to that of styrene. Protein binding by the two analogues *in vitro* was also similar (Hanzlik *et al.*, 1978).

In a series of experiments on vinyl toluene (60% *meta* and 40% *para* isomer), both short-term intraperitoneal injection of 100 or 500 mg/kg bw and inhalation of 50, 100 and

300 ppm [240, 480 and 1450 mg/m³] (6 h/day, five days/week) for up to 15 weeks caused dose- and time-dependent decreases in non-protein sulfhydryl groups and increases in the activities of 7-ethoxycoumarin *O*-deethylase and UDP-glucuronosyl transferase in the livers and kidneys of male Wistar rats, C57Bl/6 mice and Chinese hamsters. Vinyl toluene was found to bind to hepatic cytochrome P450 and to decrease the reduced glutathione content (Heinonen & Vainio, 1980, 1981; Heinonen *et al.*, 1982a,b; Heinonen, 1984).

Male Wistar rats inhaling 300 ppm [1450 mg/m³] vinyl toluene for one week or 50, 100 and 300 ppm [240, 480 and 1450 mg/m³] for 8–12 weeks showed a dose-dependent increase in the urinary excretion of thioethers (Heinonen *et al.*, 1982a,b).

After *ortho*-, *meta*- and *para*-vinyl toluenes were injected intraperitoneally into male albino Wistar rats, 11 urinary metabolites were distinguished (Fig. 1). The main metabolites were similar to the corresponding styrene metabolites and included ethylene glycol, mandelic acid, glyoxylic acid derivatives and *N*-acetylcysteine and glucuronide conjugates. Over 90% of the recovered metabolites were excreted within 24 h (Bergemalm-Rynell & Steen, 1982). *N*-Acetylcysteine derivatives substituted at carbon 8 greatly exceeded (> 80%) those substituted at carbon 9 in Sprague-Dawley rats, in spite of steric hindrance by the methyl group (Kühler, 1984).

After a single intraperitoneal dose of *para*-vinyl toluene at 50 mg/kg bw, 55% was recovered in urine within 23 h, but mainly within the first 6 h, and the main metabolites were quantified (Fig. 1). Saturation of metabolic pathways began at a dose of 250 mg/kg bw. Excretion of all metabolites of *para*-vinyl toluene was prevented by 1-phenylimidazole, an inhibitor of cytochrome P450 monooxygenases, while the excretion rates of the metabolites were increased by prior treatment with polychlorinated biphenyls (Heinonen, 1984).

4.2 Toxic effects

4.2.1 Humans

The odour threshold for vinyl toluene (55–70% *meta* and 30–45% *para* isomer) was reported to be similar to that for styrene, i.e. 50 ppm [240 mg/m³] (Wolf *et al.*, 1956). This value is much higher than the threshold cited for styrene by other sources (70 µg/m³ as a 30-min average; WHO, 1987). Strong eye and nasal irritation was observed at 400 ppm [1930 mg/m³] (Wolf *et al.*, 1956).

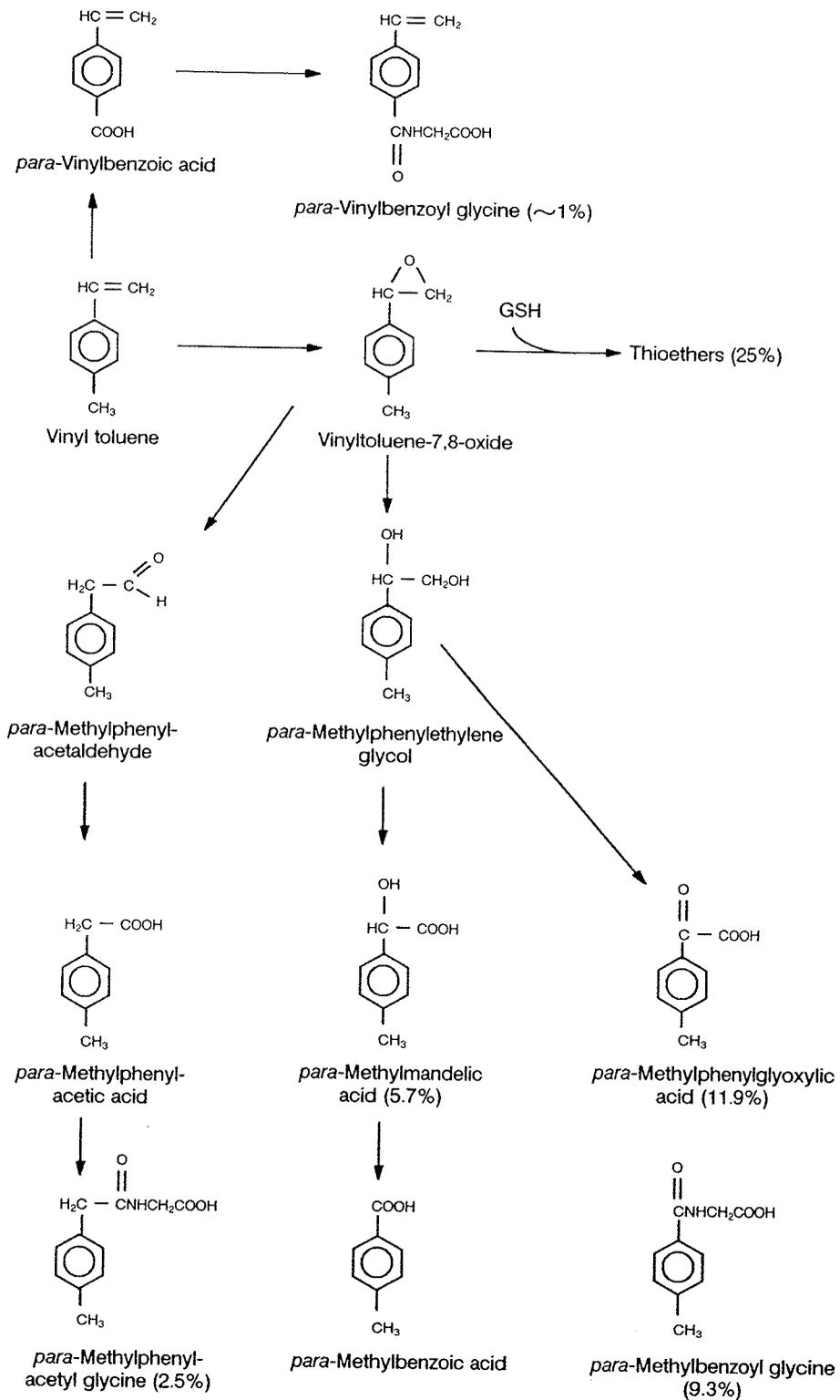
Central nervous system effects, such as depression, poor memory, slow visuomotor performance and electrophysiological changes, have often been associated with heavy occupational exposures to vinyl toluenes (Mutti & Franchini, 1987). Information is not available on human exposure to vinyl toluene alone.

In a case report on an individual with contact allergy to styrene, a cross-reaction was described with all three isomers of vinyl toluenes (Sjöborg *et al.*, 1982, 1984).

4.2.2 Experimental systems

The results of studies in experimental animals provide support for the neurotoxicity of vinyl toluene. In male Wistar and Sprague-Dawley rats exposed by inhalation to up to 300 ppm [1450 mg/m³] vinyl toluene (60–70% *meta* and 30–70% *para* isomer) for a

Fig. 1. The main metabolic pathways of vinyl toluene in rats



From Heinonen (1984). Amounts of metabolites are expressed as per cent of one intraperitoneal dose of 50 mg/kg bw

maximum of 15 weeks, decreased motor conduction velocity was observed within 12 weeks (Seppäläinen & Savolainen, 1982; Gagnaire *et al.*, 1986). Specific depletion of brain dopamine levels has been described in New Zealand rabbits (Romanelli *et al.*, 1986; Mutti *et al.*, 1988), and that is suggested to be the neurotoxic mechanism for a number of compounds with a reactive carbonyl group that can condense with dopamine to form tetrahydroisoquinolines (Mutti *et al.*, 1988; see the monograph on styrene). This effect may induce other hormonal changes that are under hypothalamic regulation which affect reproduction. Other neurochemical changes that have been observed include the release of lysosomal proteases in rat brain (Savolainen & Pfäffli, 1981).

In the subchronic studies cited above, vinyl toluene also caused cellular growth depression and hepatotoxicity in rats (Wolf *et al.*, 1956; Heinonen *et al.*, 1982b). In a chronic two-year study, rats and mice had hyperplasia of the respiratory epithelium and erosion and cyst formation in the olfactory epithelium; in mice, inflammation of the nasal passages and bronchioles was also described (US National Toxicology Program, 1990).

4.3 Reproductive and prenatal 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 2 and Appendices 1 and 2)

Vinyl toluene was not mutagenic to *Salmonella typhimurium*. It did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster*, treated either by feeding liquid vinyl toluene (up to 500 ppm [mg/kg] for one or three days) or by exposure to the gas at up to 300 ppm [1450 mg/m³] for five days.

Vinyl toluene induced forward mutations at the *tk* locus of mouse L5178Y lymphoma cells in the absence of an exogenous metabolic activation system, but only at a single, highly toxic dose.

It induced neither sister chromatid exchange nor chromosomal aberrations in Chinese hamster ovary cells; however, in human lymphocytes exposed in whole blood cultures, vinyl toluene induced both sister chromatid exchange and chromosomal aberrations in a dose-dependent manner, in the absence of exogenous metabolic activation. The induction of sister chromatid exchange was dependent on the number of erythrocytes present. Significant increases in the frequencies of sister chromatid exchange were observed in human lymphocytes exposed in whole blood cultures to *ortho*, *meta* and *para* isomers. The strongest responses were seen with the *meta* and *para* isomers, which are the dominant species in vinyl toluene (Norppa & Vainio, 1983; Norppa & Tursi, 1984).

Vinyl toluene increased the frequency of micronuclei in mouse bone-marrow erythrocytes *in vivo*.

Table 2. Genetic and related effects of vinyl toluene

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	590.0000	Norppa <i>et al.</i> (1981) (abstract)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	167.0000	Zeiger <i>et al.</i> (1987)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	590.0000	Norppa <i>et al.</i> (1981) (abstract)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	167.0000	Zeiger <i>et al.</i> (1987)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	590.0000	Norppa <i>et al.</i> (1981) (abstract)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	167.0000	Zeiger <i>et al.</i> (1987)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	590.0000	Norppa <i>et al.</i> (1981) (abstract)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	590.0000	Norppa <i>et al.</i> (1981) (abstract)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	167.0000	Zeiger <i>et al.</i> (1987)
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutation	-	-	450.0000 feeding	Norppa <i>et al.</i> (1981) (abstract)
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutation	-	-	300.0000 inhal.	Norppa <i>et al.</i> (1981) (abstract)
G5T, Gene mutation, mouse lymphoma L5178Y cells <i>in vitro</i>	(+)	0	60.0000	McGregor <i>et al.</i> (1988)
SIC, Sister chromatid exchange, Chinese hamster ovary cells <i>in vitro</i>	-	-	75.0000	US National Toxicology Program (1990)
CIC, Chromosomal aberrations, Chinese hamster ovary cells <i>in vitro</i>	-	-	50.0000	US National Toxicology Program (1990)
SHL, Sister chromatid exchange, human whole blood lymphocytes <i>in vitro</i>	+	0	40.0000	Norppa (1981a)
SHL, Sister chromatid exchange, human whole blood lymphocytes <i>in vitro</i>	+	0	118.0000	Norppa & Tursi (1984)
CHL, Chromosomal aberrations, human lymphocytes <i>in vitro</i>	+	0	320.0000	Norppa (1981a)
MVM, Micronucleus formation, mouse bone-marrow cells <i>in vivo</i>	+	-	200 bw × 1 ip	Norppa (1981b)
meta-Vinyl toluene				
SHL, Sister chromatid exchange, human whole blood lymphocytes <i>in vitro</i>	+	0	118.0000	Norppa & Vainio (1983)
para-Vinyl toluene				
SHL, Sister chromatid exchange, human whole blood lymphocytes <i>in vitro</i>	+	0	118.0000	Norppa & Vainio (1983)

^a+, positive; (+), weak positive; -, negative; 0, not tested

^bIn-vitro tests, µg/ml; in-vivo tests, mg/kg bw

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Vinyl toluene has been produced since the 1940s, as a mixture mainly of *meta* and *para* isomers, by dehydrogenation of *meta*- and *para*-ethyl toluene. It is used as a reactive monomer in the production of polymers and coatings. Few data are available on levels of occupational or environmental exposures to vinyl toluene.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Vinyl toluene (predominantly *para* isomer) was tested for carcinogenicity in one experiment in mice and one experiment in rats by intragastric intubation. The mixed isomers were tested in one experiment in mice and one experiment in rats exposed by inhalation. No increase in the incidence of tumours was observed in any of the experiments.

5.4 Other relevant data

Vinyl toluene is absorbed in rats exposed by inhalation; its neurotoxicity indicates that is distributed to the brain in both man and rat. The vinyl moiety is first metabolized to form an epoxide, which is either conjugated with glutathione or further oxidized to a number of products, including carboxylic acids, which are conjugated with glycine. The methyl group can also be oxidized to a carboxylic acid and subsequently conjugated with glycine. Saturation of metabolic pathways in rats commences at a dose of 250 mg/kg bw.

No data were available on the genetic and related effects of vinyl toluene in humans.

Vinyl toluene induces sister chromatid exchange and chromosomal aberrations in cultured human lymphocytes and micronuclei in mouse bone-marrow cells *in vivo*.

5.5 Evaluation¹

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

There is *evidence suggesting lack of carcinogenicity* of vinyl toluene in experimental animals.

Overall evaluation

Vinyl toluene is *not classifiable as to its carcinogenicity to humans (Group 3)*.

¹For definition of the italicized terms, see Preamble, pp. 27–30.

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ACRYLAMIDE

This substance was considered by a previous Working Group, in June 1985 (IARC, 1986). 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

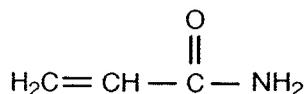
Chem. Abstr. Serv. Reg. No.: 79-06-1

Chem. Abstr. Name: 2-Propenamide

IUPAC Systematic Name: Acrylamide

Synonyms: Acrylic acid amide; acrylic amide; ethylenecarboxamide; propenamide; propenoic acid amide; vinyl amide

1.1.2 Structural and molecular formulae and relative molecular mass



$\text{C}_3\text{H}_5\text{NO}$

Relative molecular mass: 71.08

1.1.3 Chemical and physical properties of the pure substance

- (a) *Description:* White crystalline solid (monoclinic or triclinic crystal structure) (Habermann, 1991)
- (b) *Boiling-point:* 136 °C at 3.3 kPa (Habermann, 1991)
- (c) *Melting-point:* 84–85 °C (Lide, 1991)
- (d) *Density:* 1.122 g/ml at 30 °C (Habermann, 1991)
- (e) *Spectroscopy data:* Infrared [prism, 2998; grating, 8284], nuclear magnetic resonance, ultraviolet [3515] and mass spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991).
- (f) *Solubility:* Soluble in water (215.5 g/100 ml at 30 °C), acetone (63.1 g/100 ml at 30 °C), chloroform (2.7 g/100 ml at 30 °C), diethyl ether, ethanol (86.2 g/100 ml at 30 °C), ethyl acetate (12.6 g/100 ml at 30 °C) and methanol (155 g/100 ml at 30 °C); very slightly soluble in heptane (6.8 mg/100 ml at 30 °C) (Budavari, 1989; Lide, 1991)

- (g) *Volatility*: Vapour pressure, 0.9 Pa at 25 °C (Habermann, 1991)
- (h) *Stability*: The solid is stable at room temperature but polymerizes on melting or exposure to ultraviolet light (Budavari, 1989)
- (i) *Octanol-water partition coefficient (P)*: log P, -0.78 (Sangster, 1989)
- (j) *Conversion factor*: $\text{mg/m}^3 = 2.91 \times \text{ppm}^a$

1.1.4 Technical products and impurities

Acrylamide is available as a solid (either crystals or briquettes) and in aqueous solution. Most commercial preparations of acrylamide consist of a 50% aqueous solution stabilized with 25–30 ppm cupric ion or ethylenediaminetetraacetic acid, ferric ion, nitrite (Mannsville Chemical Products Corp., 1985; Habermann, 1991), hydroquinone, *tert*-butylpyrocatechol, *N*-phenyl-2-naphthylamine and other antioxidants (Budavari, 1989). Typical specifications for a 50% aqueous solution are: assay, 48–54 wt%; pH, 5.0–6.5; polymer (based on monomer), 100 ppm max.; acrylonitrile (based on solution), 100 ppm; and Cu^{++} inhibitor, 15–25 ppm max. (Habermann, 1991; Dow Chemical Co., 1992). Typical specifications for the crystalline monomer are: white, free-flowing crystal; assay, 98% min.; water, 0.8% max.; iron (as FeO), 15 ppm max.; water-insoluble material, 0.2% max.; and butanol-insoluble material, 1.5% max. (Habermann, 1991).

1.1.5 Analysis

EPA Method 8015 can be used to determine the concentrations of various non-halogenated volatile organic compounds, including acrylamide, in water, soil or sediment by gas chromatography with flame ionization detection. Aqueous process waste samples can be analysed by direct injection or the purge-and-trap method (EPA Method 5030); ground water and slightly contaminated soil and sediment samples must be analysed by the purge-and-trap method (US Environmental Protection Agency, 1986).

Biological monitoring of exposure to acrylamide involves determination of adducts of acrylamide with haemoglobin by gas chromatography-mass spectrometry, as tested experimentally (Farmer *et al.*, 1986; Bailey *et al.*, 1987) and in humans (Bergmark *et al.*, 1993).

Methods have been reported for the determination of acrylamide in water, wipe samples and polyacrylamide (Going & Thomas, 1979; Skelly & Husser, 1978). Water samples were reduced in volume by evaporation and analysed by gas chromatography with a nitrogen-selective thermionic detector. The detection limit was approximately 1 µg/L. Polyacrylamide samples were extracted with 80% methanol and 20% water (pH 3.75) for 3 h, and the extracts were analysed by high-performance liquid chromatography with an ultraviolet detector at 200 nm. The limit of detection of the monomer was approximately 0.5 µg/g. An analytical method for acrylamide in air and surface wipe samples, adapted from Skelly and Husser (1978), was described by Cummins *et al.* (1992). Air samples collected on a glass-fibre filter and XAD-7 solid sorbent tubes were desorbed with 5% methanol in water and analysed by

^aCalculated from: $\text{mg/m}^3 = (\text{relative molecular mass}/24.45) \times \text{ppm}$, assuming normal temperature (25 °C) and pressure (101.3 kPa)

high-performance liquid chromatography with ultraviolet detection. Wipe samples obtained on moistened glass-fibre filters were extracted with water prior to analysis.

1.2 Production and use

1.2.1 Production

Acrylamide has been available commercially since the mid-1950s. The principal process for making acrylamide used to be reaction of acrylonitrile with hydrated sulfuric acid and separation of the product from its sulfate salt. This process yielded satisfactory amounts of monomer, either as crystals or in solution, but it also produced unwanted sulfates and other by-products. As far as can be determined, acrylamide is not produced by this process today (Mannville Chemical Products Corp., 1985; Habermann, 1991; Smith & Oehme, 1991).

Catalytic hydration of acrylonitrile with copper metal or Raney copper catalyst was introduced in 1971. In this process, a solution of acrylonitrile in water is passed over a fixed bed of catalyst at 85 °C, and the acrylonitrile is converted directly to acrylamide. Levels of by-products are minimal, depending on the purity of the acrylonitrile. This method is now used throughout Europe, Japan and the USA (Mannville Chemical Products Corp., 1985; Habermann, 1991; Smith & Oehme, 1991).

In 1985, microorganisms began to be used for the production of acrylamide from acrylonitrile by an enzymatic hydration process. This is one of the first uses of biocatalysts in the manufacture of chemicals in the petrochemical industry (Mannville Chemical Products Corp., 1985; Habermann, 1991).

The largest volume of acrylamide is produced in Japan; there are also large production facilities in Europe and the USA. The estimated annual production capacity of the Japanese producers is 77 000 tonnes, that of the US producers, about 70 000 tonnes and that of the European producers, about 50 000 tonnes (Habermann, 1991). In Japan, 54 000 tonnes are estimated to have been produced in 1992 (Japan Petrochemical Industry Association, 1993).

Information available in 1991 indicated that acrylamide was produced by five companies in Japan, three each in Mexico and the USA, two in the United Kingdom and one each in Brazil, China, the Netherlands and Switzerland (Chemical Information Services Ltd, 1991).

1.2.2 Use

Acrylamide is a vinyl monomer which improves the aqueous solubility, adhesion and cross-linking of polymers. Water-soluble polyacrylamides are the largest application of acrylamide and are used in enhanced oil recovery as mobility control agents in water flooding, additives for oil well drilling fluids, and aids in fracturing, acidifying and other operations. Other uses of polyacrylamides are as flocculants for wastewater treatment, in the mining industry and various other process industries, soil stabilization, papermaking aids and thickeners, polymers for promoting adhesion, dye acceptors, and additives for textiles, paints, and cement. Acrylamide monomer is also used directly as a component of photopolymerization systems, in adhesives and grouts, and in cross-linking agents in vinyl polymers (WHO, 1985; Mannville Chemical Products Corp., 1985; US Environmental Protection Agency, 1990). The patterns of use for acrylamide in the USA for several years are presented in Table 1.

Table 1. End-use patterns (%) for acrylamide in the USA

Use	Year		
	1985	1988	1991
Water treatment	45	45	65
Oil drilling	20	20	5
Pulp and paper	20	20	20
Mineral processing	10	10	5
Miscellaneous ^a	5	5	5

From Anon. (1985, 1988, 1991)

^aIncludes use as monomer (e.g. cross-linking agent, grout)

1.3 Occurrence

1.3.1 *Natural occurrence*

Acrylamide is not known to occur as a natural product.

1.3.2 *Occupational exposure*

The National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health between 1981 and 1983 indicated that 10 700 employees were potentially exposed to acrylamide at work in the USA (US National Institute for Occupational Safety and Health, 1993). Of this number, 30% were estimated to be exposed to acrylamide and 70% to materials containing acrylamide. The estimate is based on a survey of US companies and did not involve measurements of actual exposures. The US Environmental Protection Agency (1990) estimated that 700–1000 workers were exposed to acrylamide in the manufacture and processing of acrylamide monomer, 1800 in soil grouting and 100 000–200 000 in laboratories using polyacrylamide gels, such as in gel electrophoresis (Ducatman & Coumbis, 1991) and chromatography (Dearfield *et al.*, 1988).

Box 1 is a list of industries in which exposure to acrylamide monomer and polymer may occur. Occupations in which there is potential exposure to acrylamide include: chemical production; construction and maintenance involving pipe grouting and sealing; soil, tunnel and dam stabilization; water and wastewater treatment; and preparation of polyacrylamide gels in the laboratory. The potential for exposure to acrylamide has been decreased by the use of commercially prepared polyacrylamide gel plates (Ducatman & Coumbis, 1991). Exposure to acrylamide polymer may lead to exposure to acrylamide, since the polymer may contain small amounts of monomer (Mallevalle *et al.*, 1984).

Box 1. Industries in which there is potential exposure to acrylamide monomer and polymer**Acrylamide monomer**

Acrylamide manufacture from acrylonitrile
Acrylamide polymer production
Adhesive and grout manufacture
Biotechnology laboratories

Acrylamide polymer

Water and wastewater treatment
Organic chemical manufacture
Inorganic chemical manufacture
Adhesive and grout manufacture
Coating applications
Moulded part manufacture
Textile products, weaving and fabric mills
Steel industry (blast furnace, metal part fabrication)
Water flocculent manufacture
Paper and pulp production
Lumber and wood production
Construction (soil and sand stabilization)
Crude oil production
Petroleum refining
Mineral processing
Concrete production
Sugar production
Hospitals
Biotechnology laboratories

Adapted from US National Institute for Occupational Safety and Health (1976, 1993)

Table 2 lists published data on exposure to acrylamide. Exposures may occur in monomer and polymer production from inhalation of dry powder or crystalline monomer, dermal contact with the monomer or dermal contact with solutions of acrylamide. Inhalation from solutions containing acrylamide is probably minimal because of their low volatility. The use of closed reactor vessels and automated addition of raw materials would be expected to reduce exposure. When acrylamide monomer is mixed or added by hand, the potential exposure is greater. Dermal exposure is known to account for a large number of cases of neurotoxic poisoning, but the dose was not given in any of the reports (Tilson, 1981; Dearfield *et al.*, 1988; He *et al.*, 1989; US Environmental Protection Agency, 1990; Myers & Macun, 1991).

Exposure may occur to either the dust or aqueous solutions of the monomer in water and wastewater treatment, preparation of chromatography and permeation gels in laboratories, underground pipe grouting operations and underground injection for soil stabilization. In these cases, the solid is mixed with water and a cross-linking or catalytic agent just prior to use. Exposures would be expected to depend strongly on the task (mixing and handling solutions) and to be intermittent.

Table 2. Occupational exposures to acrylamide

Industry, operation and process	Type of sample	No. of samples	Air concentration (mg/m ³)		Year of measurement	Country	Reference
			Mean	Range			
Acrylamide and polyacrylamide production							
Monomer and polymer production					1991	China	Bergmark <i>et al.</i> (1993); Deng <i>et al.</i> (1993); Calleman <i>et al.</i> (1994)
Polymerization room	Area	12	3.27	0.19-8.8			
Monomer synthesis	Area	18	1.07	0.11-3.01			
Monomer and polymer production					1985	China	He <i>et al.</i> (1989)
Polymerization	Area	NR	NR	5.56-9.02			
Polymerization after renovation	Area	NR	0.0324	NR			
Monomer and polymer production					1984-85	USA	Hills & Greife (1986)
Monomer operators	Personal	19	0.065 GM	0.001-0.227			
Polymer operators	Personal	27	0.031 GM	0.001-0.181			
Monomer material handlers	Personal	4	0.085 GM	0.017-0.260			
Polymer material handlers	Personal	4	0.023 GM	0.018-0.035			
Maintenance	Personal	14	0.013 GM	0.001-0.132			
Utility operators	Personal	4	0.116 GM	0.004-0.392			
Plantwide	Area	19	0.269 GM	0.014-8.291			
Plantwide	Area	18	0.029 GM	0.003-0.157			
Plantwide	Area	18	0.006 GM	0.004-0.009			
Plantwide	Area	28	0.010 GM	0.001-0.015			
Monomer and polymer production	Personal TWA	NR	NR	< 0.1	> 1970	USA	Sobel <i>et al.</i> (1986)
Continuous monomer production	Personal TWA	NR	NR	0.1-1	< 1957		
	Personal TWA	NR	NR	0.1-0.6	1957-70		
Monomer production					1976	USA	US National Institute for Occupational Safety and Health (1976)
Reactor operator	Personal 4-h	1	0.48	-			
Dryer operator	Personal 4-h	1	0.52	-			
Packing	Personal 4-h	2	0.64	0.52-0.76			
					1971-75		
Control room	Personal 8-h	NR		0.1-0.4			
Bagging room	Personal 8-h	NR		0.1-0.9			
Processing	Personal 8-h	NR		0.1-0.4			

Table 2 (contd)

Industry, operation and process	Type of sample	No. of samples	Air concentration (mg/m ³)		Year of measurement	Country	Reference
			Mean	Range			
Other uses							
Sewer line repair					1990	USA	Cummins <i>et al.</i> (1992)
Grouting operation (2 sites)	Personal	12	0.010	0.003-0.02			
Grouting operation (3 sites)	Area	18	0.022	0.002-0.05			
Sewer line repair					1985	USA	Hills & Greife (1986)
Grouting operation	Personal	2	0.005	0.002-0.007			
Grouting operation	Area	2	0.005	0.001-0.009			
Sewer line repair					1988	USA	US Environmental Protection Agency (1990)
Grouting operation	Personal	6	0.10	0.008-0.36			
Grouting operation	Area	3	0.04	< 0.004-0.08			
Coal preparation plant					1992	USA	Hoekstra & Weber (1993)
Static thickening of coal waste	Personal	2	NR	< 0.001			
Slurry	Area	12	NR	< 0.001			
Polyacrylamide flocculent manufacture					1986	South Africa	Myers & Macun (1991)
Workshop, warehouse, laboratory, powder plant	Personal	3	[0.02]				
Cleaner, closed reactor operator	Personal	1	[0.06]				
Acrylamide warehouse	Personal	2	[0.12]				
Exposed laboratory worker	Personal	1	[0.21]				
Closed reactor operator handling acrylamide	Personal	3	[0.27]				
Foreman, laboratory worker, manager	Personal	1	[0.36]				
Skip hoist operator, shooter-polymerizer, hyster driver	Personal	4	[0.48]				
Drier operator, hyster driver, making, shooting	Personal	7	[0.51]				
Making, dissolving	Personal	3	[0.75]				
Research laboratories					1991	USA	Rohwein (1991)
Preparation of polyacrylamide gels	Personal	1	1.5				
	Personal	1	0.9				
	Personal	1	0.5				
	Personal	9	N/Q				

NR, not reported; TWA, time-weighted average; GM, geometric mean; N/Q, detectable but not quantifiable

1.3.3 *Environmental occurrence*

Environmental releases of acrylamide from industrial facilities in the USA in 1991 were reported as (tonnes per year): 29.1 into air, 2.1 into water, 2100 during underground injection and 0.7 onto land (US National Library of Medicine, 1993).

Because polyacrylamide is used in water treatment, residues of acrylamide may be found in potable water. In most countries, such residues are limited to 0.25 µg/L by maintaining the concentration of acrylamide monomer in the polyacrylamide used for water treatment at < 0.05%. Concentrations of acrylamide in effluents from factories where polyacrylamide is used generally range from < 1 to 50 µg/L. In the vicinity of local grouting operations (e.g. for repair of sewer lines and soil stabilization), high levels of acrylamide may be found in wells and groundwater; a concentration of 400 mg/L was reported in one such well (WHO, 1985).

Levels of acrylamide have seldom been measured in ambient air, except as described under *Occupational exposure* above. Monitoring of concentrations in air close to six acrylamide-producing plants in the USA failed to detect any acrylamide (detection limit, 0.1 µg/m³) (WHO, 1985).

1.4 **Regulations and guidelines**

Occupational exposure limits and guidelines for acrylamide in a number of countries and regions are presented in Table 3.

Guidelines and regulations have been set for acceptable levels of acrylamide in polyacrylamide, depending on the end use (WHO, 1985). For example, the US Food and Drug Administration (1993) established regulations for the use of polymers and copolymers of acrylamide in products in contact with food (21 CFR 175.105; 175.300; 177.1010), limiting the residual acrylamide in the polymer to 0.05% for acrylate-acrylamide resins for use in food treatment or as boiler-water additives (21 CFR 173.5; 173.310), and to 0.2% in polyacrylamide used as a film former in the imprinting of soft-shell gelatin capsules and in acrylamide copolymer resins used in paper or paperboard in contact with food (21 CFR 172.255; 173.315; 176.110; 176.170; 176.180; 178.3520).

In Germany, the level of polyacrylamide used in foodstuff packaging is limited to 0.3% and the level of residual acrylamide monomer in polyacrylamide to 0.2% (WHO, 1985). In the United Kingdom, it is recommended that commercial polyelectrolytes used as coagulants during the preparation of potable waters contain no more than 0.05% acrylamide. Unregulated polyelectrolytes may be used for effluent treatment and such polyacrylamides may contain up to 5% monomer (Brown *et al.*, 1980). In Germany, the level of residual monomer in polyacrylamide used in hair sprays is limited to 0.01% (WHO, 1985).

Table 3. Occupational exposure limits and guidelines for acrylamide

Country or region	Year	Concentration (mg/m ³)	Interpretation
Argentina	1991	0.03	TWA; carcinogen; skin
Australia	> 1983	0.03	TWA; probable human carcinogen; skin
Austria	1982	0.3	TWA
Belgium	> 1984	0.03	TWA; probable human carcinogen; skin
Canada	1986	0.3	TWA; intended change
		0.6	STEL; intended change
Denmark	1988	0.3	TWA; suspected carcinogen; skin
Finland	1993	0.3	TWA
		0.9	STEL
France	1993	0.03	TWA
Germany	1993	None	Animal carcinogen and germ-cell mutagen; skin
Hungary	1978	0.3	Ceiling; suspected of having carcinogenic potential; skin; irritant
Indonesia	1978	0.3	TWA; skin
Italy	1978	0.3	TWA; skin
Japan	1983	0.3	TWA; skin
Mexico	1989	0.3	TWA; skin
Netherlands	1986	0.3	TWA; skin
Norway	1990	0.03	TWA; carcinogen; skin
Republic of Korea	1983	0.3	TWA
		0.6	STEL
Romania	1983	0.3	TWA
		0.5	Ceiling
Sweden	1991	0.3	TWA; skin
		0.9	STEL (15 min)
Switzerland	1987	0.3 (provisional)	TWA; carcinogen; skin
Taiwan	1982	0.3	TWA
United Kingdom	1992	0.3	TWA; skin
		0.6	STEL (10 min)
USA			
ACGIH (TLV)	1994	0.03	TWA; suspected human carcinogen ^a ; skin
OSHA (PEL)	1993	0.3	TWA; skin
NIOSH (REL)	1992	0.03	TWA; suspected human carcinogen; skin
Venezuela	1978	0.3	TWA; skin
		0.6	Ceiling

From Cook (1987); Arbejdstilsynet (1988); Direktoratet for Arbejdstilsynet (1990); ILO (1991); US National Institute for Occupational Safety and Health (NIOSH) (1992); American Conference of Governmental Industrial Hygienists (ACGIH) (1993); Deutsche Forschungsgemeinschaft (1993); Institut National de Recherche et de Sécurité (1993); Työministeriö (1993); UNEP (1993); US Occupational Safety and Health Administration (OSHA) (1993)

TWA, time-weighted average; STEL, short-term exposure limit; TLV, threshold limit value; PEL, permissible exposure level; REL, recommended exposure level

^aSubstance identified by other sources as a suspected or confirmed human carcinogen

2. Studies of Cancer in Humans

2.1 Cohort studies

In 1986, Sobel *et al.* reported the findings of a study on mortality among 371 employees who had had potential exposure to acrylamide during monomer and polymerization operations at a facility in Michigan, USA. The acrylamide monomer process began in 1955, and polymer production was initiated in 1965. Environmental concentrations of acrylamide in the monomer production areas had been measured using personal samplers and showed a decrease over time: before 1957, the 8-h time-weighted average concentration ranged from 0.1 to 1.0 mg/m³, and in 1957–70 it was 0.1–0.6 mg/m³; after 1970, all measured values were below 0.1 mg/m³. Potential for exposure to acrylonitrile also existed in the monomer production area. Exposure to acrylamide occurred during polymer production due to inhalation of polyacrylamide dust containing residual acrylamide. Dust concentrations were above 2 mg/m³ (time-weighted average) in packaging and drying operations and lower in other jobs; the residual amount of acrylamide in polymer dust was estimated to be about 1%. Other potential sources of acrylamide were dermal absorption and ingestion of polyacrylamide dust; however, their extent could not be assessed. Workers were identified from personnel census lists for 1955–79 as having worked in the production and pilot plants of interest. All study subjects were white; six of the 371 were women. A detailed work history was obtained for each: 76% of the workers had been employed for fewer than four years, and only 19% had started work before 1960. Fourteen subjects had also been exposed to organic dyes for five or more years. Mortality was examined from date of first potential exposure to 31 December 1982. Death certificates were obtained for all those who had died. Standardized mortality ratios (SMRs) were estimated, and expected deaths were calculated from mortality rates for US white males. Twenty-nine deaths were observed and 38 were expected (SMR, 76). The number of deaths from all cancers was slightly greater than expected (11 observed; SMR, 139; 95% confidence interval [CI], 70–249). The increase was related specifically to cancers of the digestive tract (four observed; SMR, 202; 95% CI, 57–539) and of the respiratory system (four observed; SMR, 138; 95% CI, 38–353). When workers with previous exposure to organic dyes were excluded, no increase in the rate for respiratory tract cancer was seen and two cases of digestive tract cancer were observed with 1.6 expected.

Mortality in four plants, three in the USA and one in the Netherlands, was studied in order to examine the possible cancer risk entailed by occupational exposure to acrylamide (Collins *et al.*, 1989). A total of 8854 men with potential exposure to acrylamide were employed in the plants between 1925 and 1976; 96% (8508; 7242 white and 1266 non-white) had been employed in the US facilities. Follow-up from 1925 to 1983 was complete for 94% of the cohort. Death certificates were obtained for 95% of the decedents in the USA and for 82% in the Netherlands. Exposure estimates were derived for each job in the four plants from ambient monitoring data available from 1977 onwards and from plant personnel knowledgeable about past processes and working conditions. An individual cumulative exposure index was calculated by combining the estimated average daily exposure and the number of days spent in each job held. Exposure to acrylamide was defined as cumulative exposure greater than 0.001 mg/m³-year; 2293 men were exposed. Smoking histories were available

from medical records for 35% of the cohort. SMRs were estimated on the basis of expected deaths calculated from national death rates adjusted for age, calendar time and ethnicity. Analysis by plant showed no notable excess of any of the major categories of cause of death. Among exposed workers, there was a significant deficit for mortality from all causes (SMR, 81), but weak indications of an increased risk were noted for cancer of the pancreas (eight observed; SMR, 203 [95% CI, 87–400]) and Hodgkin's disease (five observed; SMR, 129 [95% CI, 42–300]). In addition, directly standardized relative risks were estimated from internal comparisons after adjustment for smoking status and latency, in addition to age and calendar period, for four categories of cumulative exposure (< 0.001 , 0.001 – 0.03 , 0.03 – 3.0 and > 3.0 mg/m³-year). For none of the cancer sites examined did the relative risk depart significantly from unity. No trend in cancer mortality was seen with increasing cumulative exposure. In particular, for pancreatic cancer, relative risks of 0.90, 1.4, 1.1 and 1.3 were calculated by the Working Group for the four categories.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 Mouse

In a screening assay based on increased multiplicity and incidence of lung tumours in a strain of mice highly susceptible to development of this neoplasm, groups of 40 female and 40 male A/J mice, eight weeks of age, received doses of 0 (control), 6.25, 12.5 or 25.0 mg/kg bw acrylamide (purity, $> 99\%$) in 0.2 ml distilled water by oral gavage three times per week for eight weeks. Animals were killed seven months after the beginning of treatment. The authors reported a significant, dose-related increase in the number of mice with lung adenomas and in the number of lung adenomas per mouse ($p < 0.01$, logistic regression model) (Bull *et al.*, 1984a).

3.1.2 Rat

Groups of 90 male and 90 female Fischer 344 rats, five to six weeks of age, were administered 0, 0.01, 0.1, 0.5 or 2 mg/kg bw acrylamide per day in drinking-water for two years. The solutions were prepared twice a week from recrystallized acrylamide (purity, 96–99%; water content, 0.4–3.8%). Groups of 10 males and 10 females were killed at 6, 12 and 18 months, such that 60 animals of each sex were available for study. By the end of the study, survival had been reduced in groups of each sex receiving the highest dose. After adjustment for survival, there were significant increases in the incidences of thyroid gland tumours and peritoneal mesotheliomas in the region of the testis in males and tumours of the mammary gland, central nervous system, thyroid, oral cavity, uterus and clitoral gland in females (Table 4) (Johnson *et al.*, 1986).

Table 4. Numbers of Fischer 344 rats with tumours after receiving acrylamide in the drinking-water for two years

Type of tumour	Sex	Dose (mg/kg bw per day)				
		0	0.01	0.1	0.5	2.0
Thyroid gland, follicular adenomas	M	1/60	0/58	2/59	1/59	7/59*
Testis, mesotheliomas of the tunica albuginea	M	3/60	0/60	7/60	11/60*	10/60*
Adrenal gland ^a , phaeochromocytomas	M	3/60	7/59	7/60	5/60	10/60*
Mammary tumours	F	10/60	11/60	9/60	19/58	23/61*
Central nervous system, glial tumours	F	1/60	2/59	1/60	1/60	9/61*
Thyroid gland, follicular adenomas or adenocarcinomas	F	1/58	0/59	1/59	1/58	5/60*
Oral cavity, squamous papillomas	F	0/60	3/60	2/60	1/60	7/61*
Uterus, adenocarcinomas	F	1/60	2/60	1/60	0/59	5/60*
Clitoral gland, adenomas ^b	F	0/2	1/3	3/4	2/4	5/5*
Pituitary adenomas ^a	F	25/59	30/60	32/60	27/60	32/60*

From Johnson *et al.* (1986)

^aThe historical incidence of adrenal gland phaeochromocytomas in males was 8.7% (range, 1.2–14.0%); that of pituitary adenomas in females was 38.1% (range, 28.2–46.9%).

^bOnly clitoral glands with gross lesions were examined histologically.

* $p = 0.05$; pair-wise Mantel-Haenszel comparison with the control group adjusted for mortality

3.2 Intraperitoneal administration

Mouse: In a screening assay similar to that described in section 3.1.1, groups of 16 female and 16 male A/J mice, eight weeks of age, received intraperitoneal injections of 0 (control), 1, 3, 10, 30 or 60 mg/kg bw acrylamide (purity, > 99%) dissolved in distilled water three times per week for eight weeks. A further control group was left untreated. Treatment with 60 mg/kg bw acrylamide was discontinued owing to the appearance of peripheral neuropathy and poor survival; animals in the other groups survived until six months after the beginning of treatment, at which time they were killed. The incidences of lung adenomas were: males—untreated controls, 5/16; vehicle controls, 2/16; 1 mg, 8/16; 3 mg, 6/16; 10 mg, 10/17; and 20 mg, 14/15 ($p < 0.01$, logistic regression model); females—untreated controls, 7/14; vehicle controls, 1/15; 1 mg, 6/17; 3 mg, 9/17; 10 mg, 11/14; and 30 mg, 14/15 ($p < 0.01$, logistic regression model). The average numbers of lung adenomas per mouse were 0.31 in untreated male controls, 0.06 in male vehicle controls, 0.75 in males given 1 mg, 0.69 in males given 3 mg, 0.88 in males given 10 mg and 1.87 in males given 30 mg; the numbers were 0.5 in untreated female controls, 0.13 in female vehicle controls, 0.35 in females given 1 mg, 0.88 in those given 3 mg, 1.57 in those given 10 mg and 2.53 in those given 30 mg (Bull *et al.*, 1984a).

3.3 Initiation-promotion studies

Mouse: Groups of 40 female Sencar mice, six to eight weeks of age, received 12.5, 25.0 or 50.0 mg/kg bw acrylamide (purity, > 99%) six times, either by gastric intubation in 0.2 ml

distilled water, by skin application in 0.2 ml ethanol or by intraperitoneal injection in 0.2 ml distilled water over a period of two weeks (total doses, 75, 150 or 300 mg/kg bw). Two weeks later, topical applications of 1 µg 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in 0.2 ml acetone were given on the back skin three times per week for 20 weeks. Vehicle control groups received distilled water by the same schedule. Groups of 20 mice received 300 mg/kg bw acrylamide followed by acetone instead of TPA. Nearly all animals survived until 52 weeks (34–38 per group), at which time they were killed; only gross lesions of the skin were examined histologically. Dose-related increases in the incidence of skin tumours were seen when acrylamide was given in combination with TPA by all routes. The numbers of skin tumour-bearing animals (papillomas and carcinomas combined) are shown in Table 5 (Bull *et al.*, 1984a).

Table 5. Skin tumours initiated in Sencar mice by administration of acrylamide by various routes

Route	Total dose (mg/kg bw)	No. of skin tumour-bearing animals/total no. of animals
Oral	0 + TPA	2/40
	75 + TPA	12/40
	150 + TPA	23/40
	300 + TPA	30/40
	300	0/20
Intraperitoneal	0 + TPA	0/40
	75 + TPA	10/40
	150 + TPA	13/40
	300 + TPA	21/40
	300	0/20
Topical	0 + TPA	7/40
	75 + TPA	4/40
	150 + TPA	11/40
	300 + TPA	18/40
	300	0/20

From Bull *et al.* (1984a); TPA, 12-*O*-tetradecanoylphorbol 13-acetate

Groups of 40 female Swiss-ICR mice [age unspecified] were administered total doses of 0, 75, 150 or 300 mg/kg bw acrylamide (purity, > 99%), divided into six equal portions over a two-week period, in water by oral gavage. Two weeks after the last dose, animals were given skin applications of 2.5 µg/mouse TPA dissolved in 0.2 ml acetone three times per week for 20 weeks. A further group of animals received a total dose of 300 mg/kg bw acrylamide administered as described above and received skin applications of 0.2 ml acetone alone. After 52 weeks, the surviving animals were killed and skin and lung tissues were evaluated. In the different groups, 32–36 animals survived to termination of the experiment. In animals receiving 0, 75, 150 or 300 mg/kg bw acrylamide plus TPA, the numbers with skin tumours were 0/40, 4/40, 4/40 and 13/40 [$p < 0.001$, Cochran-Armitage trend test], and those with

lung tumours were 4/36, 8/34, 6/36 and 11/34 [$p > 0.05$, Cochran-Armitage trend test], respectively. The numbers of skin tumours per mouse were 0, 0.10, 0.13 and 0.43 in the control and treated groups, respectively. Increased incidences of skin and lung tumours were also observed in the group treated with 300 mg/kg bw acrylamide plus acetone; the incidence of skin tumours was 10/40 [controls, 0/40; $p < 0.001$, Fisher exact test] and that of lung tumours was 14/36 [controls, 4/36; $p = 0.06$, Fisher exact test] (Bull *et al.*, 1984b).

4. Other Data Relevant for an Evaluation of Carcinogenicity and Its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

In a study in China, haemoglobin (Hb) adducts of acrylamide and glycidamide were determined in 41 workers who were exposed to acrylamide for periods ranging between one month and 11.5 years and who had concurrent medical and neurological examinations. The levels of N-terminal valine adducts of acrylamide ranged from 0.3 to 34 nmol/g Hb, with directly proportional formation of glycidamide adducts to the same residue in Hb. Background levels of valine adducts in Hb were not found in a group of 10 controls, with one possible exception, indicating that endogenous production of or environmental contamination with acrylamide is low or nonexistent (Bergmark *et al.*, 1993). The average ratio between the doses of glycidamide and acrylamide received (the concentration of free electrophilic agents integrated over time) in this group of workers was found to be 0.3. On the basis of the Hb adduct levels and the air concentrations in two of the workshops in this study (1.1 and 3.3 mg/m³) and assuming 100% uptake of acrylamide through the air and an alveolar ventilation rate of 0.2 L/min per kg, it was concluded that there had been only minor exposure by inhalation and that dermal uptake was the dominant route of exposure.

Free acrylamide in plasma, mercapturic acids in urine (confounded by co-exposure to acrylonitrile) and several diagnostic indicators of neurotoxicity were determined in the same groups of workers described above. For workers in the acrylamide synthesis room, the average level of free acrylamide in plasma was 1.8 µmol/L and that of valine adducts was 13.4 nmol/g Hb; 64 µmol *S*-(2-carboxyethyl)cysteine were found in 24-h urine, but some was the result of exposure to acrylonitrile. The levels of free acrylamide in plasma were in good agreement with those expected on the basis of Hb adduct levels (Calleman *et al.*, 1994).

Crucial data are still lacking on the absorption and metabolism of acrylamide in humans: neither the degree of permeation through the skin nor its spectrum of urinary metabolites is known, and there are no reliable data on the rate of elimination of acrylamide.

4.1.2 Experimental systems

(a) Acrylamide

Oral absorption of acrylamide may be considered to be complete on the basis of the observation that urinary excretion of 10 mg/kg bw is similar when the compound is

administered by gavage or by intravenous injection into rats (Miller *et al.*, 1982). Frantz *et al.* (1985) reported that 26% of an aqueous solution was taken up through the skin of rats within 24 h, and a further 35% is available for further absorption. Uptake of acrylamide after inhalation has not been determined in any species.

In rats given 0.5–100 mg/kg bw of either [1-¹⁴C]- or [2,3-¹⁴C]acrylamide intravenously or orally, radioactivity was distributed rapidly throughout the body, with no selective accumulation in any tissue (Hashimoto & Aldridge, 1970; Miller *et al.*, 1982). Radioactivity was also distributed evenly among tissues of beagle dogs and miniature pigs (Ikeda *et al.*, 1987). After topical and oral administration of [2,3-¹⁴C]acrylamide to SENCAR and BALB/c mice, few strain differences were seen in either its tissue distribution or its association with DNA, RNA or proteins. Comparable concentrations were observed after administration by either route in all tissues except the skin, where the label concentration after topical administration was approximately 100 times higher than after oral dosing (Carlson & Weaver, 1985). Autoradiographic studies have shown, however, that, nine days after treatment, there is preferential retention of radioactivity in the reproductive tract of male mice. The same study also demonstrated that, 24 h after oral administration of 120 mg/kg bw acrylamide to pregnant mice on days 13.5 or 17.5 of gestation, the compound was distributed in the fetuses and, on the latter day, intense accumulation occurred in fetal skin (Marlowe *et al.*, 1986). This finding is consistent with those of previous studies that have shown that acrylamide crosses the placenta in rats, rabbits, dogs and pigs (Ikeda *et al.*, 1983, 1985).

After a single oral dose of [2,3-¹⁴C]acrylamide (10 mg/kg bw) to male Fischer 344 rats, 53–67% was excreted within 24 h and 65–82% within seven days. More than 90% of the excreted radioactivity appeared in the urine. Urinary excretion was biphasic; the initial component, presumed to represent acrylamide and its metabolites, had a half-life of about 5 h, and the terminal component, presumed to result from the release of metabolites from tissue depots, had a half-life of eight days. Faecal excretion represented 4.8 and 6% after 24 h and seven days, respectively. Radiolabel was distributed rapidly to some tissues, but an initial absorption phase was noted for fat, liver, kidney, testis and plasma. Elimination from most tissues was biphasic, with a terminal half-life of about eight days. The tissues that contained the highest amounts of total radioactivity were muscle (48%), skin (15%), blood (12%) and liver (7%), in proportion to the relative mass of these tissues. Less than 1% of the dose was located in brain, spinal cord or sciatic nerve at any time; only erythrocytes concentrated the label. After intravenous injection of [2,3-¹⁴C]acrylamide (10 mg/kg bw), the estimated first-order rate constants of elimination of the parent compound ranged from 0.23/h to 0.51/h in different tissues, with 0.4/h in the blood compartment. Only the testis exhibited an absorption phase, with a delay until peak concentration. Total recovery of acrylamide in tissues and excreta of animals was 96.8%, and no radioactivity was found in expired air (Miller *et al.*, 1982). In contrast, after administration of [1-¹⁴C]acrylamide, 5 and 6% of the dose was excreted as carbon dioxide in beagle dogs (Ikeda *et al.*, 1987) and rats (Hashimoto & Aldridge, 1970), respectively, indicating the existence of an unidentified metabolic pathway resulting in the cleavage of the 1–2 carbon bond. It must be noted that studies of the tissue distribution of acrylamide based on measurements of total radioactivity suffer from uncertainty, owing to the inclusion of all metabolites and adducts.

After administration of high doses to rats, the mercapturic acid of acrylamide, resulting from direct or glutathione *S*-transferase-catalysed reaction with glutathione (Dixit *et al.*, 1981), is the main urinary metabolite (Edwards, 1975; Miller *et al.*, 1982). Mass spectroscopic techniques have been used to show, however, that an epoxide metabolite, glycidamide, is formed *in vitro* and *in vivo* in rats (Calleman *et al.*, 1990) and that its formation is strongly dose dependent (Calleman *et al.*, 1992). The epoxide is excreted either intact as glyceramide following hydrolysis or as a mercapturic acid derivative following conjugation of either of its reactive carbons with glutathione (Sumner *et al.*, 1992) (Fig. 1 and Table 6). The fact that the proportion of urinary metabolites originating from glycidamide is higher in mice (59%) than in rats (33%) seems to indicate a higher conversion rate of acrylamide to glycidamide after an oral dose of 50 mg/kg bw in mice. Since the percentage of acrylamide metabolized *in vivo* to glycidamide increased from 13% in rats administered 100 mg/kg bw to about 50% after a dose of 5 mg/kg bw (Bergmark *et al.*, 1991), the percentage of urinary metabolites derived from glycidamide may be expected to increase and that from acrylamide, *N*-acetyl-*S*(2-carbamoyl-ethyl)cysteine, to decrease at low doses. Specific urinary metabolites of acrylamide have not been studied in any species given doses lower than 50 mg/kg bw.

Table 6. Urinary metabolites collected from three mice and three rats given 50 mg/kg bw acrylamide orally, expressed as mean percentages of the total urinary metabolites excreted in 24 h

Metabolite	Mice	Rats
<i>N</i> -Acetyl- <i>S</i> -(2-carbamoyl-ethyl)cysteine	41.2	67.4
<i>N</i> -Acetyl- <i>S</i> -(2-carbamoyl-2-hydroxyethyl)cysteine	21.3	15.7
<i>N</i> -Acetyl- <i>S</i> -(1-carbamoyl-2-hydroxyethyl)cysteine	11.7	9.0
Glycidamide	16.8	5.5
Glyceramide	5.3	2.4

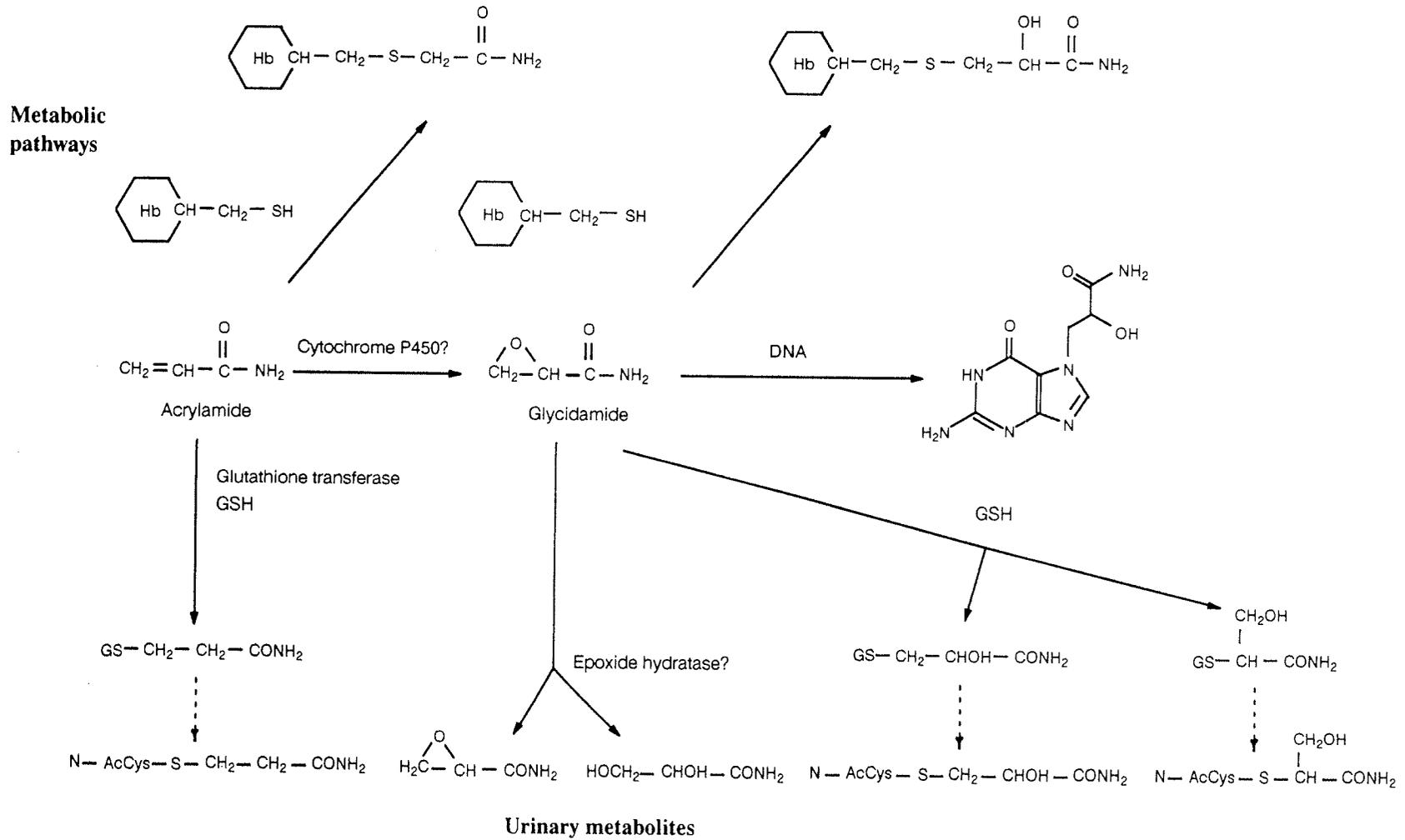
From Sumner *et al.* (1992)

Acrylamide reacts with tissue nucleophiles in Michael-type additions, and glycidamide reacts according to a nucleophilic substitution mechanism; both reactions result in covalent binding of the electrophile. Nonspecific covalent binding of radiolabelled acrylamide with proteins, RNA and DNA has been studied in different tissues of SENCAR and BALB/c mice after both topical and oral administration (Carlson & Weaver, 1985).

Covalent binding of acrylamide to central nervous system proteins may play a role in its neurotoxicity. In attempts to identify a putative target enzyme of acrylamide-induced peripheral neuropathy, inhibition of such enzymes as neuron-specific enolase (Howland *et al.*, 1980a,b), creatine kinase (Matsuoka *et al.*, 1990) and transglutaminase (Bergamini & Signorini, 1990) has been demonstrated *in vivo*, as has covalent binding to a range of proteins in the nervous system (Carrington *et al.*, 1991).

It has also been shown that a single intraperitoneal administration of acrylamide to rats of 25–100 mg/kg bw results in increased lipid peroxidation and decreased glutathione content and glutathione *S*-transferase activity in liver, while only the glutathione content was decreased in the brain (Srivastava *et al.*, 1983).

Fig. 1. Metabolism of acrylamide in mice and rats *in vivo*



Adapted from Sumner *et al.* (1992); Hb, haemoglobin; AcCys, acetylcysteine

(b) *Formation of haemoglobin adducts*

Hashimoto and Aldridge (1970) noted that a large percentage (12%) of an administered dose of acrylamide bound to Hb of red blood cells. On the basis of this observation, Bailey *et al.* (1986) developed a gas chromatographic-mass spectrometric technique to measure the formation of *S*-(2-carboxyethyl)cysteine in rats given 0.1–5 mg/kg bw acrylamide intravenously, after total hydrolysis of the protein. Using a modification of this technique, Calleman *et al.* (1990) identified the cysteine adduct, *S*-(2-hydroxy-2-carboxyethyl)cysteine, formed by reaction of glycidamide with Hb. Bergmark *et al.* (1991) developed a method to determine Hb adducts formed by both acrylamide and glycidamide with cysteine residues in rats. The Hb binding index of acrylamide with cysteine was 6400 pmol acrylamide residue/g Hb per μmol acrylamide/kg bw, which is higher than the indices for all other compounds so far studied in rats and higher than that of glycidamide, which was 1820 pmol glycidamide residue/g Hb per μmol glycidamide/kg bw after peritoneal injection at 50 mg/kg bw. Background levels of both cysteine adducts were determined in control rats. While Hb adduct formation was a linear function of dose when the metabolite glycidamide was administered to animals, Hb adduct formation by either acrylamide or glycidamide was strongly dose-dependent in rats treated with the parent compound. Thus, a cumulative dose of 100 mg/kg bw acrylamide given intraperitoneally to Sprague-Dawley rats produced Hb adduct levels ranging from 8.6 $\mu\text{mol/g}$ Hb of *S*-(2-carboxyethyl)cysteine and 0.36 $\mu\text{mol/g}$ Hb of *S*-(2-carboxy-2-hydroxy-2-ethyl)cysteine when given as a single injection to 3.6 and 0.75 $\mu\text{mol/g}$ Hb, respectively, when administered as daily doses of 3.3 mg/kg bw for 30 days. After correction for the life-span of the erythrocytes and the increase in body weight of the animals, this dose-rate effect resulted in ratios between the estimated doses received (the concentration in the blood compartment of free electrophilic agents integrated over time) of glycidamide and acrylamide of [0.08, 0.32 and 0.47], depending on whether the dose was fractionated equally over 1, 10 or 30 days, respectively.

The observed dose-rate effect was presumed to be a result of the Michaelis-Menten kinetics of the metabolic conversion of acrylamide to glycidamide, and a mathematical model has been developed to describe the formation of Hb adducts in animals treated with acrylamide (Calleman *et al.*, 1992, 1993). Using the experimental data of Bailey *et al.* (1986) and Bergmark *et al.* (1991), Calleman *et al.* (1992) estimated the maximal metabolic rate (V_{max}), the Michaelis-Menten constant (K_m) and the first-order rates of elimination of acrylamide and glycidamide from all other processes by means of the model (Table 7). At a low concentration, the ratio of the dose received (the concentration in the blood compartment of free electrophilic agents integrated over time) of glycidamide to that of acrylamide was estimated to be 0.58.

(c) *Formation of adducts with cytoskeletal proteins*

Binding of ^{14}C -acrylamide to rat neurofilament and microtubular proteins has been studied *in vitro* (Lapadula *et al.*, 1989). Binding to microtubule-associated proteins 1 and 2 was at least an order of magnitude greater than that to tubulin. Other proteins that bound acrylamide significantly were heavy- and medium-weight neurofilaments and an unidentified 53 kDa protein (Table 8).

Table 7. Pharmacokinetic parameters of acrylamide and glycidamide determined in the blood of rats *in vivo*

Parameter	Compound	
	Acrylamide	Glycidamide
Linear model		
k_{el}	0.36/h ^a	
	0.40/h ^b	
	0.37/h ^c	0.48/h ^c
	0.50/h ^d	
$t_{1/2}$	1.4–1.9/h ^{c,d}	1.5/h ^c
Non-linear model		
V_{max}	19 $\mu\text{mol/h}^e$	
K_m	66 $\mu\text{mol/h}^e$	
k'_{el}	0.21/h ^f	

k_{el} , first-order rate of elimination; $t_{1/2}$, half-life; V_{max} , maximal metabolic rate; K_m , Michaelis-Menten constant; k'_{el} , first-order rate of elimination from all processes other than metabolic conversion to glycidamide

^aCalculated by Calleman *et al.* (1992) from Edwards (1975); 100 mg/kg intravenously

^bCalculated by Calleman *et al.* (1992) from Miller *et al.* (1982); 10 mg/kg orally

^cFrom Bergmark *et al.* (1991); average for 0.5–100 mg/kg intraperitoneally

^dFrom Calleman *et al.* (1992); low-dose estimate

^eFrom Calleman *et al.* (1992); metabolic conversion of acrylamide to glycidamide

^fFrom Calleman *et al.* (1992)

Table 8. Specific activities of binding of ¹⁴C-acrylamide to cytoskeletal proteins

Protein	¹⁴ C-Acrylamide bound (mmol/mol protein)
Microtubule-associated protein-1	2.21
Microtubule-associated protein-2	0.89
Tubulin	0.04
Heavy-weight neurofilament	0.53
Medium-weight neurofilament	0.31
Light-weight neurofilament	0.06
53-kDa protein	0.43
Glial fibrillary acidic protein	0.02

From Lapadula *et al.* (1989)

(d) *Formation of adducts with protamines*

Sperm from the vasa deferentia of mice that had been injected intraperitoneally with 125 mg/kg ^{14}C -acrylamide was analysed for radioactivity in the sperm head, sperm DNA and sperm protamines at approximately daily intervals for three weeks. The radioactivity associated with purified protamine closely paralleled the total radioactivity associated with the sperm. Very little radioactivity ($< 0.5\%$) was associated with sperm DNA. The period of maximal protamine alkylation (about day 8 after dosing; Fig. 2) corresponded to the period of maximal sensitivity for induction of dominant lethal mutations. Analysis of acid-hydrolysed protamine showed that 31% of the protamine adducts co-eluted with *S*-carboxyethylcysteine (Sega *et al.*, 1989).

4.1.3 *Comparison of humans and experimental animals*

The formation of glycidamide and acrylamide adducts on the N-terminal valine of Hb is directly proportional in man and rat. Comparison of free acrylamide in plasma, valine adducts on Hb and urinary *S*-(2-carboxyethyl)cysteine indicate that the rate of elimination of acrylamide is at least five times lower in man than in rats. Therefore, since the integrated concentration-time ratio for glycidamide to acrylamide adducts in man (0.3, see p. 402) is about one-half of that for rats (0.58, see p. 402) at low doses, the tissue dose of glycidamide may be higher in man than in rats, on the basis of an equal uptake of acrylamide.

4.2 Toxic effects

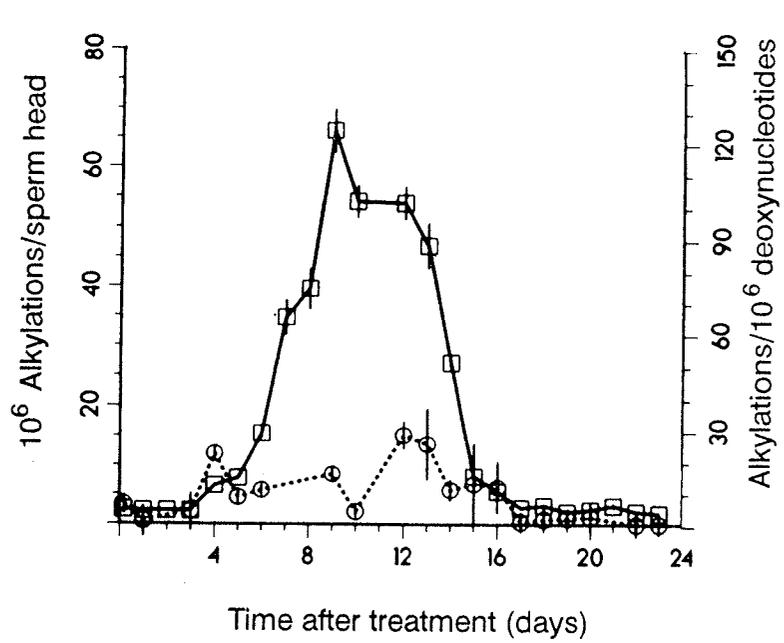
The toxicity of acrylamide has been reviewed (Dearfield *et al.*, 1988; King & Noss, 1989; US Environmental Protection Agency, 1990; Molak, 1991).

4.2.1 *Humans*

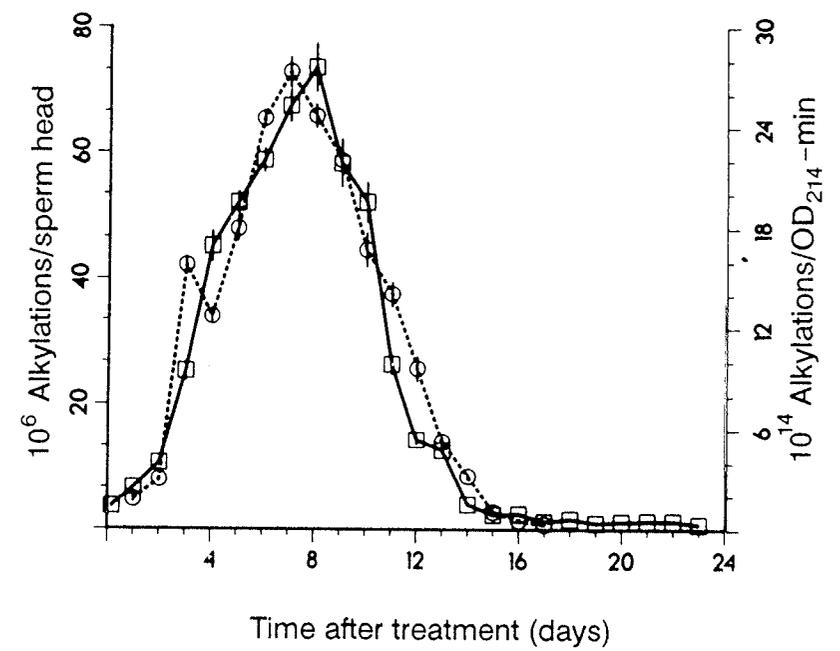
The clinical neurological signs and symptoms resulting from human exposure have been summarized (US Environmental Protection Agency, 1990). They include numbness of hands and feet, peeling of skin and impairment of sensation of vibration, touch and pain; loss of ankle reflexes, muscular atrophy, loss of body weight and ataxia are seen in severe cases. Recovery from mild forms of neuropathy is usually complete within a few months, whereas in severe cases there may never be full recovery. Recent studies of humans poisoned with acrylamide have directed attention to exposures to this substance in developing countries (He *et al.*, 1989; Myers & Macun, 1991) and prompted the development of techniques for human biomonitoring.

In the study carried out in China (described in detail in section 4.1.1; Calleman *et al.*, 1994), significant differences were found in the frequencies of signs and symptoms of neurotoxicity, and differences were seen in vibration sensitivity and electroneuromyographic measurements between the exposed group of workers and controls. These diagnostic indicators of acrylamide neurotoxicity formed the basis for a neurotoxicity index, reflecting a clinical diagnosis of peripheral neuropathy in humans. This index correlated better with biomarkers that reflect long exposure than with momentary measures of air concentrations, and the average levels of Hb adducts paralleled the average neurotoxicity indices in different workshops in the factory.

Fig. 2. Alkylation of sperm heads, sperm DNA and sperm protamine recovered during three weeks after intraperitoneal administration of ^{14}C -acrylamide at 125 mg/kg bw



Alkylation of sperm heads (squares) and DNA (circles) taken from the vasa deferentia. Error bars represent ± 1 SD.



Alkylation of sperm heads (squares) and sperm protamine (circles) recovered from the caudal epididymides. OD_{214} , optical density at 214 nm. Error bars represent ± 1 SD.

From Sega *et al.* (1989)

ACRYLAMIDE

4.2.2 *Experimental systems*

(a) *Acrylamide*

Acrylamide is a cumulative neurotoxicant in several species of experimental animals; notably, it causes peripheral neuropathy (Tilson, 1981). The disorder has been termed central-peripheral distal axonopathy (Spencer & Schaumburg, 1977), since it is characterized by distal retrograde degeneration of long, large-diameter axons in both the central and peripheral nervous systems. Treatment of experimental animals with acrylamide results in reduced water and food consumption, decreased weight gain, weakness and ataxia in hindlimbs, decrease in axonal transport, accumulation of neurofilaments and demyelination of nerves (US Environmental Protection Agency, 1987). An effect of dose rate has been suggested from studies in rats (O'Shaughnessy & Losos, 1986). The central nervous system was affected more strongly than the peripheral nervous system in rats administered acrylamide by intraperitoneal injection at doses of up to 50 mg/kg bw per day for four or 10 days than when the rats received doses of up to 12 mg/kg bw per day for 90 days. The mechanism of the neurotoxic action of acrylamide is not known, but at least three hypotheses have been proposed: inhibition of glycolytic enzymes (Spencer *et al.*, 1979), although non-neurotoxic chemicals can also inhibit these enzymes (Tanii & Hashimoto, 1985); alterations in axonal transport (Miller & Spencer, 1985; Harry, 1992); and filament degradation (Sager & Matheson, 1988; Tanii *et al.*, 1988; Carrington *et al.*, 1991). These hypotheses are not mutually exclusive. The last, in particular, may affect our understanding of the genotoxicity of acrylamide and its neoplastic properties in experimental systems. In PtK1 kidney epithelial cells, acrylamide caused networks of both vimentin and keratin filaments to collapse into a juxtannuclear aggregate (Eckert, 1986). Such effects occur at concentrations that do not affect microtubular polymerization (Sager & Matheson, 1988), and they are reversible (Denèfle & Zhu, 1989). The lack of effect of acrylamide on polymerization of microtubular protein was confirmed *in vitro*. Furthermore, the effects in cells of acrylamide and colchicine (a potent anti-microtubular agent) are additive (Shiver *et al.*, 1992).

(b) *Glycidamide*

Glycidamide depresses body weight in rats (Hashimoto *et al.*, 1988) and has a potent effect on rats undergoing the rotarod test for neurotoxicity; in contrast to acrylamide, it does not affect hindlimb splay (Costa *et al.*, 1992). Although glycidamide was as cytotoxic as acrylamide to mouse neuroblastoma cells *in vitro*, it did not cause a decrease in the number of neurites (Walum *et al.*, 1992). These findings indicate that glycidamide is not the agent that causes peripheral neuropathy, although potentially conflicting results have been reported (Abou-Donia *et al.*, 1993).

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

The reproductive, developmental and genotoxic effects of acrylamide have been reviewed (Dearfield *et al.*, 1988).

Male ddY mice receiving oral doses of 35 mg/kg bw acrylamide twice weekly for eight weeks developed testicular atrophy and degeneration of the germinal epithelium, but Sertoli and interstitial cells did not appear to be affected. Neurotoxic signs were observed, but when mice were treated concurrently with intraperitoneal injections of phenobarbital, the neurotoxic signs were significantly reduced and testicular toxicity was prevented, probably because of accelerated detoxification (Hashimoto *et al.*, 1981). Sakamoto *et al.* (1988) treated male ddY mice with a single oral dose of 100 or 150 mg/kg bw acrylamide and studied the histopathological changes in the testis over the following 10 days. In both immature and adult mice, round spermatids, especially in the Golgi phase, were sensitive to the toxic effects of acrylamide, and all other cell types (spermatogonia, spermatocytes, Sertoli cells and Leydig cells) were unchanged.

Marlowe *et al.* (1986) using autoradiography, showed that 120 mg/kg bw acrylamide administered orally to Swiss-Webster mice crossed the placenta and was distributed in the fetus on days 13.5 and 17.5 of gestation; on day 17.5, intense accumulation occurred in fetal skin. When acrylamide or its metabolites were administered to male Swiss-Webster mice, they appeared to bind to mature spermatids, since activity was observed in the seminiferous tubules and the head of the epididymis by 9 h after administration; by nine days, activity was present only in the tail of the epididymis and in the glans penis.

A reduced content of striatal dopamine receptors was seen in male but not female neonates at 14 days of age but not at 21 days after oral administration of 20 mg/kg bw acrylamide per day to pregnant Fischer 344 rats on days 7–16 of gestation (Agrawal & Squibb, 1981). Increased numbers of dopamine receptors were seen in the striatum of adult acrylamide-treated rats one day but not eight days after treatment for 10 days (Agrawal *et al.* 1981).

Reproductive toxicity was investigated in male and female Long-Evans rats by Zenick *et al.* (1986). For the studies in males, groups of 15 rats were treated with 0, 50, 100 or 200 ppm (mg/L) acrylamide in the drinking-water from 70 days of age onwards. Sexual behaviour was assessed by mating them with hormonally primed, ovariectomized females before and at two-week intervals during treatment. Sperm count, motility and morphology were assessed by recovering the ejaculate from the female reproductive tract before treatment began and after nine weeks of treatment. After 10 weeks of treatment, the control and 100-ppm groups were mated with untreated females and the females were killed on day 17 of gestation to record the number of implants. Males were killed after 11 weeks of treatment; one testis and epididymis were examined histologically and the others for spermatid and sperm counts. Severe toxicity and death were observed by week 5 in the 200-ppm group, and treatment of this group was terminated at week 6. No mortality or weight loss was observed in the other groups, but some hind limb splaying was observed in the 100-ppm group at week 8. Treatment with 50 or 100 ppm induced a significant increase in sexual activity, but reduced ejaculatory ability and reduced sperm counts were seen at 100 ppm: the sperm of only 1/15 animals reached the uterus after mating at week 9. No adverse effect on sperm count, motility

or morphology was observed at 50 ppm. Fertility at week 10 was also decreased at 100 ppm, with only 5/15 mated and 11/14 control females becoming pregnant; postimplantation loss was also increased (32% versus 8%; $p < 0.01$). On examination *post mortem* at week 11, no adverse effect was observed at 50 or 100 ppm on body or organ weights, on spermatid counts in the testis, on epididymal sperm counts or on testicular histology. The authors suggested that the reduced sperm count observed at 100 ppm in the mating trials resulted from disrupted copulatory behaviour which caused vaginal leakage of the sperm.

For the studies in females, Zenick *et al.* (1986) treated groups of 15 rats that had regular oestrous cycles with acrylamide at 0, 25, 50 or 100 ppm (equivalent to about 10–20 mg/kg bw at the highest dose) in the drinking-water; a pilot study had shown that a dose of 200 ppm was too toxic. After two weeks of treatment, the females were mated with untreated males, and exposure was continued throughout gestation and lactation; the pups were killed on postnatal day 42. Hindlimb splaying was observed during the first and second week of gestation in the 100-ppm group only, with depressed body weight from week 2 of treatment onwards. Dams in the 50-ppm group had depressed body weight only during the lactation phase. There was no adverse effect on mating performance or pregnancy rate and no significant difference in litter size or pup survival. There was a small but significant decrease in pup weight at birth in the 100-ppm group, which persisted until termination of the study on day 42. The pups from the 50-ppm group also had reduced body weight from seven days of age onwards. At 25 ppm, female pup weight was depressed on days 7 and 14 only. Vaginal opening was delayed in the pups of the 100-ppm group (day 36.0 ± 1.6 versus day 33.2 ± 1.9 ; $p < 0.01$). The cumulative intake of males at the onset of hindlimb ataxia was 544 mg/kg bw at 8–10 weeks, but copulatory behaviour was affected before ataxia was observed. The increase in postimplantation loss (up to 50%) in the mated females in the only five successful matings may indicate a dominant lethal effect (reported in other studies). It is unclear from the study whether the decrease in pup weight was secondary to toxicity in the dams or was a direct effect of acrylamide acting on the pups via the milk.

Groups of 50 male Fischer 344 rats were dosed orally by gavage with 30 mg/kg bw acrylamide daily for five days and then mated with one female each per week for 10 weeks. Pre- and post-implantation losses were significantly raised in the first four and three weeks, respectively, after exposure, both returning to control values for the remaining six weeks. This indicates that acrylamide induces dominant lethal mutations in mature spermatozoa and late-stage spermatids (Working *et al.*, 1987).

Sublet *et al.* (1989) investigated the possibility that the reduction in fertility and the increase in pre-implantation loss observed after acrylamide treatment might be due to factors other than chromosomal damage. Groups of 15 male Long-Evans hooded rats were treated orally with doses ranging from 5 to 60 mg/kg bw acrylamide for five consecutive days and mated for 4–10 weeks after exposure. Reduced fertility and pre- and post-implantation loss were observed mainly in the first three weeks after treatment. In a subsequent experiment to study factors associated with reduced implantation frequencies, decreased entry of sperm from the vagina to the uterus was observed in the first week, and there were decreased percentages of motile sperm and decreased sperm velocity in weeks 2 and 3. Ova recovered 10–14 h after copulation showed a decreased fertilization rate.

In a study of the effects of acrylamide on protein-deficient rats (Khanna *et al.*, 1988), 32 pregnant Wistar rats were fed either a normal protein diet (20% casein) or a low-protein diet (8% casein) from day 1 of gestation (day of vaginal plug). Half of each group was treated orally [presumably by gavage] from day 6 of gestation until weaning with 0.3 mg/kg bw acrylamide; controls received saline. At parturition, litters were culled to eight pups, so that each study group consisted of four litters of eight pups each. Postnatal development was studied up to weaning, on day 21 post-partum. Acrylamide had no effect on the weight of the pups of dams fed the normal diet but produced a significant reduction in the body weight of pups of dams fed the protein-deficient diet in comparison with that of their respective controls. Protein deficiency alone had no effect on the time of development of landmarks such as eye opening, pinna detachment, incisor eruption or reflex development, and acrylamide had no effect on the time of development of these landmarks in pups of the group fed the normal diet; however, acrylamide significantly retarded the appearance of the landmarks, including surface and air righting reflexes and locomotion, in pups of dams on the low-protein diet. It also reduced the numbers of dopamine and benzodiazepine binding sites in the striatum and cortex, respectively, in pups of the protein-deficient group. In a similar study (Khanna *et al.*, 1992), in which pregnant Wistar rats on high- and low-protein diets were dosed on days 6–17 of gestation, higher doses of acrylamide (3–10 mg/kg bw) given orally daily induced high mortality in dams fed the low-protein diet. In animals treated with 2 mg/kg bw acrylamide on days 6–17 and examined on day 18, there was decreased activity of brain monoamine oxidase and acetylcholine esterase; in pups of dams fed on the low-protein diet there was a decrease in striatal dopaminergic, cerebellar muscarinic and frontocortical diazepam binding sites.

In a combined study of mutagenicity and teratogenicity (Neuhäuser-Klaus & Schmahl, 1989), female T stock mice were mated with HT males and injected intraperitoneally with 75 mg/kg bw acrylamide once on day 12, or with 50 or 75 mg/kg bw on days 10, 11 and 12 of gestation; controls were injected with 10 ml/kg bw distilled water. The fetuses were examined macroscopically on day 18, and malformed fetuses and five controls were subjected to histological examination. Kinked tails and haemorrhages were observed in 4.2% of all treated fetuses and in 1.3% of controls ($p = 0.05$). The three daily doses of 75 mg/kg caused increased postimplantation loss and decreased fetal weight. Histological examination of eight treated fetuses showed hypoplasia of lymphatic organs and of centres of haematopoiesis in liver and bone marrow. Positive results were observed in the spot test in pups that were allowed to be delivered; the doubling dose was calculated to be 30 mg/kg bw acrylamide. Kinked tails were also observed in these offspring.

A full study of the teratogenicity of acrylamide in Swiss CD-1 and Sprague-Dawley rats has been reported by Field *et al.* (1990). Groups of 30 mice (25–29 pregnant at term) were dosed by gavage with 0, 3, 15 or 45 mg/kg bw acrylamide per day from days 6 to 17 gestation (vaginal plug = day 0); fetuses were removed and examined on day 17. All fetuses were examined by dissection and by staining for skeletal malformations. Maternal toxicity was observed at the highest dose only, with half the animals showing hindlimb splaying by day 17 and reduced body weight gain. No effect was seen on the numbers of implantations, resorptions or live fetuses per litter or overall, or on specific types of malformations. Fetal body weight was reduced at the highest dose. The only other significant effect was a

dose-related trend in the percentage of fetuses per litter with extra ribs, from $3.4\% \pm 1.2$ in controls to $13.9\% \pm 3.7$ at the highest dose ($p < 0.05$). Groups of 29–30 rats (23–26 pregnant at term) were dosed by gavage with 0, 2.5, 7.5 or 15 mg/kg bw acrylamide per day from day 6 to 20 of gestation (vaginal plug = day 0) and the fetuses removed for examination as described above on day 20. Maternal weight gain (minus gravid uterine weight) was reduced at 7.5 and 15 mg/kg bw, but no other clinical sign of toxicity was observed. No adverse effect was observed on the numbers of implantations, resorptions or live fetuses per litter, fetal weight or malformations. The only effect observed was a dose-related trend in the percentage of fetuses per litter with skeletal variations, which were mainly rudimentary extra ribs (from $11.1\% \pm 3.6$ in controls to $16.4\% \pm 4.0$ at the highest dose). No malformations of the tail were observed in either mice or rats. The authors commented that the extra ribs may have been related more to maternal toxicity than to a direct effect of acrylamide on the fetuses. No maternal or fetal toxicity was seen at 15 mg/kg bw per day in mice; in rats, no effect on maternal toxicity was seen at 2.5 mg/kg bw per day and probably no effect on fetal toxicity 15 mg/kg bw per day.

The reproductive toxicity of acrylamide and its epoxide metabolite, glycidamide, was studied in male Sprague-Dawley rats by Costa *et al.* (1992). Groups of eight rats were injected intraperitoneally once a day with 50 mg/kg bw acrylamide for seven days or with 50 mg/kg bw glycidamide for 14 days. Twenty-four hours after the last dose, the animals were killed and the testes removed and weighed to measure epididymal and vas deferens sperm count and viability. Neither substance affected testicular weight. Vas deferens sperm count was reduced by both substances, and glycidamide but not acrylamide reduced testicular protein content, epididymal weight and vas deferens sperm viability.

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 9 and Appendices 1 and 2)

(a) Formation of DNA adducts

Acrylamide reacts very slowly with DNA *in vitro*, with a rate constant of $[9 \times 10^{-12} \text{ L/mg DNA per h}]$ at pH 7.0 and 37 °C, estimated on the basis of all types of DNA adducts combined. The principal site of reaction of acrylamide with calf thymus DNA was the N1 of adenine, and only 14% of the reaction products was with the N7 of guanine. In some DNA adducts formed *in vitro*, the amide group of the adducted acrylamide moiety underwent spontaneous hydrolysis to a carboxylic acid group (Solomon *et al.*, 1985).

Carlson and Weaver (1985) and Segal *et al.* (1990) measured the total amount of radioactivity associated with DNA in acrylamide-treated mice. Carlson *et al.* (1986) found more radioactivity associated with DNA after oral administration of $[2,3-^{14}\text{C}]$ acrylamide than after topical administration in all tissues except the skin of SENCAR mice. This result appears to conflict with the data of Bull *et al.* (1984a), who found a higher frequency of skin tumours in the same strain of mice after oral than after topical administration. [The Working Group

noted that no attempt was made in any of these studies of tissue distribution to isolate specific DNA adducts, so that metabolic incorporation or contamination by non-covalently bound metabolites cannot be ruled out; it is not clear whether the skin samples analysed were from the application area.]

7-(2-Carbamoyl-2-hydroxyethyl)guanine formed by the metabolite glycidamide is the predominant adduct in DNA of rats and mice exposed to ^{14}C -acrylamide (Segeberäck *et al.*, 1994).

(b) *Mutation and allied effects*

Acrylamide did not increase the mutation frequency in bacteria, but it induced differential toxicity in one *rec* assay.

Somatic mutation and sex-linked recessive lethal mutations have been observed in *Drosophila melanogaster* after feeding of acrylamide to larvae. In contrast, no increase in the frequency of sex-linked recessive lethal mutations was observed in a single-injection experiment.

Unscheduled DNA synthesis in primary cultures of rat hepatocytes was not observed in two studies.

Mutations were induced at both the *tk* and *hprt* locus in mouse lymphoma L5178Y cells but not at the *hprt* locus in Chinese hamster V79H3 cells. In those studies that showed an effect, they were observed at single doses within each experiment and at relatively low (< 10%) survival.

There is consistent evidence for the induction of sister chromatid exchange and chromosomal aberrations in cultured mammalian cells, including cultures of human lymphocytes. Various mitotic disturbances have also been reported in cultured mammalian cells exposed to acrylamide. It induced significant transformation in BALB/c 3T3, C3H10T $\frac{1}{2}$ and NIH 3T3 cell lines.

[The Working Group noted that the activity of acrylamide in cultured mammalian cells was also seen in the absence of an exogenous metabolic activation system, implying that glycidamide might not be the responsible agent.]

Alkali-labile sites and DNA single-strand breakage have been observed in germ-line cells of mice. Conflicting results were reported for the induction of unscheduled DNA synthesis in rat liver *in vivo*.

In the MutaTM mouse, a transgenic mouse, three-fold and six-fold increases in mutant frequency were reported. These are considered to be equivocal results from this developing assay, since there is considerable variation in control mutation frequencies.

An intraperitoneal dose of 50 mg/kg bw acrylamide induced a significant response in the mouse spot test, in which the spots may result not only from point mutations but also from chromosomal aberrations, chromosomal loss or somatic recombination. Additionally, in the morphological specific-locus test in mice, which allows detection of both small and large genetic lesions, a significant increase in mutation rate was seen in certain stages of spermatogenesis, particularly on days 5–12 after treatment (spermatozoa and spermatids). On the basis of cytogenetic evidence, four of the six verified specific locus mutations induced postmeiotically were considered to be multi-locus lesions. Testing of spermatogonial stages

yielded negative results with a fractionated treatment regimen of 5×50 mg/kg bw in one study but positive results after a single injection of 100 mg/kg bw in another.

Acrylamide induced sister chromatid exchange in splenocytes but not in bone marrow or spermatogonia of mice. In several studies, acrylamide induced micronuclei in mouse bone-marrow cells. [Whether the micronuclei indicate structural chromosomal aberration or aneuploidy induction cannot be concluded from these data.] Micronuclei have also been observed in mouse splenocytes and spermatids following exposure to acrylamide during meiosis. In two of three studies, acrylamide given at intraperitoneal doses of 50 or 100 mg/kg bw to mice *in vivo* induced chromosomal aberrations in bone-marrow cells; however, an intraperitoneal dose of 125 mg/kg bw did not induce chromosomal aberrations in mouse splenocytes.

Except in one study, chromosomal aberrations were not induced in differentiating spermatogonia by single or repeated doses of acrylamide; however, a clastogenic effect on spermatocytes was observed in three independent studies. The induction of dominant lethal mutation in rats and mice after various treatment regimens, including one dermal application, was demonstrated in several laboratories. The stages sensitive to dominant lethal effects (late spermatids to early spermatozoa) correspond to increased DNA breakage and parallel the pattern of sperm alkylation and protamine alkylation. Heritable translocations were also induced in male mice in two independent studies.

Studies of aneuploidy induction in bone marrow and spermatogonia *in vivo* gave either negative or inconclusive results, due to the combined reporting of polyploidy and aneuploidy in one study and to protocol uncertainties in another. Hyperploidy was observed in spermatids. Moreover, approximately one-third of the micronuclei found in spermatids after acrylamide treatment of prophase cells showed kinetochore-positive staining, which might suggest a mixed breakage-aneuploidy response (Collins *et al.*, 1992).

(c) *Mutagenicity of glycidamide*

Glycidamide is mutagenic to *Salmonella typhimurium* but not to *Klebsiella pneumoniae*. It is more mutagenic than acrylamide to mouse lymphoma cells. Glycidamide induced unscheduled DNA synthesis in a cell line, in primary cultures of rat hepatocytes and in mouse spermatids *in vivo*.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Acrylamide has been produced since the 1950s by hydration of acrylonitrile. It is used mainly to produce water-soluble polyacrylamides used as flocculents for clarifying drinking-water, for treating municipal and industrial waste waters and as flow control agents in oil-well operations. Other major uses of acrylamide are in soil stabilization, in grout for repairing sewers and in acrylamide gels used in biotechnology laboratories. The major routes of exposure at the workplace appear to be dermal absorption of acrylamide monomer from solution and inhalation of dry monomer or aerosols of acrylamide solution. Exposure occurs during acrylamide and polyacrylamide manufacture, during acrylamide grouting and during laboratory preparation of polyacrylamide gels.

Table 9. Genetic and related effects of acrylamide

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BSD, <i>Bacillus subtilis</i> spore rec assay, differential toxicity	+	+	10000.0000	Tsuda <i>et al.</i> (1993)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	500.0000	Lijinsky & Andrews (1980)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	15000.0000	Bull <i>et al.</i> (1984a)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	2500.0000	Hashimoto & Tanii (1985)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	10000.0000	Knaap <i>et al.</i> (1988)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	10000.0000	Tsuda <i>et al.</i> (1993)
SA2, <i>Salmonella typhimurium</i> TA102, reverse mutation	-	-	10000.0000	Knaap <i>et al.</i> (1988)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	500.0000	Lijinsky & Andrews (1980)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	15000.0000	Bull <i>et al.</i> (1984a)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	2500.0000	Hashimoto & Tanii (1985)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	10000.0000	Knaap <i>et al.</i> (1988)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	10000.0000	Tsuda <i>et al.</i> (1993)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	500.0000	Lijinsky & Andrews (1980)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	15000.0000	Bull <i>et al.</i> (1984a)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	2500.0000	Hashimoto & Tanii (1985)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	10000.0000	Knaap <i>et al.</i> (1988)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	25000.0000	Tsuda <i>et al.</i> (1993)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	500.0000	Lijinsky & Andrews (1980)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	2500.0000	Hashimoto & Tanii (1985)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	500.0000	Lijinsky & Andrews (1980)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	15000.0000	Bull <i>et al.</i> (1984a)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	2500.0000	Hashimoto & Tanii (1985)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	10000.0000	Knaap <i>et al.</i> (1988)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	10000.0000	Tsuda <i>et al.</i> (1993)
ECW, <i>Escherichia coli</i> WP2 <i>uvrA</i> -, reverse mutation	-	-	25000.0000	Tsuda <i>et al.</i> (1993)
KPF, <i>Klebsiella pneumoniae</i> , forward mutation	-	-	10000.0000	Knaap <i>et al.</i> (1988)
DMM, <i>Drosophila melanogaster</i> , somatic mutation (and recombination)	+		70, larval feeding	Knaap <i>et al.</i> (1988)

Table 9 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DMM, <i>Drosophila melanogaster</i> , somatic mutation	+		100, larval feeding	Batiste-Alentorn <i>et al.</i> (1991)
DMM, <i>Drosophila melanogaster</i> , somatic mutation (and recombination)	+		70, larval feeding	Tripathy <i>et al.</i> (1991)
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutation	-		2800, injection	Knaap <i>et al.</i> (1988)
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutation	+		70, larval feeding	Tripathy <i>et al.</i> (1991)
URP, Unscheduled DNA synthesis, rat hepatocytes <i>in vitro</i>	-	0	700.0000	Miller & McQueen (1986)
URP, Unscheduled DNA synthesis, rat hepatocytes <i>in vitro</i>	?	0	2100.0000	Barfnecht <i>et al.</i> (1987)
URP, Unscheduled DNA synthesis, rat hepatocytes <i>in vitro</i>	-	0	70.0000	Butterworth <i>et al.</i> (1992)
GCL, Gene mutation, Chinese hamster V79H3 <i>in vitro</i> , <i>hprt</i> locus	-	0	500.0000	Tsuda <i>et al.</i> (1993)
G5T, Gene mutation, mouse lymphoma L5178Y cells <i>in vitro</i> , <i>tk</i> locus	+	0	600.0000	Moore <i>et al.</i> (1987)
G5T, Gene mutation, mouse lymphoma L5178Y cells <i>in vitro</i> , <i>tk</i> locus	(+)	(+)	7500.0000	Knaap <i>et al.</i> (1988)
G51, Gene mutation, mouse lymphoma L5178Y cells <i>in vitro</i> , <i>hprt</i> locus	+	+	700.0000	Barfnecht <i>et al.</i> (1988) (abstract)
G51, Gene mutation, mouse lymphoma L5178Y cells <i>in vitro</i> , <i>hprt</i> locus	(+)	(+)	300.0000	Knaap <i>et al.</i> (1988)
SIC, Sister chromatid exchange, Chinese hamster cells <i>in vitro</i>	+	+	300.0000	Knaap <i>et al.</i> (1988)
SIC, Sister chromatid exchange, Chinese hamster V79H3 cells <i>in vitro</i>	+	0	70.0000	Tsuda <i>et al.</i> (1993)
CIC, Chromosomal aberrations, Chinese hamster CHL cells <i>in vitro</i>	+	+	150.0000	Sofuni <i>et al.</i> (1985)
CIC, Chromosomal aberrations, Chinese hamster cells, <i>in vitro</i>	+	+	100.0000	Knaap <i>et al.</i> (1988)
CIC, Chromosomal aberrations, Chinese hamster, V79H3 cells <i>in vitro</i>	+	0	140.0000	Tsuda <i>et al.</i> (1993)
*, DNA amplification, Chinese hamster -SV40 <i>in vitro</i>	(+)	0	150.0000	Vanhorick & Moens (1983)
CIM, Chromosomal aberrations, mouse lymphoma L5178Y cells <i>in vitro</i>	+	0	750.0000	Moore <i>et al.</i> (1987)
AIA, Aneuploidy, Chinese hamster DON cells <i>in vitro</i>	+	0	1000.0000	Warr <i>et al.</i> (1990)
AIA, Aneuploidy, Chinese hamster LUC 2 cells <i>in vitro</i>	(+)	0	500.0000	Warr <i>et al.</i> (1990)
AIA, Aneuploidy, Chinese hamster V79H3 cells <i>in vitro</i>	+	0	70.0000	Tsuda <i>et al.</i> (1993)
AIA, Aneuploidy, Chinese hamster V79 cells, <i>in vitro</i>	+	0	10.0000	Adler <i>et al.</i> (1993a)
TBM, Cell transformation, BALB/c3T3 mouse cells	+	0	70.0000	Tsuda <i>et al.</i> (1993)

Table 9 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
TCM, Cell transformation, C3H10T1/2 mouse cells	+	0	50.0000	Banerjee & Segal (1986)
TCL, Cell transformation, NIH 3T3 mouse cells	+	0	12.5000	Banerjee & Segal (1986)
UIH, Unscheduled DNA synthesis, human epithelial cells <i>in vitro</i>	(+)	0	710.0000	Butterworth <i>et al.</i> (1992)
SHL, Sister chromatid exchange, human lymphocytes <i>in vitro</i>	(+)	(+)	0.0000	Norppa & Tursi (1984)
DVA, DNA strand breaks, mouse spermiogenic stages <i>in vivo</i>	+		25×1 ip	Sega & Generoso (1990)
UPR, Unscheduled DNA synthesis, rat hepatocytes <i>in vivo</i>	-		100×1 po	Butterworth <i>et al.</i> (1992)
UVA, Unscheduled DNA synthesis, rat spermatocytes <i>in vivo</i>	+		30×5 po	Butterworth <i>et al.</i> (1992)
GVA, Gene mutation, Muta mouse, bone-marrow cells <i>in vivo</i>	?		50×5 ip	Hoorn <i>et al.</i> (1993)
MST, Mouse spot test	+		50×1 ip	Neuhäuser-Klaus & Schmahl (1989)
SLP, Specific locus mutation, mouse, postspematogonia	+		50×5 ip	Russell <i>et al.</i> (1991)
SLP, Specific locus mutation, mouse, spermatozoa and spermatids	+		100×1 ip	Ehling & Neuhäuser-Klaus (1992)
SLO, Specific locus mutation, mouse, other stages	-		50×5 ip	Russell <i>et al.</i> (1991)
SLO, Specific locus mutation, mouse, spermatogonia	+		100×1 ip	Ehling & Neuhäuser-Klaus (1992)
SVA, Sister chromatid exchange, mouse bone-marrow cells <i>in vivo</i>	-		100×1 ip	Shiraishi (1978)
SVA, Sister chromatid exchange, mouse stem cells and spermatogonia treated <i>in vivo</i> , spermatogonia observed	-		100×1 ip	Shiraishi (1978)
SVA, Sister chromatid exchange, mouse splenocytes <i>in vivo</i>	+		50×1 ip	Backer <i>et al.</i> (1989)
MVM, Micronucleus formation, mouse bone-marrow cells <i>in vivo</i>	+		50×1 ip	Adler <i>et al.</i> (1988)
MVM, Micronucleus formation, mouse bone-marrow cells <i>in vivo</i>	+		100×1 ip	Čihák & Vontorková (1988)
MVM, Micronucleus formation, mouse bone-marrow cells <i>in vivo</i>	+		25×2 ip	Čihák & Vontorková (1988)
MVM, Micronucleus formation, mouse bone-marrow cells <i>in vivo</i>	+		42.5×1 ip	Čihák & Vontorková (1990)
MVM, Micronucleus formation, mouse bone-marrow cells <i>in vivo</i>	+		136×1 ip	Knaap <i>et al.</i> (1988)
MVM, Micronucleus formation, mouse splenocytes <i>in vivo</i>	+		50×1 ip	Backer <i>et al.</i> (1989)
MVM, Micronucleus formation, mouse spermatid <i>in vivo</i>	+		50×1 ip	Collins <i>et al.</i> (1992)

Table 9 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
MVM, Micronucleus formation, mouse peripheral blood erythrocytes <i>in vivo</i>	+		50×1 ip	Cao <i>et al.</i> (1993)
CBA, Chromosomal aberrations, mouse bone-marrow cells <i>in vivo</i>	-		60×3 wk diet	Shiraishi (1978)
CBA, Chromosomal aberrations, mouse bone-marrow cells <i>in vivo</i>	-		100×1 ip	Shiraishi (1978)
CBA, Chromosomal aberrations, mouse bone-marrow cells <i>in vivo</i>	+		50×1 ip	Adler <i>et al.</i> (1988)
CBA, Chromosomal aberrations, mouse bone-marrow cells <i>in vivo</i>	+		100×1 ip	Čihák & Vontorková (1988)
CVA, Chromosomal aberrations, mouse splenocytes <i>in vivo</i>	?		125×1 ip	Backer <i>et al.</i> (1989)
CCC, Chromosomal aberrations, mouse spermatocytes treated <i>in vivo</i> , spermatocytes observed	(+)		60×3 wk diet	Shiraishi (1978)
CCC, Chromosomal aberrations, mouse spermatocytes treated <i>in vivo</i> , spermatocytes observed	+		100×1 ip	Kliesch <i>et al.</i> (1989) (abstract)
CCC, Chromosomal aberrations, mouse spermatocytes treated <i>in vivo</i> , spermatocytes observed	+		100×1 ip	Adler (1990)
CGC, Chromosomal aberrations, mouse spermatogonia treated <i>in vivo</i> , spermatocytes observed	(+)		100×1 ip	Shiraishi (1978)
CGC, Chromosomal aberrations, mouse spermatogonia treated <i>in vivo</i> , spermatocytes observed	-		100×1 ip	Kliesch <i>et al.</i> (1989) (abstract)
CGC, Chromosomal aberrations, mouse spermatogonia treated <i>in vivo</i> , spermatocytes observed	-		100×1 ip	Adler (1990)
CGC, Chromosomal aberrations (reciprocal translocations), rat spermatogonial stem cells treated <i>in vivo</i> , spermatocytes observed	?		5.8×80 drin- king-water	Smith <i>et al.</i> (1986)
CGC, Chromosomal aberrations, mouse spermatogonial stem cells treated <i>in vivo</i> , spermatocytes observed	-		125×1 ip	Backer <i>et al.</i> (1989)
CGG, Chromosomal aberrations, mouse spermatogonial stem cells treated <i>in vivo</i> , spermatogonia observed	-		125×1 ip	Backer <i>et al.</i> (1989)
CGG, Chromosomal aberrations, mouse spermatogonial stem cells treated <i>in vivo</i> , spermatogonia observed	+		60×3 wk diet	Shiraishi (1978)

Table 9 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
CGG, Chromosomal aberration, mouse spermatogonial stem cells and spermatogonia treated <i>in vivo</i> , spermatogonia observed	+		100×1 ip	Shiraishi (1978)
CGG, Chromosomal aberrations, rat spermatogonia treated <i>in vivo</i> , spermatogonia observed	-		5.8×80 drinking-water	Smith <i>et al.</i> (1986)
CGG, Chromosomal aberrations, mouse spermatogonia treated <i>in vivo</i> , spermatogonia observed	-		150×1 ip	Adler <i>et al.</i> (1988)
DLM, Dominant lethal mutation, mouse	+		125×1 ip	Shelby <i>et al.</i> (1986)
DLM, Dominant lethal mutation, mouse	+		50×5 ip	Shelby <i>et al.</i> (1986)
DLM, Dominant lethal mutation, mouse	+		40×5 ip	Shelby <i>et al.</i> (1987)
DLM, Dominant lethal mutation, mouse	+		125×1 ip	Dobrzyńska <i>et al.</i> (1990)
DLM, Dominant lethal mutation, mouse	+		100×1 ip	Ehling & Neuhäuser-Klaus (1992)
DLM, Dominant lethal mutation, mouse	+		25×5 topical application	Gutierrez-Espeleta <i>et al.</i> (1992)
DLR, Dominant lethal mutation, rat	+		2.8×80 drinking-water	Smith <i>et al.</i> (1986)
DLR, Dominant lethal mutation, rat	+		30×5 gavage	Working <i>et al.</i> (1987)
MHT, Heritable translocation, mouse	+		40×5 ip	Shelby <i>et al.</i> (1987)
MHT, Heritable translocation, mouse	+		50×5 ip	Adler (1990)
AVA, Aneuploidy, mouse bone-marrow cells <i>in vivo</i>	?		60×3 wk diet	Shiraishi (1978)
AVA, Aneuploidy, mouse bone-marrow cells <i>in vivo</i>	?		100×1 ip	Shiraishi (1978)
AVA, Aneuploidy, mouse spermatogonia <i>in vivo</i>	?		60×3 wk diet	Shiraishi (1978)
AVA, Aneuploidy, mouse spermatogonia <i>in vivo</i>	?		100×1 ip	Shiraishi (1978)
AVA, Aneuploidy, mouse spermatogonia, hyperploidy	-		125×1 ip	Backer <i>et al.</i> (1989)
*, Aneuploidy, mouse spermatid micronuclei and kinetochore staining	(+)		50×1 ip	Collins <i>et al.</i> (1992)
AVA, Aneuploidy, mouse bone-marrow cells <i>in vivo</i> (mitotic delay)	-		120×1 ip	Adler <i>et al.</i> (1993b)
AVA, Aneuploidy, mouse spermatid, hyperploidy	+		120×1 ip	Adler <i>et al.</i> (1993b)
AVA, Aneuploidy, mouse spermatocytes, meiotic delay	+		120×1 ip	Adler <i>et al.</i> (1993b)
BIP, Binding (covalent) to neurofilaments and microtubule-associated proteins from rat neural cells <i>in vitro</i>	+		0.7	Lapadula <i>et al.</i> (1989)
BID, Binding (covalent) to 2'-deoxynucleosides <i>in vitro</i>	+		96000.0000	Solomon <i>et al.</i> (1985)

Table 9 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BID, Binding (covalent) to DNA from calf thymus <i>in vitro</i>	+		96000.0000	Solomon <i>et al.</i> (1985)
BVD, Binding (covalent) to DNA, mouse testis, skin, lung, liver, stomach <i>in vivo</i>	+		100×1 po	Carlson & Weaver (1985)
BVD, Binding (covalent) to DNA, mouse testis, skin, lung, liver, stomach <i>in vivo</i>	+		100×1 topical application	Carlson & Weaver (1985)
BVD, Binding (covalent) to DNA, mouse spermatocytes-spermatids <i>in vivo</i>	+		125 ip	Sega <i>et al.</i> (1989)
BVD, Binding (covalent) to DNA, mouse testis <i>in vivo</i>	+		46×1 ip	Sega <i>et al.</i> (1990)
BVD, Binding (covalent) to DNA, mouse liver <i>in vivo</i>	+		46×1 ip	Sega <i>et al.</i> (1990)
BVD, Binding (covalent) to DNA, rat liver, lung, kidney, spleen, brain and testis <i>in vivo</i>	+		50×1 ip	Segeberäck <i>et al.</i> (1994)
BVP, Binding (covalent) to RNA, mouse testis, skin, lung, liver, stomach <i>in vivo</i>	+		100×1 po	Carlson & Weaver (1985)
BVP, Binding (covalent) to RNA, mouse testis, skin, lung, liver, stomach <i>in vivo</i>	+		100×1 topical application	Carlson & Weaver (1985)
SPM, Sperm morphology, mouse <i>in vivo</i>	+		13×4 wk drin- king-water	Sakamoto & Hashimoto (1986)
Protein binding				
BVP, Binding (covalent) to proteins, mouse testis, skin, lung, liver, stomach <i>in vivo</i>	+		100×1 po	Carlson & Weaver (1985)
BVP, Binding (covalent) to proteins, mouse testis, skin, lung, liver, stomach <i>in vivo</i>	+		100×1 topical application	Carlson & Weaver (1985)
BVP, Binding (covalent) to protamines, mouse spermatid-spermatozoa <i>in vivo</i>	+		125 ip	Sega <i>et al.</i> (1989)
BVP, Binding (covalent) to haemoglobin, rat <i>in vivo</i>	+		0.5×1 iv	Bailey <i>et al.</i> (1986)
BVP, Binding (covalent) to haemoglobin, rat <i>in vivo</i>	+		50×5 ip	Calleman <i>et al.</i> (1990)
BVP, Binding (covalent) to haemoglobin, rat <i>in vivo</i>	+		1×1 ip	Bergmark <i>et al.</i> (1991)

Table 9 (contd)

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Glycidamide				
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	25.0000	Hashimoto & Tanii (1985)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	+	+	25.0000	Hashimoto & Tanii (1985)
KPF, <i>Klebsiella pneumoniae</i> , streptomycin resistance	-	0	3500.0000	Voogd <i>et al.</i> (1981)
G51, Gene mutation, mouse lymphoma L5178Y cells <i>in vitro</i> , <i>hprt</i> locus	+	-	218.0000	Barfknecht <i>et al.</i> (1988) (abstract)
URP, Unscheduled DNA synthesis, rat hepatocytes <i>in vitro</i>	-	0	348.0000	Barfknecht <i>et al.</i> (1988) (abstract)
URP, Unscheduled DNA synthesis, rat hepatocytes <i>in vitro</i>	+	0	70.0000	Butterworth <i>et al.</i> (1992)
UIH, Unscheduled DNA synthesis, human epithelial cells <i>in vitro</i>	+	0	70.0000	Butterworth <i>et al.</i> (1992)
UVM, Unscheduled DNA synthesis, mouse spermatids <i>in vivo</i>	+		0.0000	Sega <i>et al.</i> (1990)

*Not on profile

^a+, positive; (+), weak positive; -, negative; 0, not tested; ?, inconclusive (variable response within several experiments within an adequate study)

^bIn-vitro tests, µg/ml; in-vivo tests, mg/kg bw

5.2 Human carcinogenicity data

Two cohort mortality studies were conducted among workers exposed to acrylamide. The first showed no significant excess of cancer but suffered from small size, short duration of exposure and short latency. In the other study, in one Dutch and three US plants, a nonsignificant increase was seen in deaths from pancreatic cancer, but there was no trend with increasing exposure.

5.3 Animal carcinogenicity data

Acrylamide was tested for carcinogenicity in one experiment in rats by oral administration. It increased the incidences of peritoneal mesotheliomas found in the region of the testis and of follicular adenomas of the thyroid in males and of thyroid follicular tumours, mammary tumours, glial tumours of the central nervous system, oral cavity papillomas, uterine adenocarcinomas and clitoral gland adenomas in females. In screening bioassays, acrylamide, given either orally or intraperitoneally, increased both the incidence and multiplicity of lung tumours in strain A mice.

Acrylamide was also tested as an initiating agent for skin carcinogenesis after oral, intraperitoneal and topical administration to mice of one strain and after oral administration to mice of another strain, followed by topical treatment with 12-*O*-tetradecanoylphorbol 13-acetate. It induced a dose-related increase in the incidence of squamous-cell papillomas and carcinomas of the skin in all four experiments.

5.4 Other relevant data

In occupational settings, acrylamide is taken up both through the skin and by inhalation. Damage to both the central and peripheral nervous systems has been reported on several occasions in exposed humans and has been thoroughly studied in animals.

Acrylamide is metabolized *in vitro* and *in vivo* in mice, rats and humans to the epoxide, glycidamide. Both substances are equally distributed throughout the tissues and have half-lives of about 5 h in rats; acrylamide itself has also been shown to be uniformly distributed between tissues in several other species. The conversion of acrylamide to glycidamide is saturable, ranging from 50% at very low doses to 13% at 100 mg/kg bw in treated rats. Both agents are detoxified by glutathione conjugation, and glycidamide is also detoxified by hydrolysis. Both agents react directly with haemoglobin *in vivo*, but DNA adducts result only from the formation of glycidamide.

The presence of haemoglobin adducts of acrylamide was correlated with neurotoxicity in a group of highly exposed workers.

Acrylamide was not teratogenic to rats or mice after oral treatment of dams with doses up to the toxic level. It causes testicular atrophy, with damage to spermatids and mature spermatozoa. Reduced sperm motility, impaired fertility and dominant lethal mutations at the spermatozoa stage have also been reported in mice and rats. A single study in rats provides evidence that the testicular damage is not secondary to neurotoxicity, since testicular damage but not neurotoxicity was induced by injection of the reactive epoxide, glycidamide.

The genotoxicity of acrylamide has been studied extensively. It induces gene mutation, structural chromosomal aberrations, sister chromatid exchange and mitotic disturbances in mammalian cells *in vitro* in the presence or absence of exogenous metabolic systems. It induces structural chromosomal aberrations *in vivo* in both somatic and germ-line cells. Chromosomal aberrations and micronuclei were induced in mouse bone marrow and in premeiotic and postmeiotic cells. Treatment with acrylamide *in vivo* also caused somatic mutation in the spot test, heritable translocation and specific locus mutations in mice and dominant lethal mutations in both mice and rats in several studies. Acrylamide induces unscheduled DNA synthesis in rat spermatocytes *in vivo* but apparently not in rat hepatocytes; glycidamide induced unscheduled DNA synthesis in rat hepatocytes in one study *in vitro*. Acrylamide induces transformation in cultured mammalian cells. It does not induce mutation in bacteria, but glycidamide does in the absence of an exogenous metabolic system. Acrylamide induces sex-linked recessive lethal and somatic mutations in *Drosophila*.

5.5 Evaluation¹

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

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

In making the overall evaluation, the Working Group took into consideration the following supporting evidence:

- (i) Acrylamide and its metabolite glycidamide form covalent adducts with DNA in mice and rats.
- (ii) Acrylamide and glycidamide form covalent adducts with haemoglobin in exposed humans and rats.
- (iii) Acrylamide induces gene mutations and chromosomal aberrations in germ cells of mice and chromosomal aberrations in germ cells of rats and forms covalent adducts with protamines in germ cells of mice *in vivo*.
- (iv) Acrylamide induces chromosomal aberrations in somatic cells of rodents *in vivo*.
- (v) Acrylamide induces gene mutations and chromosomal aberrations in cultured cells *in vitro*.
- (vi) Acrylamide induces cell transformation in mouse cell lines.

Overall evaluation

Acrylamide is *probably carcinogenic to humans (Group 2A)*.

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¹For definition of the italicized terms, see Preamble, pp. 27-30.

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N-METHYLOLACRYLAMIDE

1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 924-42-5

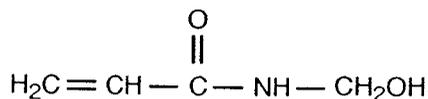
Deleted CAS Reg. No.: 90456-67-0

Chem. Abstr. Name: N-(Hydroxymethyl)-2-propenamide

IUPAC Systematic Name: N-(Hydroxymethyl)acrylamide

Synonyms: N-MAM P; N-methanolacrylamide; monomethylolacrylamide; NMA

1.1.2 Structural and molecular formulae and relative molecular mass



$\text{C}_4\text{H}_7\text{NO}_2$

Relative molecular mass: 101.1

1.1.3 Chemical and physical properties of the pure substance

- Description:* White crystalline solid (Feuer & Lynch, 1953)
- Melting-point:* 74–75 °C (Feuer & Lynch, 1953)
- Spectroscopy data:* Infrared [10698], ultraviolet and nuclear magnetic resonance spectral data have been reported (US National Toxicology Program, 1989; Sadtler Research Laboratories, 1991).
- Solubility:* Soluble in water (188 g/100 ml at 20 °C), methanol (149 g/100 ml at 30 °C), 90% ethanol (116 g/100 ml at 30 °C), isopropanol (53 g/100 ml at 30 °C) and *n*-butanol (42 g/100 ml at 30 °C) (American Cynamid Co., 1990a)
- Stability:* Aqueous solutions are highly reactive. Upon heating in the presence of acids, they are rapidly polymerized to infusible resins (Feuer & Lynch, 1953). The stability of solutions is dependent mainly upon oxygen level, contaminants, storage temperature and pH (American Cynamid Co., 1990a).
- Conversion factor:* $\text{mg}/\text{m}^3 = 4.13 \times \text{ppm}^a$

^aCalculated from: $\text{mg}/\text{m}^3 = (\text{relative molecular mass}/24.45) \times \text{ppm}$, assuming normal temperature (25 °C) and pressure (101.3 kPa)

1.1.4 *Technical products and impurities*

N-Methylolacrylamide is available commercially as a 48% aqueous solution with the following specifications: assay, 48%; water, 51–54% (typically, 52%); pH, 5.5–6.5; free formaldehyde, 1.5–< 3 wt%; acrylamide, < 5.0 wt%; copper, 2 ppm max.; methylether of hydroquinone (inhibitor), 30 ppm; and specific gravity at 25 °C, 1.10 (National Starch and Chemical Corp., 1982; American Cyanamid Co., 1990a; Cytex Industries, 1993).

1.1.5 *Analysis*

No information was available to the Working Group.

1.2 **Production and use**

1.2.1 *Production*

Acrylamide reacts readily with formaldehyde to form *N*-methylolacrylamide (Updegraff *et al.*, 1978). Information available in 1991 indicated that *N*-methylolacrylamide was produced by two companies in Japan and one each in the Netherlands, the United Kingdom and the USA (Chemical Information Services Ltd, 1991). In Japan, about 900 tonnes were produced as powder and 250 tonnes as water solution in 1992 (Japan Petrochemical Industry Association, 1993).

1.2.2 *Use*

N-Methylolacrylamide is a bifunctional monomer with reactive vinyl and hydroxymethyl groups. Thermoplastic polymers can be formed by copolymerization of *N*-methylolacrylamide with a variety of vinyl monomers by emulsion, solution and suspension techniques. The resulting products, which have pendant hydroxymethyl groups, can undergo cross-linking under moderate conditions, permitting conversion of thermoplastic backbone polymers to thermoset materials at the point of use in the absence of an external cross-linking agent. Conversely, the hydroxymethyl group can be reacted with a substrate like cellulose and subsequently cross-linked by free-radical polymerization (US National Toxicology Program, 1989; American Cyanamid Co., 1990a,b).

The uses of *N*-methylolacrylamide range from adhesives and binders in papermaking and textiles to a variety of surface coatings and resins for varnishes, films and sizing agents (American Cyanamid Co., 1990a,b; Bucher *et al.*, 1990). It can be used in wet-strength and dry-strength agents for paper, in textile finishing agents for crease resistance, in antistatic agents, in dispersing agents, in cross-linking agents and in emulsion polymers.

1.3 **Occurrence**

1.3.1 *Natural occurrence*

N-Methylolacrylamide is not known to occur as a natural product.

1.3.2 *Occupational exposure*

No data on human exposure to *N*-methylolacrylamide were available to the Working Group.

The National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health between 1981 and 1983 indicated that 20 700 US employees were potentially exposed to a product containing *N*-methylolacrylamide (US National Institute for Occupational Safety and Health, 1993). The estimate is based on a survey of US companies and did not involve measurements of actual exposures.

1.4 Regulations and guidelines

There are no reported occupational standards or guidelines for *N*-methylolacrylamide (American Conference of Governmental Industrial Hygienists, 1993; ILO, 1993; UNEP, 1993). The US Food and Drug Administration (1993) permits use of polymers of *N*-methylolacrylamide in products in contact with food.

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 Mouse

Groups of 50 male and 50 female B6C3F1 mice, eight weeks of age, were administered 0, 25 or 50 mg/kg bw *N*-methylolacrylamide (purity, approximately 98%) in deionized water by oral gavage on five days per week for 103 weeks. Surviving animals were killed at 113 weeks of age. The mean body weights of treated mice were up to 13% (males) and 25% (females) greater than those of vehicle controls. At the end of the experiment, survival rates in the control, low- and high-dose groups were 30/50, 20/50 and 21/50 males and 41/50, 35/50 and 33/50 females. The incidences of Harderian gland adenomas were increased in males given the low and high doses: control, 1/48; low-dose, 14/49; and high dose 29/50 ($p < 0.001$, logistic regression trend test) and in females given the high-dose: control, 5/47; low-dose, 8/45; and high-dose, 20/48 ($p < 0.001$, logistic regression trend test). The incidences of hepatocellular adenomas were increased in high-dose males and females: males—control, 8/50; low-dose, 4/50; and high-dose, 19/50 ($p < 0.05$, logistic regression trend test); females—control, 3/50; low-dose, 4/50; and high-dose, 17/49 ($p < 0.001$, logistic regression trend test). The incidences of hepatocellular carcinomas were marginally increased in treated male mice: control, 6/50; low-dose, 13/50; and high-dose, 12/50 ($p = 0.023$, incidental tumour test for comparison between low-dose and control). The incidence of hepatocellular adenomas and carcinomas (combined) showed a positive trend, and the incidences in high-dose males and females were higher than those in the vehicle controls: males—control, 12/50; low-dose, 17/50; and high-dose, 26/50 ($p < 0.001$, logistic regression trend test); females—control, 6/50; low-dose, 7/50; and high-dose, 17/49 ($p = 0.002$, logistic regression trend test). In high-dose males, the incidences of alveolar-

bronchiolar adenomas (control, 3/49; low-dose, 6/50; and high-dose, 11/50; $p < 0.05$, logistic regression trend test) and carcinomas were increased (control, 2/49; low-dose, 4/50; and high-dose, 10/50; $p < 0.05$, logistic regression trend test). The incidence of alveolar–bronchiolar adenomas and carcinomas (combined) showed a positive trend in male mice (control, 5/49; low-dose, 10/50; and high-dose, 18/50; $p < 0.001$, logistic regression trend test). The incidence of alveolar–bronchiolar adenomas and carcinomas (combined) was increased in high-dose females (control, 6/50; low-dose, 8/50; and high-dose, 13/49; $p < 0.05$, logistic regression trend test). The incidences of benign granulosa-cell tumours of the ovary were increased in treated groups (control, 0/50; low-dose, 5/45; and high-dose, 5/47; $p < 0.05$, logistic regression trend test) (US National Toxicology Program, 1989; Bucher *et al.*, 1990).

3.1.2 Rat

Groups of 50 male and 50 female Fischer 344/N rats, seven weeks of age, were administered 0, 6 or 12 mg/kg bw *N*-methylolacrylamide (purity, approximately 98%) in deionized water by oral gavage on five days per week for 103 weeks. Surviving animals were killed at 112 weeks of age. The mean body weights of treated rats were slightly lower than those of vehicle controls. At the end of the experiment, the survival rates in the control, low-dose and high-dose groups, respectively, were: males—28/50, 22/50 and 27/50; females, 35/50, 22/50 and 33/50. No neoplastic lesion was seen that was attributable to administration of *N*-methylolacrylamide (US National Toxicology Program, 1989; Bucher *et al.*, 1990).

4. Other Data Relevant for 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 its intravenous administration at 140 mg/kg bw to rats, *N*-methylolacrylamide was distributed rapidly in total body water, with a first-order rate of elimination of 0.45/h from the blood compartment. Evidence for glutathione conjugation with *N*-methylolacrylamide in the bile was found in studies with the substance labelled in the methylene carbon, but no evidence was found for conversion to acrylamide *in vivo*. It is not known whether *N*-methylolacrylamide, like acrylamide, is also converted to an epoxide metabolite. No data were available on urinary metabolites (Edwards, 1975a).

N-Methylolacrylamide is an α,β -unsaturated carbonyl compound which reacts with nucleophilic atoms in Michael-type additions. It modified glycolytic enzymes in brain *in vitro* (Sakamoto & Hashimoto, 1985). Hashimoto and Aldridge (1970) found similar rates for the reaction of *N*-methylolacrylamide and acrylamide with glutathione *in vitro*; they also found

that both compounds react with protein sulfhydryls and haemoglobin in rats *in vivo*. The patterns of distribution of the two compounds between different tissues and subcellular organelles were also similar following oral administration to rats of equal doses of substances labelled in the carbonyl carbon.

4.2 Toxic effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

N-Methylolacrylamide given in large doses was found to be neurotoxic (Barnes, 1970). Edwards (1974, 1975b) confirmed the neurotoxicity of *N*-methylolacrylamide and demonstrated that its administration to rats hastened the onset of neurotoxicity induced by acrylamide. In mice (Hashimoto *et al.*, 1981) and rats (Tanii & Hashimoto, 1983), *N*-methylolacrylamide induced peripheral neuropathy of the same type as that induced by acrylamide but at a potency about 20–30% that of acrylamide. Neurotoxicity occurred in rats exposed to 25 mg/kg bw or more, as shown by both neurobehavioural and morphological examinations (US National Toxicology Program, 1989).

4.3 Reproductive and prenatal effects

No data were available to the Working Group.

4.4 Genetic and related effects (see also Table 1 and Appendices 1 and 2)

4.4.1 Humans

No data were available to the Working Group.

4.4.2 Experimental systems

Few studies are available for evaluation. *N*-Methylolacrylamide did not induce gene mutation in *Salmonella typhimurium*. In single studies with Chinese hamster ovary cells *in vitro*, it induced chromosomal aberrations but only a weakly increased frequency of sister chromatid exchange. Micronuclei were not observed in bone-marrow cells of mice exposed *in vivo*.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

N-Methylolacrylamide is a bifunctional monomer used in the production of thermoplastic polymers and as a cross-linking agent in adhesives and binders for paper products and textiles. No data were available on occupational exposure to this compound.

Table 1. Genetic and related effects of *N*-methylolacrylamide

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	2500.0000	Hashimoto & Tanii (1985)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	5000.0000	Zeiger <i>et al.</i> (1988)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	2500.0000	Hashimoto & Tanii (1985)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	5000.0000	Zeiger <i>et al.</i> (1988)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	2500.0000	Hashimoto & Tanii (1985)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	2500.0000	Hashimoto & Tanii (1985)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	2500.0000	Hashimoto & Tanii (1985)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	5000.0000	Zeiger <i>et al.</i> (1988)
SAS, <i>Salmonella typhimurium</i> TA97, reverse mutation	-	-	5000.0000	Zeiger <i>et al.</i> (1988)
SIC, Sister chromatid exchange, Chinese hamster ovary cells <i>in vitro</i>	(+)	(+)	250.0000	US National Toxicology Program (1989)
CIC, Chromosomal aberrations, Chinese hamster ovary cells <i>in vitro</i>	+	+	250.0000	US National Toxicology Program (1989)
MVM, Micronucleus formation, mouse bone-marrow cells <i>in vivo</i>	-	-	150.0000 × 2 ip	US National Toxicology Program (1989)

^a +, positive; (+), weak positive; -, negative

^b In-vitro tests, µg/ml; in-vivo tests, mg/kg bw

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

N-Methylolacrylamide was tested by oral gavage in one experiment in mice and one experiment in rats. In mice, it increased the incidences of Harderian gland adenomas, hepatocellular adenomas and carcinomas and alveolar-bronchiolar lung adenomas and carcinomas in animals of each sex and the incidence of benign granulosa-cell tumours of the ovary in females. In rats, no increase in tumour incidence was observed.

5.4 Other relevant data

N-Methylolacrylamide is absorbed by rats and mice after oral administration; no information was available regarding dermal application or inhalation. *N*-Methylolacrylamide administered to rats intravenously was distributed rapidly in body water; its distribution in tissues and subcellularly is similar to that of acrylamide. *N*-Methylolacrylamide reacts with glutathione, protein sulfhydryls and haemoglobin at rates similar to those of acrylamide, but it is not known if it is converted to acrylamide or an epoxide. Neurotoxicity developed in rats and mice exposed subchronically to *N*-methylolacrylamide.

No data were available on the genetic and related effects of *N*-methylolacrylamide in humans.

N-Methylolacrylamide did not induce micronuclei in mouse bone marrow *in vivo* but did induce chromosomal aberrations in Chinese hamster ovary cells *in vitro* and weakly increased the frequency of sister chromatid exchange. It was not mutagenic to *Salmonella typhimurium*.

5.5 Evaluation¹

There is *inadequate evidence* in humans for the carcinogenicity of *N*-methylolacrylamide.

There is *limited evidence* in experimental animals for the carcinogenicity of *N*-methylolacrylamide.

Overall evaluation

N-Methylolacrylamide is not classifiable as to its carcinogenicity to humans (Group 3).

6. References

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¹For definition of the italicized terms, see Preamble, pp. 27-30.

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METHYL METHACRYLATE

This substance was considered by a previous Working Group, in February 1978 (IARC, 1979). 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

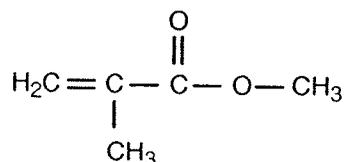
Chem. Abstr. Serv. Reg. No.: 80-62-6

Chem. Abstr. Name: 2-Methyl-2-propenoic acid, methyl ester

IUPAC Systematic Name: Methacrylic acid, methyl ester

Synonyms: 2-(Methoxycarbonyl)-1-propene; methyl 2-methylacrylate; methyl 2-methyl-2-propenoate; MMA

1.1.2 Structural and molecular formulae and relative molecular mass



$\text{C}_5\text{H}_8\text{O}_2$

Relative molecular mass: 100.12

1.1.3 Chemical and physical properties of the pure substance

- (a) *Description:* Colourless liquid (CYRO Industries, 1987), with a fruity, pungent odour (American Conference of Governmental Industrial Hygienists, 1991)
- (b) *Boiling-point:* 100–101 °C (Lide, 1991)
- (c) *Melting-point:* –48 °C (Lide, 1991)
- (d) *Density:* 0.9440 at 20 °C/4 °C (Lide, 1991)
- (e) *Spectroscopy data:* Infrared [2226], ultraviolet, nuclear magnetic resonance and mass spectral data have been reported (Sadtler Research Laboratories, 1991; US National Library of Medicine, 1993a).
- (f) *Solubility:* Slightly soluble in water (1.6 g/100 ml at 20 °C), glycerine and ethylene glycol (CYRO Industries, 1987; Bauer, 1990); soluble in acetone, diethyl ether and ethanol (Lide, 1991)

- (g) *Volatility*: Vapour pressure, 3.87 kPa at 20 °C (Bauer, 1990; Rohm & Haas Co., 1993); relative vapour density (air = 1), 3.45 (Verschueren, 1983)
- (h) *Stability*: Highly inflammable vapours (Mannsville Chemical Products Corp., 1987); lower explosive limit, 2.1 vol. % in air (CYRO Industries, 1987)
- (i) *Reactivity*: Monomer can be polymerized by light, heat, oxygen or ionizing radiation and by benzoyl peroxide (American Conference of Governmental Industrial Hygienists, 1991)
- (j) *Octanol-water partition coefficient (P)*: log P, 1.38 (Sangster, 1989)
- (k) *Conversion factor*: $\text{mg/m}^3 = 4.1 \times \text{ppm}^a$

1.1.4 Technical products and impurities

The purity of commercial methyl methacrylate is typically 99.9% [specification, 99.8% min.]; it contains traces of acidity as methacrylic acid (0.003% max.; specification, 0.005% max.) and water (0.03% max.; specification, 0.05% max.). Inhibitors added for storage and transportation are usually 10–50 ppm [specification, 9–55 ppm] methyl ether of hydroquinone and 25–60 ppm hydroquinone, although other phenolic inhibitors, such as dimethyl *tert*-butyl phenol, can also be used (Degussa AG, 1988; Bauer, 1990; CYRO Industries, 1992; Rohm & Haas Co., 1993). Phenothiazine has been used for this purpose because it acts both anaerobically and aerobically, but it is not commonly used in products intended for use as polymer intermediates (Bauer, 1990).

1.1.5 Analysis

Methyl methacrylate can be determined in air by gas chromatography with flame ionization detection. The sample is adsorbed on fused silica (XAD-2 resin) or charcoal coated with 4-*tert*-butylcatechol and desorbed with carbon disulfide or toluene. The estimated limit of detection is 0.01 mg per sample (Eller, 1989; US Occupational Safety and Health Administration, 1990; Harper, 1992). A method involving desorption with 5% isopropanol in carbon disulfide from charcoal has a detection limit of 0.8 mg/m³ (Kollár *et al.*, 1988).

1.2 Production and use

1.2.1 Production

Methyl methacrylate was first produced commercially in Germany in 1933. The original process was a variant of the current acetone-cyanohydrin process, in which acetone and hydrogen cyanide are reacted to produce acetone cyanohydrin; this is treated with concentrated sulfuric acid to form methacrylamide sulfate, which is reacted directly with methanol to form crude methyl methacrylate and ammonium bisulfate. The crude methyl methacrylate is purified by distillation (Mannsville Chemical Products Corp., 1987; Bauer, 1990).

^aCalculated from: $\text{mg/m}^3 = (\text{relative molecular mass}/24.45) \times \text{ppm}$, assuming normal temperature (25 °C) and pressure (101.3 kPa)

In the isobutylene (isobutene) process, introduced in Japan in 1983, methyl methacrylate is made by oxidation of isobutylene to methacrylic acid with nitric acid and subsequent esterification with methanol. *tert*-Butanol is used as the starting material. In another process based on isobutylene used in Japan, the methacrylonitrile process, isobutylene is converted by ammoxidation to methylacrylonitrile, which is then hydrolysed with sulfuric acid and reacted with methanol to produce methyl methacrylate (Bauer, 1990).

Some methyl methacrylate monomer is recovered by 'cracking' (depolymerizing) polymethyl methacrylate scrap (Mannsville Chemical Products Corp., 1987).

Production of methyl methacrylate monomer in Japan was 403 thousand tonnes in 1990, 401 thousand tonnes in 1991 and 384 thousand tonnes in 1992 (Japan Chemical Week, 1991, 1992; Japan Petrochemical Industry Association, 1993). Production of methyl methacrylate in the USA was (thousand tonnes) 536 in 1990, 500 in 1991 and 380 in 1992 (preliminary figures) (Anon., 1993); in the years 1960, 1965, 1970, 1975, 1980 and 1981, production was 54, 113, 202, 248, 354 and 404 thousand tonnes, respectively (US International Trade Commission, 1982; Mannsville Chemical Products Corp., 1987). Capacities for the production of methyl methacrylate in 1988 in several countries are presented in Table 1. Global production was approximately 1400 thousand tonnes.

Table 1. Capacity for production of methyl methacrylate in several countries, 1988

Country	No. of facilities	Process	Capacity (thousand tonnes/year)
France	1	Acetone cyanohydrin	60
Germany	2	Acetone cyanohydrin	160
	1 ^a	Ethylene	-
Italy	1	Acetone cyanohydrin	50
Japan	3	Acetone cyanohydrin	131
	3 ^a	Isobutylene	135
	1	Methacrylonitrile	60
Spain	2	Acetone cyanohydrin	50
United Kingdom	1	Acetone cyanohydrin	105
USA	3	Acetone cyanohydrin	514
Other countries			about 150

From Bauer (1990)

^aOne in construction

Information available in 1991 indicated that methyl methacrylate was produced by six companies in Japan, three each in China, Mexico and the USA, two each in Germany and Spain, and one each in Argentina, Brazil, the former Czechoslovakia, France, Italy, Poland and the United Kingdom (Chemical Information Services Ltd, 1991).

1.2.2 Use

Methyl methacrylate, methacrylic acid and other methacrylates readily polymerize to form long-chain homopolymers and copolymers. Methyl methacrylate monomer is the most

important ester of methacrylic acid commercially. Acrylic sheeting, made by casting, moulding or extruding polymethyl methacrylate or modified polymers, is the largest application for methyl methacrylate. Cast sheeting, used, for instance, for safety glazing, panels and lighting, is the major type of sheeting produced. Polymethyl methacrylate resins are also used to make moulded and extruded products that require resins with good optical clarity and stability, such as plumbing fixtures and outdoor lighting. Methyl methacrylate-butadiene-styrene resins are being made increasingly for use as impact modifiers for clear rigid polyvinyl chloride, particularly for making bottles (Mannsville Chemical Products Corp., 1987; Bauer, 1990).

Methyl methacrylate polymers and copolymers are used in waterborne, solvent and undissolved coatings. Exterior latex paint based on emulsions containing methyl methacrylate is the surface coating in which the monomer is used most widely. Solvent reducible polymers containing methyl methacrylate are used for industrial finishes, metal and foil coatings and a variety of overlays for special purposes. Solvent and emulsion polymers containing methacrylates are also used in adhesives, sealants, leather coatings, paper coatings, inks, floor polishes and textile finishes (Mannsville Chemical Products Corp., 1987; Bauer, 1990). Engineering adhesives are undissolved, liquid, reactive, durable adhesives for bonding durable substrates, and consist primarily of methyl methacrylate monomer with polymethyl methacrylate and other polymers (Gehman, 1990).

Special methacrylate polymers are used for dental prostheses, surgical bone cements and leaded acrylic radiation shields. Polymer concretes based on methyl methacrylate and Portland cement are used to patch highways and bridges. Methyl methacrylate is also used in the production of polymers added to lubricating oils (Mannsville Chemical Products Corp., 1987; Bauer, 1990).

Dental prosthesis fabrication involves preparation of impression trays, orthodontic appliances and dentures. A powder containing small, prepolymerized, spherical acrylate particles—usually polymethyl methacrylate or mixed acrylate copolymers—is combined with a liquid, the main component of which is methyl methacrylate. The liquid also contains either a cross-linking polyfunctional monomer or a self-curing activator. The solid and liquid components are combined and handled manually while the material is moulded into plastic or metal impression trays (Ruyter & Sjøvik, 1981; Rajaniemi, 1986). Bone cements are also usually two-component mixtures prepared just before use (Darre *et al.*, 1987).

Methyl methacrylate is also used in the preparation of synthetic fingernails (IARC, 1993) and orthotic shoe inserts (Gunter & Schulenberg, 1982).

Typical use patterns for methyl methacrylate in the USA are presented in Table 2. In Europe in 1992, 31% methyl methacrylate was used in the manufacture of acrylic sheet and moulding powders, 50% for surface coating and emulsion polymers, 13% for other methacrylates and 6% for miscellaneous uses (European Chemical Industry Council, 1993).

1.3 Occurrence

1.3.1 *Natural occurrence*

Methyl methacrylate is not known to occur in nature.

Table 2. End use patterns for methyl methacrylate in the USA (%)

Use	Year			
	1981	1985	1988	1991
Cast and extruded sheet	32	30	25	24
Moulding powders and resins	25	25	25	21
Surface coatings	32	33	20	18
Exports	10	10	8	11
Impacts modifiers	-	-	10	10
Emulsion polymers	-	-	6	8
Mineral-based sheet	-	-	-	3
Higher methacrylates	-	-	2	2
Polyester modifiers	-	-	4	1
Miscellaneous ^a	1	2	-	2

From Anon. (1981, 1985, 1988, 1991); -, not reported

^aIncludes synthetic fibre modification

1.3.2 Occupational exposure

The National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health between 1981 and 1983 indicated that 170 082 US employees were potentially occupationally exposed to methyl methacrylate (US National Institute for Occupational Safety and Health, 1993). Of this number, 9% were estimated to be exposed to methyl methacrylate and 91% to materials containing methyl methacrylate. The estimate is based on a survey of US companies and did not involve measurements of actual exposures.

Industries in which exposures to methyl methacrylate may occur include: monomer production, polymer production and plastics manufacture, thermoplastics processing, plastics cutting, hospitals and dental clinics. Occupations in which there is potential exposure to methyl methacrylate include: chemical process operator, surgeon and surgical assistant, operating room nurse, dental technician and hygienist, and beauty technician applying synthetic fingernails. Air concentrations of methyl methacrylate in a variety of industries are given in Table 3. Most of the data on exposure are from the chemical industry during production or use of the monomer as a chemical intermediate in production of acrylic plastics and polymers, and from dental clinics and laboratories during dental prosthesis manufacture and repair.

(a) Chemical industry

In two US chemical plants, the concentrations in personal air samples in 1960–83 ranged from not detected to 7.83 ppm [32 mg/m³] during methyl methacrylate production and from 0.05 to 11.5 ppm [0.2–47 mg/m³] during acrylic fibre production from the monomer. Workers were also potentially exposed to acrylamide, acrylonitrile, epichlorohydrin and

Table 3. Occupational exposures to methyl methacrylate

Industry (country)	Operation/Process	Type of sample	No. of samples	Air concentration (mg/m ³)		Year of measurement	Reference
				Mean	Range		
Monomer, primary production and use							
Methyl methacrylate manufacture (USA)	Various	Personal	NR	[2.83]	[0-32]	1965-69	Collins <i>et al.</i> (1989)
	Various	Personal	NR	[1.44]	[0-26]	1975-79	
	Various	Personal	NR	[0.53]	[0-5.8]	1980-83	
Acrylic fibre manufacture (USA)	Various	Personal	NR	[3.4]	[0.2-47]	1975-79	Collins <i>et al.</i> (1989)
	Various	Personal	NR	[3.0]	[0.2-22]	1980-83	
Acrylic ester-styrene copolymer production (USA)	Solution polymer reactor (open hatch batch process)	Personal	20	[0.29]	[ND ^a -1.55]	[1981]	Samimi & Falbo (1982)
	Solution polymer reactor (closed batch reactor)	Personal	13	[0.42]	[ND ^a -1.53]		
	Polymer flake reactor (closed continuous reactor)	Personal	6	ND	ND		
	All production areas	Area	57	[~1.0]	[ND ^a -13.5]		
	Unloading docks	Personal	11	[0.074]	[ND ^a -0.38]		
Thermoplastics processing (Finland)	Injection moulding, 235 °C	Area	4	0.06	SD 0.05	NR	Vainiotalo & Pfäffli (1989)
	Thermoforming, 100 °C	Area	12	1.0	SD 0.3		
	Thermoforming, 160 °C	Area	8	4.6	SD 1.4		
	Extrusion, 220-270 °C	Area	11	1.8	SD 0.4		
Plastics processing (Denmark)	Cleaning	Personal	4	452	NR-450	1983-89	ATABAS (1994)
	Moulding	Personal	13	57	NR-195		
	Mechanical mixing	Personal	24	180	NR-453		
	Manual mixing	Personal	3	501	NR-754		
	Filling	Personal	11	164	NR-337		
Monomer production (Slovakia)	NR	Personal	6	42	20.9-133	NR	Kollár <i>et al.</i> (1988)
Polymethyl methacrylate sheet manufacture (USA) (five plants)	NR, 8-h TWA	Personal	169 workers	105	4.1-713	1975	Cromer & Kronoveter (1976)

Table 3 (contd)

Industry (country)	Operation/Process	Type of sample	No. of samples	Air concentration (mg/m ³)		Year of measurement	Reference
				Mean	Range		
Polymethyl methacrylate production (Russia)	Polymerization	Area	} > 800	NR	8-60	NR	Blagodatin <i>et al.</i> (1970)
	Block glass	Area		NR	75-150		
	Ornamental	Area		NR	10-65		
Polymer production (Russia)	Process reactor	Area	NR	NR	100-600	1969	Dobrinskij (1970)
Polymethyl methacrylate production (China)	Purification	Area	75	28.2	NR	1976-78	Lang <i>et al.</i> (1986)
	Prepolymer	Area	89	95.5	NR		
	Polymerization	Area	115	62.4	NR		
	Extrusion	Area	62	33.1	NR		
	Model making	Area	61	21.3	NR		
	Pouring glass surface	Area	48	11.3	NR	1982-83	Lang <i>et al.</i> (1986)
	Prepolymer	Area	265	155.5	NR		
	Polymerization	Area	366	203.2	NR		
	Extrusion	Area	138	32.2	NR		
	Model making	Area	183	19.3	NR		
Monomer production (Poland)	Various	Area	378	11	0.2-382	NR	Jedrychowski (1982)
Dental clinics							
Dental clinic (USA)	Denture fabrication	Personal	8	2.4	ND ^b -5.3	1980	Boiano (1980a)
	Orthodontics fabrication	Personal	4	0.55	0.4-0.84		
	Grinding	Personal	2	ND ^b	-		
Dental laboratory (Norway)	Denture fabrication	Area	4	[50]	[10-140]	NR	Brune & Beltesbrekke (1981)
Dental clinic (USA)	Denture fabrication	Personal	4	[0.28]	[0.08-0.78]	1981	Lewis & Shoemaker (1981)
	Denture fabrication	Area	1		[0.08]		
Dental clinic (USA)	Denture fabrication/repair	Personal	1	16.3	-	1991	McCammon (1992)
		Area	3	17.5	[< 0.25]-50		

METHYL METHACRYLATE

Table 3 (contd)

Industry (country)	Operation/Process	Type of sample	No. of samples	Air concentration (mg/m ³)		Year of measurement	Reference
				Mean	Range		
Dental laboratory (USA)	Denture fabrication	Personal	4	3.8	2.2-5.6	1982-83	Rom <i>et al.</i> (1984)
Dental clinics (Denmark)	Denture fabrication	Personal	21	30	NR-121	1983-89	ATABAS (1994)
Dental laboratories (United Kingdom)	Denture fabrication (modern laboratory)	Area	4	[11]	[0.8-25]	1986	Money <i>et al.</i> (1987)
	Denture fabrication (special laboratory)		5	[180]	[14-590]	1986	
	Denture fabrication (no ventilation)		4	[273]	[98-420]	1986	
Other uses							
Hospital (USA)	Mixing and application of surgical bone cement	Personal	11	[7.2]	[< 1.6-12.7]	1983	Apol & Helgerson (1984)
Optical lens manufacture (USA)	Acrylic lens production	Area	4	[6.3]	[3.7-8.2]	1980	Boiano (1980b)
Newspaper printing (USA)	Letter-flex platemaking	Personal	10	ND ^b	-	1982	Gunter (1982)
Orthopaedic clinic (USA)	Orthotic inserts fabrication	Personal	3	67	17-110	1982	Gunter & Schulenberg (1982)
		Area	3	253	23-417		
Research laboratory (Finland)	Cutting acrylic with laser	Area	2	246	206-286	NR	Hietanen <i>et al.</i> (1992)
Plastic furniture manufacture (USA)	Furniture construction	Personal	25	5.6	0.3-18	1976	Hollett (1977)
	Band saw	Area	2	3.8	2.5-5		
	Joiner	Area	3	1.8	0.8-2.7		
	Gluing	Area	4	2.95	0.4-5.3		
Beauty salon (USA)	Artificial fingernail preparation	Personal	3	[87.5]	[61.5-102.5]	1976	Kronoveter (1977a)
		Area	2	[53]	[53 and 53]1976		

Table 3 (contd)

Industry (country)	Operation/Process	Type of sample	No. of samples	Air concentration (mg/m ³)		Year of measurement	Reference
				Mean	Range		
Beauty salon (USA)	Artificial fingernail preparation	Personal, short-term	25	[83.2]	SD [16]	NR	Froines & Garabrant (1986)
		Personal, 8-h	59	[21.7]	SD [1.6]	NR	
Hospital (USA)	Mixing surgical bone cement	Personal	19	[114]	[8.2-316]	1977	Kronoveter (1977b)
Hospital (Denmark)	Knee replacement	Area	NR	NR	[0-205]	NR	Darre <i>et al.</i> (1992)
	Hip replacement	Area	NR	NR	[0-410]		

NR, not reported; SD, standard deviation; ND, not detected; 8-h, 8-hour time-weighted average

^a < 0.004 mg/m³

^b < 0.01 mg/sample

formaldehyde during methyl methacrylate production, and to acrylonitrile and vinylidene chloride during acrylic fibre production (Collins *et al.*, 1989).

Area samples for the analysis of methyl methacrylate were collected at four Finnish plants where polymethyl methacrylate was processed (Vainiotalo & Pfäffli, 1989). The average concentrations of methyl methacrylate in air during polymethyl methacrylate degradation were 0.06–4.6 mg/m³. Other compounds present included oligomers of methyl methacrylate of higher relative molecular mass, including free radicals.

The exposures of workers and area concentrations of styrene and several acrylates, including methyl methacrylate, were monitored in an acrylic ester–styrene copolymer production facility in the USA where solution polymers, emulsion polymers and polymer flakes were produced (Samimi & Falbo, 1982). Concentrations in personal samples ranged from not detected to 0.378 ppm [≤ 1.55 mg/m³] and those in area samples up to 3.3 ppm [13.5 mg/m³]. The highest concentrations of methyl methacrylate were found at the batch reactors and the unloading dock. Other compounds measured included ethyl acrylate, *n*-butyl acrylate, styrene, α -methyl styrene (*ortho*-vinyltoluene, see monograph, p. 373) and 2-ethylhexyl acrylate (see monograph, p. 475).

(b) *Dental clinics and laboratories*

The concentrations of methyl methacrylate in the air of dental clinics vary widely depending on the facility, production volume and type of ventilation (see Table 3). The preparation of dental prostheses and orthodontic devices involves manual handling (Rajaniemi & Tola, 1985), and dermal exposure to methyl methacrylate may occur even when gloves are used because of the permeability of glove materials to methyl methacrylate (Rajaniemi, 1986). In a Finnish study of 163 dental technicians and technical assistants who reported daily dermal contact with methyl methacrylate-containing compounds, only three subjects wore protective gloves during acrylic moulding and only 15 subjects wore gloves while performing other tasks that may have involved exposure to methyl methacrylate (Rajaniemi & Tola, 1985). Methyl methacrylate comprised 3–5% of the autopolymerizing acrylic compound used for repair and less than 1% of the polymerized hot-cure acrylic denture base handled by these workers. Other exposures in dental laboratories include phenolic inhibitors, formaldehyde mercury vapour and dusts containing gold, chromium, nickel and cobalt alloys, silicon carbide, and corundum (Al₂O₃).

In a separate study of dental technicians, monitoring of methacrylate in the urine of workers exposed dermally to methyl methacrylate showed that percutaneous uptake had occurred. Urine specimens were collected from 11 dental technicians exposed to methyl methacrylate during construction and repair of dental prostheses and from 10 unexposed controls over a 24-h period which included a normal working day. The actual durations of exposure were 30–240 min. The highest urinary concentrations of methacrylate in the exposed group ranged from 16 to 373 nmol/mmol creatinine (mean, 81 [standard deviation, 102; geometric mean, 53; geometric standard deviation, 2.4]). There was no clear relationship between duration of exposure and urinary output. The primary route of uptake was presumed to be percutaneous, although the concentrations in air were not measured (Rajaniemi *et al.*, 1989).

(c) Other occupational exposures

During the fabrication of orthotic shoe inserts, short-term personal exposures to methyl methacrylate ranged from 17 to 110 mg/m³; the concentrations in area samples were 23–417 mg/m³ (Gunter & Schulenberg, 1982).

Methyl methacrylate is released as a pyrolysis product when acrylic plastics are cut with a carbon dioxide laser beam. Air concentrations of methyl methacrylate measured 20 cm from a cutting surface over a period of 15–20 min were found to be 206 and 286 mg/m³ (Hietanen *et al.*, 1992). Other pyrolysis products measured included anthracene, fluoranthrene, pyrene, benzene, toluene, methyl acrylate and ethyl acrylate.

Operating room personnel employed in mixing and applying bone cement were exposed to concentrations of < 1.6–316 mg/m³ (Kronoveter, 1977b; Apol & Helgersen, 1984). Other compounds to which they were exposed included nitrous oxide and halogenated anaesthetic gases.

1.3.3 *Environmental occurrence*

No data were available to the Working Group on concentrations of methyl methacrylate in ambient air or water. The annual total air emissions, water releases, underground injection releases and land releases of methyl methacrylate in the USA, reported to the US Environmental Protection Agency by industrial facilities from 1987 through 1991, are presented in Table 4.

Table 4. Annual air, water, underground injection and land releases of methyl methacrylate in the USA, 1987–91

Year	No. of locations	Releases (tonnes)			
		Air	Water	Underground injection	Land
1987	194	1650	11	103	5
1988	217	1600	13	148	4
1989	248	1430	13	89	2
1990	258	1200	12	95	< 1
1991	240	1200	3	123	2

From US National Library of Medicine (1993b)

1.3.4 *Food*

Methyl methacrylate was found at levels of 180 and 275 ppb (µg/L) in maple syrup that had been contaminated by its plastic container. The residues in the container may have resulted from incomplete polymerization of methyl methacrylate–styrene–butadiene copolymer resin or from decomposition of the plastic when the container was formed (Hollifield *et al.*, 1980).

Migration of methyl methacrylate from polymethyl methacrylate containers to food-simulated solvents was investigated by gas chromatography with a detection limit of 0.05 ppm

[0.05 mg/L]. Residual amounts found in commercial polymethyl methacrylate wares ranged from 0.03 to 1.0%; no methyl methacrylate appeared to migrate to water or to 4% acetic acid. In a similar study, migration of methyl methacrylate into 20% ethanol was 1 ppm [1 mg/L] after one day and 10 ppm [10 mg/L] after 90 days at 25 °C (Inoue *et al.*, 1981a,b).

1.3.5 Tissues and body fluids

Methyl methacrylate was detected in the saliva of subjects wearing autopolymerized dental appliances at a maximum concentration of 45 µg/ml in whole saliva and 180 µg/ml in the salivary film on the fitting surface. Methyl methacrylate was detected for up to one week after insertion of the appliance. It was not detected in blood or urine and not in dental appliances made from conventionally heat-cured acrylic resins (Baker *et al.*, 1988).

Blood levels of monomeric methyl methacrylate in eight patients during knee replacement operations were 0.10–1.44 µg/ml after tourniquet release. In a ninth patient, an exceptionally high concentration (119.8 µg/ml) was seen immediately after tourniquet release (Svartling *et al.*, 1986). Methyl methacrylate was detected in the blood of patients following use of polymethyl methacrylate bone cement during hip replacement surgery at concentrations ranging from 0.24 to 15.1 µg/ml (Crout *et al.*, 1979).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for methyl methacrylate are presented in Table 5. The US Food and Drug Administration (1993) has established regulations for the use of monomers, polymers, copolymers and homopolymers of methyl methacrylate in products intended for use in contact with food. The monomer content in styrene-methyl methacrylate copolymers used as components of paper and paperboard in contact with fatty foods is limited to 0.5%.

Table 5. Occupational exposure limits and guidelines for methyl methacrylate

Country or region	Year	Concentration (mg/m ³)	Interpretation
Argentina	1991	410	TWA
Australia	1983	410	TWA; sensitizer
		510	STEL
Austria	1982	410	TWA
Belgium	1984	410	TWA
		510	STEL
Brazil	1978	320	TWA
Canada	1986	410	TWA
Chile	1983	328	TWA
Denmark	1988	307	TWA
Finland	1993	410	TWA
		615	STEL
France	1990	410	TWA
		820	STEL

Table 5 (contd)

Country or region	Year	Concentration (mg/m ³)	Interpretation
Germany	1993	210	TWA; sensitizer; local irritant; PR3
Hungary	1978	50	TWA; skin; sensitizer; irritant
		250	STEL
Indonesia	1978	410	TWA
Mexico	1989	410	TWA
Netherlands	1986	410	TWA
Poland	1982	50	TWA
Romania	1975	300	Average
		500	Maximum
Sweden	1991	200	TWA; skin; sensitizer
		600	STEL
Switzerland	After 1987	210	TWA; sensitizer; PR3
		420	STEL
Taiwan	1981	410	TWA
United Kingdom	1992	410	TWA
		510	STEL (10-min)
USA			
ACGIH (TLV)	1994	410	TWA
OSHA (PEL)	1992	410	TWA
NIOSH (REL)	1992	410	TWA
Venezuela	1978	410	TWA
		510	Ceiling

From Cook (1987); Arbejdstilsynet (1988); ILO (1991); US National Institute for Occupational Safety and Health (NIOSH) (1992); US Occupational Safety and Health Administration (OSHA) (1992); American Conference of Governmental Industrial Hygienists (ACGIH) (1993); Deutsche Forschungsgemeinschaft (1993); Institut National de Recherche et de Sécurité (1993); Työministeriö (1993); UNEP (1993)

TWA, time-weighted average; STEL, short-term exposure limit; TLV, threshold limit value; PEL, permissible exposure level; REL, recommended exposure level; PR3, there is no reason to fear a risk of damage to the developing embryo or fetus when exposure limits are adhered to (Group C); skin, absorption through the skin may be a significant source of exposure

2. Studies of Cancer in Humans

2.1 Cohort studies

Following unpublished reports that indicated a significant excess risk for colorectal cancer among employees exposed to both ethyl acrylate and methyl methacrylate, a mortality study was designed at two US plants to investigate whether exposure to methyl methacrylate was associated with an increased cancer risk (Collins *et al.*, 1989). In total, 2671 men (2473 whites and 198 non-whites) who had worked in either plant from their inception (1951 and 1957, respectively) until 1974 were included in the study; 1302 had worked in a plant

manufacturing methyl methacrylate and 1361 in a facility using methyl methacrylate for the manufacture of acrylic fibres; eight men had worked in both plants. The follow-up period was from plant inception to 31 December 1983 and was 98% complete. A job-exposure matrix was developed on the basis of environmental monitoring data and interviews of plant employees in order to characterize the exposure histories of individual workers. In the two plants, 25 and 15 job titles, respectively, were considered to have involved exposure to methyl methacrylate. In the latter plant, all 15 jobs also entailed exposure to acrylonitrile and vinylidene chloride. In the former plant, possible exposure to acrylamide, acrylonitrile, epichlorohydrin and formaldehyde was reported. In both plants, the mean 8-h time-weighted exposure to methyl methacrylate was below 1.0 ppm between 1960 and 1983; reported peak levels were 11.5 ppm in the plant for the manufacture of acrylic fibre and 7.8 ppm in the other facility. Cumulative exposure to methyl methacrylate was calculated for each worker. Information on smoking habits was available from medical records for nearly 60% of the cohort members. The cohort was divided into unexposed (cumulative exposure, < 0.1 ppm-year) and exposed (cumulative exposure, > 0.1 ppm-year). Indirectly standardized mortality ratios (SMRs) and directly standardized relative risks (SRRs) were calculated. SMRs were adjusted by age, period and ethnicity, as were SRRs, which were also adjusted for latency and smoking (smokers, nonsmokers, unknown). The exposed cohort (28 021 person-years) showed significantly decreased mortality from all causes (114 deaths; SMR, 67) and nonsignificantly increased mortality from all cancers (35 deaths; SMR, 104); six deaths from digestive cancer were observed (8.1 expected), one of which was due to cancer of the large intestine (2.6 expected). Cancer mortality was also analysed according to attained cumulative exposure (0, 0-0.19, 0.20-2.0 and > 2.0 ppm-years). For none of the cancer sites was a significantly increasing risk found in parallel with increasing dose.

Walker *et al.* (1991) reviewed and analysed (with some additional new material) all of the data in the original unpublished reports on colorectal cancer and exposure to ethyl acrylate and methyl methacrylate of three cohorts in two US plants. One cohort (I) comprised 3934 white men employed between 1933 and 1945 in a plant where ethyl acrylate production began in 1933 and use of methyl methacrylate in 1936; the second (II) comprised 6548 white men from the same plant who had been hired between 1946 and 1982; the third cohort (III) comprised 3381 white men employed between 1943 and 1982 in another, similar facility where production of acrylic sheet began in 1943. Exposure in the plants was to ethyl acrylate and methyl methacrylate simultaneously, as well as to other agents, including ethylene dichloride, methylene chloride and acrylonitrile. Methyl methacrylate was the most extensively used chemical (88-100%). Exposure of cohort members was estimated on the basis of a job-specific semi-quantitative rating scale, which did not distinguish between ethyl acrylate and methyl methacrylate. In the three cohorts combined, overall mortality for 1933-86 was below that expected on the basis of mortality rates for US white males (4106 deaths observed; SMR, 96). The ratio for deaths from all cancers was slightly increased (924 observed; SMR, 102), as was that for deaths from cancer of the large intestine (99 observed; SMR, 121). Detailed analyses of colorectal cancer mortality were performed for each of the three cohorts. Expected deaths were calculated from the mortality rates of the counties relevant to each plant. Table 6 presents results for those workers classified as having been exposed to methyl methacrylate and ethyl acrylate. Mortality from colon cancer was

significantly increased in cohort I (SMR, 150; 95% confidence interval [CI], 106–205) and nonsignificantly increased in cohort III (SMR, 152; 95% CI, 92–238). The rate for rectal cancer was increased in cohort I (SMR, 192; 95% CI, 92–340). Mortality from cancers of the colon and rectum was further analysed among exposed workers in cohort I (Table 7).

Table 6. Mortality from colon and rectal cancer among workers exposed to ethyl acrylate and methyl methacrylate in two US plants at different periods (see text for definition of cohorts)

Cohort	Colon			Rectum		
	O/E	SMR	95% CI	O/E	SMR	95% CI
I	38/25.4	150		10/5.2	192	
II	7/7.1	99		0/1.3	0	
III	19/12.5	152		1/3.1	32	
Total	64/44.9	143	110–182	11/9.6	115	57–205

From Walker *et al.* (1991); O, observed; E, expected; SMR, standardized mortality ratio; CI, confidence interval

Table 7. Dose-response analysis of risks for colon and rectal cancer among workers in cohort I

Exposure	Colon		Rectum	
	No. of deaths	SMR	No. of deaths	SMR
Achieved exposure score				
≥ 1	38	150	NR	NR
≥ 5	21	146		
≥ 10	13	159		
≥ 15	12	215		
Exposure score ≥ 20 years since first employment				
1–4	13	139	6	252
5–9	6	116	0	0
10–14	1	45	1	185
≥ 15	11	240	3	283

From Walker *et al.* (1991); SMR, standardized mortality ratio; NR, not reported

No regular increase according to years elapsed since first exposure was observed for colon cancer. Analysis by achieved exposure score showed the greatest increase in the category of highest exposure (SMR, 215; 95% CI, 111–376). Analysis by exposure score 20 or more years after first employment did not indicate a consistently increasing risk for either colon or rectal cancer. The risk for colon cancer was highest in the ≥ 15 category (SMR, 240; 95% CI, 133–434). Analysis by maximal intensity of exposure also did not reveal an increasing risk with increasing maximal exposure to ethyl acrylate or methyl methacrylate.

2.2 Case-control studies

In the case-control study conducted in Montréal (see the monograph on styrene, p. 258), methyl methacrylate was one of the exposures assessed. The prevalence of exposure was 0.4%. The only cancer site for which there was a significantly increased risk was lung (any exposure: odds ratio, 5.9, 90% CI, 1.7–21; five exposed cases). No significant increase in risk for colorectal cancer was observed (Siemiatycki, 1991).

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

Rat: Groups of 25 male and 25 female young Wistar rats were administered 0, 6, 60 or 2000 ppm [mg/L] methyl methacrylate ([purity unspecified] containing 10 ppm monomethyl ether of *tert*-butylhydroquinone to inhibit polymerization) in the drinking-water for two years. At the start of the fifth month of treatment, the 6- and 60-mg/L dose levels were raised to 7 and 70 mg/L. Survival at two years in the control, low-, mid- and high-dose groups was: 13, 18, 15 and 13 males and 16, 18, 18 and 15 females, respectively. No treatment-related increase in the incidence of tumours was reported (Borzelleca *et al.*, 1964). [The Working Group noted the small number of animals and the inadequate reporting.]

3.2 Inhalation

3.2.1 Mouse

Groups of 50 male and 50 female B6C3F1 mice, eight to nine weeks of age, were exposed by inhalation to 0, 500 or 1000 ppm [2050 or 4100 mg/m³] methyl methacrylate (purity, > 99%; containing 10 ppm monomethyl ether of hydroquinone as an inhibitor of polymerization) for 6 h a day on five days a week for 102 weeks. Animals were killed at 113–114 weeks of age. During most of the second year of the study, the mean body weights of treated male mice and high-dose female mice were 10–18% lower than those of the controls. Survival in the control, low-dose and high-dose groups at the end of the experiment was 44, 42 and 47 males and 27, 26 and 33 females. No tumour related to the treatment was found. Alveolar-bronchiolar adenomas and carcinomas (combined) occurred in a significant negative trend in males (control, 11/50; low-dose, 1/50; and high-dose, 4/50; $p = 0.017$, incidental tumour test), as did hepatocellular adenomas and carcinomas (combined) (control,

16/50; low-dose, 7/48; and high-dose, 7/49; $p = 0.043$, incidental tumour test). Pituitary adenomas and carcinomas (combined) occurred at a significant negative trend in females (control, 12/49; low-dose, 3/44; and high-dose, 2/39; $p = 0.004$, incidental tumour test). A dose-dependent increase in the incidence of nasal cavity inflammation and epithelial hyperplasia was observed (US National Toxicology Program, 1986; Chan *et al.*, 1988).

3.3.2 Rat

Groups of 50 male and 50 female Fischer 344/N rats, seven to eight weeks of age, were exposed by inhalation to 0, 500 or 1000 ppm [2050 or 4100 mg/m³] (males) and 0, 250 or 500 ppm [1025 or 2050 mg/m³] (females) methyl methacrylate (purity, > 99%, containing 10 ppm monomethyl ether of hydroquinone as an inhibitor of polymerization) for 6 h a day on five days a week for 102 weeks. Animals were killed at 111–112 weeks of age. Survival rates in the control, low- and high-dose groups at the end of the experiment were 26, 29 and 28 males and 30, 27 and 29 females. A marginal increase in the incidence of mononuclear-cell leukaemia was observed in female rats (control, 11/50; low-dose, 13/50; high-dose, 20/50; $p = 0.051$, life-table trend test). The historical incidence of mononuclear-cell leukaemia in females exposed by inhalation at the same laboratory was 29.3% (range, 22–36%). In males, significant, dose-related decreases were observed in the incidences of adenomas and carcinomas (combined) of the pituitary gland (control, 24/45; low-dose, 20/47; high-dose, 13/48; $p = 0.004$, incidental tumour trend test) and preputial gland (control, 5/50; low-dose, 4/50; high-dose, 0/50; $p = 0.029$, incidental tumour trend test). A dose-dependent increase in the incidence of nasal cavity inflammation was observed (US National Toxicology Program, 1986; Chan *et al.*, 1988). [The Working Group considered that the marginally increased incidence of mononuclear-cell leukaemia observed in female rats was not biologically significant, as it fell within the range of values seen in historical controls.]

4. Other Data Relevant for an Evaluation of Carcinogenicity and Its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

Methyl methacrylate can be absorbed through the skin (Rajaniemi, 1986). Blood levels of monomeric methyl methacrylate were studied in 11 patients after total hip arthroplasty; its elimination was characterized by a biphasic pattern, with a half-life of 0.3 min for the initial portion and 3.0 min for the terminal phase. The peak concentration of the monomer occurred after 1.7–2.0 min (Gentil *et al.*, 1993). A widely different half-life for methyl methacrylate was reported by Svartling *et al.* (1986). The presence of methacrylic acid has been demonstrated in the blood of patients during hip replacement (Crout *et al.*, 1979); and dental technicians exposed to methyl methacrylate excreted 19–200 nmol methacrylate in urine collected over 24 h, pre-shift concentrations being about 10 times lower (Rajaniemi *et al.*, 1989).

4.1.2 *Experimental systems*

A high proportion of methyl methacrylate is fully oxidized in rats: thus, irrespective of whether administration was oral or intravenous, up to 65% of a single dose (5.7 mg/kg bw) of [1,3-¹⁴C]methyl methacrylate was expired as ¹⁴CO₂ within 2 h and 84–88% within 10 days. The same pattern of excretion was seen after oral or intravenous administration of [2-¹⁴C]methyl methacrylate. Less than 2% of the dose was exhaled as the parent compound and 4–7% remained in the carcass 10 days after treatment (Bratt & Hathway, 1977).

After intraperitoneal injection of methyl methacrylate into rats, a mercapturic acid and a methacrylic acid were identified as metabolites. Only after co-administration of the esterase inhibitor tri-*ortho*-tolyl phosphate was there a strong, significant increase in urinary thioether excretion over that in controls (rising from zero to 11%), showing that hydrolysis by carboxylesterase is an important metabolic route for this substance (Delbressine *et al.*, 1981). The initial rate of pulmonary excretion of ¹⁴CO₂ was the same whatever carbon was labelled, implying that the three propylene carbons are metabolized simultaneously. On this basis, it has been proposed that, following hydrolysis of the parent compound, methacrylic acid complexes with coenzyme A to enter the pathway indicated in Figure 1 in which methyl-malonyl coenzyme A, which is also formed in valine catabolism, is converted to succinyl coenzyme A. Hence, all four carbons would enter the citric acid cycle (Bratt & Hathway, 1977).

Formation of formaldehyde from methyl methacrylate via methanol has been observed *in vitro* in the presence of rat liver microsomes as the metabolizing system (Kotlovskii *et al.*, 1988).

Methyl methacrylate is an α,β -unsaturated carbonyl compound which reacts in Michael-type additions with nucleophilic atoms; however, no data are available on adduct formation with proteins or DNA *in vivo*. Although formation of an epoxide metabolite from methyl methacrylate has been predicted (Boylard & Chasseaud, 1970), it has not been demonstrated.

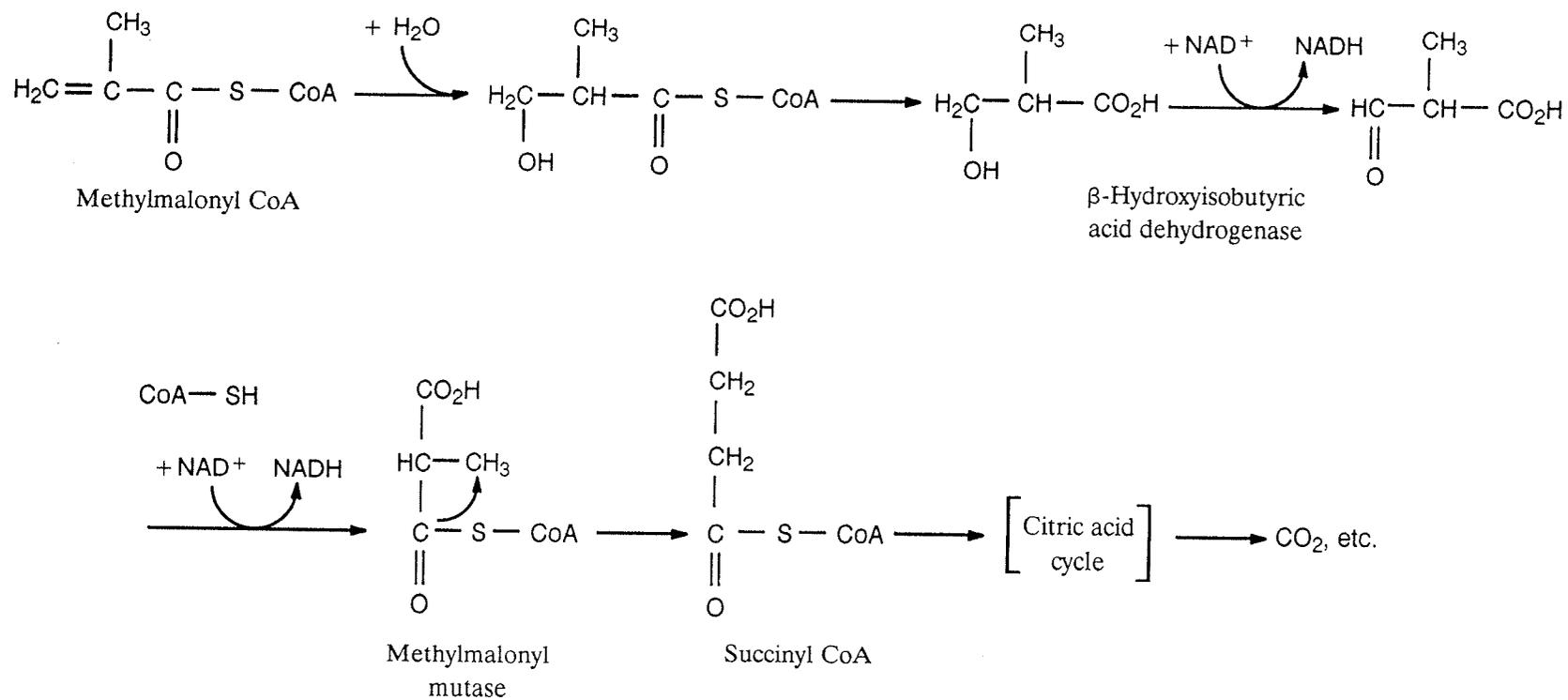
4.2 Toxic effects

4.2.1 *Humans*

Several adverse effects on health have been reported in people exposed either occupationally or during surgery to methyl methacrylate. Among the subjective neurological symptoms described by dental technicians are numbness, pain and whitening of the fingers (Rajaniemi & Tola, 1985). Several other symptoms, including intoxication and loss of appetite, memory and ability to concentrate, have also been reported (Steendahl *et al.*, 1992). Local neurotoxicity, as demonstrated by a significant decrease in distal sensory conduction velocities from the digits (Seppäläinen & Rajaniemi, 1984) and generalized peripheral neuropathy (Donaghy *et al.*, 1991), has also been diagnosed in dental technicians.

Methyl methacrylate is known to cause contact dermatitis (Farli *et al.*, 1990), asthma (Pickering *et al.*, 1986) and effects on the gastrointestinal (Sharova, 1989), cardiovascular (Schuh *et al.*, 1973; Marez *et al.*, 1992) and respiratory systems (Marez *et al.*, 1993). In two groups of workers exposed for up to 26 years to 11–33 and 100–200 mg/m³ time-weighted

Fig. 1. Scheme for the degradation of methyl methacrylate in mammals



From Bratt & Hathway (1977)

average concentrations of methyl methacrylate, dose-dependent increases in the incidences of neurasthenia, laryngitis and hypotension were reported (Lang *et al.*, 1986).

Interactions of methyl methacrylate with the endocrine system, resulting in altered levels of insulin, somatotrophic hormone and prolactin, were thought to be the cause of the adipogenicity observed in female but not male workers (Makarov *et al.*, 1981).

4.2.2 *Experimental systems*

Methyl methacrylate affects the endocrine system in rats (Stepanov *et al.*, 1991), the respiratory system in sheep (Fairman *et al.*, 1984), the cardiovascular system in dogs (Waters *et al.*, 1992) and the nervous system in rats (Husain *et al.*, 1985). Sensitization has been demonstrated in guinea-pigs (van der Walle & Bensink, 1982).

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Three groups of five pregnant Sprague-Dawley rats were treated intraperitoneally with 0.13, 0.27 or 0.44 ml/kg bw (0.1, 0.2 or 0.4 g/kg bw) methyl methacrylate on days 5, 10 and 15 of gestation and 43–50 fetuses per group were examined. Significantly reduced fetal body weights were observed in all three groups, and increased numbers of haematomas were seen at various sites in the animals given the two higher doses. No increase in the incidence of skeletal defects was observed (Singh *et al.*, 1972).

McLaughlin *et al.* (1978) exposed 18 ICR mice to 1330 ppm [5453 mg/m³] methyl methacrylate by whole-body inhalation for 2 h twice daily from days 6 to 15 of gestation, and examined the fetuses on day 18. No adverse effect on the pregnancies or on fetal development was observed. The fetal weights were increased slightly.

Nicholas *et al.* (1979) exposed groups of 22 and 27 pregnant Sprague-Dawley rats to 110 mg/L [26 800 ppm] methyl methacrylate vapour (head only), for 17 and 54 min per day (about 25 and 75% of the time to death of 50% of animals after a single exposure of 72.2 min), respectively, from days 6 to 15 of gestation. The fetuses were examined on day 20 for gross and skeletal malformations only. Both doses were toxic to the dams, as shown by loss of body weight during the first few days of treatment and decreased food intake throughout. The highest dose caused a small but significant increase in early fetal deaths and both doses reduced fetal weight and crown–rump length. The highest dose induced increased incidences of haematomas and retarded ossification.

In a well-conducted study, groups of 27 Crl:CD rats were exposed to 99, 304, 1178 or 2028 ppm [406, 1246, 4830 or 8315 mg/m³] methyl methacrylate vapour for 6 h per day on days 6–15 of gestation and the fetuses examined on day 20. Transient reduction in body weight gain was observed in all groups of dams, which persisted at the two highest doses throughout the treatment period. No adverse effect on pregnancies, on embryofetal

development or on malformation frequency was observed at any dose level (Solomon *et al.*, 1993).

4.4 Genetic and related effects (see also Table 8 and Appendices 1 and 2)

4.4.1 Humans

No data were available to the Working Group.

4.4.2 Experimental systems

Methyl methacrylate did not cause gene mutation in the commonly used reversion strains of *Salmonella typhimurium* in the presence or absence of an exogenous metabolic system. A significant response for forward mutation was observed in *S. typhimurium* in the presence of an exogenous metabolic system.

Methyl methacrylate was shown to induce gene mutation, micronuclei and chromosomal aberrations in mouse lymphoma L5178Y cells, and chromosomal aberrations and sister chromatid exchange in Chinese hamster ovary cells. No sister chromatid exchange was observed in human lymphocytes *in vitro*. [The Working Group noted that both tests were performed with inadequate exposure.]

In vivo, methyl methacrylate was reported to induce chromosomal aberrations in rat bone-marrow cells but not micronuclei in mouse bone marrow cells.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Methyl methacrylate is produced mainly by a process based on the reaction of acetone with hydrogen cyanide. It is an important monomer used mainly in the production of acrylic sheeting, moulding powders and resins and surface coatings. Occupational exposures have been measured during its production and during its use in polymers, as a component of surgical bone cement, in denture fabrication and during the preparation of artificial fingernails.

5.2 Human carcinogenicity data

A large mortality study was conducted of workers in acrylic sheet manufacture in two US plants. A significant increase in mortality from colon cancers was seen in one plant and a nonsignificant increase in the other; a nonsignificant increase in mortality from rectal cancer was found in the first plant. The increases were most evident among workers employed during the earliest production period and in jobs entailing the highest exposure. Exposure was predominantly to methyl methacrylate, but workers were also exposed to ethyl acrylate and to volatile by-products of the polymerization process.

Another US study examined the mortality of workers employed in methyl methacrylate manufacture and polymerization and found no significant increase in the number of cancer deaths.

Table 8. Genetic and related effects of methyl methacrylate

Test system	Result ^a		Dose ^b (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SAF, <i>Salmonella typhimurium</i> TM677, forward mutation (8-aza ^r)	-	+	5000.0000	Poss <i>et al.</i> (1979)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	500.0000	Lijinsky & Andrews (1980)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	5000.0000	Hachitani <i>et al.</i> (1981)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	2300.0000	Waegemaekers & Bensink (1984)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	5000.0000	Zeiger <i>et al.</i> (1987)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	500.0000	Lijinsky & Andrews (1980)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	2300.0000	Hachitani <i>et al.</i> (1981)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	5000.0000	Waegemaekers & Bensink (1984)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	1700.0000	Zeiger <i>et al.</i> (1987)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	500.0000	Lijinsky & Andrews (1980)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	2300.0000	Hachitani <i>et al.</i> (1981)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	5000.0000	Waegemaekers & Bensink (1984)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	5000.0000	Zeiger <i>et al.</i> (1987)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	500.0000	Lijinsky & Andrews (1980)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	2300.0000	Hachitani <i>et al.</i> (1981)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	-	-	5000.0000	Waegemaekers & Bensink (1984)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	500.0000	Lijinsky & Andrews (1980)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	2300.0000	Hachitani <i>et al.</i> (1981)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	5000.0000	Waegemaekers & Bensink (1984)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	5000.0000	Zeiger <i>et al.</i> (1987)
SAS, <i>Salmonella typhimurium</i> TA97, reverse mutation	-	-	1700.0000	Zeiger <i>et al.</i> (1987)
G5T, Gene mutation, mouse lymphoma L5178Y cells <i>in vitro</i> , <i>tk</i> locus	+	0	2200.0000	Doerr <i>et al.</i> (1989)

Table 8 (contd)

Test system	Result ^a		Dose ^b LED/HID	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
G5T, Gene mutation, mouse lymphoma L5178Y cells <i>in vitro</i> , <i>tk</i> locus	+	0	2000.0000	Moore <i>et al.</i> (1988)
G5T, Gene mutation, mouse lymphoma L5178Y cells <i>in vitro</i> , <i>tk</i> locus	+	+	250.0000	Myhr <i>et al.</i> (1990)
G5T, Gene mutation, mouse lymphoma L5178Y cells <i>in vitro</i> , <i>tk</i> locus	+	+	500.0000	Dearfield <i>et al.</i> (1991)
MIA, Micronucleus formation, mouse lymphoma L5178Y cells <i>in vitro</i>	(+)	0	2200.0000	Doerr <i>et al.</i> (1989)
SIC, Sister chromatid exchange, Chinese hamster ovary cells <i>in vitro</i>	+	+	16.0000	Anderson <i>et al.</i> (1990)
CIC, Chromosomal aberrations, Chinese hamster ovary cells <i>in vitro</i>	+	(+)	1600.0000	Anderson <i>et al.</i> (1990)
CIM, Chromosomal aberrations, mouse lymphoma L5178Y cells <i>in vitro</i>	(+)	0	2200.0000	Doerr <i>et al.</i> (1989)
SHL, Sister chromatid exchange, human lymphocytes <i>in vitro</i>	?	0	0.1000	Cannas <i>et al.</i> (1987)
SHL, Sister chromatid exchange, human lymphocytes <i>in vitro</i>	-	0	0.0000	Bigatti <i>et al.</i> (1989)
MVM, Micronucleus formation, mouse bone-marrow cells <i>in vivo</i>	-		4500 × 1 ip	Hachitani <i>et al.</i> (1981)
MVM, Micronucleus formation, mouse bone-marrow cells <i>in vivo</i>	-		1100 × 4 ip	Hachitani <i>et al.</i> (1981)
CBA, Chromosomal aberrations, rat bone-marrow cells <i>in vivo</i>	+		1300 × 1 ip	Fedyukovich & Egorova (1991)
CBA, Chromosomal aberrations, rat bone-marrow cells <i>in vivo</i>	+		650 × 2/wk, up to 8 wk	Fedyukovich & Egorova (1991)

^a+, positive; (+), weak positive; -, negative; 0, not tested; ?, inconclusive (variable response within several experiments within an adequate study)

^bIn-vitro tests, µg/ml; in-vivo tests, mg/kg bw

5.3 Animal carcinogenicity data

Methyl methacrylate was tested for carcinogenicity in one experiment in mice and one experiment in rats exposed by inhalation. No significant treatment-related increase in tumour incidence occurred. One study in rats by oral administration was inadequate for evaluation.

5.4 Other relevant data

Methyl methacrylate can be absorbed through the skin and is rapidly metabolized in man. In rats, it is first hydrolysed, and the dominant metabolic pathway is to fully oxidized carbons which are exhaled as carbon dioxide; a very small proportion is excreted as thioethers in the urine. Methyl methacrylate produces a number of toxic effects in man and experimental animals.

Exposure of mice and rats to methyl methacrylate by inhalation had no adverse reproductive effects.

No data were available on the genetic and related effects of methyl methacrylate in humans.

It caused chromosomal aberrations in rat bone marrow but did not induce micronuclei in mouse bone marrow *in vivo*. Gene mutation, sister chromatid exchange, micronuclei and chromosomal aberrations were induced in mammalian cells *in vitro*. Methyl methacrylate did not cause reverse gene mutation in bacteria but induced forward gene mutation in *Salmonella typhimurium* in a single study in the presence of an exogenous metabolic activation system.

5.5 Evaluation¹

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

There is *evidence suggesting lack of carcinogenicity* of methyl methacrylate in experimental animals.

Overall evaluation

Methyl methacrylate *is not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

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¹For definition of the italicized terms, see Preamble, pp. 27–30.

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2-ETHYLHEXYL ACRYLATE

1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 103-11-7

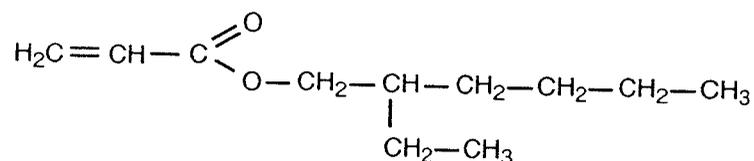
Deleted CAS Reg. No.: 78733-32-1; 84948-57-2; 93460-77-6

Chem. Abstr. Name: 2-Propenoic acid, 2-ethylhexyl ester

IUPAC Systematic Name: Acrylic acid, 2-ethylhexyl ester

Synonyms: 2-Ethylhexyl 2-propenoate

1.1.2 Structural and molecular formulae and relative molecular mass



$\text{C}_{11}\text{H}_{20}\text{O}_2$

Relative molecular mass: 184.28

1.1.3 Chemical and physical properties of the pure substance

- (a) *Description:* Colourless liquid (Hoechst Celanese Corp., 1992, undated)
- (b) *Boiling-point:* 213.5 °C (Hoechst Celanese Corp., 1992, undated)
- (c) *Melting-point:* -90 °C (Ohara *et al.*, 1985)
- (d) *Density:* Specific gravity, 0.8865 at 20 °C/20 °C (Hoechst Celanese Corp., 1992, undated)
- (e) *Spectroscopy data:* Infrared, nuclear magnetic resonance and mass spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991).
- (f) *Solubility:* Slightly soluble in water (0.01 wt% at 20 °C); soluble in alcohols, ethers and many organic solvents (acetone, benzene, ethyl ether, heptane, methanol, carbon tetrachloride) (Union Carbide Corp., 1982)
- (g) *Volatility:* Vapour pressure, 0.14 mm Hg [19 Pa] at 20 °C; relative vapour density (air = 1), 6.4 at 20 °C (Hoechst Celanese Corp., 1992, undated)
- (h) *Stability:* Flash-point, 92 °C (open cup); rapid, uncontrolled polymerization can cause explosion (Tyler, 1993)

- (i) *Octanol-water partition coefficient (P)*: log P, 3.67 (Beratergremium für umwelt-relevante Altstoffe, 1993); 4.32 (Tyler & Smock, 1993)
- (j) *Conversion factor*: $\text{mg/m}^3 = 7.54 \times \text{ppm}^a$

1.1.4 *Technical products and impurities*

2-Ethylhexyl acrylate is available as a commercial product with the following specifications: assay, 99.5 wt% min. (this value includes up to 0.4% 2-ethyl-4-methylpentyl acrylate, which is equivalent to 2-ethylhexyl acrylate in reactivity and performance in use); water, 0.05–0.10 wt% max.; acidity (as acrylic acid), 0.009 wt% max.; hydroquinone (inhibitor), 40–160 ppm; monomethyl ether of hydroquinone (inhibitor), 10–220 ppm (Union Carbide Corp., 1982; Hoechst Celanese Corp., 1988).

1.1.5 *Analysis*

Methods for sampling and analysing air have been developed for vapours of acrylate monomers, including 2-ethylhexyl acrylate. The acrylate monomer vapour is adsorbed on activated silica gel or charcoal, desorbed in acetone or carbon disulfide and analysed by gas chromatography with flame ionization detection (Bosserman & Ketcham, 1980; Samimi & Falbo, 1982). The limit of sensitivity is 0.01 ppm [0.075 mg/m³] (Bosserman & Ketcham, 1980). Steichen (1976) described a head-space method for the determination of residual 2-ethylhexyl acrylate monomer in the polymer by gas chromatography–flame ionization detection, with a limit of detection of 5 ppm [mg/kg].

1.2 **Production and use**

1.2.1 *Production*

Direct, acid-catalysed esterification of acrylic acid with 2-ethylhexanol is the principal method for the manufacture of 2-ethylhexyl acrylate. The commonest catalysts are sulfuric and *para*-toluenesulfonic acid and sulfonic acid functional cation-exchange resins. The monomethyl ether of hydroquinone is added as a polymerization inhibitor, and the esters are used in this form in most industrial applications (Ohara *et al.*, 1985; Bauer, 1991; Tyler, 1993).

Estimated production volumes of 2-ethylhexyl acrylate in the USA in 1980, 1985, 1990 and 1991 were 31, 36, 53 and 48 thousand tonnes, respectively (Mannville Chemical Products Corp., 1984; US International Trade Commission, 1986, 1991, 1993). In 1990, about 50 thousand tonnes were manufactured in Germany (Beratergremium für umwelt-relevante Altstoffe, 1993).

1.2.2 *Use*

Acrylic esters are used in the production of polymers and copolymers with a wide range of applications. As a plasticizing co-monomer, 2-ethylhexyl acrylate is used in the production

^aCalculated from: $\text{mg/m}^3 = (\text{relative molecular mass}/24.45) \times \text{ppm}$, assuming normal temperature (25 °C) and pressure (101.3 kPa)

of resins for pressure-sensitive adhesives, latex, paints, textile and leather finishes and coatings for paper. 2-Ethylhexyl acrylate can also be used as a co-monomer in solution polymers for industrial metal finishing (Mannsville Chemical Products Corp., 1984; Ohara *et al.*, 1985; Tyler, 1993; Hoechst Celanese Corp., undated).

The major current use of 2-ethylhexyl acrylate is in acrylic pressure-sensitive adhesives, of which it is a major component. The typical composition of an adhesive for general-purpose tape is 75% 2-ethylhexyl acrylate, 20% vinyl acetate, 4% acrylic acid and 1% *N*-methylolacrylamide (see monograph, p. 435) (Temin, 1990).

2-Ethylhexyl acrylate is also used in ultraviolet-curable coatings without solvents, which provide a glossy, abrasion-resistant finish, e.g. on book covers and record albums. A typical ultraviolet-cured formulation might include 20% trimethylpropane triacrylate, 70% acrylated polyurethane oligomer, 10% 2-ethylhexyl acrylate diluent monomer and small amounts of photoinitiator. A liquid coating or ink is spread on the surface of the substrate, and the coating is exposed to ultraviolet light for less than 1 sec and is completely cured (Mannsville Chemical Products Corp., 1984).

The estimated use patterns of acrylic esters, including 2-ethylhexyl acrylate, in Japan, western Europe and the USA are presented in Table 1 (Ohara *et al.*, 1985).

Table 1. Estimated distribution of uses of acrylic esters (% of total)

Use	Japan	Western Europe	USA
Surface coatings	34	35	42
Textiles	16	18	23
Acrylic fibres	14	7	6
Adhesives	20	15	5
Other	16	25	24

From Ohara *et al.* (1985)

1.3 Occurrence

1.3.1 Natural occurrence

2-Ethylhexyl acrylate is not known to occur as a natural product.

1.3.2 Occupational exposure

The National Occupational Exposure Survey conducted by the National Institute for Occupational Safety and Health between 1981 and 1983 indicated that 11 300 US employees were potentially exposed to 2-ethylhexyl acrylate (US National Institute for Occupational Safety and Health, 1993). Of this number, 53% were estimated to be exposed to pure 2-ethylhexyl acrylate and 47% to products containing it. The estimate is based on a survey of US companies and did not involve measurements of actual exposures.

Few data have been reported on occupational exposure to 2-ethylhexyl acrylate. Exposures of workers to styrene (see p. 239) and several acrylates, including 2-ethylhexyl acrylate, and area concentrations were monitored in a US facility where acrylic ester-styrene copolymers were produced. The concentrations of 2-ethylhexyl acrylate in 11 personal samples collected for various times at a process reactor which had an opening hatch for addition of starting products (reactor A) ranged from not detectable to 2 ppb [$15 \mu\text{g}/\text{m}^3$] (mean, 0.4 ppb [$3 \mu\text{g}/\text{m}^3$]); nine personal samples taken at a similar reactor contained no detectable concentration, but 13 personal samples collected on workers tending a completely closed reactor contained no detectable compound to 5 ppb [$38 \mu\text{g}/\text{m}^3$] (mean, 1 ppb [$7.5 \mu\text{g}/\text{m}^3$]). No detectable concentrations were found in six personal samples taken from workers at a closed polymer flake continuous reactor, and those in 11 personal samples collected at the unloading docks ranged from not detectable to 5 ppb [$38 \mu\text{g}/\text{m}^3$] (mean, 2 ppb [$15 \mu\text{g}/\text{m}^3$]). In all cases, exposures to ethyl acrylate, *n*-butyl acrylate, methyl methacrylate (see monograph, p. 445), styrene (see monograph, p. 233) and *alpha*-methylstyrene exceeded the levels of 2-ethylhexyl acrylate. All 49 area samples taken in the same production areas, except reactor A, had no detectable levels of 2-ethylhexyl acrylate, but eight area samples taken at reactor A had levels ranging from not detectable to 161 ppb [$1.2 \text{ mg}/\text{m}^3$] (mean, 30 ppb [$226 \mu\text{g}/\text{m}^3$]) (Samimi & Falbo, 1982).

Data on exposure of workers during manufacture of 2-ethylhexyl acrylate in four US plants are summarized in Table 2. Since 2-ethylhexyl acrylate is used primarily as an intermediate in closed process reactors, the concentrations are generally expected to be low. Exposure by inhalation would also be expected to be low in view of the vapour pressure of this compound (19 Pa); however, dermal exposures may occur during spills or leaks (Björkner *et al.*, 1980).

1.3.3 Water

2-Ethylhexyl acrylate was detected at concentrations ranging from 0.6 to 11 ppb ($\mu\text{g}/\text{L}$) (mean, 4 ppb) in the effluent from the last stage of an on-site waste-treatment facility which received water from a large petrochemical plant on the US coast of the Gulf of Mexico. The influent untreated wastewater contained 0.55–5.6 ppm (mg/L) (mean, 2.0 ppm) (Berglund & Whipple, 1987).

1.4 Regulations and guidelines

Occupational standards or guidelines have not been established for 2-ethylhexyl acrylate (ILO, 1993; American Conference of Governmental Industrial Hygienists, 1993; UNEP, 1993). Russia has a short-term exposure limit of $1 \text{ mg}/\text{m}^3$ (ILO, 1993). Union Carbide, a major supplier of 2-ethylhexyl acrylate, has adopted a threshold limit value of 5 ppm [$38 \text{ mg}/\text{m}^3$] for its internal operations (Samimi & Falbo, 1982).

The US Food and Drug Administration (1993) has established regulations for the use of monomers, polymers and copolymers of 2-ethylhexyl acrylate in products in contact with food.

Table 2. Concentrations of 2-ethylhexyl acrylate to which workers are exposed during manufacture

Operation	No. of samples	Concentration in personal air samples			
		Geometric mean		Range	
		ppb	mg/m ³	ppb	mg/m ³
Full-shift time-weighted average (> 336 min)					
Operations A	12 ^a	500	3.8	100-900	0.75-6.8
Operations B	60	50	0.4	1-1100	0.01-8.3
Quality assurance	9	30	0.2	< 10-120	< 0.1-0.9
Maintenance	63	140	1.1	3-460	0.02-3.5
Loading and unloading	20	160	1.2	100-600	0.75-4.5
Short-term (< 15 min)					
Operations A	1	500	3.8	500	3.8
Operations B	5 ^a	220	1.7	130-280	1.0-2.1
Maintenance and use	1	100	0.75	100	0.75
Loading and unloading	1	< 100	< 0.75	< 100	< 0.75

From Tyler (1993)

^aArea samples

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3.1 Skin application

Mouse: A group of 40 male C3H/HeJ mice, 7-10 weeks of age, received skin applications of about 20 mg/mouse 75% 2-ethylhexyl acrylate (purity, 99%) dissolved in acetone three times per week for life. Two groups of 40 mice were given applications of acetone alone (negative control), and one group of 40 mice was given about 0.03 mg/mouse 0.2% 3-methylcholanthrene (positive control) by the same dose schedule. The numbers of mice alive in the two acetone control groups and in the 2-ethylhexyl acrylate-treated group were 33, 19 and 30 at one year and 22, 13 and 15 at 1.5 years, respectively. Six skin tumours (two squamous-cell carcinomas and four papillomas) developed in the group treated with 2-ethylhexyl acrylate but in neither of the acetone control groups [$p = 0.001$, Fisher exact test]; 35/40 animals in the positive control group developed skin tumours, 34 of which were malignant (DePass, 1982; DePass *et al.*, 1985).

Groups of 80 male C3H/HeJ mice, six weeks of age, received skin applications of 2.5, 21, 43 or 86.5% (w/w) 2-ethylhexyl acrylate (purity, 99.5%) in 25 μ l acetone three times per week

for life, except that treatment of the group given 43% was stopped at 24 weeks (stop test). Two groups of 80 mice served as untreated or acetone-treated controls. The mean body weights were slightly greater in the treated groups than in the controls. No treatment-related effect on survival was observed: median survival was 97–102 weeks in treated mice and 105–108 weeks in controls. Skin tumours were seen in the groups treated with 21 and 86.5%: papillomas in 5 and 10 mice, respectively [$p < 0.001$ when compared with none in control groups]; squamous-cell carcinomas in 20 [$p < 0.001$, Fisher exact test] and 16 mice [$p < 0.001$ when compared with none in control groups, Fisher exact test]; malignant melanomas in 7 [$p = 0.001$] and 9 mice [$p < 0.0015$ when compared with none in control groups, Fisher exact test]. One basal-cell carcinoma and five fibrosarcomas were also seen in the 21% group and one haemangioma in the 86.5% group. No skin tumour occurred in the group given 2.5% or in the stop-test group. Scaling and scabbing were observed in all treated groups and persisted throughout the treatment period. Regression of the lesions was reported to have occurred within seven weeks after cessation of treatment of the group with 43%. Hyperkeratosis and hyperplasia had occurred in all groups, however, by the end of treatment (Wenzel-Hartung *et al.*, 1989).

Groups of 80 male NMRI mice, seven weeks of age, received skin applications of 0, 21.5, 43 or 85% (w/w) 2-ethylhexyl acrylate (purity, > 99.7%) in 25 μ l acetone three times per week on clipped dorsal skin. A positive control group of 80 mice received 0.015% benzo[*a*]pyrene in 25 μ l acetone by the same dose schedule. After seven months of treatment, each group, including the positive control group, was divided into two subgroups. One continued to receive the original treatment for the remainder of the two-year study period; in the second subgroup, treatment was discontinued, and, after two months, the mice were treated with 5 μ g/animal 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in 0.1 ml acetone twice a week for 20 weeks and then observed for the remainder (about nine months) of the two-year study. Body weights and survival were not affected by treatment with 2-ethylhexyl acrylate, with or without TPA. At termination of the study, 13–31% of the mice treated with 2-ethylhexyl acrylate and 17–19% of the acetone controls with or without TPA treatment were still alive. No skin tumour was seen in the groups treated with 2-ethylhexyl acrylate without TPA or in the acetone controls. One squamous-cell papilloma of the skin was seen in each of the groups painted with 21.5, 43 and 85% 2-ethylhexyl acrylate and TPA. Squamous-cell carcinomas were observed only in the benzo[*a*]pyrene-treated mice treated with and without TPA. Hyperkeratosis and hyperplasia occurred in all treated groups (Mellert *et al.*, 1994).

4. Other Data Relevant for 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

Elimination of total radioactivity from tissues was measured in two studies after administration of 2-ethylhexyl [2,3-¹⁴C]acrylate to groups of Wistar rats (Gut *et al.*, 1988; Sapota, 1988). In the study of Gut *et al.* (1988), doses of 10 mg/kg bw were administered either intraperitoneally or intravenously. One hour after dosing, only small amounts of metabolized compound were found in blood and other tissues; the tissues with the highest percentages of total radioactivity after intravenous administration were kidney (25.5%), liver (12.8%), brain (7.7%), thymus (7.4%), spleen (6.4%) and blood (2.6%). Elimination of radioactivity from blood was bi-exponential and was dependent on route of administration and age (weight) of the rats. The half-lives for the initial phase after intravenous and intraperitoneal administration were 30 min and 60 min in four-month-old (265–295 g) rats and 115 min and 130 min in seven-month-old (450–500 g) rats, respectively. The corresponding half-lives for the terminal phase were 5 and 6 h and 14 and 14 h. Elimination from other tissues followed a pattern similar to that of blood. Less than 0.01% of an administered dose was excreted in the faeces and 13.5% of an intravenous dose and 7.2% of an intraperitoneal dose were excreted in the urine within 24 h; about 2% of an intraperitoneal dose of 1 mmol/kg bw was excreted as thioethers. About 50% of the administered dose was expired, mostly as carbon dioxide, during the first 24 h after dosing, when about two-thirds of the radioactivity could be accounted for.

In the study of Sapota (1988), doses of 100 mg/kg bw were administered either orally or by intraperitoneal injection. Direct comparison with the results of Gut *et al.* (1988) is not possible, but about 75% of the administered radioactivity was found within 24 h in exhaled air after intraperitoneal treatment and 50% after oral treatment. Elimination from erythrocytes was biphasic, whereas elimination from plasma was monophasic and had a half-life of about 22 h. [The difference of the results from those of Gut *et al.* may be due to use of a higher dose, which would have saturated elimination pathways. Studies of distribution and elimination of total radioactivity are generally of limited relevance for toxicological evaluations.]

2-Ethylhexyl acrylate is believed to undergo carboxylesterase-catalysed metabolism, like other acrylate esters (Miller *et al.*, 1981). It is excreted in the urine both as *N*-acetyl-*S*-(2-carboxyethyl)cysteine and as *N*-acetyl-*S*-2-(2-ethylhexyloxycarbonyl)ethylcysteine (Kopecký *et al.*, 1985). Two unidentified metabolites were detected in the bile of rats (Cikrt *et al.*, 1986). In rats exposed by inhalation, the percentage of a dose of the acrylate excreted in urine as thioethers over 24 h was dose dependent and decreased from 8.0 to 3.0% as the 6-h exposure concentration in air increased from 250 to 1000 mg/m³, indicating saturable metabolism along this pathway. A decrease in the number of non-protein -SH groups was also observed in the blood and liver of these animals (Vodička *et al.*, 1990).

2-Ethylhexyl acrylate significantly increased bile flow (Cikrt *et al.*, 1986) and blood glucose level (Vodička *et al.*, 1990) in rats.

2-Ethylhexyl acrylate is an α,β -unsaturated carbonyl compound which reacts in Michael-type additions with nucleophiles (Tyler, 1993). No study is available, however, on the identity of putative adducts formed by 2-ethylhexyl acrylate or any of its metabolites with proteins or DNA *in vivo*.

4.2 Toxic effects

The toxicology of 2-ethylhexyl acrylate has been reviewed (Beratergremium für umwelt-relevante Altstoffe, 1993).

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

Sensitization was observed when guinea-pigs were treated with 2-ethylhexyl acrylate in Freund's complete adjuvant (Waegemaekers & van der Walle, 1983).

4.3 Reproductive and prenatal 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 3 and Appendices 1 and 2)

2-Ethylhexylacrylate did not induce mutation in *Salmonella typhimurium*. Equivocal results for mutation at the *tk* and *hprt* loci were seen in Chinese hamster ovary and L5178Y mouse lymphoma cells *in vitro*.

It is unclear whether 2-ethylhexylacrylate is clastogenic. Equivocal results were obtained for small colony formation and chromosomal aberrations in L5178Y mouse lymphoma cells. It did not induce micronuclei in these cells.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

2-Ethylhexyl acrylate is produced by acid-catalysed esterification of acrylic acid with 2-ethylhexanol. Its major uses are in pressure-sensitive adhesives, in resins for latex paints and paper coatings and in the finishing of textiles. Occupational exposure to 2-ethylhexyl acrylate has been reported during its production and use.

5.2 Human carcinogenicity data

No data were available to the Working Group.

Table 3. Genetic and related effects of 2-ethylhexyl acrylate

Test system	Result ^a		Dose ^b (LED/ HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	5000	Zeiger <i>et al.</i> (1985)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	5000	Zeiger <i>et al.</i> (1985)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	5000	Zeiger <i>et al.</i> (1985)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	5000	Zeiger <i>et al.</i> (1985)
GCO, Gene mutation, Chinese hamster ovary cells <i>in vitro</i> , <i>hprt</i> locus (suspension assay)	-	0	26	Moore <i>et al.</i> (1991)
GCO, Gene mutation, Chinese hamster ovary cells <i>in vitro</i> , <i>hprt</i> locus	?	0	80	Moore <i>et al.</i> (1991)
G5T, Gene mutation, mouse lymphoma L5178Y cells, <i>tk</i> locus	?	0	34	Dearfield <i>et al.</i> (1989)
MIA, Micronucleus formation, L5178Y mouse lymphoma cells	-	0	34	Dearfield <i>et al.</i> (1989)
CIM, Chromosome aberrations, L5178Y mouse lymphoma cells	?	0	34	Dearfield <i>et al.</i> (1989)

^a+, positive; -, negative; 0, not tested; ?, inconclusive (variable response within several experiments within an adequate study)

^bIn-vitro tests, µg/ml; in-vivo tests, mg/kg bw

5.3 Animal carcinogenicity data

2-Ethylhexyl acrylate was tested by skin application in three experiments in mice. It increased the incidence of squamous-cell carcinomas of the skin in two experiments and of malignant melanomas in one experiment. In the third experiment, in a different strain of mice, 2-ethylhexyl acrylate did not increase skin tumour incidence, with or without subsequent application of 12-*O*-tetradecanoylphorbol 13-acetate.

5.4 Other relevant data

2-Ethylhexylacrylate is rapidly metabolized in rats; a small proportion is exhaled as carbon dioxide within 24 h, and a small proportion is excreted as thioethers in urine. No data were available on the genetic and related effects of 2-ethylhexylacrylate in humans. There is very little evidence for or against its genotoxicity in experimental systems.

5.5 Evaluation¹

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

There is *limited evidence* in experimental animals for the carcinogenicity of ethylhexyl acrylate.

Overall evaluation

Ethylhexyl acrylate *is not classifiable as to its carcinogenicity to humans (Group 3)*.

6. References

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¹For definition of the italicized terms, see Preamble, pp. 27-30.

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SUMMARY OF FINAL EVALUATIONS

Agent	Degree of evidence of carcinogenicity		Overall evaluation of carcinogenicity to humans
	Human	Animal	
Ethylene	I	I	3
Ethylene oxide	L	S	1 ^b
Propylene	I	I	3
Propylene oxide	I	S	2B
Isoprene	I ^a	S	2B
Styrene	I	L	2B ^b
Styrene-7,8-oxide	I ^a	S	2A ^b
4-Vinylcyclohexene	I ^a	S	2B
4-Vinylcyclohexene diepoxide	I ^a	S	2B
Vinyl toluene	I ^a	ESL	3
Acrylamide	I	S	2A ^b
N-Methylolacrylamide	I ^a	L	3
Methyl methacrylate	I	ESL	3
2-Ethylhexyl acrylate	I ^a	L	3

S, sufficient evidence; L, limited evidence; I, inadequate evidence; ESL, evidence suggesting lack of carcinogenicity; for definitions of categories and groups, see preamble, pp. 27-30

^aNo data available

^bOther relevant data taken into account in making the overall evaluation

APPENDIX 1

**SUMMARY TABLES OF
GENETIC AND RELATED EFFECTS**

Summary table of genetic and related effects of 4-vinylcyclohexene

Nonmammalian systems													Mammalian systems																													
Proka-ryotes			Lower eukaryotes				Plants			Insects			In vitro						In vivo																							
													Animal cells			Human cells			Animals			Humans																				
D	G		D	R	G	A	D	G	C	R	G	C	A	D	G	S	M	C	A	T	I	D	G	S	M	C	A	T	I	D	G	S	M	C	DL	A	D	S	M	C	A	

A, aneuploidy; C, chromosomal aberrations; D, DNA damage; DL, dominant lethal mutation; G, gene mutation; I, inhibition of intercellular communication; M, micronuclei; R, mitotic recombination and gene conversion; S, sister chromatid exchange; T, cell transformation

In completing the tables, the following symbols indicate the consensus of the Working Group with regard to the results for each endpoint:

- + considered to be positive for the specific endpoint and level of biological complexity
- +¹ considered to be positive, but only one valid study was available to the Working Group
- considered to be negative
- ¹ considered to be negative, but only one valid study was available to the Working Group
- ? considered to be equivocal or inconclusive (e.g., there were contradictory results from different laboratories; there were confounding exposures; the results were equivocal)

Summary table of genetic and related effects of methyl methacrylate

Nonmammalian systems														Mammalian systems																														
Prokaryotes		Lower eukaryotes				Plants				Insects				In vitro							In vivo																							
														Animal cells							Human cells																							
D	G	D	R	G	A	D	G	C	R	G	C	A	D	G	S	M	C	A	T	I	D	G	S	M	C	A	T	I	D	G	S	M	C	DL	A	D	S	M	C	A				
-													+	+ ¹	? ¹	+					-								- ¹	+ ¹														

A, aneuploidy; C, chromosomal aberrations; D, DNA damage; DL, dominant lethal mutation; G, gene mutation; I, inhibition of intercellular communication; M, micronuclei; R, mitotic recombination and gene conversion; S, sister chromatid exchange; T, cell transformation

In completing the tables, the following symbols indicate the consensus of the Working Group with regard to the results for each endpoint:

- + considered to be positive for the specific endpoint and level of biological complexity
- +¹ considered to be positive, but only one valid study was available to the Working Group
- considered to be negative
- ¹ considered to be negative, but only one valid study was available to the Working Group
- ? considered to be equivocal or inconclusive (e.g., there were contradictory results from different laboratories; there were confounding exposures; the results were equivocal)

APPENDIX 2

**ACTIVITY PROFILES FOR
GENETIC AND RELATED EFFECTS**

APPENDIX 2

ACTIVITY PROFILES FOR GENETIC AND RELATED EFFECTS

Methods

The x-axis of the activity profile (Waters *et al.*, 1987, 1988) represents the bioassays in phylogenetic sequence by endpoint, and the values on the y-axis represent the logarithmically transformed lowest effective doses (LED) and highest ineffective doses (HID) tested. The term 'dose', as used in this report, does not take into consideration length of treatment or exposure and may therefore be considered synonymous with concentration. In practice, the concentrations used in all the in-vitro tests were converted to $\mu\text{g/ml}$, and those for in-vivo tests were expressed as mg/kg bw . Because dose units are plotted on a log scale, differences in molecular weights of compounds do not, in most cases, greatly influence comparisons of their activity profiles. Conventions for dose conversions are given below.

Profile-line height (the magnitude of each bar) is a function of the LED or HID, which is associated with the characteristics of each individual test system—such as population size, cell-cycle kinetics and metabolic competence. Thus, the detection limit of each test system is different, and, across a given activity profile, responses will vary substantially. No attempt is made to adjust or relate responses in one test system to those of another.

Line heights are derived as follows: for negative test results, the highest dose tested without appreciable toxicity is defined as the HID. If there was evidence of extreme toxicity, the next highest dose is used. A single dose tested with a negative result is considered to be equivalent to the HID. Similarly, for positive results, the LED is recorded. If the original data were analysed statistically by the author, the dose recorded is that at which the response was significant ($p < 0.05$). If the available data were not analysed statistically, the dose required to produce an effect is estimated as follows: when a dose-related positive response is observed with two or more doses, the lower of the doses is taken as the LED; a single dose resulting in a positive response is considered to be equivalent to the LED.

In order to accommodate both the wide range of doses encountered and positive and negative responses on a continuous scale, doses are transformed logarithmically, so that effective (LED) and ineffective (HID) doses are represented by positive and negative

numbers, respectively. The response, or logarithmic dose unit (LDU_{ij}), for a given test system i and chemical j is represented by the expressions

$$LDU_{ij} = -\log_{10}(\text{dose}), \text{ for HID values; } LDU \leq 0$$

and (1)

$$LDU_{ij} = -\log_{10}(\text{dose} \times 10^{-5}), \text{ for LED values; } LDU \geq 0.$$

These simple relationships define a dose range of 0 to -5 logarithmic units for ineffective doses (1-100 000 $\mu\text{g/ml}$ or mg/kg bw) and 0 to +8 logarithmic units for effective doses (100 000-0.001 $\mu\text{g/ml}$ or mg/kg bw). A scale illustrating the LDU values is shown in Figure 1. Negative responses at doses less than 1 $\mu\text{g/ml}$ (mg/kg bw) are set equal to 1. Effectively, an LED value $\geq 100\ 000$ or an HID value ≤ 1 produces an $LDU = 0$; no quantitative information is gained from such extreme values. The dotted lines at the levels of log dose units 1 and -1 define a 'zone of uncertainty' in which positive results are reported at such high doses (between 10 000 and 100 000 $\mu\text{g/ml}$ or mg/kg bw) or negative results are reported at such low dose levels (1 to 10 $\mu\text{g/ml}$ or mg/kg bw) as to call into question the adequacy of the test.

Fig. 1. Scale of log dose units used on the y-axis of activity profiles

Positive ($\mu\text{g/ml}$ or mg/kg bw)	Log dose units	
0.001	8	-----
0.01	7	----
0.1	6	---
1.0	5	---
10	4	---
100	3	---
1000	2	---
10 000	1	---
100 000	0	-----
..... 10	-1	---
..... 100	-2	---
..... 1000	-3	---
..... 10 000	-4	---
..... 100 000	-5	-----
Negative ($\mu\text{g/ml}$ or mg/kg bw)		

LED and HID are expressed as $\mu\text{g/ml}$ or mg/kg bw .

In practice, an activity profile is computer generated. A data entry programme is used to store abstracted data from published reports. A sequential file (in ASCII) is created for each compound, and a record within that file consists of the name and Chemical Abstracts Service number of the compound, a three-letter code for the test system (see below), the qualitative test result (with and without an exogenous metabolic system), dose (LED or HID), citation number and additional source information. An abbreviated citation for each publication is stored in a segment of a record accessing both the test data file and the citation

file. During processing of the data file, an average of the logarithmic values of the data subset is calculated, and the length of the profile line represents this average value. All dose values are plotted for each profile line, regardless of whether results are positive or negative. Results obtained in the absence of an exogenous metabolic system are indicated by a bar (-), and results obtained in the presence of an exogenous metabolic system are indicated by an upward-directed arrow (†). When all results for a given assay are either positive or negative, the mean of the LDU values is plotted as a solid line; when conflicting data are reported for the same assay (i.e., both positive and negative results), the majority data are shown by a solid line and the minority data by a dashed line (drawn to the extreme conflicting response). In the few cases in which the numbers of positive and negative results are equal, the solid line is drawn in the positive direction and the maximal negative response is indicated with a dashed line.

Profile lines are identified by three-letter code words representing the commonly used tests. Code words for most of the test systems in current use in genetic toxicology were defined for the US Environmental Protection Agency's GENE-TOX Program (Waters, 1979; Waters & Auletta, 1981). For *IARC Monographs* Supplement 6, Volume 44 and subsequent volumes, including this publication, codes were redefined in a manner that should facilitate inclusion of additional tests. Naming conventions are described below.

Data listings are presented in the text and include endpoint and test codes, a short test code definition, results [either with (M) or without (NM) an exogenous activation system], the associated LED or HID value and a short citation. Test codes are organized phylogenetically and by endpoint from left to right across each activity profile and from top to bottom of the corresponding data listing. Endpoints are defined as follows: A, aneuploidy; C, chromosomal aberrations; D, DNA damage; F, assays of body fluids; G, gene mutation; H, host-mediated assays; I, inhibition of intercellular communication; M, micronuclei; P, sperm morphology; R, mitotic recombination or gene conversion; S, sister chromatid exchange; and T, cell transformation.

Dose conversions for activity profiles

Doses are converted to $\mu\text{g/ml}$ for in-vitro tests and to mg/kg bw per day for in-vivo experiments.

1. In-vitro test systems

- (a) Weight/volume converts directly to $\mu\text{g/ml}$.
- (b) Molar (M) concentration \times molecular weight = $\text{mg/ml} = 10^3 \mu\text{g/ml}$; mM concentration \times molecular weight = $\mu\text{g/ml}$.
- (c) Soluble solids expressed as % concentration are assumed to be in units of mass per volume (i.e., 1% = 0.01 g/ml = 10 000 $\mu\text{g/ml}$; also, 1 ppm = 1 $\mu\text{g/ml}$).
- (d) Liquids and gases expressed as % concentration are assumed to be given in units of volume per volume. Liquids are converted to weight per volume using the density (D) of the solution ($D = \text{g/ml}$). Gases are converted from volume to mass using the ideal gas law, $PV = nRT$. For exposure at 20–37°C at standard atmospheric pressure, 1% (v/v) = 0.4 $\mu\text{g/ml} \times$ molecular weight of the gas. Also, 1 ppm (v/v) = $4 \times 10^{-5} \mu\text{g/ml} \times$ molecular weight.

- (e) In microbial plate tests, it is usual for the doses to be reported as weight/plate, whereas concentrations are required to enter data on the activity profile chart. While remaining cognisant of the errors involved in the process, it is assumed that a 2-ml volume of top agar is delivered to each plate and that the test substance remains in solution within it; concentrations are derived from the reported weight/plate values by dividing by this arbitrary volume. For spot tests, a 1-ml volume is used in the calculation.
- (f) Conversion of particulate concentrations given in $\mu\text{g}/\text{cm}^2$ are based on the area (A) of the dish and the volume of medium per dish; i.e., for a 100-mm dish: $A = \pi R^2 = \pi \times (5 \text{ cm})^2 = 78.5 \text{ cm}^2$. If the volume of medium is 10 ml, then $78.5 \text{ cm}^2 = 10 \text{ ml}$ and $1 \text{ cm}^2 = 0.13 \text{ ml}$.

2. In-vitro systems using in-vivo activation

For the body fluid–urine (BF–) test, the concentration used is the dose (in mg/kg bw) of the compound administered to test animals or patients.

3. In-vivo test systems

- (a) Doses are converted to mg/kg bw per day of exposure, assuming 100% absorption. Standard values are used for each sex and species of rodent, including body weight and average intake per day, as reported by Gold *et al.* (1984). For example, in a test using male mice fed 50 ppm of the agent in the diet, the standard food intake per day is 12% of body weight, and the conversion is dose = $50 \text{ ppm} \times 12\% = 6 \text{ mg/kg bw per day}$.

Standard values used for humans are: weight—males, 70 kg; females, 55 kg; surface area, 1.7 m^2 ; inhalation rate, 20 l/min for light work, 30 l/min for mild exercise.

- (b) When reported, the dose at the target site is used. For example, doses given in studies of lymphocytes of humans exposed *in vivo* are the measured blood concentrations in $\mu\text{g}/\text{ml}$.

Codes for test systems

For specific nonmammalian test systems, the first two letters of the three-symbol code word define the test organism (e.g., SA– for *Salmonella typhimurium*, EC– for *Escherichia coli*). If the species is not known, the convention used is –S–. The third symbol may be used to define the tester strain (e.g., SA8 for *S. typhimurium* TA1538, ECW for *E. coli* WP2uvrA). When strain designation is not indicated, the third letter is used to define the specific genetic endpoint under investigation (e.g., –D for differential toxicity, –F for forward mutation, –G for gene conversion or genetic crossing-over, –N for aneuploidy, –R for reverse mutation, –U for unscheduled DNA synthesis). The third letter may also be used to define the general endpoint under investigation when a more complete definition is not possible or relevant (e.g., –M for mutation, –C for chromosomal aberration).

For mammalian test systems, the first letter of the three-letter code word defines the genetic endpoint under investigation: A– for aneuploidy, B– for binding, C– for chromosomal aberration, D– for DNA strand breaks, G– for gene mutation, I– for inhibition of intercellular communication, M– for micronucleus formation, R– for DNA

repair, S-- for sister chromatid exchange, T-- for cell transformation and U-- for unscheduled DNA synthesis.

For animal (i.e., non-human) test systems *in vitro*, when the cell type is not specified, the code letters -IA are used. For such assays *in vivo*, when the animal species is not specified, the code letters -VA are used. Commonly used animal species are identified by the third letter (e.g., --C for Chinese hamster, --M for mouse, --R for rat, --S for Syrian hamster).

For test systems using human cells *in vitro*, when the cell type is not specified, the code letters -IH are used. For assays on humans *in vivo*, when the cell type is not specified, the code letters -VH are used. Otherwise, the second letter specifies the cell type under investigation (e.g., -BH for bone marrow, -LH for lymphocytes).

Some other specific coding conventions used for mammalian systems are as follows: BF- for body fluids, HM- for host-mediated, --L for leukocytes or lymphocytes *in vitro* (-AL, animals; -HL, humans), -L- for leukocytes *in vivo* (-LA, animals; -LH, humans), --T for transformed cells.

Note that these are examples of major conventions used to define the assay code words. The alphabetized listing of codes must be examined to confirm a specific code word. As might be expected from the limitation to three symbols, some codes do not fit the naming conventions precisely. In a few cases, test systems are defined by first-letter code words, for example: MST, mouse spot test; SLP, mouse specific locus test, postspermatogonia; SLO, mouse specific locus test, other stages; DLM, dominant lethal test in mice; DLR, dominant lethal test in rats; MHT, mouse heritable translocation test.

The genetic activity profiles and listings were prepared in collaboration with Environmental Health Research and Testing Inc. (EHRT) under contract to the US Environmental Protection Agency; EHRT also determined the doses used. The references cited in each genetic activity profile listing can be found in the list of references in the appropriate monograph.

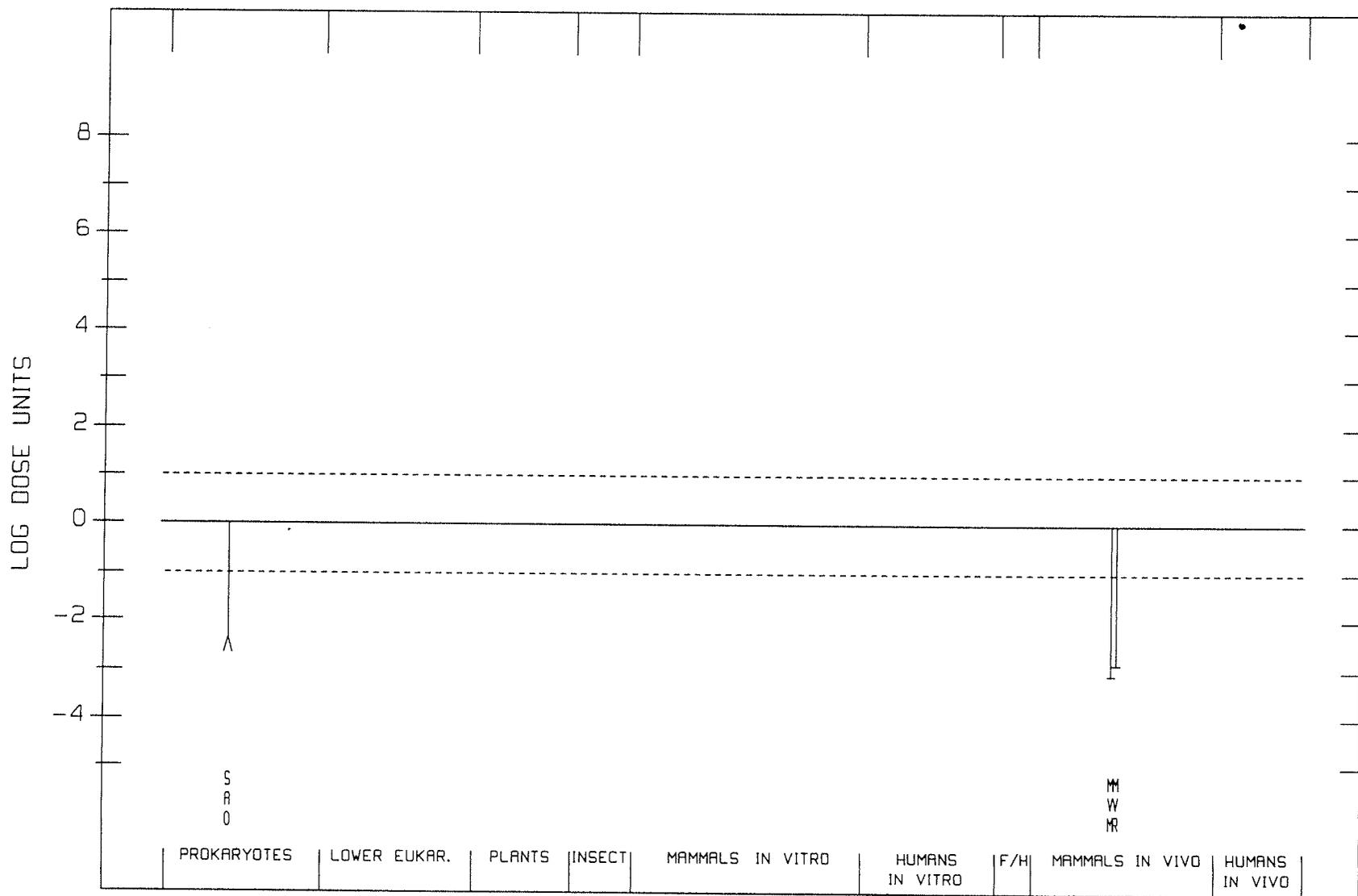
References

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Waters, M.D., Stack, H.F., Brady, A.L., Lohman, P.H.M., Haroun, L. & Vainio, H. (1988) Use of computerized data listings and activity profiles of genetic and related effects in the review of 195 compounds. *Mutat. Res.*, 205, 295-312

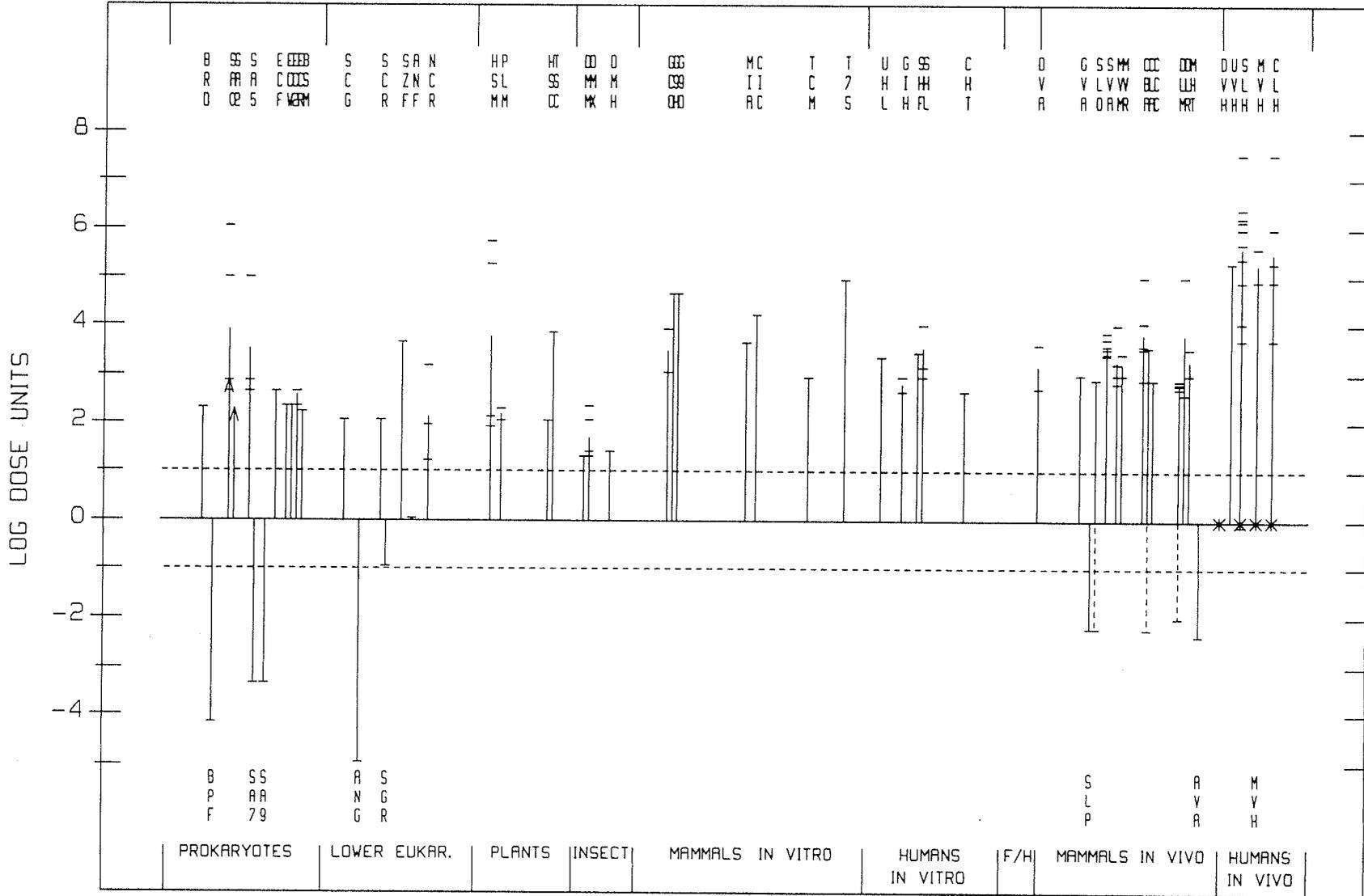
ETHYLENE

74-85-1



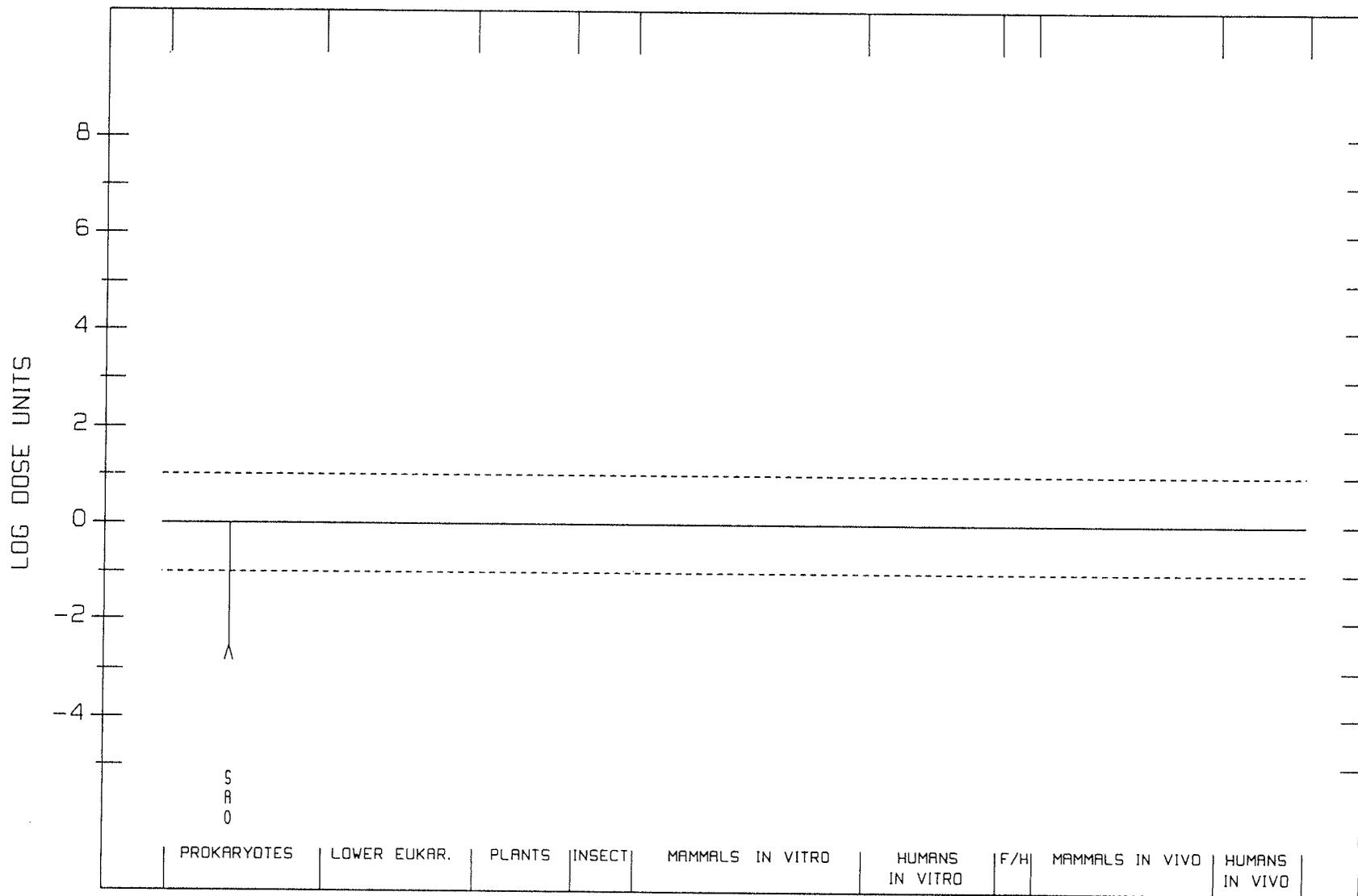
ETHYLENE OXIDE

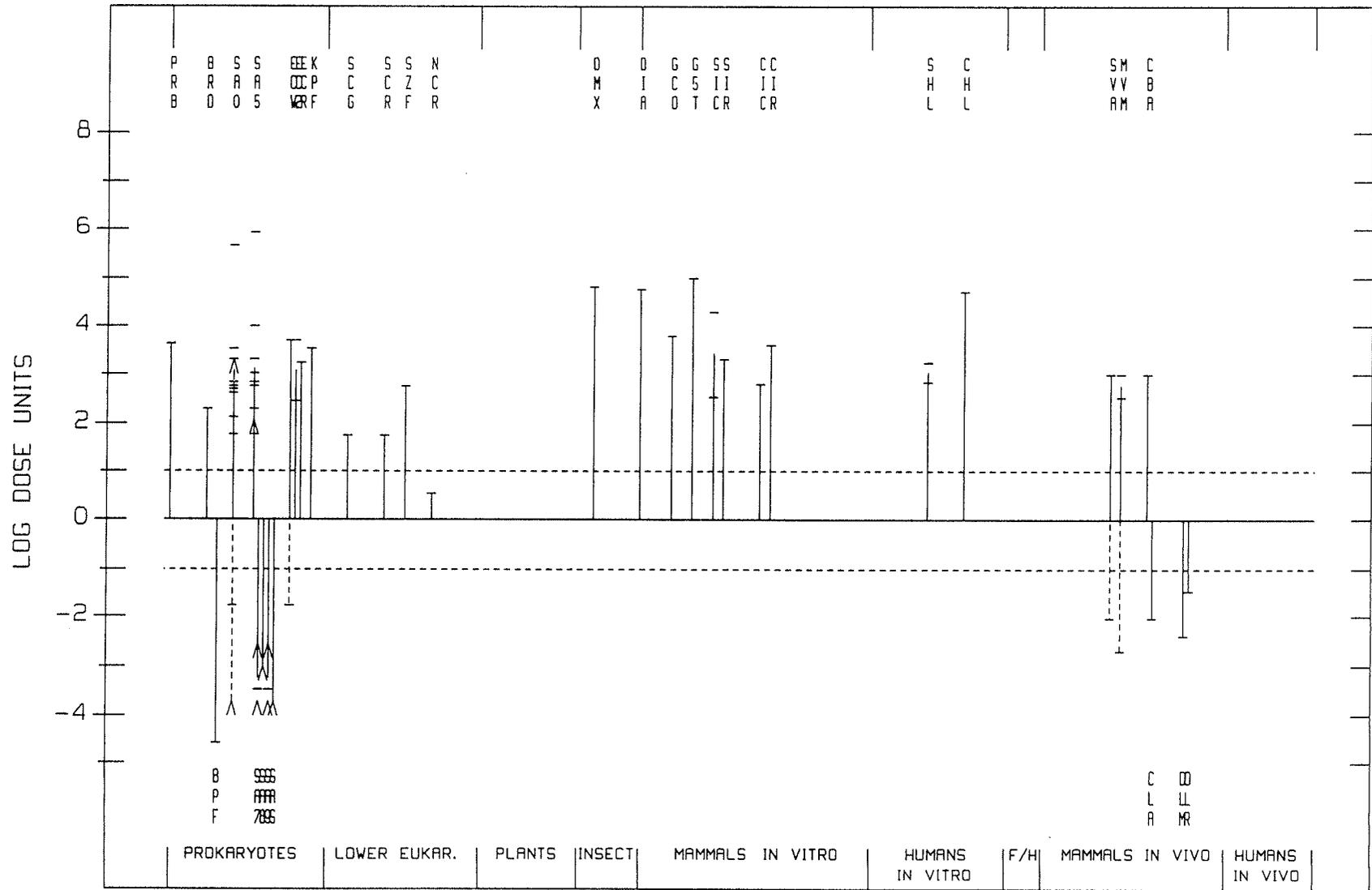
75-21-8



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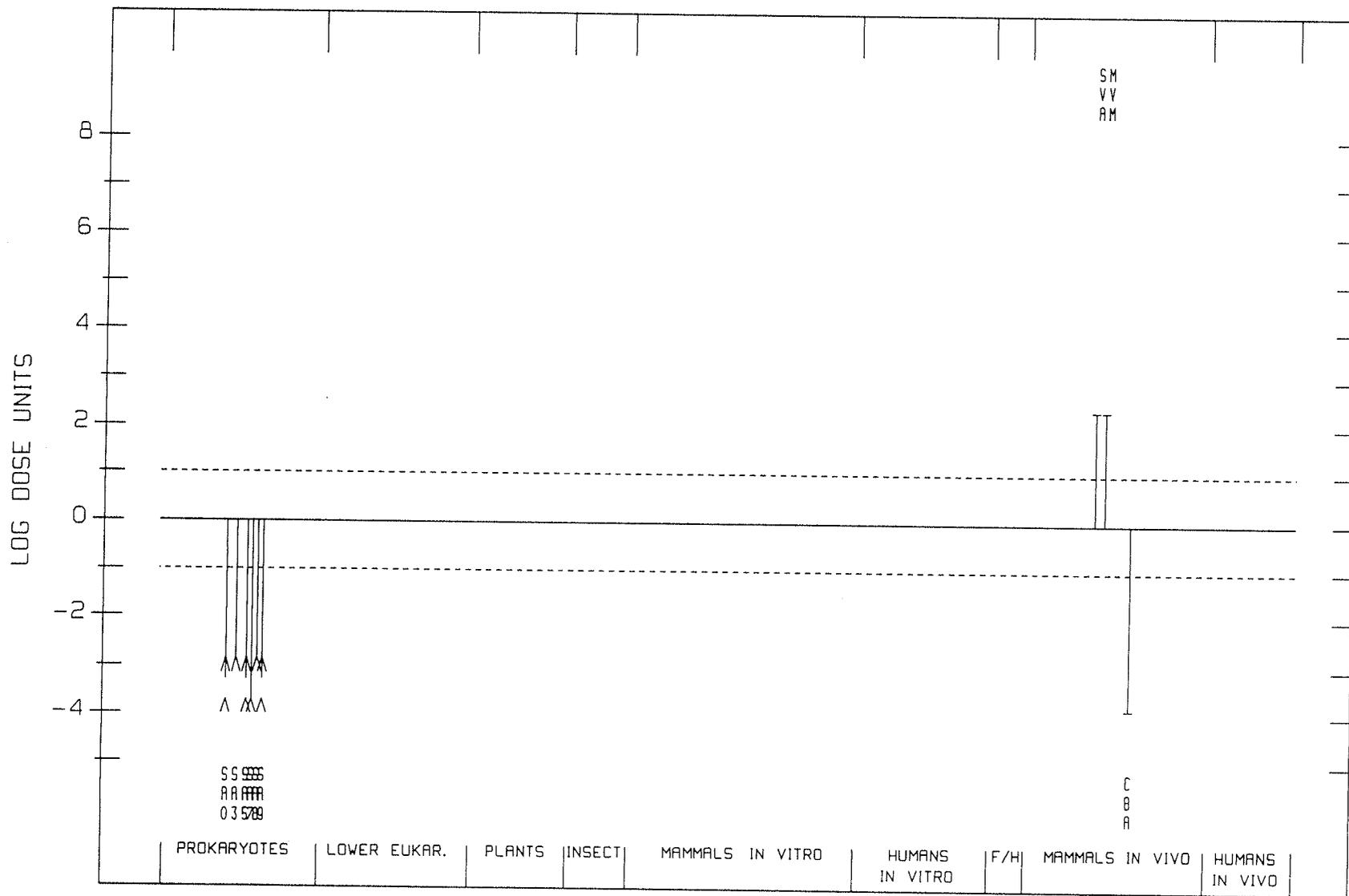
115-07-1





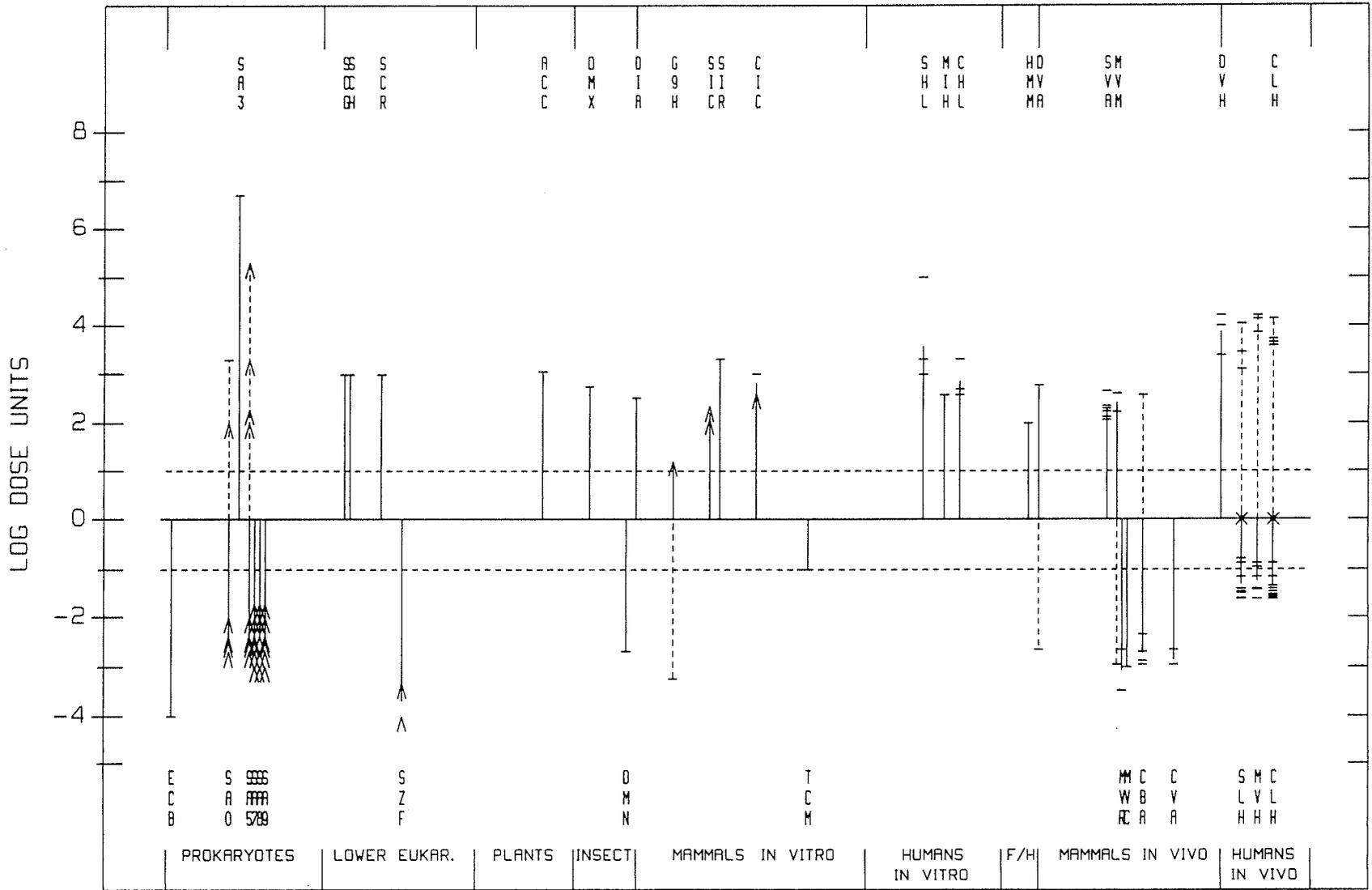
ISOPRENE

78-79-5



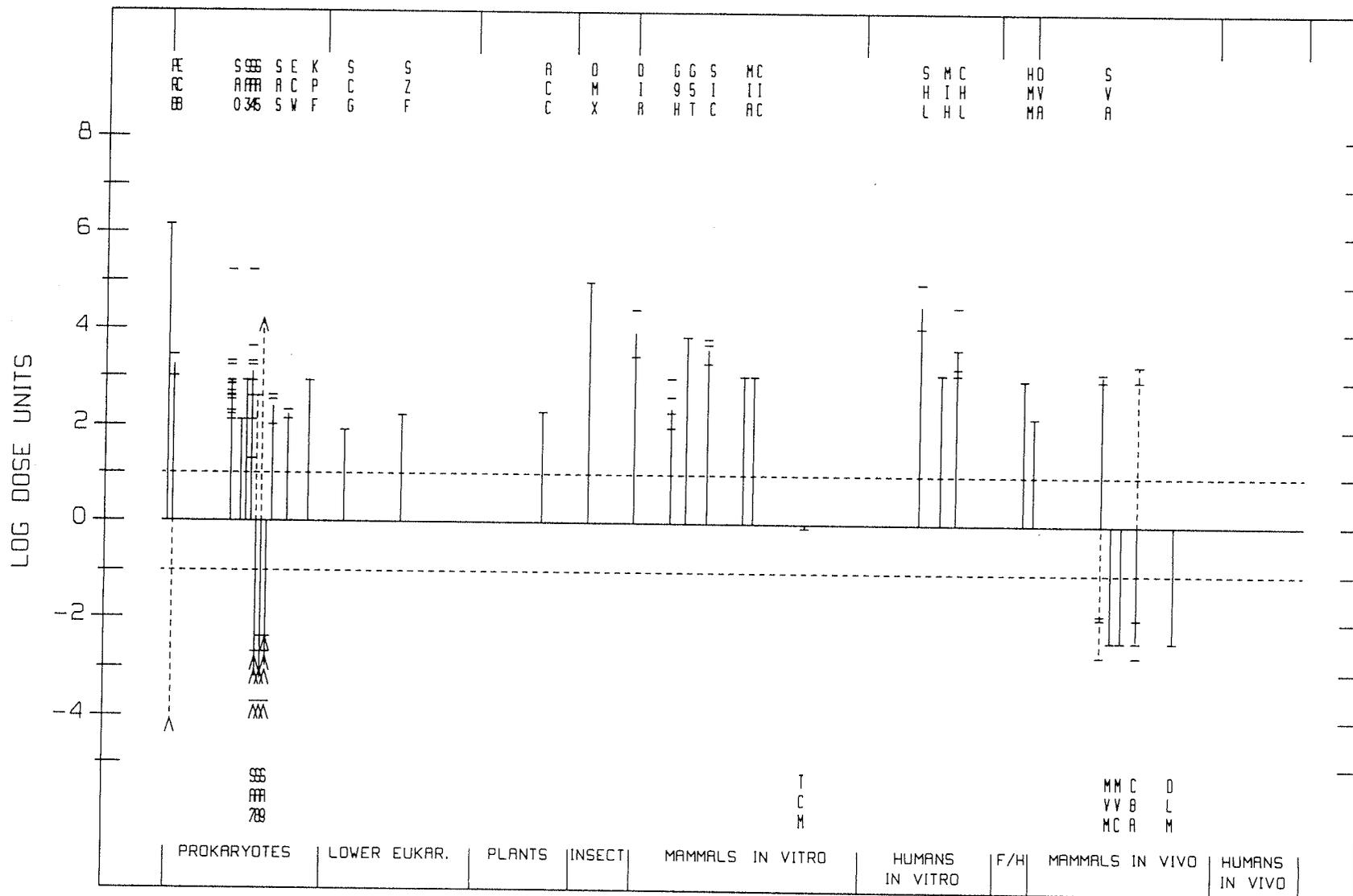
STYRENE

100-42-5



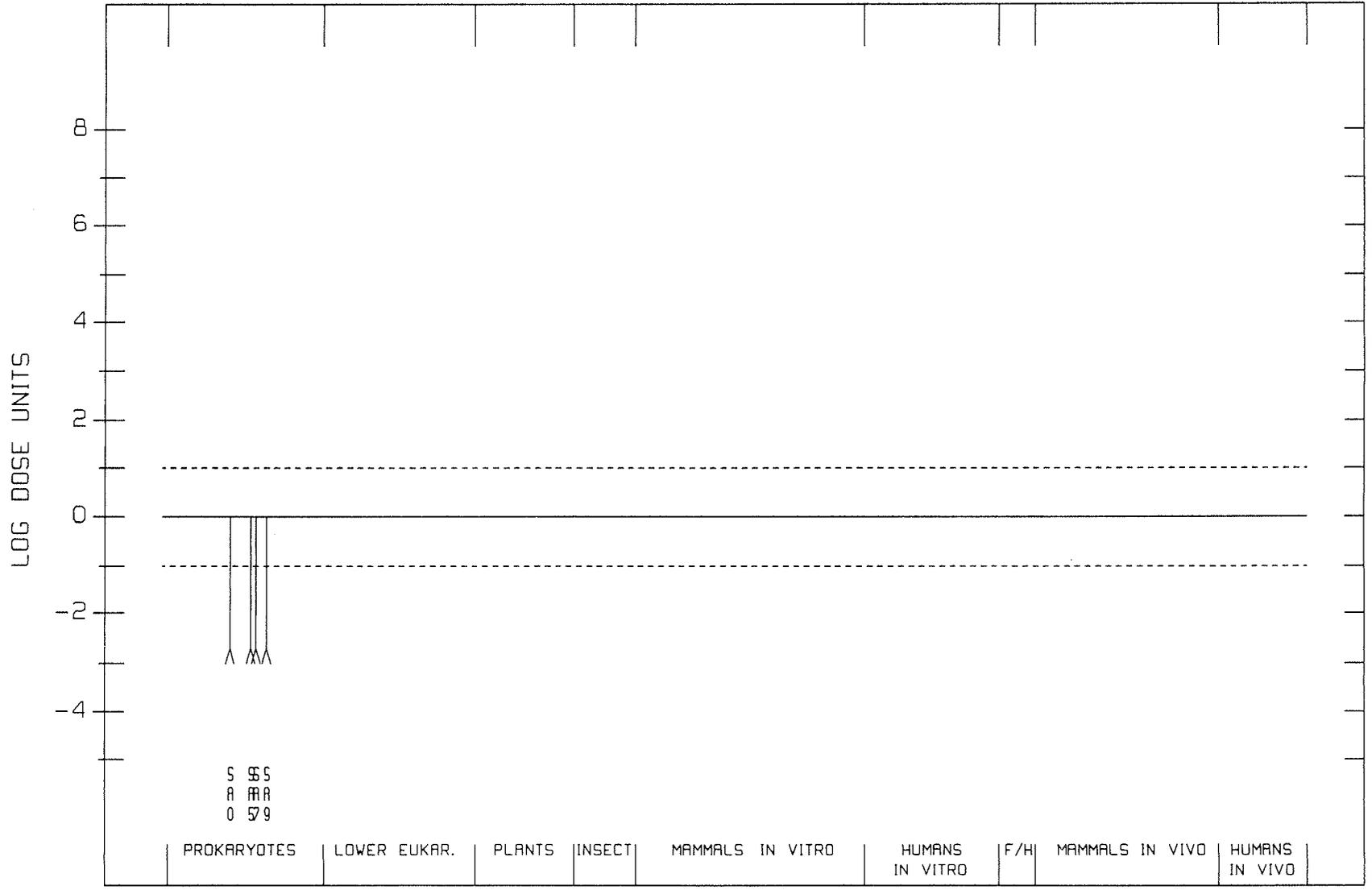
STYRENE OXIDE

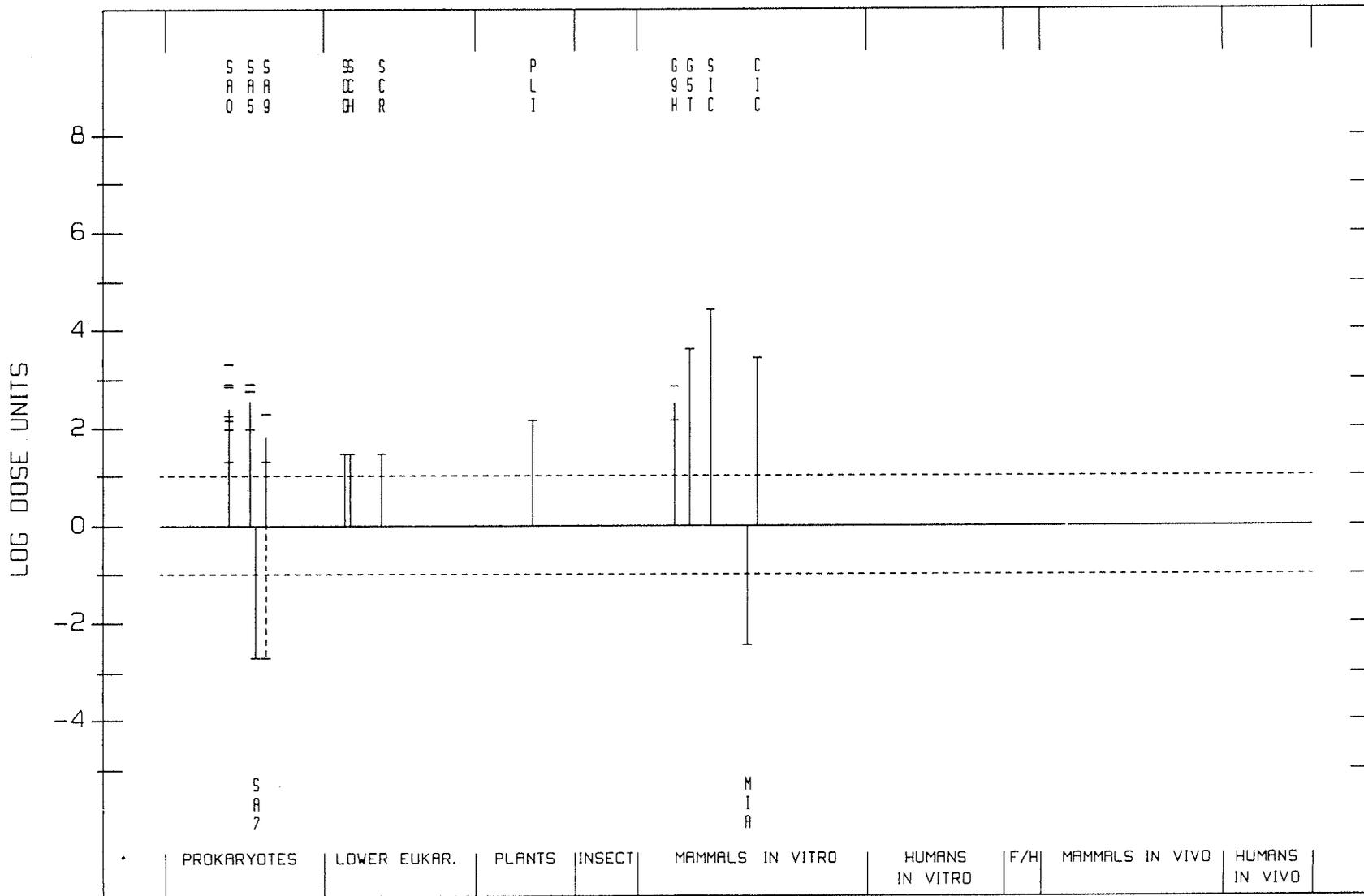
96-09-3



VINYL CYCLOHEXENE, 4-

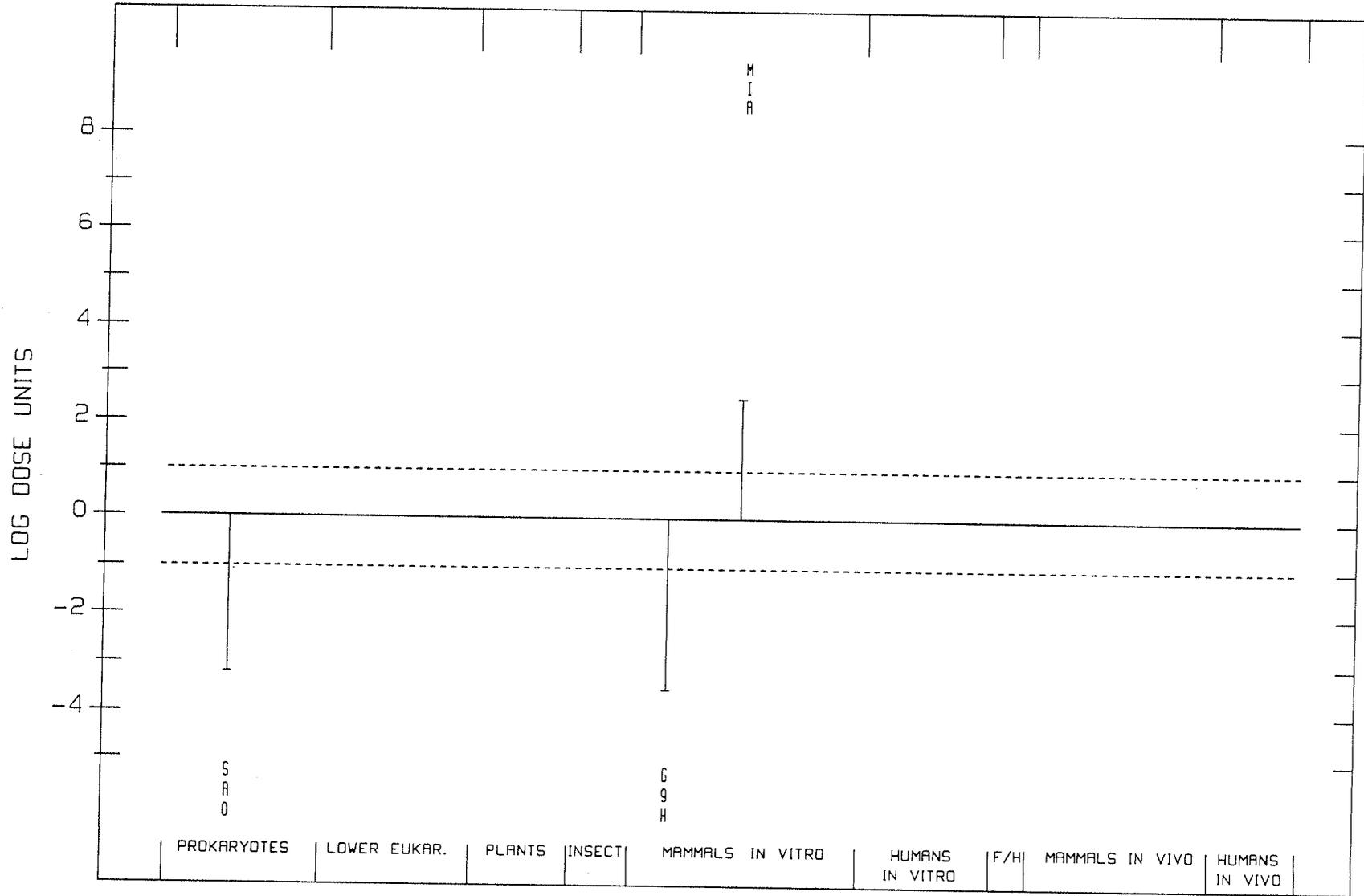
100-40-3





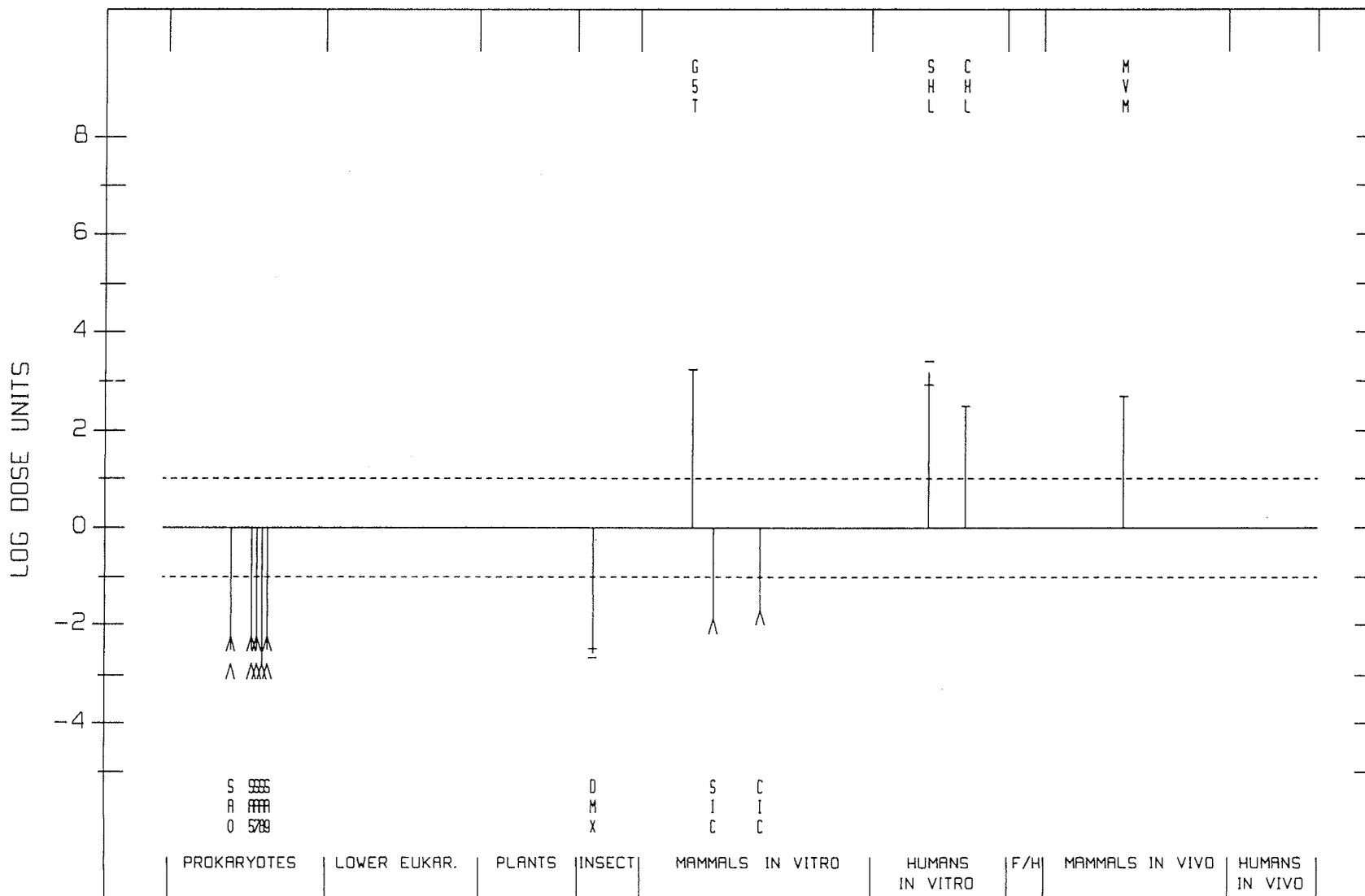
EPOXYETHYLCYCLOHEXANE-1,2-DIOL

NO CASRN



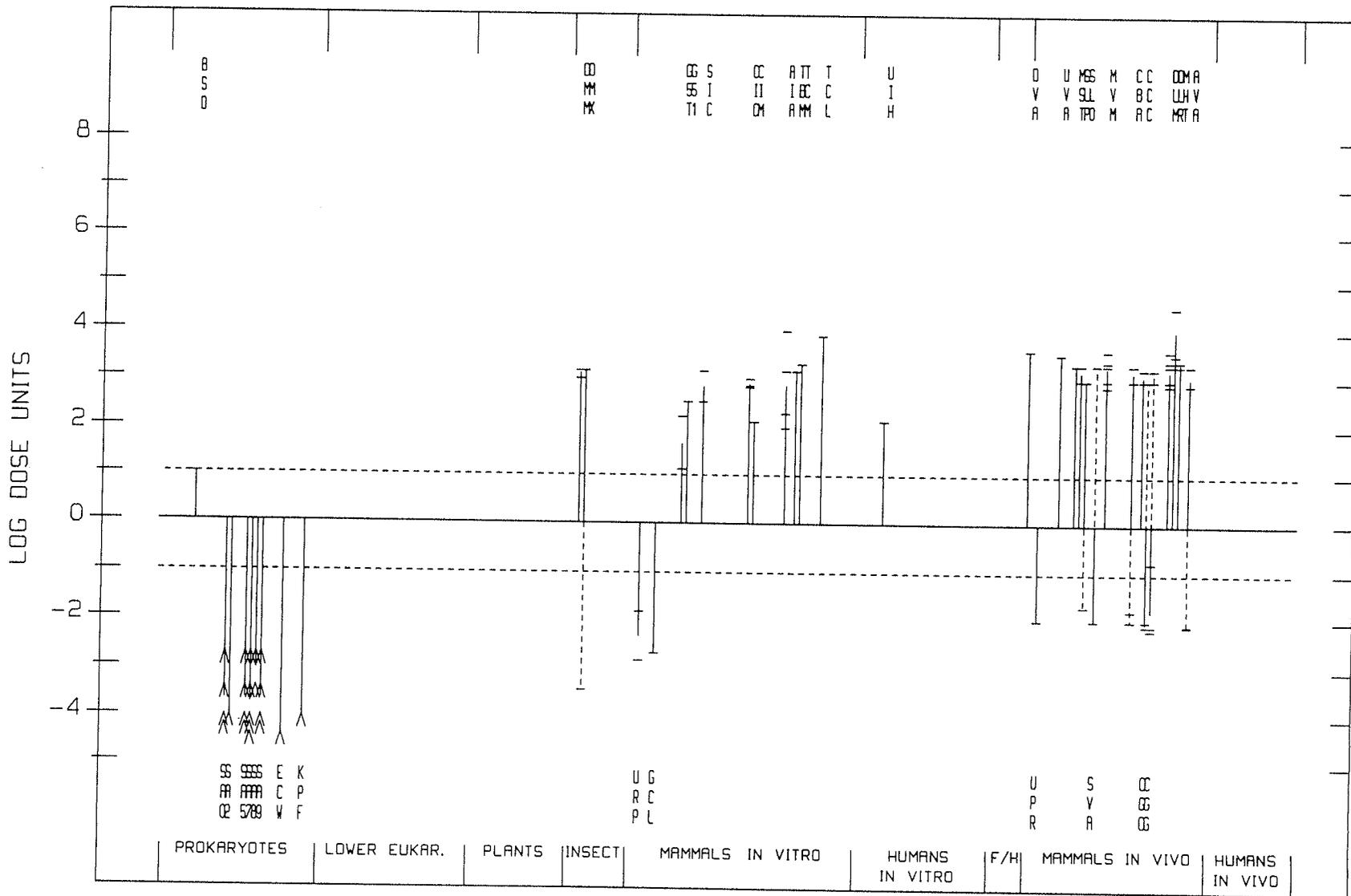
VINYL TOLUENE

25013-15-4



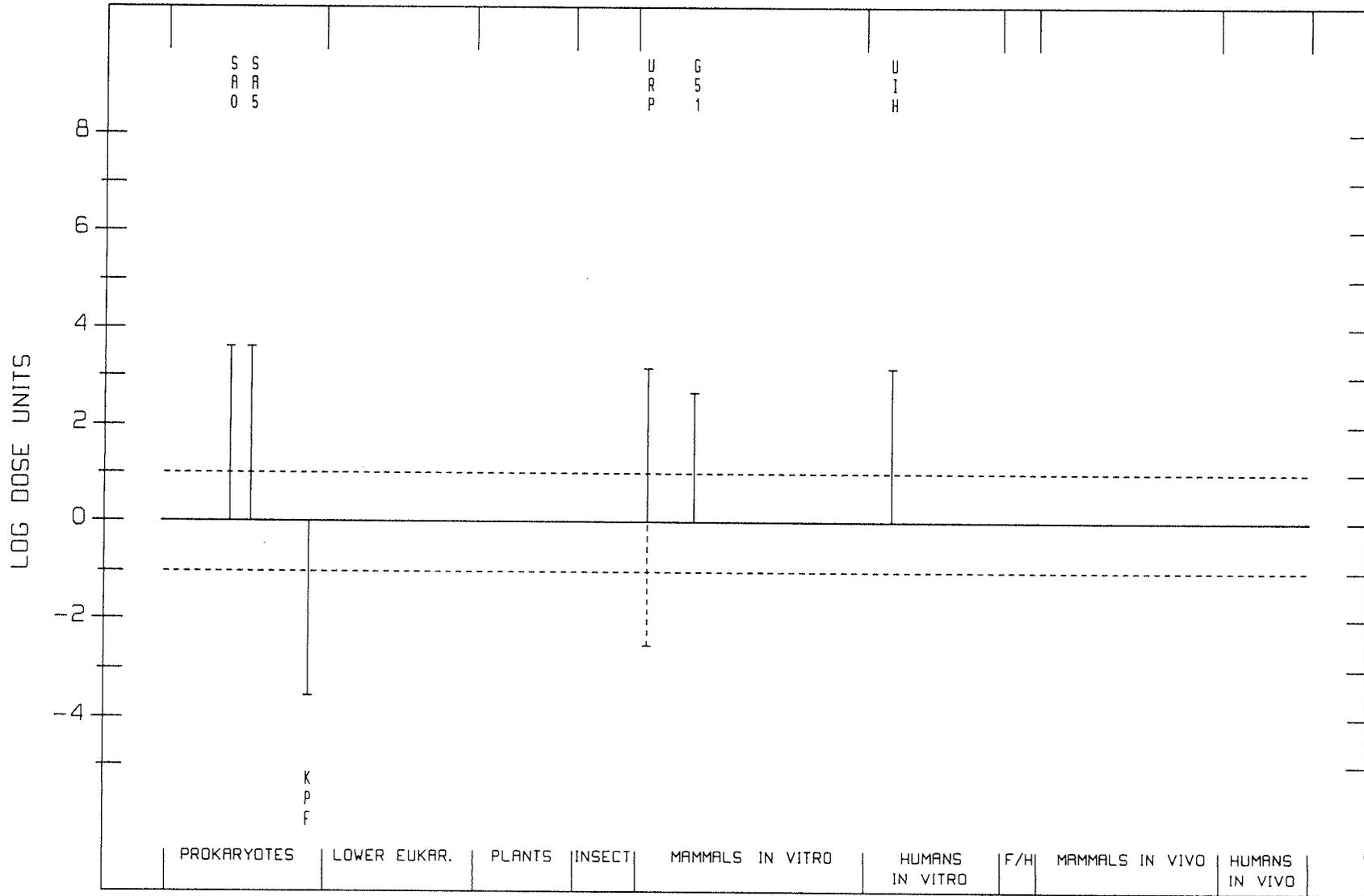
ACRYLAMIDE

79-06-1



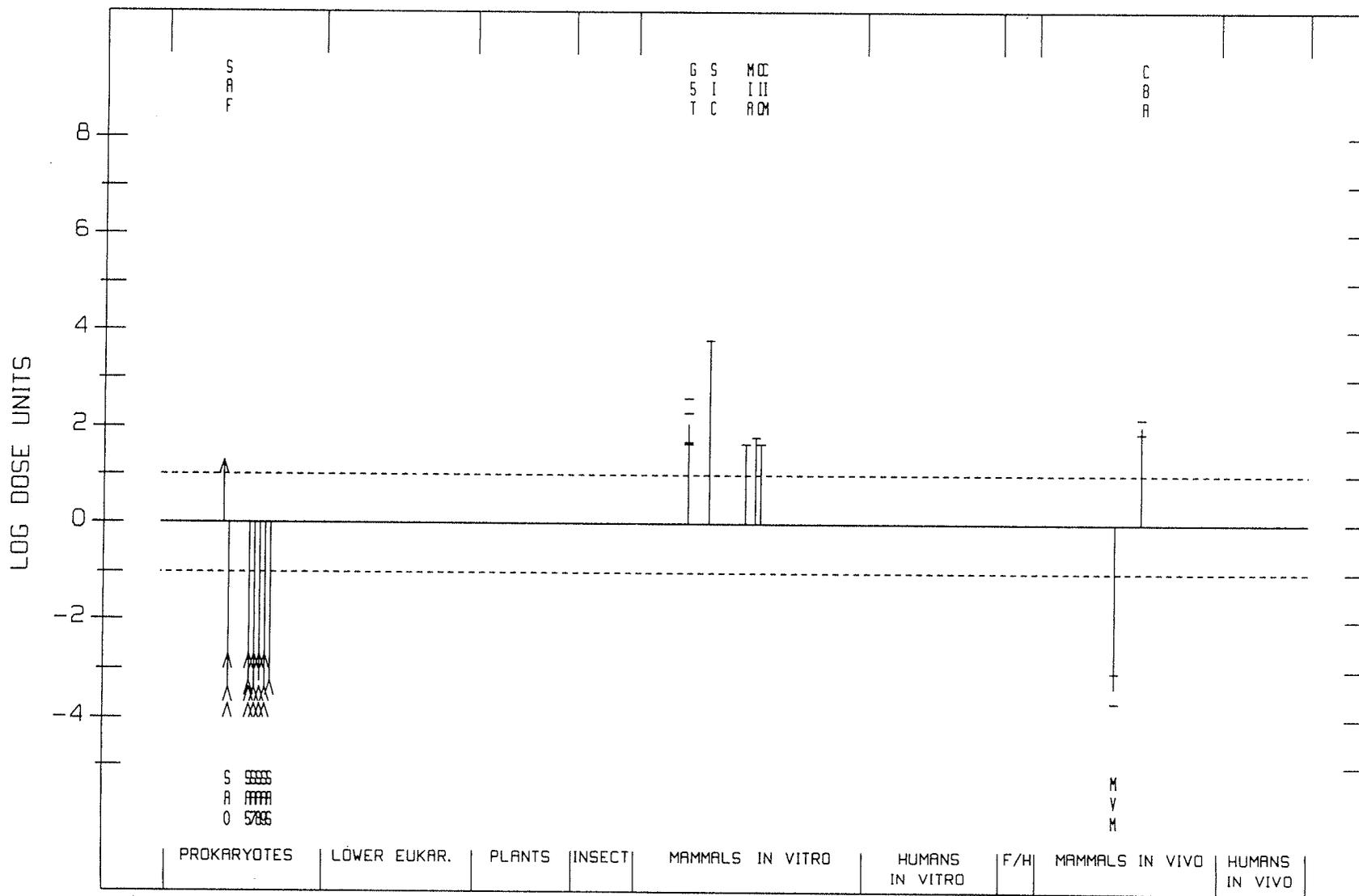
GLYCIDAMIDE

5694-00-8



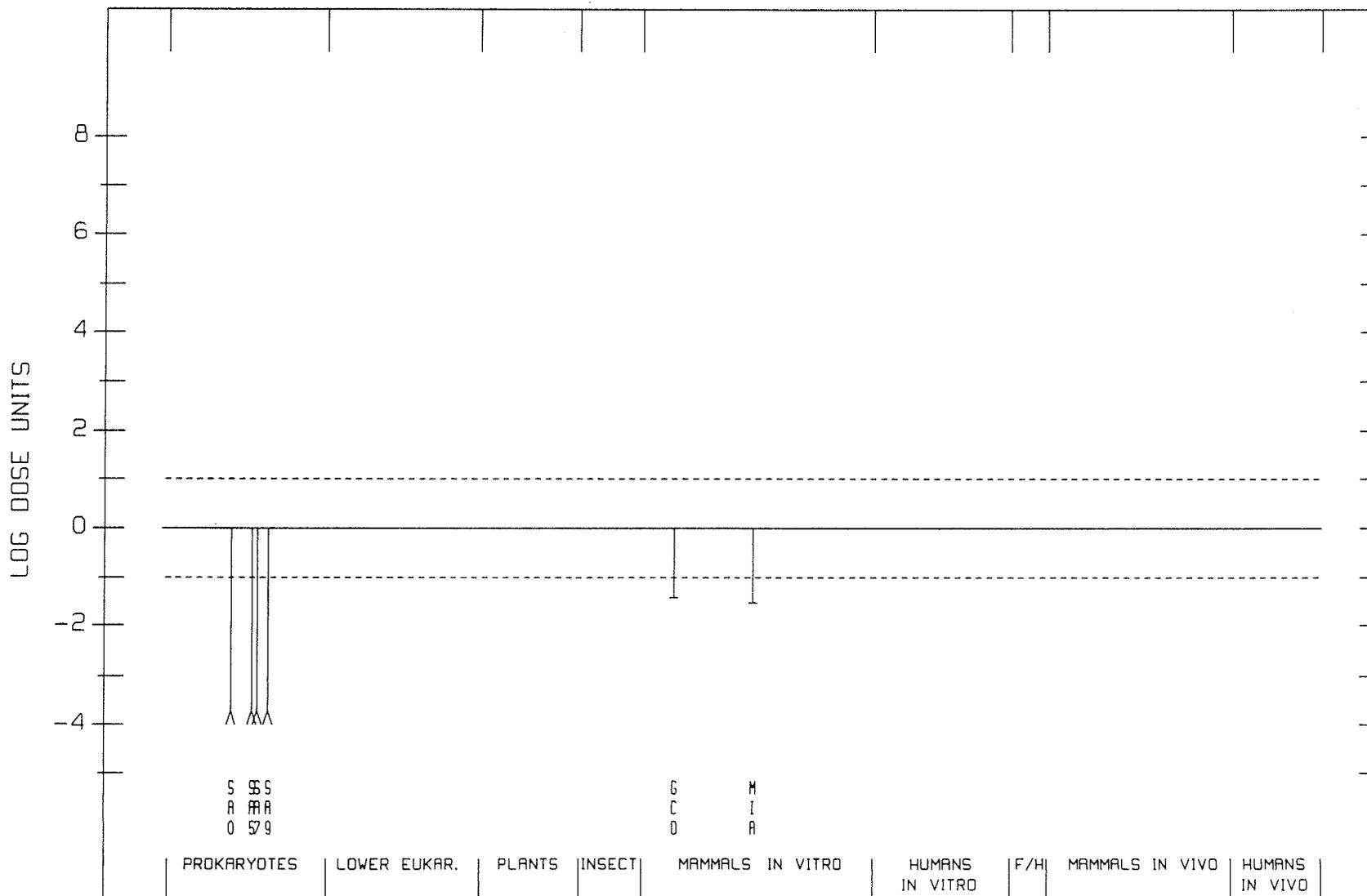
METHYL METHACRYLATE

80-62-6



ETHYLHEXYLACRYLATE, 2-

103-11-7



CUMULATIVE CROSS INDEX TO IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

The volume, page and year of publication are given. References to corrigenda are given in parentheses.

A

A- α -C	40, 245 (1986); <i>Suppl.</i> 7, 56 (1987)
Acetaldehyde	36, 101 (1985) (<i>corr.</i> 42, 263); <i>Suppl.</i> 7, 77 (1987)
Acetaldehyde formylmethylhydrazone (<i>see</i> Gyromitrin)	
Acetamide	7, 197 (1974); <i>Suppl.</i> 7, 389 (1987)
Acetaminophen (<i>see</i> Paracetamol)	
Acridine orange	16, 145 (1978); <i>Suppl.</i> 7, 56 (1987)
Acriflavinium chloride	13, 31 (1977); <i>Suppl.</i> 7, 56 (1987)
Acrolein	19, 479 (1979); 36, 133 (1985); <i>Suppl.</i> 7, 78 (1987)
Acrylamide	39, 41 (1986); <i>Suppl.</i> 7, 56 (1987); 60, 389 (1994)
Acrylic acid	19, 47 (1979); <i>Suppl.</i> 7, 56 (1987)
Acrylic fibres	19, 86 (1979); <i>Suppl.</i> 7, 56 (1987)
Acrylonitrile	19, 73 (1979); <i>Suppl.</i> 7, 79 (1987)
Acrylonitrile-butadiene-styrene copolymers	19, 91 (1979); <i>Suppl.</i> 7, 56 (1987)
Actinolite (<i>see</i> Asbestos)	
Actinomycins	10, 29 (1976) (<i>corr.</i> 42, 255); <i>Suppl.</i> 7, 80 (1987)
Adriamycin	10, 43 (1976); <i>Suppl.</i> 7, 82 (1987)
AF-2	31, 47 (1983); <i>Suppl.</i> 7, 56 (1987)
Aflatoxins	1, 145 (1972) (<i>corr.</i> 42, 251); 10, 51 (1976); <i>Suppl.</i> 7, 83 (1987); 56, 245 (1993)
Aflatoxin B ₁ (<i>see</i> Aflatoxins)	
Aflatoxin B ₂ (<i>see</i> Aflatoxins)	
Aflatoxin G ₁ (<i>see</i> Aflatoxins)	
Aflatoxin G ₂ (<i>see</i> Aflatoxins)	
Aflatoxin M ₁ (<i>see</i> Aflatoxins)	
Agaritine	31, 63 (1983); <i>Suppl.</i> 7, 56 (1987)
Alcohol drinking	44 (1988)
Aldicarb	53, 93 (1991)
Aldrin	5, 25 (1974); <i>Suppl.</i> 7, 88 (1987)
Allyl chloride	36, 39 (1985); <i>Suppl.</i> 7, 56 (1987)
Allyl isothiocyanate	36, 55 (1985); <i>Suppl.</i> 7, 56 (1987)
Allyl isovalerate	36, 69 (1985); <i>Suppl.</i> 7, 56 (1987)

- Aluminium production 34, 37 (1984); *Suppl.* 7, 89 (1987)
- Amaranth 8, 41 (1975); *Suppl.* 7, 56 (1987)
- 5-Aminoacenaphthene 16, 243 (1978); *Suppl.* 7, 56 (1987)
- 2-Aminoanthraquinone 27, 191 (1982); *Suppl.* 7, 56 (1987)
- para*-Aminoazobenzene 8, 53 (1975); *Suppl.* 7, 390 (1987)
- ortho*-Aminoazotoluene 8, 61 (1975) (*corr.* 42, 254);
Suppl. 7, 56 (1987)
- para*-Aminobenzoic acid 16, 249 (1978); *Suppl.* 7, 56 (1987)
- 4-Aminobiphenyl 1, 74 (1972) (*corr.* 42, 251);
Suppl. 7, 91 (1987)
- 2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline (*see* MeIQ)
- 2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (*see* MeIQx)
- 3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (*see* Trp-P-1)
- 2-Aminodipyrido[1,2-*a*:3',2'-*d*]imidazole (*see* Glu-P-2)
- 1-Amino-2-methylanthraquinone 27, 199 (1982); *Suppl.* 7, 57 (1987)
- 2-Amino-3-methylimidazo[4,5-*f*]quinoline (*see* IQ)
- 2-Amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole (*see* Glu-P-1)
- 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (*see* PhIP)
- 2-Amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (*see* MeA- α -C)
- 3-Amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (*see* Trp-P-2)
- 2-Amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole 7, 143 (1974); *Suppl.* 7, 57 (1987)
- 4-Amino-2-nitrophenol 16, 43 (1978); *Suppl.* 7, 57 (1987)
- 2-Amino-4-nitrophenol 57, 167 (1993)
- 2-Amino-5-nitrophenol 57, 177 (1993)
- 2-Amino-5-nitrothiazole 31, 71 (1983); *Suppl.* 7, 57 (1987)
- 2-Amino-9*H*-pyrido[2,3-*b*]indole (*see* A- α -C)
- 11-Aminoundecanoic acid 39, 239 (1986); *Suppl.* 7, 57 (1987)
- Amitrole 7, 31 (1974); 41, 293 (1986) (*corr.* 52, 513; *Suppl.* 7, 92 (1987))
- Ammonium potassium selenide (*see* Selenium and selenium compounds)
- Amorphous silica (*see also* Silica) 42, 39 (1987); *Suppl.* 7, 341 (1987)
- Amosite (*see* Asbestos)
- Ampicillin 50, 153 (1990)
- Anabolic steroids (*see* Androgenic (anabolic) steroids)
- Anaesthetics, volatile 11, 285 (1976); *Suppl.* 7, 93 (1987)
- Analgesic mixtures containing phenacetin (*see also* Phenacetin) *Suppl.* 7, 310 (1987)
- Androgenic (anabolic) steroids *Suppl.* 7, 96 (1987)
- Angelicin and some synthetic derivatives (*see also* Angelicins) 40, 291 (1986)
- Angelicin plus ultraviolet radiation (*see also* Angelicin and some synthetic derivatives) *Suppl.* 7, 57 (1987)
- Angelicins *Suppl.* 7, 57 (1987)
- Aniline 4, 27 (1974) (*corr.* 42, 252);
27, 39 (1982); *Suppl.* 7, 99 (1987)
- ortho*-Anisidine 27, 63 (1982); *Suppl.* 7, 57 (1987)
- para*-Anisidine 27, 65 (1982); *Suppl.* 7, 57 (1987)
- Anthanthrene 32, 95 (1983); *Suppl.* 7, 57 (1987)
- Anthophyllite (*see* Asbestos)
- Anthracene 32, 105 (1983); *Suppl.* 7, 57 (1987)
- Anthranilic acid 16, 265 (1978); *Suppl.* 7, 57 (1987)
- Antimony trioxide 47, 291 (1989)
- Antimony trisulfide 47, 291 (1989)
- ANTU (*see* 1-Naphthylthiourea)

- Apholate 9, 31 (1975); *Suppl.* 7, 57 (1987)
 Aramite® 5, 39 (1974); *Suppl.* 7, 57 (1987)
 Areca nut (*see* Betel quid)
 Arsanilic acid (*see* Arsenic and arsenic compounds)
 Arsenic and arsenic compounds 1, 41 (1972); 2, 48 (1973);
 23, 39 (1980); *Suppl.* 7, 100 (1987)
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 Arsenic trioxide (*see* Arsenic and arsenic compounds)
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 106 (1987) (*corr.* 45, 283)
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 Atrazine 42, 159 (1987); *Suppl.* 7, 117 (1987)
 Attapulgit 1, 69 (1972) (*corr.* 42, 251); *Suppl.* 7,
 118 (1987)
 Auramine, manufacture of (*see also* Auramine, technical-grade) *Suppl.* 7, 118 (1987)
 Aurothioglucose 13, 39 (1977); *Suppl.* 7, 57 (1987)
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 5-Azacytidine (*see* Azacitidine)
 Azaserine 10, 73 (1976) (*corr.* 42, 255);
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 Azathioprine 26, 47 (1981); *Suppl.* 7, 119 (1987)
 Aziridine 9, 37 (1975); *Suppl.* 7, 58 (1987)
 2-(1-Aziridinyl)ethanol 9, 47 (1975); *Suppl.* 7, 58 (1987)
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- Barium chromate (*see* Chromium and chromium compounds)
 Basic chromic sulfate (*see* Chromium and chromium compounds)
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 Benz[*a*]acridine 32, 123 (1983); *Suppl.* 7, 58 (1987)
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Suppl. 7, 58 (1987)
 Benzene 7, 203 (1974) (*corr.* 42, 254); 29, 93,
 391 (1982); *Suppl.* 7, 120 (1987)
 Benzidine 1, 80 (1972); 29, 149, 391 (1982);
Suppl. 7, 123 (1987)
 Benzidine-based dyes *Suppl.* 7, 125 (1987)
 Benzo[*b*]fluoranthene 3, 69 (1973); 32, 147 (1983);
Suppl. 7, 58 (1987)
 Benzo[*j*]fluoranthene 3, 82 (1973); 32, 155 (1983); *Suppl.* 7,
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 Benzo[*k*]fluoranthene 32, 163 (1983); *Suppl.* 7, 58 (1987)
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- Benzo[*a*]fluorene 32, 177 (1983); *Suppl.* 7, 58 (1987)
- Benzo[*b*]fluorene 32, 183 (1983); *Suppl.* 7, 58 (1987)
- Benzo[*c*]fluorene 32, 189 (1983); *Suppl.* 7, 58 (1987)
- Benzo[*ghi*]perylene 32, 195 (1983); *Suppl.* 7, 58 (1987)
- Benzo[*c*]phenanthrene 32, 205 (1983); *Suppl.* 7, 58 (1987)
- Benzo[*a*]pyrene 3, 91 (1973); 32, 211 (1983);
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- Benzo[*e*]pyrene 3, 137 (1973); 32, 225 (1983);
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- para*-Benzoquinone dioxime 29, 185 (1982); *Suppl.* 7, 58 (1987)
- Benzotrichloride (*see also* α -Chlorinated toluenes) 29, 73 (1982); *Suppl.* 7, 148 (1987)
- Benzoyl chloride 29, 83 (1982) (*corr.* 42, 261); *Suppl.* 7,
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- Benzoyl peroxide 36, 267 (1985); *Suppl.* 7, 58 (1987)
- Benzyl acetate 40, 109 (1986); *Suppl.* 7, 58 (1987)
- Benzyl chloride (*see also* α -Chlorinated toluenes) 11, 217 (1976) (*corr.* 42, 256); 29,
49 (1982); *Suppl.* 7, 148 (1987)
- Benzyl violet 4B 16, 153 (1978); *Suppl.* 7, 58 (1987)
- Bertrandite (*see* Beryllium and beryllium compounds)
- Beryllium and beryllium compounds 1, 17 (1972); 23, 143 (1980) (*corr.* 42,
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- Beryllium acetate, basic (*see* Beryllium and beryllium compounds)
- Beryllium-aluminium alloy (*see* Beryllium and beryllium compounds)
- Beryllium carbonate (*see* Beryllium and beryllium compounds)
- Beryllium chloride (*see* Beryllium and beryllium compounds)
- Beryllium-copper alloy (*see* Beryllium and beryllium compounds)
- Beryllium-copper-cobalt alloy (*see* Beryllium and beryllium compounds)
- Beryllium fluoride (*see* Beryllium and beryllium compounds)
- Beryllium hydroxide (*see* Beryllium and beryllium compounds)
- Beryllium-nickel alloy (*see* Beryllium and beryllium compounds)
- Beryllium oxide (*see* Beryllium and beryllium compounds)
- Beryllium phosphate (*see* Beryllium and beryllium compounds)
- Beryllium silicate (*see* Beryllium and beryllium compounds)
- Beryllium sulfate (*see* Beryllium and beryllium compounds)
- Beryl ore (*see* Beryllium and beryllium compounds)
- Betel quid 37, 141 (1985); *Suppl.* 7, 128 (1987)
- Betel-quid chewing (*see* Betel quid)
- BHA (*see* Butylated hydroxyanisole)
- BHT (*see* Butylated hydroxytoluene)
- Bis(1-aziridinyl)morpholinophosphine sulfide 9, 55 (1975); *Suppl.* 7, 58 (1987)
- Bis(2-chloroethyl)ether 9, 117 (1975); *Suppl.* 7, 58 (1987)
- N,N*-Bis(2-chloroethyl)-2-naphthylamine 4, 119 (1974) (*corr.* 42, 253);
Suppl. 7, 130 (1987)
- Bischloroethyl nitrosourea (*see also* Chloroethyl nitrosoureas) 26, 79 (1981); *Suppl.* 7, 150 (1987)
- 1,2-Bis(chloromethoxy)ethane 15, 31 (1977); *Suppl.* 7, 58 (1987)
- 1,4-Bis(chloromethoxymethyl)benzene 15, 37 (1977); *Suppl.* 7, 58 (1987)
- Bis(chloromethyl)ether 4, 231 (1974) (*corr.* 42, 253);
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- Bis(2-chloro-1-methylethyl)ether 41, 149 (1986); *Suppl.* 7, 59 (1987)
- Bis(2,3-epoxycyclopentyl)ether 47, 231 (1989)

- Bisphenol A diglycidyl ether (*see* Glycidyl ethers)
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 Boot and shoe manufacture and repair 25, 249 (1981); *Suppl.* 7, 232 (1987)
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 1,3-Butadiene 39, 155 (1986) (*corr.* 42, 264);
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n-Butyl acrylate 39, 67 (1986); *Suppl.* 7, 59 (1987)
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Suppl. 7, 59 (1987)
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- Cabinet-making (*see* Furniture and cabinet-making)
 Cadmium acetate (*see* Cadmium and cadmium compounds)
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 255); *Suppl.* 7, 139 (1987); 58, 119
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 Cadmium chloride (*see* Cadmium and cadmium compounds)
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 Carbazole 32, 239 (1983); *Suppl.* 7, 59 (1987)
 3-Carbethoxyorsoralen 40, 317 (1986); *Suppl.* 7, 59 (1987)
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- Carbon tetrachloride 1, 53 (1972); 20, 371 (1979);
Suppl. 7, 143 (1987)
- Carmoisine 8, 83 (1975); *Suppl.* 7, 59 (1987)
- Carpentry and joinery 25, 139 (1981); *Suppl.* 7, 378 (1987)
- Carrageenan 10, 181 (1976) (*corr.* 42, 255); 31,
79 (1983); *Suppl.* 7, 59 (1987)
- Catechol 15, 155 (1977); *Suppl.* 7, 59 (1987)
- CCNU (*see* 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea)
- Ceramic fibres (*see* Man-made mineral fibres)
- Chemotherapy, combined, including alkylating agents (*see* MOPP and
other combined chemotherapy including alkylating agents)
- Chlorambucil 9, 125 (1975); 26, 115 (1981);
Suppl. 7, 144 (1987)
- Chloramphenicol 10, 85 (1976); *Suppl.* 7, 145 (1987);
50, 169 (1990)
- Chlordane (*see also* Chlordane/Heptachlor) 20, 45 (1979) (*corr.* 42, 258)
- Chlordane/Heptachlor *Suppl.* 7, 146 (1987); 53, 115 (1991)
- Chlordecone 20, 67 (1979); *Suppl.* 7, 59 (1987)
- Chlordimeform 30, 61 (1983); *Suppl.* 7, 59 (1987)
- Chlorendic acid 48, 45 (1990)
- Chlorinated dibenzodioxins (other than TCDD) 15, 41 (1977); *Suppl.* 7, 59 (1987)
- Chlorinated drinking-water 52, 45 (1991)
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- α -Chlorinated toluenes *Suppl.* 7, 148 (1987)
- Chlormadinone acetate (*see also* Progestins; Combined oral
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- Chlornaphazine (*see* *N,N*-Bis(2-chloroethyl)-2-naphthylamine)
- Chloroacetonitrile (*see* Halogenated acetonitriles)
- para*-Chloroaniline 57, 305 (1993)
- Chlorobenzilate 5, 75 (1974); 30, 73 (1983);
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- Chlorodibromomethane 52, 243 (1991)
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- Chloroethane 52, 315 (1991)
- 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea (*see also* Chloroethyl
nitrosoureas) 26, 137 (1981) (*corr.* 42, 260);
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- 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (*see also*
Chloroethyl nitrosoureas) *Suppl.* 7, 150 (1987)
- Chloroethyl nitrosoureas *Suppl.* 7, 150 (1987)
- Chlorofluoromethane 41, 229 (1986); *Suppl.* 7, 60 (1987)
- Chloroform 1, 61 (1972); 20, 401 (1979);
Suppl. 7, 152 (1987)
- Chloromethyl methyl ether (technical-grade) (*see also*
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- (4-Chloro-2-methylphenoxy)acetic acid (*see* MCPA)
- Chlorophenols *Suppl.* 7, 154 (1987)
- Chlorophenols (occupational exposures to) 41, 319 (1986)
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- 4-Chloro-*ortho*-phenylenediamine 27, 81 (1982); *Suppl.* 7, 60 (1987)
- 4-Chloro-*meta*-phenylenediamine 27, 82 (1982); *Suppl.* 7, 60 (1987)

- Chloroprene 19, 131 (1979); *Suppl.* 7, 160 (1987)
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Chloroquine 13, 47 (1977); *Suppl.* 7, 60 (1987)
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(*see also* Chlordimeform) 16, 277 (1978); 30, 65 (1983);
Suppl. 7, 60 (1987); 48, 123 (1990)
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2-Chloro-1,1,1-trifluoroethane 41, 253 (1986); *Suppl.* 7, 60 (1987)
Chlorozotocin 50, 65 (1990)
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- Chromic acetate (*see* Chromium and chromium compounds)
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Chromium sulfate (*see* Chromium and chromium compounds)
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8, 91 (1975); *Suppl.* 7, 169 (1987)
- Chrysoidine
Chrysotile (*see* Asbestos)
- CI Acid Orange 3 57, 121 (1993)
CI Acid Red 114 57, 247 (1993)
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Ciclosporin 50, 77 (1990)
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Cinnamyl anthranilate 16, 287 (1978); 31, 133 (1983);
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57, 259 (1993)
- CI Pigment Red 3
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Cisplatin 26, 151 (1981); *Suppl.* 7, 170 (1987)
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- Clofibrate 21, 551 (1979); *Suppl.* 7, 172 (1987)
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Cobalt[III] acetate (*see* Cobalt and cobalt compounds)
Cobalt-aluminium-chromium spinel (*see* Cobalt and cobalt compounds)
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- Cobalt[III] chloride (*see* Cobalt and cobalt compounds)
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 Copper 8-hydroxyquinoline 15, 103 (1977); *Suppl.* 7, 61 (1987)
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 Cycasin 1, 157 (1972) (*corr.* 42, 251); 10, 121 (1976); *Suppl.* 7, 61 (1987)
 Cyclamates 22, 55 (1980); *Suppl.* 7, 178 (1987)
 Cyclamic acid (*see* Cyclamates)
 Cyclochlorotine 10, 139 (1976); *Suppl.* 7, 61 (1987)
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 Cyclopropane (*see* Anaesthetics, volatile)
 Cyclophosphamide 9, 135 (1975); 26, 165 (1981); *Suppl.* 7, 182 (1987)
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 Dacarbazine 26, 203 (1981); *Suppl.* 7, 184 (1987)
 Dantron 50, 265 (1990) (*corr.* 59, 257)
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- Deltamethrin 53, 251 (1991)
- Deoxynivalenol (*see* Toxins derived from *Fusarium graminearum*,
F. culmorum and *F. crookwellense*)
- Diacetylaminoazotoluene 8, 113 (1975); *Suppl.* 7, 61 (1987)
- N,N'*-Diacetylbenzidine 16, 293 (1978); *Suppl.* 7, 61 (1987)
- Diallate 12, 69 (1976); 30, 235 (1983);
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- 2,4-Diaminoanisole 16, 51 (1978); 27, 103 (1982);
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- 4,4'-Diaminodiphenyl ether 16, 301 (1978); 29, 203 (1982);
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- 1,2-Diamino-4-nitrobenzene 16, 63 (1978); *Suppl.* 7, 61 (1987)
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- 2,6-Diamino-3-(phenylazo)pyridine (*see* Phenazopyridine
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- 2,4-Diaminotoluene (*see also* Toluene diisocyanates) 16, 83 (1978); *Suppl.* 7, 61 (1987)
- 2,5-Diaminotoluene (*see also* Toluene diisocyanates) 16, 97 (1978); *Suppl.* 7, 61 (1987)
- ortho*-Dianisidine (*see* 3,3'-Dimethoxybenzidine)
- Diazepam 13, 57 (1977); *Suppl.* 7, 189 (1987)
- Diazomethane 7, 223 (1974); *Suppl.* 7, 61 (1987)
- Dibenz[*a,h*]acridine 3, 247 (1973); 32, 277 (1983);
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- Dibenz[*a,c*]anthracene 32, 289 (1983) (*corr.* 42, 262);
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- Dibenz[*a,h*]anthracene 3, 178 (1973) (*corr.* 43, 261);
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- 7*H*-Dibenzo[*c,g*]carbazole 3, 260 (1973); 32, 315 (1983);
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- Dibenzodioxins, chlorinated (other than TCDD)
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- Dibenzo[*a,e*]fluoranthene 32, 321 (1983); *Suppl.* 7, 61 (1987)
- Dibenzo[*h,rs'*]pentaphene 3, 197 (1973); *Suppl.* 7, 62 (1987)
- Dibenzo[*a,e*]pyrene 3, 201 (1973); 32, 327 (1983);
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- Dibenzo[*a,h*]pyrene 3, 207 (1973); 32, 331 (1983);
Suppl. 7, 62 (1987)
- Dibenzo[*a,i*]pyrene 3, 215 (1973); 32, 337 (1983);
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- Dibromoacetonitrile (*see* Halogenated acetonitriles)
- 1,2-Dibromo-3-chloropropane 15, 139 (1977); 20, 83 (1979);
Suppl. 7, 191 (1987)
- Dichloroacetonitrile (*see* Halogenated acetonitriles)
- Dichloroacetylene 39, 369 (1986); *Suppl.* 7, 62 (1987)
- ortho*-Dichlorobenzene 7, 231 (1974); 29, 213 (1982);
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- para*-Dichlorobenzene 7, 231 (1974); 29, 215 (1982);
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- 3,3'-Dichlorobenzidine 4, 49 (1974); 29, 239 (1982);
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- trans*-1,4-Dichlorobutene 15, 149 (1977); *Suppl.* 7, 62 (1987)
- 3,3'-Dichloro-4,4'-diaminodiphenyl ether 16, 309 (1978); *Suppl.* 7, 62 (1987)
- 1,2-Dichloroethane 20, 429 (1979); *Suppl.* 7, 62 (1987)
- Dichloromethane 20, 449 (1979); 41, 43 (1986);
Suppl. 7, 194 (1987)
- 2,4-Dichlorophenol (*see* Chlorophenols; Chlorophenols,
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- (2,4-Dichlorophenoxy)acetic acid (*see* 2,4-D)
- 2,6-Dichloro-*para*-phenylenediamine 39, 325 (1986); *Suppl.* 7, 62 (1987)
- 1,2-Dichloropropane 41, 131 (1986); *Suppl.* 7, 62 (1987)
- 1,3-Dichloropropene (technical-grade) 41, 113 (1986); *Suppl.* 7, 195 (1987)
- Dichlorvos 20, 97 (1979); *Suppl.* 7, 62 (1987);
53, 267 (1991)
- Dicofol 30, 87 (1983); *Suppl.* 7, 62 (1987)
- Dicyclohexylamine (*see* Cyclamates)
- Dieldrin 5, 125 (1974); *Suppl.* 7, 196 (1987)
- Dienoestrol (*see also* Nonsteroidal oestrogens) 21, 161 (1979)
- Diepoxybutane 11, 115 (1976) (*corr.* 42, 255); *Suppl.* 7,
62 (1987)
- Diesel and gasoline engine exhausts 46, 41 (1989)
- Diesel fuels 45, 219 (1989) (*corr.* 47, 505)
- Diethyl ether (*see* Anaesthetics, volatile)
- Di(2-ethylhexyl)adipate 29, 257 (1982); *Suppl.* 7, 62 (1987)
- Di(2-ethylhexyl)phthalate 29, 269 (1982) (*corr.* 42, 261); *Suppl.* 7,
62 (1987)
- 1,2-Diethylhydrazine 4, 153 (1974); *Suppl.* 7, 62 (1987)
- Diethylstilboestrol 6, 55 (1974); 21, 173 (1979)
(*corr.* 42, 259); *Suppl.* 7, 273 (1987)
- Diethylstilboestrol dipropionate (*see* Diethylstilboestrol)
- Diethyl sulfate 4, 277 (1974); *Suppl.* 7, 198 (1987);
54, 213 (1992)
- Diglycidyl resorcinol ether 11, 125 (1976); 36, 181 (1985);
Suppl. 7, 62 (1987)
- Dihydrosafrole 1, 170 (1972); 10, 233 (1976);
Suppl. 7, 62 (1987)
- 1,8-Dihydroxyanthraquinone (*see* Dantron)
- Dihydroxybenzenes (*see* Catechol; Hydroquinone; Resorcinol)
- Dihydroxymethylfuratrizine 24, 77 (1980); *Suppl.* 7, 62 (1987)
- Diisopropyl sulfate 54, 229 (1992)
- Dimethisterone (*see also* Progestins; Sequential oral
contraceptives) 6, 167 (1974); 21, 377 (1979)
- Dimethoxane 15, 177 (1977); *Suppl.* 7, 62 (1987)
- 3,3'-Dimethoxybenzidine 4, 41 (1974); *Suppl.* 7, 198 (1987)
- 3,3'-Dimethoxybenzidine-4,4'-diisocyanate 39, 279 (1986); *Suppl.* 7, 62 (1987)
- para*-Dimethylaminoazobenzene 8, 125 (1975); *Suppl.* 7, 62 (1987)
- para*-Dimethylaminoazobenzenediazo sodium sulfonate 8, 147 (1975); *Suppl.* 7, 62 (1987)
- trans*-2-[(Dimethylamino)methylimino]-5-[2-(5-nitro-2-furyl)-
vinyl]-1,3,4-oxadiazole 7, 147 (1974) (*corr.* 42, 253); *Suppl.* 7,
62 (1987)

- 4,4'-Dimethylangelicin plus ultraviolet radiation (*see also*
Angelicin and some synthetic derivatives) *Suppl.* 7, 57 (1987)
- 4,5'-Dimethylangelicin plus ultraviolet radiation (*see also*
Angelicin and some synthetic derivatives) *Suppl.* 7, 57 (1987)
- 2,6-Dimethylaniline 57, 323 (1993)
- N,N*-Dimethylaniline 57, 337 (1993)
- Dimethylarsinic acid (*see* Arsenic and arsenic compounds)
- 3,3'-Dimethylbenzidine 1, 87 (1972); *Suppl.* 7, 62 (1987)
- Dimethylcarbamoyl chloride 12, 77 (1976); *Suppl.* 7, 199 (1987)
- Dimethylformamide 47, 171 (1989)
- 1,1-Dimethylhydrazine 4, 137 (1974); *Suppl.* 7, 62 (1987)
- 1,2-Dimethylhydrazine 4, 145 (1974) (*corr.* 42, 253); *Suppl.* 7, 62 (1987)
- Dimethyl hydrogen phosphite 48, 85 (1990)
- 1,4-Dimethylphenanthrene 32, 349 (1983); *Suppl.* 7, 62 (1987)
- Dimethyl sulfate 4, 271 (1974); *Suppl.* 7, 200 (1987)
- 3,7-Dinitrofluoranthene 46, 189 (1989)
- 3,9-Dinitrofluoranthene 46, 195 (1989)
- 1,3-Dinitropyrene 46, 201 (1989)
- 1,6-Dinitropyrene 46, 215 (1989)
- 1,8-Dinitropyrene 33, 171 (1984); *Suppl.* 7, 63 (1987);
46, 231 (1989)
- Dinitrosopentamethylenetetramine 11, 241 (1976); *Suppl.* 7, 63 (1987)
- 1,4-Dioxane 11, 247 (1976); *Suppl.* 7, 201 (1987)
- 2,4'-Diphenyldiamine 16, 313 (1978); *Suppl.* 7, 63 (1987)
- Direct Black 38 (*see also* Benzidine-based dyes) 29, 295 (1982) (*corr.* 42, 261)
- Direct Blue 6 (*see also* Benzidine-based dyes) 29, 311 (1982)
- Direct Brown 95 (*see also* Benzidine-based dyes) 29, 321 (1982)
- Disperse Blue 1 48, 139 (1990)
- Disperse Yellow 3 8, 97 (1975); *Suppl.* 7, 60 (1987);
48, 149 (1990)
- Disulfiram 12, 85 (1976); *Suppl.* 7, 63 (1987)
- Dithranol 13, 75 (1977); *Suppl.* 7, 63 (1987)
- Divinyl ether (*see* Anaesthetics, volatile)
- Dulcin 12, 97 (1976); *Suppl.* 7, 63 (1987)

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- Endrin 5, 157 (1974); *Suppl.* 7, 63 (1987)
- Enflurane (*see* Anaesthetics, volatile)
- Eosin 15, 183 (1977); *Suppl.* 7, 63 (1987)
- Epichlorohydrin 11, 131 (1976) (*corr.* 42, 256);
Suppl. 7, 202 (1987)
- 1,2-Epoxybutane 47, 217 (1989)
- 1-Epoxyethyl-3,4-epoxycyclohexane (*see* 4-Vinylcyclohexene diepoxide)
- 3,4-Epoxy-6-methylcyclohexylmethyl-3,4-epoxy-6-methyl-
cyclohexane carboxylate 11, 147 (1976); *Suppl.* 7, 63 (1987)
- cis*-9,10-Epoxy stearic acid 11, 153 (1976); *Suppl.* 7, 63 (1987)
- Erionite 42, 225 (1987); *Suppl.* 7, 203 (1987)
- Ethinylloestradiol (*see also* Steroidal oestrogens) 6, 77 (1974); 21, 233 (1979)
- Ethionamide 13, 83 (1977); *Suppl.* 7, 63 (1987)

- Ethyl acrylate 19, 57 (1979); 39, 81 (1986);
Suppl. 7, 63 (1987)
- Ethylene 19, 157 (1979); *Suppl.* 7, 63 (1987);
60, 45 (1994)
- Ethylene dibromide 15, 195 (1977); *Suppl.* 7, 204 (1987)
- Ethylene oxide 11, 157 (1976); 36, 189 (1985)
(*corr.* 42, 263); *Suppl.* 7, 205 (1987);
60, 73 (1994)
- Ethylene sulfide 11, 257 (1976); *Suppl.* 7, 63 (1987)
- Ethylene thiourea 7, 45 (1974); *Suppl.* 7, 207 (1987)
- 2-Ethylhexyl acrylate 60, 475 (1994)
- Ethyl methanesulfonate 7, 245 (1974); *Suppl.* 7, 63 (1987)
- N*-Ethyl-*N*-nitrosoourea 1, 135 (1972); 17, 191 (1978);
Suppl. 7, 63 (1987)
- Ethyl selenac (*see also* Selenium and selenium compounds) 12, 107 (1976); *Suppl.* 7, 63 (1987)
- Ethyl tellurac 12, 115 (1976); *Suppl.* 7, 63 (1987)
- Ethinodiol diacetate (*see also* Progestins; Combined oral
contraceptives) 6, 173 (1974); 21, 387 (1979)
- Eugenol 36, 75 (1985); *Suppl.* 7, 63 (1987)
- Evans blue 8, 151 (1975); *Suppl.* 7, 63 (1987)
- F**
- Fast Green FCF 16, 187 (1978); *Suppl.* 7, 63 (1987)
- Fenvalerate 53, 309 (1991)
- Ferbam 12, 121 (1976) (*corr.* 42, 256);
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- Ferric oxide 1, 29 (1972); *Suppl.* 7, 216 (1987)
- Ferrochromium (*see* Chromium and chromium compounds)
- Fluometuron 30, 245 (1983); *Suppl.* 7, 63 (1987)
- Fluoranthene 32, 355 (1983); *Suppl.* 7, 63 (1987)
- Fluorene 32, 365 (1983); *Suppl.* 7, 63 (1987)
- Fluorescent lighting (exposure to) (*see* Ultraviolet radiation)
- Fluorides (inorganic, used in drinking-water) 27, 237 (1982); *Suppl.* 7, 208 (1987)
- 5-Fluorouracil 26, 217 (1981); *Suppl.* 7, 210 (1987)
- Fluorspar (*see* Fluorides)
- Fluosilicic acid (*see* Fluorides)
- Fluroxene (*see* Anaesthetics, volatile)
- Formaldehyde 29, 345 (1982); *Suppl.* 7, 211 (1987)
- 2-(2-Formylhydrazino)-4-(5-nitro-2-furyl)thiazole 7, 151 (1974) (*corr.* 42, 253);
Suppl. 7, 63 (1987)
- Frusemide (*see* Furosemide)
- Fuel oils (heating oils) 45, 239 (1989) (*corr.* 47, 505)
- Fumonisin B₁ (*see* Toxins derived from *Fusarium moniliforme*)
- Fumonisin B₂ (*see* Toxins derived from *Fusarium moniliforme*)
- Furazolidone 31, 141 (1983); *Suppl.* 7, 63 (1987)
- Furniture and cabinet-making 25, 99 (1981); *Suppl.* 7, 380 (1987)
- Furosemide 50, 277 (1990)
- 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (*see* AF-2)
- Fusarenon-X (*see* Toxins derived from *Fusarium graminearum*,
F. culmorum and *F. crookwellense*)

Fusarenone-X (*see* Toxins derived from *Fusarium graminearum*,
F. culmorum and *F. crookwellense*)

Fusarin C (*see* Toxins derived from *Fusarium moniliforme*)

G

- Gasoline 45, 159 (1989) (*corr.* 47, 505)
- Gasoline engine exhaust (*see* Diesel and gasoline engine exhausts)
- Glass fibres (*see* Man-made mineral fibres)
- Glass manufacturing industry, occupational exposures in 58, 347 (1993)
- Glasswool (*see* Man-made mineral fibres)
- Glass filaments (*see* Man-made mineral fibres)
- Glu-P-1 40, 223 (1986); *Suppl.* 7, 64 (1987)
- Glu-P-2 40, 235 (1986); *Suppl.* 7, 64 (1987)
- L-Glutamic acid, 5-[2-(4-hydroxymethyl)phenylhydrazide]
(*see* Agaritine)
- Glycidaldehyde 11, 175 (1976); *Suppl.* 7, 64 (1987)
- Glycidyl ethers 47, 237 (1989)
- Glycidyl oleate 11, 183 (1976); *Suppl.* 7, 64 (1987)
- Glycidyl stearate 11, 187 (1976); *Suppl.* 7, 64 (1987)
- Griseofulvin 10, 153 (1976); *Suppl.* 7, 391 (1987)
- Guinea Green B 16, 199 (1978); *Suppl.* 7, 64 (1987)
- Gyromitrin 31, 163 (1983); *Suppl.* 7, 391 (1987)

H

- Haematite 1, 29 (1972); *Suppl.* 7, 216 (1987)
- Haematite and ferric oxide *Suppl.* 7, 216 (1987)
- Haematite mining, underground, with exposure to radon 1, 29 (1972); *Suppl.* 7, 216 (1987)
- Hairdressers and barbers (occupational exposure as) 57, 43 (1993)
- Hair dyes, epidemiology of 16, 29 (1978); 27, 307 (1982);
52, 269 (1991)
- Halogenated acetonitriles 52, 269 (1991)
- Halothane (*see* Anaesthetics, volatile)
- HC Blue No. 1 57, 129 (1993)
- HC Blue No. 2 57, 143 (1993)
- α -HCH (*see* Hexachlorocyclohexanes)
- β -HCH (*see* Hexachlorocyclohexanes)
- γ -HCH (*see* Hexachlorocyclohexanes)
- HC Red No. 3 57, 153 (1993)
- HC Yellow No. 4 57, 159 (1993)
- Heating oils (*see* Fuel oils)
- Hepatitis B virus 59, 45 (1994)
- Hepatitis C virus 59, 165 (1994)
- Hepatitis D virus 59, 223 (1994)
- Heptachlor (*see also* Chlordane/Heptachlor) 5, 173 (1974); 20, 129 (1979)
- Hexachlorobenzene 20, 155 (1979); *Suppl.* 7, 219 (1987)
- Hexachlorobutadiene 20, 179 (1979); *Suppl.* 7, 64 (1987)
- Hexachlorocyclohexanes 5, 47 (1974); 20, 195 (1979) (*corr.* 42,
258); *Suppl.* 7, 220 (1987)
- Hexachlorocyclohexane, technical-grade (*see* Hexachloro-
cyclohexanes)
- Hexachloroethane 20, 467 (1979); *Suppl.* 7, 64 (1987)

Hexachlorophene	20, 241 (1979); <i>Suppl.</i> 7, 64 (1987)
Hexamethylphosphoramide	15, 211 (1977); <i>Suppl.</i> 7, 64 (1987)
Hexoestrol (<i>see</i> Nonsteroidal oestrogens)	
Hycanthone mesylate	13, 91 (1977); <i>Suppl.</i> 7, 64 (1987)
Hydralazine	24, 85 (1980); <i>Suppl.</i> 7, 222 (1987)
Hydrazine	4, 127 (1974); <i>Suppl.</i> 7, 223 (1987)
Hydrochloric acid	54, 189 (1992)
Hydrochlorothiazide	50, 293 (1990)
Hydrogen peroxide	36, 285 (1985); <i>Suppl.</i> 7, 64 (1987)
Hydroquinone	15, 155 (1977); <i>Suppl.</i> 7, 64 (1987)
4-Hydroxyazobenzene	8, 157 (1975); <i>Suppl.</i> 7, 64 (1987)
17 α -Hydroxyprogesterone caproate (<i>see also</i> Progestins)	21, 399 (1979) (<i>corr.</i> 42, 259)
8-Hydroxyquinoline	13, 101 (1977); <i>Suppl.</i> 7, 64 (1987)
8-Hydroxysenkirkine	10, 265 (1976); <i>Suppl.</i> 7, 64 (1987)
Hypochlorite salts	52, 159 (1991)

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Indeno[1,2,3- <i>cd</i>]pyrene	3, 229 (1973); 32, 373 (1983); <i>Suppl.</i> 7, 64 (1987)
Inorganic acids (<i>see</i> Sulfuric acid and other strong inorganic acids, occupational exposures to mists and vapours from)	
Insecticides, occupational exposures in spraying and application of IQ	53, 45 (1991) 40, 261 (1986); <i>Suppl.</i> 7, 64 (1987); 56, 165 (1993)
Iron and steel founding	34, 133 (1984); <i>Suppl.</i> 7, 224 (1987)
Iron-dextran complex	2, 161 (1973); <i>Suppl.</i> 7, 226 (1987)
Iron-dextrin complex	2, 161 (1973) (<i>corr.</i> 42, 252); <i>Suppl.</i> 7, 64 (1987)
Iron oxide (<i>see</i> Ferric oxide)	
Iron oxide, saccharated (<i>see</i> Saccharated iron oxide)	
Iron sorbitol-citric acid complex	2, 161 (1973); <i>Suppl.</i> 7, 64 (1987)
Isatidine	10, 269 (1976); <i>Suppl.</i> 7, 65 (1987)
Isoflurane (<i>see</i> Anaesthetics, volatile)	
Isoniazid (<i>see</i> Isonicotinic acid hydrazide)	
Isonicotinic acid hydrazide	4, 159 (1974); <i>Suppl.</i> 7, 227 (1987)
Isophosphamide	26, 237 (1981); <i>Suppl.</i> 7, 65 (1987)
Isoprene	60, 215 (1994)
Isopropanol	5, 223 (1977); <i>Suppl.</i> 7, 229 (1987)
Isopropanol manufacture (strong-acid process) (<i>see also</i> Isopropyl alcohol; Sulfuric acid and other strong inorganic acids, occupational exposures to mists and vapours from)	<i>Suppl.</i> 7, 229 (1987)
Isopropyl oils	15, 223 (1977); <i>Suppl.</i> 7, 229 (1987)
Isosafrole	1, 169 (1972); 10, 232 (1976); <i>Suppl.</i> 7, 65 (1987)

J

Jacobine	10, 275 (1976); <i>Suppl.</i> 7, 65 (1987)
Jet fuel	45, 203 (1989)
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- Kaempferol 31, 171 (1983); *Suppl.* 7, 65 (1987)
Kepone (*see* Chlordecone)

L

- Lasiocarpine 10, 281 (1976); *Suppl.* 7, 65 (1987)
Lauroyl peroxide 36, 315 (1985); *Suppl.* 7, 65 (1987)
Lead acetate (*see* Lead and lead compounds)
Lead and lead compounds 1, 40 (1972) (*corr.* 42, 251); 2, 52, 150 (1973); 12, 131 (1976); 23, 40, 208, 209, 325 (1980); *Suppl.* 7, 230 (1987)
Lead arsenate (*see* Arsenic and arsenic compounds)
Lead carbonate (*see* Lead and lead compounds)
Lead chloride (*see* Lead and lead compounds)
Lead chromate (*see* Chromium and chromium compounds)
Lead chromate oxide (*see* Chromium and chromium compounds)
Lead naphthenate (*see* Lead and lead compounds)
Lead nitrate (*see* Lead and lead compounds)
Lead oxide (*see* Lead and lead compounds)
Lead phosphate (*see* Lead and lead compounds)
Lead subacetate (*see* Lead and lead compounds)
Lead tetroxide (*see* Lead and lead compounds)
Leather goods manufacture 25, 279 (1981); *Suppl.* 7, 235 (1987)
Leather industries 25, 199 (1981); *Suppl.* 7, 232 (1987)
Leather tanning and processing 25, 201 (1981); *Suppl.* 7, 236 (1987)
Ledate (*see also* Lead and lead compounds) 12, 131 (1976)
Light Green SF 16, 209 (1978); *Suppl.* 7, 65 (1987)
d-Limonene 56, 135 (1993)
Lindane (*see* Hexachlorocyclohexanes)
The lumber and sawmill industries (including logging) 25, 49 (1981); *Suppl.* 7, 383 (1987)
Luteoskyrin 10, 163 (1976); *Suppl.* 7, 65 (1987)
Lynoestrenol (*see also* Progestins; Combined oral contraceptives) 21, 407 (1979)

M

- Magenta 4, 57 (1974) (*corr.* 42, 252); *Suppl.* 7, 238 (1987); 57, 215 (1993)
Magenta, manufacture of (*see also* Magenta) *Suppl.* 7, 238 (1987)
Malathion 30, 103 (1983); *Suppl.* 7, 65 (1987)
Maleic hydrazide 4, 173 (1974) (*corr.* 42, 253); *Suppl.* 7, 65 (1987)
Malonaldehyde 36, 163 (1985); *Suppl.* 7, 65 (1987)
Maneb 12, 137 (1976); *Suppl.* 7, 65 (1987)
Man-made mineral fibres 43, 39 (1988)
Mannomustine 9, 157 (1975); *Suppl.* 7, 65 (1987)
Mate 51, 273 (1991)
MCPA (*see also* Chlorophenoxy herbicides; Chlorophenoxy herbicides, occupational exposures to) 30, 255 (1983)
MeA- α -C 40, 253 (1986); *Suppl.* 7, 65 (1987)

- Medphalan 9, 168 (1975); *Suppl.* 7, 65 (1987)
- Medroxyprogesterone acetate 6, 157 (1974); 21, 417 (1979) (*corr.* 42, 259); *Suppl.* 7, 289 (1987)
- Megestrol acetate (*see also* Progestins; Combined oral contraceptives)
- MeIQ 40, 275 (1986); *Suppl.* 7, 65 (1987); 56, 197 (1993)
- MeIQx 40, 283 (1986); *Suppl.* 7, 65 (1987) 56, 211 (1993)
- Melamine 39, 333 (1986); *Suppl.* 7, 65 (1987)
- Melphalan 9, 167 (1975); *Suppl.* 7, 239 (1987)
- 6-Mercaptopurine 26, 249 (1981); *Suppl.* 7, 240 (1987)
- Mercuric chloride (*see* Mercury and mercury compounds)
- Mercury and mercury compounds 58, 239 (1993)
- Merphalan 9, 169 (1975); *Suppl.* 7, 65 (1987)
- Mestranol (*see also* Steroidal oestrogens) 6, 87 (1974); 21, 257 (1979) (*corr.* 42, 259)
- Metabisulfites (*see* Sulfur dioxide and some sulfites, bisulfites and metabisulfites)
- Metallic mercury (*see* Mercury and mercury compounds)
- Methanearsonic acid, disodium salt (*see* Arsenic and arsenic compounds)
- Methanearsonic acid, monosodium salt (*see* Arsenic and arsenic compounds)
- Methotrexate 26, 267 (1981); *Suppl.* 7, 241 (1987)
- Methoxsalen (*see* 8-Methoxypsoralen)
- Methoxychlor 5, 193 (1974); 20, 259 (1979); *Suppl.* 7, 66 (1987)
- Methoxyflurane (*see* Anaesthetics, volatile)
- 5-Methoxypsoralen 40, 327 (1986); *Suppl.* 7, 242 (1987)
- 8-Methoxypsoralen (*see also* 8-Methoxypsoralen plus ultraviolet radiation) 24, 101 (1980)
- 8-Methoxypsoralen plus ultraviolet radiation *Suppl.* 7, 243 (1987)
- Methyl acrylate 19, 52 (1979); 39, 99 (1986); *Suppl.* 7, 66 (1987)
- 5-Methylangelicin plus ultraviolet radiation (*see also* Angelicin and some synthetic derivatives) *Suppl.* 7, 57 (1987)
- 2-Methylaziridine 9, 61 (1975); *Suppl.* 7, 66 (1987)
- Methylazoxymethanol acetate 1, 164 (1972); 10, 131 (1976); *Suppl.* 7, 66 (1987)
- Methyl bromide 41, 187 (1986) (*corr.* 45, 283); *Suppl.* 7, 245 (1987)
- Methyl carbamate 12, 151 (1976); *Suppl.* 7, 66 (1987)
- Methyl-CCNU [*see* 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea]
- Methyl chloride 41, 161 (1986); *Suppl.* 7, 246 (1987)
- 1-, 2-, 3-, 4-, 5- and 6-Methylchrysenes 32, 379 (1983); *Suppl.* 7, 66 (1987)
- N*-Methyl-*N*,4-dinitrosoaniline 1, 141 (1972); *Suppl.* 7, 66 (1987)
- 4,4'-Methylene bis(2-chloroaniline) 4, 65 (1974) (*corr.* 42, 252); *Suppl.* 7, 246 (1987); 57, 271 (1993)
- 4,4'-Methylene bis(*N,N*-dimethyl)benzenamine 27, 119 (1982); *Suppl.* 7, 66 (1987)
- 4,4'-Methylene bis(2-methylaniline) 4, 73 (1974); *Suppl.* 7, 248 (1987)

- 4,4'-Methylenedianiline 4, 79 (1974) (*corr.* 42, 252);
39, 347 (1986); *Suppl.* 7, 66 (1987)
- 4,4'-Methylenediphenyl diisocyanate 19, 314 (1979); *Suppl.* 7, 66 (1987)
- 2-Methylfluoranthene 32, 399 (1983); *Suppl.* 7, 66 (1987)
- 3-Methylfluoranthene 32, 399 (1983); *Suppl.* 7, 66 (1987)
- Methylglyoxal 51, 443 (1991)
- Methyl iodide 15, 245 (1977); 41, 213 (1986);
Suppl. 7, 66 (1987)
- Methylmercury chloride (*see* Mercury and mercury compounds)
- Methylmercury compounds (*see* Mercury and mercury compounds)
- Methyl methacrylate 19, 187 (1979); *Suppl.* 7, 66 (1987);
60, 445 (1994)
- Methyl methanesulfonate 7, 253 (1974); *Suppl.* 7, 66 (1987)
- 2-Methyl-1-nitroanthraquinone 27, 205 (1982); *Suppl.* 7, 66 (1987)
- N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine 4, 183 (1974); *Suppl.* 7, 248 (1987)
- 3-Methylnitrosaminopropionaldehyde [*see* 3-(*N*-Nitrosomethylamino)-
propionaldehyde]
- 3-Methylnitrosaminopropionitrile [*see* 3-(*N*-Nitrosomethylamino)-
propionitrile]
- 4-(Methylnitrosamino)-4-(3-pyridyl)-1-butanal [*see* 4-(*N*-Nitrosomethyl-
amino)-4-(3-pyridyl)-1-butanal]
- 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone [*see* 4-(*N*-Nitrosomethyl-
amino)-1-(3-pyridyl)-1-butanone]
- N*-Methyl-*N*-nitrosourea 1, 125 (1972); 17, 227 (1978);
Suppl. 7, 66 (1987)
- N*-Methyl-*N*-nitrosourethane 4, 211 (1974); *Suppl.* 7, 66 (1987)
- N*-Methylolacrylamide 60, 435 (1994)
- Methyl parathion 30, 131 (1983); *Suppl.* 7, 392 (1987)
- 1-Methylphenanthrene 32, 405 (1983); *Suppl.* 7, 66 (1987)
- 7-Methylpyrido[3,4-*c*]psoralen 40, 349 (1986); *Suppl.* 7, 71 (1987)
- Methyl red 8, 161 (1975); *Suppl.* 7, 66 (1987)
- Methyl selenac (*see also* Selenium and selenium compounds) 12, 161 (1976); *Suppl.* 7, 66 (1987)
- Methylthiouracil 7, 53 (1974); *Suppl.* 7, 66 (1987)
- Metronidazole 13, 113 (1977); *Suppl.* 7, 250 (1987)
- Mineral oils 3, 30 (1973); 33, 87 (1984) (*corr.* 42,
262); *Suppl.* 7, 252 (1987)
- Mirex 5, 203 (1974); 20, 283 (1979) (*corr.* 42,
258); *Suppl.* 7, 66 (1987)
- Mitomycin C 10, 171 (1976); *Suppl.* 7, 67 (1987)
- MNNG [*see N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine]
- MOCA [*see* 4,4'-Methylene bis(2-chloroaniline)]
- Modacrylic fibres 19, 86 (1979); *Suppl.* 7, 67 (1987)
- Monocrotaline 10, 291 (1976); *Suppl.* 7, 67 (1987)
- Monuron 12, 167 (1976); *Suppl.* 7, 67 (1987);
53, 467 (1991)
- MOPP and other combined chemotherapy including
alkylating agents *Suppl.* 7, 254 (1987)
- Morpholine 47, 199 (1989)
- 5-(Morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-
oxazolidinone 7, 161 (1974); *Suppl.* 7, 67 (1987)
- Mustard gas 9, 181 (1975) (*corr.* 42, 254);
Suppl. 7, 259 (1987)

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N

- Nafenopin 24, 125 (1980); *Suppl.* 7, 67 (1987)
- 1,5-Naphthalenediamine 27, 127 (1982); *Suppl.* 7, 67 (1987)
- 1,5-Naphthalene diisocyanate 19, 311 (1979); *Suppl.* 7, 67 (1987)
- 1-Naphthylamine 4, 87 (1974) (*corr.* 42, 253);
Suppl. 7, 260 (1987)
- 2-Naphthylamine 4, 97 (1974); *Suppl.* 7, 261 (1987)
- 1-Naphthylthiourea 30, 347 (1983); *Suppl.* 7, 263 (1987)
- Nickel acetate (*see* Nickel and nickel compounds)
- Nickel ammonium sulfate (*see* Nickel and nickel compounds)
- Nickel and nickel compounds 2, 126 (1973) (*corr.* 42, 252); 11, 75
(1976); *Suppl.* 7, 264 (1987)
(*corr.* 45, 283); 49, 257 (1990)
- Nickel carbonate (*see* Nickel and nickel compounds)
- Nickel carbonyl (*see* Nickel and nickel compounds)
- Nickel chloride (*see* Nickel and nickel compounds)
- Nickel-gallium alloy (*see* Nickel and nickel compounds)
- Nickel hydroxide (*see* Nickel and nickel compounds)
- Nickelocene (*see* Nickel and nickel compounds)
- Nickel oxide (*see* Nickel and nickel compounds)
- Nickel subsulfide (*see* Nickel and nickel compounds)
- Nickel sulfate (*see* Nickel and nickel compounds)
- Niridazole 13, 123 (1977); *Suppl.* 7, 67 (1987)
- Nithiazide 31, 179 (1983); *Suppl.* 7, 67 (1987)
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