WORLD HEALTH ORGANIZATION
INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

VOLUME 77
SOME INDUSTRIAL CHEMICALS

2000
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IARC MONOGRAPHS

ON THE

EVALUATION OF CARCINOGENIC RISKS TO HUMANS

Some Industrial Chemicals

VOLUME 77

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 2000

2000

IARC MONOGRAPHS

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

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

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

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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.

IARC WORKING GROUP ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS: SOME INDUSTRIAL CHEMICALS

Lyon, 15–22 February 2000

LIST OF PARTICIPANTS

Members

S.J. Borghoff, CIIT, PO Box 12137, Research Triangle Park, NC 27709, United States

J. Caldwell, Division of Biomedical Sciences, Imperial College School of Medicine, Sir Alexander Fleming Building, South Kensington, London SW7 2AZ, United Kingdom

R.C. Cattley, Amgen, MS-15-2-B, One Amgen Center Drive, Thousand Oaks, CA 91320, United States

S. Cordier, Unit of Epidemiological and Statistical Research on the Environment and Health, INSERM–U 170, 16 avenue Paul Vaillant Couturier, 94807 Villejuif Cedex, France

N. Danford, Microptic Cytogenetic Services, 2 Langland Close, Mumbles, Swansea SA3 4LY, United Kingdom

P.A. Demers, School of Occupational and Environmental Hygiene, University of British Columbia, 2206 East Mall, 3rd floor, Vancouver BC V6T 1Z3, Canada

T.A. Dragani, Istituto Nazionale per lo Studio e la Cura dei Tumori, via G. Venezian 1, 20133 Milan, Italy

J.K. Dunnick, National Institute of Environmental Health Sciences, PO Box 12233, Research Triangle Park, NC 27709, United States

E. Dybing, National Institute of Public Health, Department of Environmental Medicine, Postboks 4404 Torshov, 0403 Oslo, Norway (*Chairman*)

E. Elovaara, Finnish Institute of Occupational Health, Topeliuksenkatu 41 a A, 00250 Helsinki, Finland

L.R. Ferguson, Auckland Cancer Society, Research Centre, Private Bag 92019, Auckland 1000, New Zealand

L. Fishbein, 4320 Ashford Lane, Fairfax, VA 22032, United States

M. Gérin, University of Montréal, Occupational and Environmental Health, Faculty of Medicine, CP 6128–Centre-Ville, Montreal, Quebec H3C 3J7, Canada (*Vice-Chairman*)

G.C. Hard, American Health Foundation, 1 Dana Road, Valhalla, NY 10595, United States

U. Hass, Institute of Food Safety and Toxicology, Mørkhøj Bygade 19, 2860 Søborg, Denmark

K.S. Hougaard, Danish National Institute of Occupational Health, Department of Chemical Working Environment, Lersø Parkalle 105, 2100 Copenhagen Ø, Denmark

T. Kauppinen, Finnish Institute of Occupational Health, Topeliuksenkatu 41 a A, 00250 Helsinki, Finland

H. Kromhout, Environmental and Occupational Health Group, Wageningen University, PO Box 238, 6700 AAE Wageningen, The Netherlands

L. Levy, MRC Institute for Environment and Health, University of Leicester, 94 Regent Road, Leicester LE1 7DD, United Kingdom

M.E. McManus, Faculty of Biological and Chemical Sciences, University of Queensland, Brisbane Qld 4072, Australia

F.E. Mirer, UAW Health and Safety Department, 8000 East Jefferson Avenue, Detroit, MI 48214, United States

G.J. Mulder, Sylvius Laboratories, Wassenaarseweg 72, PO Box 9503, 2300 RA Leiden, The Netherlands

S. Olin, International Life Sciences Institute, 1126 Sixteenth Street NW, Washington DC 20036, United States

E. Parry, School of Biological Sciences, University of Wales, Singleton Park, Swansea SA2 8PP, United Kingdom

D.H. Phillips, Section of Molecular Carcinogenesis, Haddow Laboratories, Institute of Cancer Research, 15 Cotswold Road, Sutton Surrey SM2 5NG, United Kingdom

A. Pintér, Fodor József National Public Health Centre, National Institute of Environmental Health, Gyáli ut 2-6, 1097 Budapest, Hungary

D. Schrenk, Universität Kaiserslautern, Lebensmittelchemie und Umwelttoxikologie, Erwin-Schrödinger-Strasse, Postfach 3049, 67653 Kaiserslautern, Germany

P. Stewart, Division of Cancer Epidemiology and Genetics, National Cancer Institute, EPS 8102, 6120 Executive Blvd, Mail Stop 7240, Bethesda, MD 20892-7240, United States

E. Ward, Industrywide Studies Branch, Division of Surveillance, Hazard Evaluations and Field Studies, National Institute for Occupational Safety and Health, R.A. Taft Laboratories, 4676 Columbia Parkway, Cincinnati, OH 45226, United States

Representatives/Observers

Representative of the National Cancer Institute

D.G. Longfellow, Chemical and Physical Carcinogenesis Branch, Division of Cancer Biology, National Cancer Institute, 6006 Executive Blvd, Suite 220, MSC 7055, Rockville, MD 20892-7055, United States

American Industrial Health Council

R.M. David, Health and Environment Laboratories, Eastman Kodak Company, Rochester, NY 14652-6272, United States

L. Lehman-McKeeman, Human and Environmental Safety Division, Miami Valley Laboratories, Procter and Gamble Co., PO Box 538707, Cincinnati, OH 45253, United States

European Centre for Ecotoxicology and Toxicology of Chemicals
G. Gans, BASF Aktiengesellschaft, Z 470, D-67056 Ludwigshafen, Germany

Technical Resources International Incorporated
T. Junghans, Technical Resources Inc., 6500 Rock Spring Drive, Suite 650, Bethesda, MD 20817, United States

IARC Secretariat
R.A. Baan, Unit of Carcinogen Identification and Evaluation
I. Burstyn, Environmental Cancer Epidemiology
J. Cheney (*Editor*)
M. Friesen, Unit of Nutrition and Cancer
Y. Grosse, Unit of Carcinogen Identification and Evaluation
J. Korte, Environmental Cancer Epidemiology
V. Krutovskikh, Unit of Gene–Environment Interactions
C. Malaveille, Unit of Endogenous Cancer Risk Factors
A. t' Mannetje, Environmental Cancer Epidemiology
D. McGregor, Unit of Carcinogen Identification and Evaluation (*Responsible Officer*)[1]
C. Partensky, Unit of Carcinogen Identification and Evaluation
J. Rice, Unit of Carcinogen Identification and Evaluation (*Head of Programme*)
J. Wilbourn, Unit of Carcinogen Identification and Evaluation

Technical assistance
S. Egraz
M. Lézère
A. Meneghel
D. Mietton
J. Mitchell
E. Perez
S. Reynaud

[1] Present address: 102 rue Duguesclin, 69006 Lyon, France

PREAMBLE

IARC MONOGRAPHS PROGRAMME ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

PREAMBLE

1. BACKGROUND

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

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

2. OBJECTIVE AND SCOPE

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

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

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

Some epidemiological and experimental studies indicate that different agents may act at different stages in the carcinogenic process, and several mechanisms may be involved. The aim of the *Monographs* has been, from their inception, to evaluate evidence of carcinogenicity at any stage in the carcinogenesis process, independently of the underlying mechanisms. Information on mechanisms may, however, be used in making the overall evaluation (IARC, 1991a; Vainio *et al.*, 1992; see also pp. 25–27).

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

The *IARC Monographs* are recognized as an authoritative source of information on the carcinogenicity of a wide range of human exposures. A survey of users in 1988 indicated that the *Monographs* are consulted by various agencies in 57 countries. About 3000 copies of each volume are printed, for distribution to governments, regulatory bodies and interested scientists. The Monographs are also available from IARC*Press* in Lyon and via the Distribution and Sales Service of the World Health Organization in Geneva.

3. SELECTION OF TOPICS FOR MONOGRAPHS

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

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

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

4. DATA FOR MONOGRAPHS

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

With regard to biological and epidemiological data, only reports that have been published or accepted for publication in the openly available scientific literature are reviewed by the working groups. In certain instances, government agency reports that have undergone peer review and are widely available are considered. Exceptions may be made on an ad-hoc basis to include unpublished reports that are in their final form and publicly available, if their inclusion is considered pertinent to making a final evaluation (see pp. 25–27). In the sections on chemical and physical properties, on analysis, on production and use and on occurrence, unpublished sources of information may be used.

5. THE WORKING GROUP

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

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

6. WORKING PROCEDURES

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

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

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

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

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

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

7. EXPOSURE DATA

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

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

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

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

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

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

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

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

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

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

8. STUDIES OF CANCER IN HUMANS

(*a*) *Types of studies considered*

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

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

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

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

(*b*) *Quality of studies considered*

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

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

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

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

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

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

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

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

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

(*d*) *Criteria for causality*

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

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

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

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

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

9. STUDIES OF CANCER IN EXPERIMENTAL ANIMALS

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

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

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

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

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

(*a*) *Qualitative aspects*

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

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

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

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

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

(*b*) *Quantitative aspects*

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

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

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

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

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

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

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

In coming to an overall evaluation of carcinogenicity in humans (see pp. 25–27), the Working Group also considers related data. The nature of the information selected for the summary depends on the agent being considered.

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

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

Tests of genetic and related effects are described in view of the relevance of gene mutation and chromosomal damage to carcinogenesis (Vainio *et al.*, 1992; McGregor *et al.*, 1999). The adequacy of the reporting of sample characterization is considered and, where necessary, commented upon; with regard to complex mixtures, such comments are similar to those described for animal carcinogenicity tests on p. 18. The available data are interpreted critically by phylogenetic group according to the end-points detected, which may include DNA damage, gene mutation, sister chromatid exchange, micronucleus formation, chromosomal aberrations, aneuploidy and cell transformation. The concentrations employed are given, and mention is made of whether use of an exogenous metabolic system *in vitro* affected the test result. These data are given as listings of test systems, data and references. The Genetic and Related Effects data presented in the *Monographs* are also available in the form of Graphic Activity Profiles (GAP) prepared in collaboration with the United States Environmental Protection Agency (EPA) (see also

Waters *et al.*, 1987) using software for personal computers that are Microsoft Windows® compatible. The EPA/IARC GAP software and database may be downloaded free of charge from *www.epa.gov/gapdb*.

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

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

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

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

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

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

11. SUMMARY OF DATA REPORTED

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

(*a*) *Exposure*

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

(*b*) *Carcinogenicity in humans*

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

(*c*) *Carcinogenicity in experimental animals*

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

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

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

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

Structure–activity relationships are mentioned when relevant.

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

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

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

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

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

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

12. EVALUATION

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

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

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

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

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

(i) *Carcinogenicity in humans*

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

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

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

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

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

Inadequate evidence of carcinogenicity: The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association between exposure and cancer, 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, the chemical composition and the potential contribution of carcinogens known to be present are considered by the Working Group in its overall evaluation of human carcinogenicity. The Working Group also determines the extent to which the materials tested in experimental systems are related to those to which humans are exposed.

(*c*) *Overall evaluation*

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

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

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

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

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

Group 2

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

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

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

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

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

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

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

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

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

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

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

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GENERAL REMARKS ON THE SUBSTANCES CONSIDERED

Introduction

This seventy-seventh volume of the *IARC Monographs* reviews sixteen industrial organic chemicals, seven of which have previously been evaluated one or more times. For these seven substances, the most recent previous evaluations are summarized below. Since these previous evaluations, new data have become available, and these have been incorporated into the monographs and taken into consideration in the present evaluations.

Table 1. Previous evaluations of compounds covered in this volume

	Human	Animal	Overall evaluation	*Monograph* volume (year)
4-Chloro-*ortho*-toluidine	L	S	2A	48 (1990)
Cinnamyl anthranilate	ND	L	3	Suppl. 7 (1987)
Coumarin	ND	L	3	Suppl. 7 (1987)
Di(2-ethylhexyl) adipate	ND	L	3	Suppl. 7 (1987)
Di(2-ethylhexyl) phthalate	ND	S	2B	Suppl. 7 (1987)
N-Nitrosodiethanolamine	ND	S	2B	Suppl. 7 (1987)
ortho-Toluidine	I	S	2B	Suppl. 7 (1987)

L, limited evidence; S, sufficient evidence; ND, no data; I, inadequate evidence; 2A, probably carcinogenic to humans; 2B, possibly carcinogenic to humans; 3, cannot be classified as to its carcinogenicity to humans

Exposures

This volume includes evaluations of the carcinogenicity of several chemical intermediates or additives to which a large number of workers are exposed in various industries. Information on the extent of occupational exposures to many of these compounds in the United States was available from the National Occupational Exposure Survey (NOES) conducted by the United States National Institute for Occupational Safety and Health (NIOSH). NOES was a nationwide observational survey conducted in a sample of 4490 establishments from 1981 to 1983. The target population was defined as employees working in establishments or job sites in the United States of America employing eight or more workers in a defined list of Standard Industrial Classifications.

Generally, these classifications emphasized coverage of construction, manufacturing, transportation, private and business service and hospital industries. The NOES had little or no coverage of agriculture, mining, wholesale/retail trade, finance/real estate, or government operations. NOES addressed recordable potential exposure that had to meet two criteria: (1) a chemical, physical or biological agent or tradename product had to be observed in sufficient proximity to an employee such that one or more physical phases of that agent or product were likely to come into contact with or enter the body of the employee; and (2) the duration of the potential exposure had to meet the minimum duration guidelines, i.e., it must have presented a potential exposure for at least 30 min per week (on an annual average) or be used at least once per week for 90% of the weeks of the work year (NOES, 1999). No exposure measurements were made.

The Toxics Release Inventory (TRI) is a compilation of data reported by chemical manufacturers and processors to the United States Environmental Protection Agency under a regulation promulgated in 1986. TRI, which is updated every year, contains data on the reported annual releases of toxic chemicals from industrial facilities in the United States to the environment—air, water, soil, underground injection; amounts transferred off-site for waste treatment; and source reduction and recycling data. Data reported from selected categories of the TRI database are reported in these monographs. These categories include air emissions (fugitive or non-point emissions plus stack or point emissions), surface water discharges, underground injection and releases to land. Categories typically not included in the monographs are classified as transfers (recycling, energy recovery, treatment, POTWs [Publicly Owned Treatment Works], disposal and movement to other off-site locations). Due to changes in reporting requirements, apparent trends in reported releases do not always indicate an actual increase or decrease in quantities released.

Aromatic amines such as those evaluated in this volume are sometimes used commercially and/or in laboratory studies as their strong acid (e.g., hydrochloride) salts. The carcinogenicity of an amine and that of its strong acid salts are expected to be qualitatively similar, as the salts dissociate readily under physiological conditions.

Although epidemiological studies of some of the industries where exposure to chemicals considered in this volume occurs have been conducted, exposure to many of these chemicals has rarely been specifically assessed for epidemiological purposes. Quantitative estimates of historical exposure are often not available and therefore it is difficult to identify highly exposed subgroups or to estimate individual exposures. Without such data, it is difficult to evaluate exposure–response relationships which might, in some cases, allow cancer excesses to be attributed to specific agents when there is mixed exposure.

The presence of chemicals as intermediates, additives or contaminants in the workplace implies co-exposure to complex, often varying mixtures of potential carcinogens other than the compound of interest. Thus, when epidemiological studies of populations with mixed and complex exposures find positive results, it is often difficult to ascribe causality to a single agent, and for many compounds it may never

be possible to establish sufficient evidence of carcinogenicity in humans using traditional epidemiological data. In these cases, additional evidence from biomarkers of exposure or effect such as haemoglobin or DNA adducts in exposed humans to demonstrate and quantify genotoxic effects or other relevant mechanisms may allow a more definitive classification of potential carcinogenicity to humans. Such data were not available to the Working Group for most of the agents evaluated in this volume.

Metalworking fluids and ethanolamines

Epidemiological studies relevant to the evaluation of di- and triethanolamine and *N*-nitrosodiethanolamine involve occupational cohorts exposed to metalworking fluids. Metalworking fluids can be divided into four broad categories: straight oils, soluble oils, semi-synthetic and synthetic fluids.

Straight oils are generally mineral oils (60–100%) and do not contain ethanolamines as additives. Untreated and mildly treated mineral oils containing polycyclic aromatic hydrocarbons that were used in the past are recognized as human carcinogens (IARC, 1984). Straight oils may contain elemental sulfur, sulfur compounds and chlorinated compounds such as chlorinated paraffins, some of which are carcinogenic (National Toxicology Program, 1986a,b; IARC, 1990). Before the 1940s, metalworking fluids were predominantly straight oils. Refined straight oils continue to be used in lower-production operations and those requiring lubrication.

'Soluble oils' are emulsions of highly refined petrochemicals (30–85%) typically diluted 1:5 to 1:40 with water for use. Oils were not always highly refined before the 1970s. Emulsifiers are petroleum sulfonates and other detergents. Soluble oils may frequently contain ethanolamines and antimicrobial agents of various types. In the past, nitrites were occasionally added to soluble oils. *N*-Nitrosodiethanolamine has been detected in some bulk samples of soluble oils (National Institute for Occupational Safety and Health, 1998). By the 1950s, water-soluble fluids were being increasingly used in high-production operations.

Semi-synthetic fluids contain smaller amounts of oil than soluble oils (3–30%), along with the same mixture of additives mentioned below for synthetic fluids. They are typically diluted 1:10 to 1:40 for use. This category has not been examined separately in epidemiological studies. One study (Sullivan *et al.*, 1998) grouped semi-synthetic oil exposure with soluble oil exposures.

Synthetic fluids contain no oil, but rather are primarily water with additives including buffers such as ethanolamines, organic polyglycols, organic esters, phosphates and antimicrobial agents. Mono- and diethanolamine may be present in concentrations of 2–3% and triethanolamine may be present in concentrations of up to 25% of undiluted fluids. They are typically diluted 1:10 to 1:40 for use. Diethanolamine may be converted to *N*-nitrosodiethanolamine in the presence of nitrosating agents. The semi-synthetic and synthetic fluids became more common in the 1960s and 1970s.

In addition to the chemicals evaluated in this volume, some synthetic and soluble fluids may contain chlorinated paraffins, formaldehyde (see IARC, 1995a)-releasing biocides, microbial contaminants and metal and metal alloy contaminants (National Institute for Occupational Safety and Health, 1998).

Genetically modified animal models

For three chemicals reviewed in this volume (diethanolamine, triethanolamine and pyridine), carcinogenicity experiments have been performed using genetically modified mice designed to be particularly susceptible to the induction of tumours at certain organ sites through specific mechanisms. The use of such animals for evaluation of the carcinogenicity of chemicals has been discussed (McGregor *et al*., 1999). The transgenic Tg.AC mice carry the v-Ha-*ras* gene under a zeta-globin promoter and are specifically designed to detect chemical carcinogens and tumour promoters in skin carcinogenesis experiments. Genetically modified mice which lack one copy of an essential tumour-suppressor gene such as *p53* model the situation in which a functioning copy of the suppressor gene has been lost in somatic cells of a normal individual. The transgenic and/or knock-out models, therefore, may also be useful not only for carcinogen identification but also for studies aimed at identifying the mode of action of chemicals and, in particular, to test genetic targets of carcinogenicity. However, because of the limited available database on the responses of any particular genetically modified mice to chemical carcinogens, bioassays using these animals must be interpreted with caution.

Peroxisome proliferation

Three of the compounds evaluated in this volume (di(2-ethylhexyl) phthalate, di(2-ethylhexyl) adipate and cinnamyl anthranilate) are carcinogenic to the liver in mice and/or rats, and have been proposed to act by a mechanism involving peroxisomal proliferation in hepatocytes in those species. The role of peroxisome proliferation in evaluating carcinogenicity in humans has been discussed (IARC, 1995b). When, for any chemical, the relationship between peroxisome proliferation and liver tumours in rats or mice has been established, this should be considered relevant information in the evaluation of the possible risks for cancer in humans, taking into account the following:

(a) whether information is available to exclude mechanisms of carcinogenesis other than those related to peroxisome proliferation;

(b) whether peroxisome proliferation (increases in peroxisome volume density or fatty acid β-oxidation activity) and hepatocellular proliferation have been demonstrated under the conditions of the bioassay;

(c) whether such effects have been looked for and have not been found in adequately designed and conducted investigations of human groups and systems.

Two primary responses have been proposed to account for liver carcinogenesis in rats and mice by peroxisome proliferators. These are (i) induction of peroxisome

proliferation and (ii) increased hepatocellular proliferation (Lake, 1995a; Cattley *et al*., 1998). Such responses, together with the ability of peroxisome proliferators to inhibit apoptosis in rat and mouse hepatocytes and their properties as liver tumour promoters in these rodents, account for peroxisome proliferator-induced liver tumour formation in these species (Popp & Cattley, 1993; Ashby *et al*., 1994; Lake, 1995a,b; Roberts, 1996; Cattley *et al*., 1998). Details are presented in the monograph on di(2-ethylhexyl) phthalate.

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THE MONOGRAPHS

DI(2-ETHYLHEXYL) PHTHALATE

This substance was considered by previous Working Groups, in October 1981 (IARC, 1982) 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.

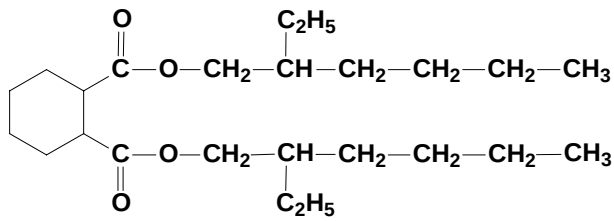
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 117-81-7
Deleted CAS Reg. Nos: 8033-53-2; 40120-69-2; 50885-87-5; 126639-29-0; 137718-37-7; 205180-59-2
Chem. Abstr. Name: 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
IUPAC Systematic Names: Bis(2-ethylhexyl) phthalate; phthalic acid, bis(2-ethylhexyl) ester
Synonyms: Bis(2-ethylhexyl) 1,2-benzenedicarboxylate; bis(2-ethylhexyl) *ortho*-phthalate; DEHP; dioctyl phthalate; di-sec octyl phthalate; ethylhexyl phthalate; 2-ethylhexyl phthalate; octyl phthalate; phthalic acid di(2-ethylhexyl) ester; phthalic acid dioctyl ester

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{24}H_{38}O_4$ Relative molecular mass: 390.56

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Light-coloured liquid with a slight odour (Agency for Toxic Substances and Disease Registry, 1993; Verschueren, 1996)
(*b*) *Boiling-point*: 384 °C (Lide, 1999)
(*c*) *Melting-point*: –55 °C (Lide, 1999)
(*d*) *Density*: 0.981 g/cm^3 at 25 °C (Lide, 1999)
(*e*) *Spectroscopy data*: Infrared (prism [28]; grating [18401]), ultraviolet [22080], nuclear magnetic resonance (proton [9392]; C-13 [5201]) and mass [NIST, 43511] spectral data have been reported (Sadtler Research Laboratories, 1980; Lide & Milne, 1996)
(*f*) *Solubility*: Slightly soluble in water (0.285 mg/L at 24 °C)[1]; slightly soluble in carbon tetrachloride (Environmental Protection Agency, 1998; Lide, 1999)
(*g*) *Volatility*: Vapour pressure, 8.6×10^{-4} Pa at 25 °C, 160 Pa at 200 °C; relative vapour density (air = 1), 13.4 (Howard *et al.*, 1985; Verschueren, 1996)
(*h*) *Octanol/water partition coefficient (P)*: log P, 7.45 (Hansch *et al.*, 1995)
(*i*) *Conversion factor*[2]: mg/m^3 = 16.0 × ppm

1.1.4 *Technical products and impurities*

Di(2-ethylhexyl) phthalate is available in a variety of technical grades (including a special grade for capacitor applications and a low residuals grade). Typical specifications are: minimal ester content, 99.0–99.6%; maximal moisture content, 0.1%; and acidity (as acetic acid or phthalic acid), 0.007–0.01% (Aristech Chemical Corp., 1992; WHO, 1992).

Trade names for di(2-ethylhexyl) phthalate include: Bisoflex; Compound 889; Diacizer DOP; DOP; Eastman DOP Plasticizer; Ergoplast; Etalon; Eviplast; Fleximel; Flexol DOP; Good-rite GP 264; Hatco-DOP; Kodaflex DOP; Monocizer DOP; Octoil; Palatinol AH; Pittsburgh PX 138; Plasthall DOP; Platinol AH; Reomol D 79P; Sicol 150; Staflex DOP; Truflex DOP; Vestinol AH; Vinycizer; Witcizer 312 (National Toxicology Program, 1991; WHO, 1992; American Conference of Governmental Industrial Hygienists, 1999).

1.1.5 *Analysis*

Detection and quantification of very low levels of di(2-ethylhexyl) phthalate are seriously limited by the presence of this compound as a contaminant in almost all laboratory equipment and reagents. Plastics, glassware, aluminium foil, cork, rubber, glass wool, solvents and Teflon® sheets have all been found to be contaminated (Agency for Toxic Substances and Disease Registry, 1993).

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[1] Lower values have been proposed, based on models (Staples *et al.*, 1997).
[2] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

Determination of di(2-ethylhexyl) phthalate in air, water, soil/sediments and food is usually accomplished by gas chromatographic analysis; a high-performance liquid chromatography method for food has also been developed. Di(2-ethylhexyl) phthalate is usually extracted from environmental samples with an organic solvent such as hexane, chloroform, dichloromethane or acetonitrile. Air samples are drawn through a solid sorbent material and desorbed with carbon disulfide. A purge-and-trap method has been developed for separation of di(2-ethylhexyl) phthalate from the fat in foods (Agency for Toxic Substances and Disease Registry, 1993).

Selected methods for the analysis of di(2-ethylhexyl) phthalate in various matrices are presented in Table 1.

1.2 Production

The worldwide production of di(2-ethylhexyl) phthalate has been increasing during recent decades and in the late 1980s amounted to approximately 1 million tonnes per year (WHO, 1992). Production of di(2-ethylhexyl) phthalate in the United States increased during the 1980s, from approximately 114 000 tonnes in 1982 to over 130 000 tonnes in 1986 (Environmental Protection Agency, 1998). In 1994, production of di(2-ethylhexyl) phthalate in the United States was 117 500 tonnes; production in Japan in 1995 was 298 000 tonnes; production in Taiwan in 1995 was 207 000 tonnes, down from 241 000 tonnes in 1994 (Anon., 1996).

Di(2-ethylhexyl) phthalate is produced commercially by the reaction of excess 2-ethylhexanol with phthalic anhydride in the presence of an acid catalyst such as sulfuric acid or *para*-toluenesulfonic acid. It was first produced in commercial quantities in Japan around 1933 and in the United States in 1939 (IARC, 1982).

Information available in 1999 indicated that di(2-ethylhexyl) phthalate was produced by 30 companies in China, 15 companies in India, 12 companies in Japan, eight companies in Mexico, seven companies in Taiwan, five companies each in Germany and the Russian Federation, four companies each in Argentina, Brazil, the Philippines and the United States, three companies each in Canada, Chile, Spain, Thailand, Turkey and Venezuela, two companies each in Belgium, Colombia, Ecuador, France, Indonesia, Iran, Italy, Korea (Republic of), Malaysia and Poland, and one company each in Albania, the Czech Republic, Finland, Kazakhstan, Pakistan, Peru, Romania, South Africa, Sweden, Ukraine, the United Kingdom and Viet Nam (Chemical Information Services, 1999).

1.3 Use

Di(2-ethylhexyl) phthalate is widely used as a plasticizer in flexible vinyl products. Plastics may contain from 1 to 40% di(2-ethylhexyl) phthalate by weight and are used in consumer products such as imitation leather, rainwear, footwear, upholstery, flooring, wire and cable, tablecloths, shower curtains, food packaging materials and children's

Table 1. Selected methods for the analysis of di(2-ethylhexyl) phthalate

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Collect on cellulose ester membrane filter; desorb with carbon disulfide	GC/FID	10 µg/sample	Eller (1994) [Method 5020]
Drinking-water, and source water	Extract in liquid–solid extractor; elute with dichloromethane; concentrate by evaporation	GC/MS	0.5 µg/L	Environmental Protection Agency (1995a) [Method 525.2]
Drinking-water	Extract in liquid–liquid extractor; isolate; dry; concentrate	GC/PID	2.25 µg/L	Environmental Protection Agency (1995b) [Method 506]
Wastewater, municipal and industrial	Extract with dichloromethane; dry; exchange to hexane and concentrate	GC/ECD	2.0 µg/L	Environmental Protection Agency (1999a) [Method 606]
	Extract with dichloromethane; dry; concentrate	GC/MS	2.5 µg/L	Environmental Protection Agency (1999b) [Method 625]
	Add isotope-labelled analogue; extract with dichloromethane; dry over sodium sulfate; concentrate	GC/MS	10 µg/L	Environmental Protection Agency (1999c) [Method 1625]
Groundwater, leachate, soil, sludge, sediment	Aqueous sample: extract with dichloromethane; elute with acetonitrile; exchange to hexane; Solid sample: extract with dichloromethane/acetone (1:1) or hexane/acetone (1:1); clean-up	GC/ECD	0.27 µg/L (aqueous)	Environmental Protection Agency (1996) [Method 8061A]

[a] Abbreviations: GC, gas chromatography; ECD, electron capture detection; FID, flame ionization detection; MS, mass spectrometry; PID, photoionization detection

toys. Poly(vinyl chloride) (PVC) containing di(2-ethylhexyl) phthalate is also used for tubing and containers for blood products and transfusions. Di(2-ethylhexyl) phthalate is also used as a hydraulic fluid and as a dielectric fluid (a non-conductor of electric current) in electrical capacitors (Agency for Toxic Substances and Disease Registry, 1989). Other uses are in rubbing alcohol, liquid detergents, decorative inks, munitions, industrial and lubricating oils and defoaming agents during paper and paperboard manufacture (Environmental Protection Agency, 1998). It is also used as an acaricide in

orchards, an inert ingredient in pesticides, a detector for leaks in respirators, in the testing of air filtration systems and as a component of cosmetic products (National Toxicology Program, 1991).

World consumption of phthalates in the early 1990s was estimated to be 3.25 million tonnes, of which di(2-ethylhexyl) phthalate accounted for approximately 2.1 million tonnes. The estimated total consumption of di(2-ethylhexyl) phthalate by geographical region was (thousand tonnes): western Europe, 465; North America, 155; eastern Asia, 490; Japan, 245; and others, 765 (Towae *et al.*, 1992).

1.4 Occurrence

Concern regarding exposure to di(2-ethylhexyl) phthalate rose to prominence when Jaeger and Rubin (1970) reported its presence in human blood that had been stored in PVC bags. The same authors later reported the presence of di(2-ethylhexyl) phthalate in tissue samples of the lung, liver and spleen from patients who had received blood transfusions before death (Jaeger & Rubin, 1972). While occupational inhalation is a significant potential route of exposure, medical procedures such as haemodialysis, extracorporeal membrane oxygenation, blood transfusion, umbilical catheterization and short-term cardiopulmonary by-pass can also result in high exposures (Huber *et al.*, 1996; Karle *et al.*, 1997). Patients undergoing haemodialysis are considered to have the highest exposure, due to the chronic nature of the treatment. Further, because of the widespread use of di(2-ethylhexyl) phthalate in plastic containers and its ability to leach out of PVC, humans are exposed to this substance on a regular basis. The extensive manufacture of di(2-ethylhexyl) phthalate-containing plastics has resulted in its becoming a ubiquitous environmental contaminant (Huber *et al.*, 1996).

1.4.1 *Natural occurrence*

Di(2-ethylhexyl) phthalate is not known to occur naturally.

1.4.2 *Occupational exposure*

According to the 1981–83 US National Occupational Exposure Survey, approximately 341 000 workers in the United States were potentially exposed to di(2-ethylhexyl) phthalate. Occupational exposure to di(2-ethylhexyl) phthalate may occur during its manufacture and its use mostly as a plasticizer of PVC (compounding, calendering and coating operations). Printing and painting occupations account also for a large number of workers potentially exposed (NOES, 1999). Occupational exposure to di(2-ethylhexyl) phthalate occurs by inhalation, essentially in the form of an aerosol (mist) because of the very low vapour pressure of the substance (Fishbein, 1992). Indeed, di(2-ethylhexyl) phthalate aerosols are used to test the efficiency of high-efficiency particulate air filters during their manufacture (Roberts, 1983).

Few data are available on levels of occupational exposure to di(2-ethylhexyl) phthalate (Table 2). Huber *et al.* (1996) observed that concentrations in air reported in older studies were well above (up to 60 mg/m^3) those determined later; these older studies, however, reported concentrations of total phthalates.

Urinary levels of di(2-ethylhexyl) phthalate, its metabolites and total phthalates have been shown in a few studies to be higher in di(2-ethylhexyl) phthalate-exposed workers than in non-exposed workers and in post-shift samples than in pre-shift samples. No standard method has been proposed for biological monitoring of exposure to di(2-ethylhexyl) phthalate (Liss *et al.*, 1985; Nielsen *et al.*, 1985; Dirven *et al.*, 1993a).

Other exposures may occur concurrently with exposure to di(2-ethylhexyl) phthalate, e.g., phthalic anhydride, other phthalates and hydrogen chloride, depending on the type of industry (Liss *et al.*, 1985; Nielsen *et al.*, 1985; Vainiotalo & Pfäffli, 1990).

1.4.3 *Environmental occurrence*

The environmental fate of phthalate esters has been reviewed (Staples *et al.*, 1997).

Di(2-ethylhexyl) phthalate is considered a priority and/or hazardous pollutant in the United States (Environmental Protection Agency, 1984; Kelly *et al.*, 1994), Canada (Meek & Chan, 1994; Meek *et al.*, 1994; Environment Canada, 1997) and the Netherlands (Wams, 1987) because of the very large quantities that have been emitted during its production, use and disposal and its ubiquitous occurrence and stability in the environment. It is known to be widely distributed, generally at very low levels, in air, precipitation, water, sediment soil and biota (with the highest levels found in industrial areas), in food samples and in human and animal tissues (Peterson & Freeman, 1982; Giam *et al.*, 1984; Wams, 1987; WHO, 1992; Agency for Toxic Substances and Disease Registry, 1993; Kelly *et al.*, 1994; Sharman *et al.*, 1994; Huber *et al.*, 1996). The principal route by which it enters the environment is via transport in air or via leaching from plastics and plasticizer plants or other sources such as sewage treatment plants, paper and textile mills and refuse incinerators. Patients receiving blood products or undergoing treatments requiring extracorporeal blood circulation may be exposed by leaching of di(2-ethylhexyl) phthalate from PVC bags and tubing (Wams, 1987; WHO, 1992; Agency for Toxic Substances and Disease Registry, 1993). Human daily intakes of di(2-ethylhexyl) phthalate from various exposure pathways have been estimated (Table 3) (Meek & Chan, 1994).

(*a*) *Air*

According to the Toxics Release Inventory (Environmental Protection Agency, 1999d), air emissions of di(2-ethylhexyl) phthalate from 298 industrial facilities in the United States amounted to 107 tonnes in 1997.

In Canada, 27 tonnes of di(2-ethylhexyl) phthalate were released to air in 1995, according to the Canadian National Pollutant Release Inventory (Environment Canada, 1997).

Table 2. Workplace air levels of di(2-ethylhexyl) phthalate

Production	Country	Air concentration (mg/m^3)		Sampling	No. of samples	Reference
		Mean	Range			
Di(2-ethylhexyl) phthalate manufacturing plant	USA			Personal—whole-shift		Liss & Hartle (1983)
Chemical operators, technicians and maintenance workers			ND–4.11[a]		50[b]	
Poly(vinyl chloride) processing industry	Sweden			Personal—2-h		Nielsen *et al.* (1985)
Thick film department: calender operators/machine attendants		0.4[c]	0.1–0.8		16	
Poly(vinyl chloride) processing plants	Finland			Area—1.5–3 h		Vainiotalo & Pfäffli (1990)
Extrusion		0.05	± 0.03		4	
Extrusion		0.3	± 0.2		5	
Calendering		0.5	± 0.5		7	
Hot embossing		0.05	± 0.02		5	
Welding		0.3	± 0.05		4	
Injection moulding		0.02	± 0.01		2	
Compounding		0.02	± 0.01		5	
Thermoforming		0.02	± 0.02		2	
High-frequency welding		< 0.02				
Poly(vinyl chloride) processing plants	Netherlands			Personal—2-h		Dirven *et al.* (1993a)
Boot factory						
Mixing process		0.26	0.1–1.2		16	
Extruder process		0.12	0.05–0.28		11	
Cable factory						
Mixing process		0.18	0.01–0.81		8	
Extruder process		0.24	0.01–1.27		13	

Table 2 (contd)

Production	Country	Air concentration (mg/m^3)		Sampling	No. of samples	Reference
		Mean	Range			
Various plants	USA			Personal—4–5 h		Roberts (1983)
Di(2-ethylhexyl) phthalate manufacture		ND			8	
Two aerosol filter testing facilities			0.01–0.14		4	
Poly(vinyl chloride) sheet processing plant			0.06–0.29		3	

[a] ND, not detected
[b] Only six measurements were above the detection limit
[c] Presented as total phthalates, but where di(2-ethylhexyl) phthalate was the main plasticizer

Table 3. Estimated daily intake of di(2-ethylhexyl) phthalate by the population of Canada

Substrate/medium	Estimated intake for various age ranges (ng/kg bw per day)				
	0–0.5 years[a]	0.5–4 years[b]	5–11 years[c]	12–19 years[d]	20–70 years[e]
Ambient air: Great Lakes region	0.03–0.3	0.03–0. 3	0.04–0.4	0.03–0.3	0.03–0.3
Indoor air	860	990	1200	950	850
Drinking-water	130–380	60–180	30–100	20–70	20–60
Food	7900	18 000	13 000	7200	4900
Soil	0.064	0.042	0.014	0.04	0.03
Total estimated intake	8900–9100	19 000	14 000	8200	5800

From Meek & Chan (1994)
[a] Assumed to weigh 6 kg, breathe 2 m^3 air, drink 0.75 L water and ingest 35 mg soil
[b] Assumed to weigh 13 kg, breathe 5 m^3 air, drink 0.8 L water and ingest 50 mg soil
[c] Assumed to weigh 27 kg, breathe 12 m^3 air, drink 0.9 L water and ingest 35 mg soil
[d] Assumed to weigh 57 kg, breathe 21 m^3 air, drink 1.3 L water and ingest 20 mg soil
[e] Assumed to weigh 70 kg, breathe 23 m^3 air, drink 1.5 L water and ingest 20 mg soil

Di(2-ethylhexyl) phthalate concentrations of up to 790 ng/m^3 have been found in urban and polluted air, but usually the levels in ambient air are well below 100 ng/m^3 (WHO, 1992: Agency for Toxic Substances and Disease Registry, 1993).

Di(2-ethylhexyl) phthalate released into air can be carried for long distances in the troposphere and it has been detected over the Atlantic and Pacific Oceans; wash-out by rain appears to be a significant removal process (Atlas & Giam, 1981; Giam *et al.*, 1984; WHO, 1992).

Di(2-ethylhexyl) phthalate in air has been monitored in the North Atlantic, the Gulf of Mexico and on Enewetak Atoll in the North Pacific and levels ranged from not detectable to 4.1 ng/m^3 (Giam *et al.*, 1978, 1980; Atlas & Giam, 1981). Concentrations of di(2-ethylhexyl) phthalate in the atmosphere of the northwestern Gulf of Mexico averaged 1.16 ng/m^3 for ten samples, with 57% of the compound measured in the vapour phase only. The concentration was one to two orders of magnitude lower in maritime air than in continental atmospheres (Giam *et al.*, 1978, 1980).

Somewhat similar levels in air, between 0.5 and 5 ng/m^3 (mean, 2 ng/m^3) of di(2-ethylhexyl) phthalate have been found in the Great Lakes ecosystem (Canada and United States). The concentration of di(2-ethylhexyl) phthalate in precipitation ranged from 4 to 10 ng/L (mean, 6 ng/L). Atmospheric fluxes to the Great Lakes are a combination of dry and wet removal processes. The total deposition of di(2-ethylhexyl) phthalate into Lakes Superior, Michigan, Huron, Erie and Ontario was estimated to amount to 16, 11, 12, 5.0 and 3.7 tonnes per year, respectively (Eisenreich *et al.*, 1981).

In Sweden, air concentrations of di(2-ethylhexyl) phthalate were measured at 14 monitoring stations (53 samples). The median air concentration was 2.0 ng/m^3 (range, 0.3–77 ng/m^3) and the average fallout was 23.8 µg/m^2 per month (range, 6.0–195.5 µg/m^2 per month). The total annual fallout of di(2-ethylhexyl) phthalate in Sweden was estimated to be 130 tonnes (Thurén & Larsson, 1990).

During 1995, four sets of samples from two monitoring stations of the breathable fraction of atmospheric particulates including phthalates were measured in the air of Rieti urban area in Italy. The concentrations of di(2-ethylhexyl) phthalate ranged from 20.5 to 31 ng/m^3 (normalized) and from 34.8 to 503.5 ng/m^3 (normalized), respectively, at the two monitoring stations (Guidotti *et al*., 1998).

The concentration of di(2-ethylhexyl) phthalate in air at Lyngby, Denmark was calculated to be 22 ng/m^3 based on the analysis of snow samples (Løkke & Rasmussen, 1983). Levels of 26–132 ng/m^3 were measured in four samples from a residential area in Antwerp, Belgium (Cautreels *et al*., 1977). The yearly average concentrations at three air sampling stations in New York City in 1978 ranged from 10 to 17 ng/m^3, respectively (Bove *et al*., 1978).

There is a paucity of data concerning concentrations of di(2-ethylhexyl) phthalate concentrations in indoor air, although its volatilization from plastic products has been noted (Wams, 1987; Agency for Toxic Substances and Disease Registry, 1993).

Di(2-ethylhexyl) phthalate has been shown to account for 69 and 52% of the total amount of phthalates adsorbed to sedimented dust and particulate matter, respectively, in a number of Oslo dwellings. It was found at levels of 11–210 µg/100 mg [110–2100 mg/kg] sedimented dust in 38 dwellings and at levels of 24–94 µg/100 mg [240–940 mg/kg] suspended particulate matter (mean ± SD, 60 ± 30) in six dwellings. It was suggested that suspended particulate exposure to di(2-ethylhexyl) phthalate is one- to three-fold higher than the estimated vapour phase exposure (Øie *et al*., 1997).

(*b*) *Water and sediments*

In general, concentrations of di(2-ethylhexyl) phthalate in fresh waters are in the range of < 0.1–10 µg/L, although occasionally much higher values have been observed (~ 100 µg/L) when water basins are surrounded by heavy concentrations of industry (WHO, 1992; Agency for Toxic Substances and Disease Registry, 1993).

Surface water discharges of di(2-ethylhexyl) phthalate from 298 industrial facilities in 1994 in the United States amounted to 264 kg, as reported in the Environmental Protection Agency Toxics Release Inventory (Environmental Protection Agency, 1999d).

Di(2-ethylhexyl) phthalate has been detected in 24% of 901 surface water supplies at a median concentration of 10 µg/L and in 40% of 367 sediment samples at a median concentration of 1000 µg/kg in samples recorded in the STORET database in the United States (Staples *et al*., 1985). Di(2-ethylhexyl) phthalate concentrations in water from Galveston Bay, Texas, ranged from < 2 to 12 000 ng/L (average, 600 ng/L) (Murray *et al*., 1981), somewhat higher than those found earlier for the Mississippi Delta

(23–225 ng/L; average, 70 ng/L) and the Gulf of Mexico coast (6–316 ng/L; average, 130 ng/L) (Giam *et al*., 1978). Levels of di(2-ethylhexyl) phthalate up to 720 ng/L were found in two sampling stations of the Mississippi River in the summer of 1984 (DeLeon *et al*., 1986).

Levels of dissolved di(2-ethylhexyl) phthalate in samples from the River Mersey estuary, Liverpool, United Kingdom, ranged from 0.125 to 0.693 µg/L (Preston & Al-Omran, 1989).

Levels of up to 1.9 µg/L di(2-ethylhexyl) phthalate were found in rivers of the greater Manchester area, United Kingdom (Fatoki & Vernon, 1990) and at unspecified levels as contaminants of the Elbe River and its tributaries in Germany during the period 1992–94 (Franke *et al*., 1995).

Levels of di(2-ethylhexyl) phthalate in two rivers in southern Sweden were 0.32–3.10 µg/L and 0.39–1.98 µg/L. The highest value was in samples taken near an industrial effluent discharge (Thurén, 1986).

In a 12-day survey, di(2-ethylhexyl) phthalate at levels ranging from 0.2 to 0.6 µg/L was found in the River Rhine near Lobith and levels ranging from < 0.1 to 0.3 ng/L were found in the IJsselmeer, The Netherlands (Ritsema *et al*., 1989).

Levels of di(2-ethylhexyl) phthalate in water samples from 12 stations in the Klang River Basin in central Malaysia ranged from 3.1 to 64.3 µg/L between January 1992 and February 1993. The highest levels of phthalates in the water and sediment samples were collected near industrial areas where direct discharge points were found (Tan, 1995).

Di(2-ethylhexyl) phthalate has been reported in the leachate from municipal and industrial landfills at levels ranging from < 0.01 to 150 µg/mL (Ghassemi *et al*., 1984). It has also been detected in 13% of 86 samples of urban storm water runoff evaluated for the National Urban Runoff Program at concentrations ranging from 7 to 39 µg/L (Cole *et al*., 1984).

Since di(2-ethylhexyl) phthalate is lipophilic, it tends to be absorbed onto sediment, which serves as a sink (WHO, 1992). Di(2-ethylhexyl) phthalate has been measured in rivers and lake sediments in Europe (Schwartz *et al*., 1979; Giam & Atlas, 1980; Thurén, 1986; Preston & Al-Omeron, 1989; Ritsema *et al*., 1989) and in river and bay sediments in the United States (Peterson & Freeman, 1982; Ray *et al*., 1983; Hollyfield & Sharma, 1995). Concentrations ranged from 0.029 to 70 mg/kg. Near direct discharge points from industry in Sweden and Malaysia, concentrations of di(2-ethylhexyl) phthalate in sediments were above 1000 mg/kg (Thurén, 1986; Tan, 1995), and ranged from 190 to 700 µg/kg near industrial discharges in marine sediments around coastal Taiwan (Jeng, 1986).

In experimental studies of a marine environment of Narragansett Bay, Rhode Island, United States, it was shown that biodegradation by the surface microlayer biota accounted for at least 30% of the removal of di(2-ethylhexyl) phthalate (Davey *et al*., 1990).

Water solubility is a major factor limiting the degradation of phthalate esters under methanogenic conditions. In a study of the degradation of di(2-ethylhexyl) phthalate

and its intermediate hydrolysis products, 2-ethylhexanol and mono(2-ethylhexyl) phthalate in a methanogenic phthalic acid ester-degrading enrichment culture at 37 °C, the culture readily degraded 2-ethylhexanol via 2-ethylhexanoic acid to methane, mono(2-ethylhexyl) phthalate was degraded to stoichiometric amounts of methane with phthalic acid as a transient intermediate, while di(2-ethylhexyl) phthalate remained unaffected throughout the 330-day experimental period (Ejlertsson & Svensson, 1996; Ejlertsson *et al*., 1997).

(*c*) *Soil*

The principal source of di(2-ethylhexyl) phthalate release to land is disposal of industrial and municipal waste to landfills (Agency for Toxic Substances and Disease Registry, 1993; Bauer & Herrmann, 1997). Additionally, di(2-ethylhexyl) phthalate from various sources such as food wraps is released to municipal waste. Waste disposal of PVC products containing varying amounts of di(2-ethylhexyl) phthalate to landfills is another source (Swedish Environmental Protection Agency, 1996). Releases of di(2-ethylhexyl) phthalate to land from 298 industrial facilities in the United States in 1997 amounted to 32 137 kg (Environmental Protection Agency, 1999d). According to the Canadian National Pollutant Release Inventory, 33 tonnes of di(2-ethylhexyl) phthalate were released from Canadian facilities to land (Environment Canada, 1997).

Five soils and leachate-sprayed soils from the Susquehanna River basin in Pennsylvania and New York had levels of di(2-ethylhexyl) phthalate ranging from 0.001 to 1.2 mg/kg (Russell & McDuffie, 1983). Contaminated soil in the Netherlands was found to contain up to 1.5 mg/kg dry matter (Wams, 1987). Residues of di(2-ethylhexyl) phthalate in soil collected in the vicinity of a di(2-ethylhexyl) phthalate manufacturing plant amounted to up to 0.5 mg/kg (Persson *et al*., 1978).

(*d*) *Foods*

The most common route of human exposure to di(2-ethylhexyl) phthalate is through food contamination. The average daily exposure from food in the United States has been estimated to be about 0.3 mg/day per individual, with a maximum exposure of 2 mg/day (WHO, 1992; Agency for Toxic Substances and Disease Registry, 1993).

Di(2-ethylhexyl) phthalate has been found at generally low levels in a broad variety of foods, including milk, cheese, margarine, butter, meat, cereals, fish and other seafood (Cocchieri, 1986; Giam & Wong, 1987; Castle *et al*., 1990; Petersen, 1991; WHO, 1992; Agency for Toxic Substances and Disease Registry, 1993; Gilbert, 1994). It can originate from PVC wrapping materials, manufacturing processes or from the animals which had produced the milk or meat (Giam & Wong, 1987; Gilbert, 1994; Sharman *et al*., 1994). The highest levels of di(2-ethylhexyl) phthalate have been measured in milk products, meat and fish as well as in other products which have a high fat content. The use of di(2-ethylhexyl) phthalate in food contact applications is reported to be decreasing (Page & Lacroix, 1995).

Di(2-ethylhexyl) phthalate was determined in milk, cream, butter and cheese samples from a variety of sources from Norway, Spain and the United Kingdom. Samples of Norwegian milk obtained at various stages during collection, transportation and packaging operations showed no apparent trends in phthalate contamination, with total phthalate levels (expressed as di(2-ethylhexyl) phthalate equivalents) in the raw milk of between 0.12 and 0.28 μg/kg. On processing the di(2-ethylhexyl) phthalate was concentrated in the cream at levels up to 1.93 μg/g, whereas low-fat milk contained from < 0.01 to 0.07 μg/g [mg/L]. In the United Kingdom, pooled milk samples from doorstep delivery in different regions of the country contained < 0.01–0.09 μg/g [mg/L] di(2-ethylhexyl) phthalate. Concentrations of di(2-ethylhexyl) phthalate in 10 samples of retail cream and 10 samples of butter obtained in the United Kingdom ranged from 0.2 to 2.7 μg/g and 2.5 to 7.4 μg/g, respectively. Thirteen retail milk and cream products from Spain had levels of di(2-ethylhexyl) phthalate ranging from < 0.01 to 0.55 μg/g (Sharman *et al.*, 1994).

Milk samples were collected from a dairy in Norway at various stages of the milking process to determine the extent of migration of di(2-ethylhexyl) phthalate from plasticized tubing used in commercial milking equipment. Concentrations for each individual cow averaged 0.03 μg/g [mg/L] and rose to 0.05 μg/g [mg/L] in the central collecting tank. In control milk samples obtained by hand-milking, the concentration of di(2-ethylhexyl) phthalate was below 0.005 μg/g [mg/L]. In Norway and the United Kingdom, di(2-ethylhexyl) phthalate in milk tubing has been replaced by other types of plasticizers, such as di(2-ethylhexyl) adipate (see monograph in this volume) and diisodecyl phthalate (Castle *et al.*, 1990).

An investigation of residues of di(2-ethylhexyl) phthalate in retail whole milk samples from 14 Danish dairies about six months after the use of di(2-ethylhexyl) phthalate-plasticized milk tubing was banned in Denmark in August 1989 revealed a mean concentration lower than 50 μg/L (Petersen, 1991).

Retail samples of Canadian butter and margarine wrapped in aluminium foil–paper laminate were found to contain di(2-ethylhexyl) phthalate at levels up to 11.9 mg/kg. Ten samples of butter (454 g each) had levels of di(2-ethylhexyl) phthalate ranging from 2.9 to 11.9 mg/kg and six samples of margarine (454 g each) had levels ranging from 0.8 to 11.3 mg/kg. Analysis of the wrappers showed little correlation between the levels of di(2-ethylhexyl) phthalate in the total wrapper and the corresponding food. When di(2-ethylhexyl) phthalate was not present in the wrapper, a background level of di(2-ethylhexyl) phthalate from about 3 to 7 mg/kg was found in butter while, with di(2-ethylhexyl) phthalate present in the wrapper, an average level in the butter of 9.4 mg/kg of the phthalate was found (Page & Lacroix, 1992).

Di(2-ethylhexyl) phthalate was found in both the packaging and in a number of contacted foods sampled in a 1985–89 survey as part of the Canadian Health Protection Branch Total Diet Program. Low levels (65 μg/kg [L] average in beverages and 29 μg/kg average in foods) associated with the use of di(2-ethylhexyl) phthalate-plasticized cap or lid seals were found in a variety of glass-packaged foods. It was

found in 14 types of cheese at levels up to 5.5 mg/kg [average, 2.2 mg/kg] and, on a butter–fat basis, these levels averaged about 8 mg/kg di(2-ethylhexyl) phthalate. Levels in factory-packaged fish were 0.2 mg/kg in halibut and 2.1 mg/kg in pollack and in two smoked salmon samples were 0.3 and 3.9 mg/kg. Di(2-ethylhexyl) phthalate was found in nine varieties of factory-packaged non-frozen meats at levels that ranged from 0.1 to 3.7 mg/kg (Page & Lacroix, 1995).

Analysis of dairy food composite samples showed the presence of di(2-ethylhexyl) phthalate in all samples at 0.1–3.4 mg/kg. The levels in total diet samples of meat, poultry and fish ranged from 0.1 to 2.6 mg/kg and, in total diet cereal products, ranged from 0.02 to 1.5 mg/kg. Low incidence and low levels of di(2-ethylhexyl) phthalate were found in total diet samples of fruits and vegetables (mostly not detected to 0.07 mg/kg) (Page & Lacroix, 1995).

Di(2-ethylhexyl) phthalate was detected in 80, 71, 84 and 52% of Italian plastic packaged salted meat, jam, baby food and milk samples, respectively, and in all the cheese and vegetable soups samples. The mean di(2-ethylhexyl) phthalate levels ranged between 0.21 and 2.38 mg/kg (Cocchieri, 1986).

A German study in which 22 samples of baby milk, baby food, mothers' milk and cows' milk were analysed for their content of phthalates found a relatively narrow range of 50–210 mg/kg di(2-ethylhexyl) phthalate, with hardly any differences between the food items (Gruber *et al*., 1998).

Di(2-ethylhexyl) phthalate was found (mg/kg wet weight) in the following commercial fish (pooled samples of 10 individuals each): herring (fillets), 4.71; mackerel (fillets), 6.50; cod (liver), 5.19; plaice (fillets), < 0.010; and redfish (fillets), < 0.010 (Musial *et al*., 1981).

In an investigation of 2-ethyl-1-hexanol as a contaminant in some samples of bottled Italian drinking-water, di(2-ethylhexyl) phthalate was also found in 12 glass bottled drinking-water samples (sealed with caps with plastic internal gasket) at levels ranging from 2.4 to 17.7 μg/L (mean, 6.0 μg/L). It was also found in 13 poly(ethylene terephthalate) bottled drinking-water samples (sealed with caps with plastic internal gasket) at levels ranging from 2.7 to 31.8 μg/L (mean, 10.5 μg/L) (Vitali *et al*., 1993).

(*e*) *Exposure from medical devices*

Di(2-ethylhexyl) phthalate at concentrations up to 40% by weight is generally used as a plasticizer in PVC materials which have been widely used for a variety of medical purposes (e.g., infusion–transfusion, dialysis systems or feeding tubes and catheters in disposable medical devices). It is known to leach from PVC blood packs into whole blood, platelet concentrates and plasma during storage; the concentration of di(2-ethylhexyl) phthalate increases with storage time and it is converted by a plasma enzyme to a more toxic metabolite, mono(2-ethylhexyl) phthalate (Rock *et al.*, 1978). Di(2-ethylhexyl) phthalate has been detected in the blood and tissues of patients receiving blood transfusions and haemodialysis treatments (Jaeger & Rubin, 1972; Rock *et al*., 1978; Sasakawa & Mitomi, 1978; Cole *et al*., 1981; Rock *et al*.,

1986; Christensson *et al*., 1991; Dine *et al*., 1991; Huber *et al*., 1996; Mettang *et al*., 1996a).

Di(2-ethylhexyl) phthalate was detected in whole blood at levels ranging from 16.8 to 46.1 µg/mL [mg/L] and in packed cells at levels ranging from 32.6 to 55.5 µg/mL [mg/L] in PVC blood bags stored at 5 °C. These levels increased with storage. The average content was 6.7 ± 4.6 µg/mL in cryoprecipitate and 7.4 ± 2.8 µg/mL in fresh frozen plasma. Both values were independent of the storage period (Sasakawa & Mitomi, 1978).

The accumulation of di(2-ethylhexyl) phthalate in platelet-poor plasma stored for seven and 14 days in PVC bags sterilized by steam, ethylene oxide or irradiation revealed seven-day storage levels of di(2-ethylhexyl) phthalate of 378 ± 19, 362 ± 10 and 275 ± 15 mg/L, respectively, and 14-day storage levels of 432 ± 24, 428 ± 22 and 356 ± 23 mg/L, respectively (Dine *et al*., 1991).

In one study of newborn infants who received exchange transfusion, the plasma levels of di(2-ethylhexyl) phthalate in six patients varied between 3.4 and 11.1 mg/L, while mono(2-ethylhexyl) phthalate levels in the corresponding samples ranged from 2.4 to 15.1 mg/L. In newborn infants subjected to a single exchange transfusion, concentrations of di(2-ethylhexyl) phthalate in plasma from the blood taken from the transfusion set varied between 36.8 and 84.9 mg/L, while mono(2-ethylhexyl) phthalate in these samples ranged between 3.0 and 15.6 mg/mL (Sjöberg *et al*., 1985a). The concentrations in blood of both di- and mono(2-ethylhexyl) phthalates were similar in PVC bags stored for four days or less (Rock *et al*., 1986).

In an additional study to investigate further the disposition of di(2-ethylhexyl) phthalate and mono(2-ethylhexyl) phthalate during a single exchange transfusion in four newborn infants, the amounts of di(2-ethylhexyl) phthalate and mono(2-ethylhexyl) phthalate infused ranged from 0.8 to 3.3 and 0.05 to 0.20 mg/kg bw, respectively. There were indications that about 30% of the infused di(2-ethylhexyl) phthalate originated from parts of the transfusion set other than the blood bag. Approximately 30% of the infused amount of di(2-ethylhexyl) phthalate was withdrawn during the course of each transfusion. Immediately after the transfusions, the plasma levels of di(2-ethylhexyl) phthalate levels ranged between 5.8 and 19.6 mg/L and subsequently declined rapidly (reflecting its distribution within the body), followed by a slower elimination phase. The half-life of this phase was approximately 10 h (Sjöberg *et al*., 1985b).

Serum levels of di(2-ethylhexyl) phthalate in 16 newborn infants undergoing exchange transfusion indicated an undetectable level (< 1 mg/L) before exchange but levels ranging from 6.1 to 21.6 mg/L serum (average, 12.5 ± 6.2 mg/L) after a single exchange transfusion. In 13 newborn infants receiving a second blood unit, the serum levels of di(2-ethylhexyl) phthalate ranged from 12.3 to 87.8 mg/L and, in six patients receiving a third blood unit, the serum levels ranged from 24.9 to 93.1 mg/L (Plonait *et al*., 1993).

The degree of exposure to di(2-ethylhexyl) phthalate was assessed in 11 patients undergoing haemodialysis for treatment of renal failure and showed that on average,

an estimated 105 mg di(2-ethylhexyl) phthalate was extracted from the dialyser during a single 4-h dialysis session, with a range of 23.8–360 mg. Time-averaged circulating concentrations of mono(2-ethylhexyl) phthalate during the session (1.33 ± 0.58 mg/L) were similar to those of di(2-ethylhexyl) phthalate (1.91 ± 2.11 mg/L). Assuming a schedule of treatment three times per week, the average patient in the study would have received approximately 16 g di(2-ethylhexyl) phthalate over the course of a year, with a range of 3.7–56 g (Pollack *et al*., 1985).

Di(2-ethylhexyl) phthalate was found at concentrations ranging from 0.8 to 4.2 mg/L serum in 17 haemodialysis patients after dialysis and 0.1–0.9 mg/L in four of seven continuous ambulatory peritoneal dialysis (CAPD) patients. In three of the CAPD patients and in all of the predialysis patients, di(2-ethylhexyl) phthalate was not detected (< 0.1 mg/L); in no case could the hydrolysis product mono(2-ethylhexyl) phthalate be detected (< 0.4 mg/L) (Nässberger *et al*., 1987).

A comparative evaluation of haemodialysis tubing plasticized with di(2-ethylhexyl) phthalate and with tri-2-ethylhexyl trimellitate was made in 11 patients (10 men, one woman) with chronic renal failure on haemodialysis for a period of six months. During treatment with tubing containing di(2-ethylhexyl) phthalate, the plasma level of di(2-ethylhexyl) phthalate rose from 0.10 mg/L (range, < 0.05–0.17) to 0.70 mg/L (range, 0.30–1.6) (detection limit, 0.05 mg/L) (Christensson *et al*., 1991).

The degree of exposure to and the fate of di(2-ethylhexyl) phthalate and derived mono(2-ethylhexyl) phthalate, 2-ethylhexanol and phthalic acid in seven elderly patients undergoing regular CAPD were compared with those in six aged-matched healthy controls during a 4-h dwell period. Serum concentrations of di(2-ethylhexyl) phthalate and phthalic acid were significantly higher ($p = 0.027$ and $p = 0.026$, respectively) in patients (median, 0.079 mg/L; range, 0.032–0.210 mg/L; and 0.167 mg/L; range, 0.097–0.231 mg/L, respectively) than in controls (median, 0.0195 mg/L; range, 0.016–0.025 mg/L; and 0.012 mg/L; range, 0.006–0.034 mg/L, respectively). The concentration of mono(2-ethylhexyl) phthalate in the fluid of CAPD bags before use was four times higher than that of di(2-ethylhexyl) phthalate. During the first 4 h of dwell time, the concentrations of mono(2-ethylhexyl) phthalate and 2-ethylhexanol in dialysate consistently decreased from 0.177 mg/L (range, 0.137–0.239 mg/L) to 0.022 mg/L (range, 0.005–0.058 mg/L) ($p = 0.017$), and from 0.087 mg/L (range, 0.075–0.097 mg/L) to 0.05 mg/mL (range, 0.023–0.064 mg/L) ($p = 0.017$), respectively, while the concentration of di(2-ethylhexyl) phthalate remained stable. Remarkably high concentrations of phthalic acid (0.129 mg/L; range, 0.038–0.466 mg/L) were found in the CAPD bags before use, and these concentrations tended to increase during dwell time but without statistical significance (0.135 mg/L; range, 0.073–0.659 mg/L; $p = 0.062$) (Mettang *et al.*, 1996a).

Levels of di(2-ethylhexyl) phthalate ranging from < 1 to 4100 μg/mL [mg/L] in the condensate from water traps of six respirators have been reported. Estimation of the inhalatory di(2-ethylhexyl) phthalate exposure to five artificially ventilated preterm infants over a 24-h period yielded values ranging between 1 μg/h and 4200 μg/h. Di(2-

ethylhexyl) phthalate (0.23 mg/kg wet weight) was found in the lung tissue of one infant who died of pneumothorax soon after birth following artificial ventilation (Roth *et al*., 1988)

Serum samples and autopsy specimens were examined from two infants with congenital diaphragmatic hernia who had received life support with extracorporeal membrane oxygenation (ECMO). The serum levels of di(2-ethylhexyl) phthalate after 14 and 24 days of ECMO support were 26.8 and 33.5 mg/L respectively, and levels of 3.5, 1.0 and 0.4 mg/kg di(2-ethylhexyl) phthalate were found in liver, heart and testicular tissues, respectively, and trace quantities were found in the brain. The rate of di(2-ethylhexyl) phthalate extraction from the model PVC circuits was linear with time (rate, 3.5 and 4.1 mg/L per hour). The exposure to di(2-ethylhexyl) phthalate for a 4-kg infant on ECMO support for 3–10 days was estimated to be 42–140 mg/kg (Shneider *et al*., 1989).

A more recent study of 18 infants on ECMO life support also reported leaching of di(2-ethylhexyl) phthalate from the PVC circuits at linear rates that were dependent on the surface area of the circuit. For standard 3–10-day treatment courses, the mean peak plasma concentration of di(2-ethylhexyl) phthalate was 8.3 ± 5.7 mg/L, and the estimated exposure over 3–10 days was 10–35 mg/kg bw. No leaching of di(2-ethylhexyl) phthalate from heparin-coated PVC circuits was detected (Karle *et al.*, 1997).

Exposure of children to di(2-ethylhexyl) phthalate by migration from PVC toys and other articles into saliva has been reported. Until the early 1980s, di(2-ethylhexyl) phthalate was the predominant plasticizer used in soft PVC children's products. Since then, it has been replaced in most countries by other plasticizers, in particular di(iso-nonyl) phthalate (Steiner *et al*., 1998; Wilkinson & Lamb, 1999).

1.5 Regulations and guidelines

Occupational exposure limits for di(2-ethylhexyl) phthalate are given in Table 4.

The World Health Organization has established an international drinking-water guideline for di(2-ethylhexyl) phthalate of 8 µg/L (WHO, 1993). The Environmental Protection Agency (1998) has set the maximum contaminant level (MCL) for di(2-ethylhexyl) phthalate in drinking-water at 6 µg/L in the United States.

The Czech Republic has set a maximum limit for plastic materials for di(2-ethyl-hexyl) phthalate of 50 mg/g as a component of plastic products permitted for contact with food (UNEP, 1999).

The Food and Drug Administration (1999) permits the use of di(2-ethylhexyl) phthalate in the United States as a component of adhesives used in food packaging, as a plasticizer in resinous and polymeric coatings used in food packaging, as a component of defoaming agents used in the manufacture of paper and paperboard used in food packaging, as a flow promoter in food contact surfaces not to exceed 3 wt% based on monomers, as a component of cellophane where total phthalates do

not exceed 5%, as a component of surface lubricants used in the manufacture of metallic articles that contact food and as a food-packaging plasticizer for foods of high water content.

The European Pharmacopoeia identifies di(2-ethylhexyl) phthalate as a substance that may be used in the manufacture of PVC plasticized containers and tubing for human blood and blood components, at a level of not more than 40% in the plastic (Council of Europe, 1997).

Table 4. Occupational exposure limits for di(2-ethylhexyl) phthalate[a]

Country[b]	Year	Concentration (mg/m^3)[b]	Interpretation[b]
Argentina	1991	5	TWA
		10	STEL
Australia	1993	5	TWA
		10	STEL
Belgium	1993	5	TWA
		10	STEL
Canada	1994	5	TWA
		10	STEL
Czech Republic	1993	5	TWA
		10	STEL
Denmark	1993	5 (Ca)	TWA
Finland	1998	5 (sk)	TWA
		10	STEL
France	1993	5	TWA
Germany	1999	10	TWA
Hungary	1993	5 (sk)	TWA
		10	STEL
Ireland	1997	5	TWA
		10	STEL
Japan	1998	5	TWA
Netherlands	1993	5	TWA
Philippines	1993	5	TWA
Poland	1998	1	TWA
		5	STEL
Russian Federation	1993	1	STEL
Slovakia	1993	5	TWA
		10	STEL
Sweden	1993	3	TWA
		5	STEL
Switzerland	1993	5	TWA
United Kingdom	1993	5	TWA
		10	STEL

Table 4 (contd)

Country[b]	Year	Concentration (mg/m^3)[b]	Interpretation[b]
United States			
OSHA (PEL)	1999	5	TWA
NIOSH (REL)	1997	5 (Ca)	TWA
		10	STEL
ACGIH[c] (TLV)	1999	5 (A3)	TWA

[a] From Finnish Ministry of Social Affairs and Health (1998); National Library of Medicine (1998); Deutsche Forschungsgemeinschaft (1999); Occupational Safety and Health Standards (1999); UNEP (1999)
[b] Abbreviations: TWA, time-weighted average; STEL, short-term exposure limit; A3, animal carcinogen; C, suspected of being a carcinogen; Ca, potential occupational carcinogen; PEL, permissible exposure limit; REL, recommended exposure limit; sk, skin designation; TLV, threshold limit value
[c] The following countries follow the exposure limits suggested by the ACGIH: Bulgaria, Colombia, Jordan, New Zealand, Republic of Korea, Singapore and Viet Nam

In the United States, there is a voluntary industry standard that states that pacifiers, rattles and teethers shall not intentionally contain di(2-ethylhexyl) phthalate [ASTM F 963-96a] (American Society of Testing and Materials, 1997).

The European Union has temporarily banned the use of six phthalates, including di(2-ethylhexyl) phthalate, in toys and other articles intended for children aged under three years of age and designed to be put in the mouth. Several countries in Europe also have proposed, or are considering, restrictions on use of phthalates as plasticizers (softeners) in PVC toys and baby care items (Anon., 1999).

2. Studies of Cancer in Humans

Cohort study

Occupational exposure

The mortality of 221 workers in a di(2-ethylhexyl) phthalate production plant in Germany was followed between 1940 and 1976. Most subjects (135/221) were hired after 1965 and the process was completely enclosed in 1966. No information on level of exposure was provided. Information on vital status for foreigners [number not stated] was obtained for only 55% of them, but appeared to be complete for the remaining cohort. Reference rates were obtained from local populations (the city of Ludwigshafen, the Rheinhessen-Pfalz *land*) and national rates. Altogether, eight deaths

occurred during the follow-up period versus 15.9 expected using local rates [standardized mortality ratio, 0.50; 95% confidence interval, 0.22–0.99] and 17.0 expected using national rates. One death from pancreatic cancer (0.13 expected) and one from bladder papilloma (0.01 expected) occurred among workers with a long exposure time (≥ 20 years). No further report on a longer follow-up for this cohort was available to the Working Group (Thiess *et al.*, 1978). [The Working Group noted that the majority of the cohort members were employed after exposure levels had been considerably reduced, and that the methods for this study were poorly described.]

Dialysis patients

Long-term dialysis patients are likely to experience elevated exposures to di(2-ethylhexyl) phthalate, through frequent and protracted exposure to substances leached from surgical tubing during dialysis (see Section 1.4.3(*e*)).

Cancer risk among dialysis patients has been specifically studied because of concern about medical conditions (for example, immunodeficiency) or incidental exposures from treatment (viruses, drugs) (Inamoto *et al.*, 1991). However, exposure to di(2-ethylhexyl) phthalate resulting from treatment has not been studied as such in relation to cancer risk. Due to the medical condition of this population, follow-up is usually very short and incompatible with induction of chemical carcinogenesis. In conclusion, the Working Group was not aware of any study of dialysis patients for which study methods were suitable for the evaluation of carcinogenic risk associated with di(2-ethylhexyl) phthalate.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F$_1$ mice, six weeks of age, were fed diets containing 3000 or 6000 mg/kg diet (ppm) di(2-ethylhexyl) phthalate (> 99% pure) for 103 weeks. All surviving mice were killed at 104–105 weeks. There was a clear dose-related decrease in body weight gain in females. Survival at the end of the study was more than 60% in males and more than 50% in females. High-dose males had a slightly decreased body weight gain. In male mice, significant increases in the incidence of hepatocellular carcinomas were observed (control, 9/50; low-dose, 14/48; high-dose, 19/50; $p = 0.022$, Fisher's exact test). The Cochran–Armitage test also indicated a significant trend ($p = 0.018$). The incidence of hepatocellular adenomas and carcinomas combined was also increased in males (control, 14/50; low-dose, 25/48, $p = 0.013$; high-dose, 29/50, $p = 0.002$, Fisher's exact test). In females, significant increases in the incidence of hepatocellular carcinomas were seen (control, 0/50; low-dose, 7/50, $p = 0.006$;

high-dose, 17/50, $p < 0.001$, Fisher's exact test) and of hepatocellular adenoma and carcinoma combined (control, 1/50; low-dose, 12/50; high-dose, 18/50, $p < 0.001$, trend and Fisher's exact tests) (Kluwe *et al.*,1982; National Toxicology Program, 1982; Kluwe *et al*., 1983, 1985).

Groups of 65 male and 65 female B6C3F_1 mice, six weeks of age, were fed diets containing 0, 100, 500, 1500 or 6000 ppm di(2-ethylhexyl) phthalate (purity, 99.7%) for 104 weeks. Another group of 65 male and 65 female mice received the highest concentration for 78 weeks and then control diet for a further 26 weeks (recovery group). Ten of these animals per group and sex were killed at week 105 for biochemical analyses of peroxisome proliferation. Two subgroups of 15 additional mice in the 0 and 6000 ppm groups were designated for measurement of cell proliferation, biochemical analysis for peroxisome proliferation and histopathological evaluation and were killed at week 79. These additional animals were included in the final analysis. All surviving mice were killed at 105 weeks for histopathological examination. There was a clear dose-related decrease in body weight gain in females. The survival of male mice treated with 6000 ppm was significantly lower than that of the controls; about 80% of the untreated controls and treated animals in the other groups survived. In females, more than 60% of the animals survived until the end of the study. Hepatocellular tumour incidences in males were: 8/70 (control), 14/60 (100 ppm), 21/65 (500 ppm), 27/65 (1500 ppm), 37/70 (6000 ppm) and 15/55 (recovery group). All but the 100 ppm group differed significantly ($p < 0.05$, Fisher's exact test) from the control group. The respective incidences in females were 3/70, 4/60, 7/65, 19/65, 44/70 and 30/55, with the 1500-ppm, 6000-ppm and recovery groups differing significantly from the concurrent control group ($p < 0.05$, Fisher's exact test) (David *et al*., 1999)

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, five to six weeks of age, were fed diets containing 6000 or 12 000 mg/kg diet (ppm) di(2-ethylhexyl) phthalate (> 99% pure) for 103 weeks. All surviving rats were killed at 104–105 weeks. Other groups of 50 males and 50 females served as controls. There was a dose-related decrease in body weight gain in both sexes but no effect on survival. More than 60% of the animals survived to the end of the study. High-dose male rats had significant increases ($p = 0.01$, Fisher's exact test) in the combined incidence of hepatocellular carcinomas and neoplastic nodules (control, 3/50; low-dose, 6/49; high-dose, 12/49). The Cochran–Armitage test also indicated a significant trend ($p = 0.007$). [The Working Group noted that the term neoplastic nodule is now generally assumed to represent hepatocellular adenomas.] The incidence of hepatocellular carcinomas alone or neoplastic nodules alone was not significantly increased. In female rats, the incidence of hepatocellular carcinomas was increased in high-dose rats (8/50; $p = 0.003$, Fisher's exact test) compared with controls (0/50) and that of neoplastic nodules was also

increased in high-dose females (5/50; $p < 0.028$) compared with controls (0/50). The incidence of hepatocellular carcinomas and neoplastic nodules combined was also increased in low-dose (6/49; $p = 0.012$) and high-dose (13/50; $p < 0.001$) females compared with controls (0/50) (Kluwe *et al*., 1982; National Toxicology Program, 1982; Kluwe *et al*., 1983, 1985).

Groups of 65 male and 65 female Fischer 344 rats, six weeks of age, were fed diets containing 0, 100, 500, 2500 or 12 500 ppm di(2-ethylhexyl) phthalate (purity, 99.7%) for up to 104 weeks. Another group of 65 male and 65 female rats received the highest concentration for 78 weeks and then control diet for a further 26 weeks (recovery group). Ten of these animals per group and sex were killed at week 105 for biochemical analyses of peroxisome proliferation. Fifteen additional rats in the 0-, 2500- and 12 500-ppm groups were designated for measurement of cell proliferation, biochemical analyses for peroxisome proliferation and histopathological evaluation and were killed at 79 weeks, and these animals were included in the final tumour analyses. About 65% of males and females survived until the end of the study. All surviving rats were killed at 105 weeks for histopathological examination. For group comparison, the Fisher's exact test was used. Hepatocellular tumour incidences in males were: 5/80 (control), 5/50 (100 ppm), 4/55 (500 ppm), 11/65 (2500 ppm, $p < 0.05$), 34/80 (12 500 ppm, $p < 0.05$) and 18/55 (recovery group, $p < 0.05$). The respective incidences in females were 0/80, 4/50 ($p < 0.05$), 1/55, 3/65, 22/80 ($p < 0.05$) and 10/55 ($p < 0.05$) (David *et al*., 1999).

Several studies using smaller numbers of animals have also been reported, some of which were not designed for carcinogenicity testing. These studies are reviewed below.

Groups of 20 female Fischer 344 rats, eight weeks of age, were fed a diet containing 0 (control), 0.03, 0.10 or 1.2% di(2-ethylhexyl) phthalate [purity not specified] for two years. Neoplastic nodules or hepatocellular carcinomas were seen in 0/18 control, 1/18 low-dose, 1/19 mid-dose and 6/20 high-dose rats ($p < 0.01$). Di(2-ethylhexyl) phthalate did not induce foci of altered hepatocytes as judged by basophilia, ATPase-deficiency or glucose-6-phosphatase-deficiency (Cattley *et al*., 1987).

Groups of 10 male Fischer 344 rats, six weeks of age, were fed a diet containing 2% di(2-ethylhexyl) phthalate (purity, 98%) for 95 weeks. Neoplastic nodules and/or hepatocellular carcinomas were found in 0/18 controls and 6/10 rats fed di(2-ethylhexyl) phthalate ($p < 0.005$). Both the neoplastic nodules and hepatocellular carcinomas were negative for γ-glutamyltranspeptidase (γ-GT) (Rao *et al*., 1987).

Groups of 10–14 male Fischer 344 rats were fed a diet containing 0 (control) or 2% di(2-ethylhexyl) phthalate (98% pure) for 108 weeks. All liver lobes were sliced at 1–2-mm intervals and the number and size of grossly visible lesions recorded. Neoplastic nodules and/or hepatocellular carcinomas were observed in 1/10 controls and 11/14 treated rats [no statistical analysis given] (Rao *et al*., 1990). [The authors suggest that their finding of a high incidence of liver tumours was due to their gross slicing technique, although they do not report a comparison of their findings with conventional liver trimming and sectioning techniques].

A total of 520 male Sprague-Dawley rats (180 g) were fed diets containing 0, 0.02, 0.2 or 2% (200, 2000 or 20 000 ppm) di(2-ethylhexyl) phthalate (purity, > 99%) for 102 weeks [group sizes not specified]. There was a significant, dose-dependent decrease in body weights in the mid- and high-dose groups, but not in the low-dose animals. Data on survival were not reported. For biochemical assays and histopathological examinations, 7–18 rats were killed at weeks 24, 48, 72 and 96. The number of animals at terminal sacrifice was not given. No hyperplastic nodules or hepatocellular carcinomas were reported in either the control or treated animals (Ganning *et al*., 1991) [The Working Group noted the inadequate reporting.]

As part of a larger experiment for studying the characteristics of hepatocarcinogenesis, 17 male Fischer 344 rats were fed a diet containing 2% (20 000 ppm) di(2-ethylhexyl) phthalate [purity unspecified] for up to 78 weeks. A group of 18 untreated animals served as controls. At 52 weeks, no liver tumours had developed in 10 di(2-ethylhexyl) phthalate-treated rats or in 10 controls, while at 78 weeks, hepatocellular carcinomas or neoplastic nodules were found in 3/7 di(2-ethylhexyl) phthalate-treated rats and 0/8 controls (Hayashi *et al*., 1994).

3.2 Inhalation exposure

Hamster: Groups of 65–80 male or female Syrian golden hamsters, 12 weeks of age, were exposed by whole-body inhalation to 15 μg/m^3 di(2-ethylhexyl) phthalate vapour (> 99% pure) for 24 h per day on five days per week until natural death. Total exposure over the lifetime was 7–10 mg/kg bw. No difference in survival was seen between controls and the di(2-ethylhexyl) phthalate-treated groups. Median survival in controls was 709 days in males and 507 days in females and that in treated animals was 703 days in males and 522 days in females. Tumour incidence was not increased in di(2-ethylhexyl) phthalate-treated hamsters (Schmezer *et al*., 1988). [The Working Group noted the low concentration and that it was selected to simulate occupational exposure.]

3.3 Intraperitoneal administration

Hamster: Groups of 50 male and 50 female Syrian golden hamsters, six weeks of age, were administered 0 (control) or 3 g/kg bw di(2-ethylhexyl) phthalate (> 99% pure) by intraperitoneal injection once per week, once per two weeks or once per month (total doses, 24–54 g/kg bw). Hamsters were maintained for their natural lifespan. There were no differences in survival between groups (range of median survival being 629–686 days for males and 465–495 days for females). The incidence of tumours was not increased by treatment (Schmezer *et al*., 1988). [The Working Group noted limitations in the dosing schedule.]

3.4 Administration with known carcinogens and modifying agents

There are numerous reports of studies of the initiating or promoting activities of di(2-ethylhexyl) phthalate given in combination with known carcinogens or promoting agents. Selected studies (complex protocols involving multiple promoting agents and special procedures were excluded) are summarized below and in Tables 5 and 6.

3.4.1 *Mouse*

Liver: Male B6C3F_1 mice, four weeks of age, received a single intraperitoneal injection of 80 mg/kg bw *N*-nitrosodiethylamine (NDEA) in tricaprylin. Two weeks later, the mice were fed diets containing 0, 3000, 6000 or 12 000 ppm di(2-ethylhexyl) phthalate for up to six months. Groups of 10 mice were killed at two, four and six months after NDEA treatment. Few hepatocellular foci were seen at two, four or six months in mice treated with NDEA alone or di(2-ethylhexyl) phthalate alone, while numerous foci and neoplasms were seen in mice given di(2-ethylhexyl) phthalate after NDEA. No tumours were found at six months in mice receiving NDEA alone. By the end of the study, the number of foci per unit volume of liver was similar in mice at all doses of di(2-ethylhexyl) phthalate, but there was an increase in the volume of the foci (0, 1.4, 0.6, 9.4 mm^3 for the control, 3000-, 6000- and 12 000-ppm groups, respectively) (Ward *et al.*, 1983).

The differential effects of short- or long-term exposure to di(2-ethylhexyl) phthalate were studied in male B6C3F_1 mice. Mice were given an intraperitoneal injection of 80 mg/kg bw NDEA at four weeks of age. At five weeks of age, the mice were fed diets containing 3000 ppm di(2-ethylhexyl) phthalate for periods of one, seven, 28, 84 or 168 days and were killed at 168 days. When di(2-ethylhexyl) phthalate was fed after NDEA treatment for 28 or more days, there was an increase in incidences of hepatocellular foci (45, 50, 67%) and adenomas (20.6, 17.8 and 46.6%) compared with those in mice receiving NDEA alone (foci, 20%; adenomas, 6.6%). There was also an increase in lesion number and size (Ward *et al.*, 1984).

Male B6C3F_1 mice, four weeks of age, received a single intraperitoneal injection of 80 mg/kg bw NDEA in tricaprylin and, two weeks later, were fed diets containing 0, 3000, 6000 or 12 000 ppm di(2-ethylhexyl) phthalate (purity, 99%) for up to 18 months. Groups of 10–20 mice were killed at two, four, six or 18 months. The numbers of mice with hepatocellular foci, adenomas and carcinomas were determined. All doses of di(2-ethylhexyl) phthalate increased the numbers of all lesions at the time periods studied compared with mice receiving NDEA alone. At 18 months, carcinomas were found in 3/10 mice treated with NDEA alone and in 10/10 and 18/20 mice treated with NDEA + di(2-ethylhexyl) phthalate at the low and mid doses, respectively. All mice given 12 000 ppm di(2-ethylhexyl) phthalate with NDEA initiation died by nine months and 11/20 had liver carcinomas. In mice treated with di(2-ethylhexyl) phthalate alone, 2/30 had liver carcinomas (Ward *et al.*, 1986).

Table 5. Selected promotion studies on di(2-ethylhexyl) phthalate (DEHP) with known carcinogens and modifying factors

Tumour type Species/strain (sex)	Known carcinogen (initiator)	Route of adminis-tration	Interval between initiator and promoter	Dose and duration of DEHP	Route of adminis-tration	Promoting activity for DEHP	Reference
Liver							
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	2 weeks	3000 mg/kg diet/6 months	Oral	+	Ward *et al.* (1983)
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	2 weeks	6000 mg/kg diet/6 months	Oral	+	Ward *et al.* (1983)
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	2 weeks	12 000 mg/kg diet/6 months	Oral	+	Ward *et al.* (1983)
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	1 week	3000 mg/kg diet/28 days	Oral	+	Ward *et al.* (1984)
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	1 week	3000 mg/kg diet/84 days	Oral	+	Ward *et al.* (1984)
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	1 week	3000 mg/kg diet/168 days	Oral	+	Ward *et al.* (1984)
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	2 weeks	3000 mg/kg diet/18 months	Oral	+	Ward *et al.* (1986)
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	2 weeks	6000 mg/kg diet/18 months	Oral	+	Ward *et al.* (1986)
$B6C3F_1$ mice (M)	80 mg/kg bw NDEA	i.p.	2 weeks	12 000 mg/kg diet/18 months	Oral	+	Ward *et al.* (1986)
C3H/HeNCr mice (M,F)	5 mg/kg bw NDEA	i.p.	2 weeks	12 000 mg/kg diet/24 weeks	Oral	+	Weghorst *et al.* (1993/94)
Fischer 344 rats (M)	150 mg/kg bw NDEA	i.p.	3 weeks	12 000 mg/kg diet/6 months	Oral	–	Popp *et al.* (1985)
Fischer 344 rats (M)	282 mg/kg bw NDEA	i.p.	2 weeks	12 000 mg/kg diet/14 weeks	Oral	–	Ward *et al.* (1986)
Fischer 344 rats (M)	200 mg/kg diet AAF 7 weeks	Oral	4 weeks	12 000 mg/kg diet/31 weeks	Oral	–	Williams *et al.* (1987)
Fischer 344 rats (M)	PH/200 mg/kg NDEA	i.p.	2 weeks	3000 mg/kg diet/6 weeks	Oral	–	Ito *et al.* (1988)
Sprague-Dawley rats (F)	8 mg/kg NDEA	i.g.	1 week	10 mg/kg 3 × weekly/ 11 weeks	i.g.	–	Oesterle & Deml. (1988)
Sprague-Dawley rats (F)	8 mg/kg NDEA	i.g.	1 week	100 mg/kg 3 × weekly/ 11 weeks	i.g.	–	Oesterle & Deml (1988)
Sprague-Dawley rats (F)	8 mg/kg NDEA	i.g.	1 week	200 mg/kg 3 × weekly/ 11 weeks	i.g.	+	Oesterle & Deml (1988)
Sprague-Dawley rats (F)	8 mg/kg NDEA	i.g.	1 week	500 mg/kg 3 × weekly/ 11 weeks	i.g.	+	Oesterle & Deml (1988)
Fischer 344 rats (M)	200 ppm AAF 7 weeks	Oral	4 weeks	12 000 ppm /24 weeks	Oral	–	Maruyama *et al.* (1990)

Table 5 (contd)

Tumour type Species/strain (sex)	Known carcinogen (initiator)	Route of adminis-tration	Interval between initiator and promoter	Dose and duration of DEHP	Route of adminis-tration	Promoting activity for DEHP	Reference
Kidney							
Fischer 344 rats (M)	500 mg/kg diet EHEN/2 weeks	Oral	0 weeks	12 000 ppm/24 weeks	Oral	+	Kurokawa *et al*. (1988)
Urinary bladder							
Fischer 344 rats (M)	5000 ppm BBN weeks 1–4; 30 000 ppm uracil weeks 8–11	Oral	0 weeks	3000 ppm/16 weeks	Oral	–	Hagiwara *et al*. (1990)

AAF, 2-acetylaminofluorene; BBN, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine; EHEN, *N*-ethyl-*N*-hydroxyethylnitrosamine; NDEA, *N*-nitrosodiethylamine; F, female; M, male; i.p., intraperitoneal injection; i.g., intragastric; PH, partial hepatectomy

Table 6. Initiation studies on di(2-ethylhexyl) phthalate (DEHP) with promoting agents

Species/strain	DEHP initiation	Route of administration	Interval between initiation and promotion	Dose and duration of promoter	Route of administration	Initiating activity of DEHP	Reference
Mouse $B6C3F_1$ (M)	25 or 50 g/kg bw	Gavage	2 weeks	500 mg/L phenobarbital 6 or 18 months	Drinking-water	– –	Ward *et al.* (1986)
Fischer 344 rats (F)	10 g/kg bw DEHP 6, 12, 24 h after PH	Oral	2 weeks	200 ppm AAF 2 weeks 1.5 mL/kg CCl_4 once	Diet Gavage		Garvey *et al.* (1987)
Fischer 344 rats (F)	12 000 mg/kg diet DEHP 12 weeks	Oral	2 weeks	500 mg/kg diet phenobarbital, 39 weeks	Diet	–	Garvey *et al.* (1987)

PH, partial hepatectomy; AAF, 2-acetylaminofluorene; CCl_4, carbon tetrachloride

In a study to test di(2-ethylhexyl) phthalate for initiating activity, groups of 7–20 male B6C3F$_1$ mice, four weeks of age, were given a single intragastric dose of 25 or 50 g/kg bw di(2-ethylhexyl) phthalate (99% pure). Groups of 10–17 controls were used. Two weeks later, phenobarbital was given as a promoting agent at a concentration of 500 mg/L in the drinking-water for six or 18 months. At 18 months, hepatocellular carcinomas were found in 0/7 mice given 50 g/kg di(2-ethylhexyl) phthalate, 2/15 mice given 50 g/kg bw di(2-ethylhexyl) phthalate + phenobarbital, 1/10 mice given 25 g/kg di(2-ethylhexyl) phthalate, 2/20 mice given 25 g/kg di(2-ethylhexyl) phthalate + phenobarbital, 3/17 mice given phenobarbital alone and 0/10 untreated mice [statistical analysis not given]. Thus the study showed no evidence of initiating activity of di(2-ethylhexyl) phthalate (Ward *et al*., 1986).

Groups of 10 male and five female C3H/HeNCr mice, 15 days of age, received either a single intraperitoneal injection of 5 mg/kg bw NDEA or saline. At weaning (four weeks of age), mice were divided into two groups and fed diets containing either 0 or 12 000 ppm di(2-ethylhexyl) phthalate [purity unspecified] for 24 weeks. All mice were killed at 28 weeks of age and the number and size of hepatic foci were measured. Di(2-ethylhexyl) phthalate in combination with NDEA increased the average numbers of foci per liver (NDEA-treated males, 176; NDEA + di(2-ethylhexyl) phthalate-treated males, 366; NDEA-treated females, 47; NDEA + di(2-ethylhexyl) phthalate-treated females, 169). The numbers of adenomas per liver were also increased (NDEA-treated males, 7; NDEA + di(2-ethylhexyl) phthalate-treated males, 15.8; NDEA-treated females, 0; NDEA + di(2-ethylhexyl) phthalate-treated females, 2). In male mice, treatment with NDEA + di(2-ethylhexyl) phthalate yielded larger adenomas than those seen in mice treated with NDEA alone (2.4 mm^3 versus 1.3 mm^3) (Weghorst *et al*., 1993/94).

Skin: Groups of 25 female SENCAR mice, seven weeks of age, received a single application of 20 µg 7,12-dimethylbenz[*a*]anthracene (DMBA) in 0.2 mL acetone on the skin of the back. One week later, mice received applications of 100 mg per animal di(2-ethylhexyl) phthalate (99% pure) twice weekly for 28 weeks. 12-*O*-Tetradecanoylphorbol 13-acetate (TPA) control groups received 2 µg TPA. To test di(2-ethylhexyl) phthalate as a second-stage promoter, mice received TPA for two weeks followed by di(2-ethylhexyl) phthalate for 26 weeks. Appropriate acetone, TPA and di(2-ethylhexyl) phthalate controls were included. Di(2-ethylhexyl) phthalate, when tested as a complete promoter (28 weeks of exposure), enhanced only slightly the numbers of papillomas (0.88 per mouse versus DMBA alone 0 per mouse) but significantly ($p < 0.01$) enhanced papillomas when given for 26 weeks after two weeks of TPA first-stage promotion (6.44 versus 2.2). The authors concluded that di(2-ethylhexyl) phthalate was a second-stage promoter (Diwan *et al*., 1985; Ward *et al*., 1986).

3.4.2 *Rat*

Liver: Groups of 10 female Fischer 344 rats, six to eight weeks of age, received a single intraperitoneal injection of 150 mg/kg bw NDEA followed three weeks later by

a diet containing 1.2% di(2-ethylhexyl) phthalate (99.5% pure) for three or six months. No neoplasms or nodules were identified. Di(2-ethylhexyl) phthalate did not increase the number of foci or the mean volume of the foci, as identified by five different histological markers (Popp *et al*.,1985).

The initiating activity of di(2-ethylhexyl) phthalate was examined after single and sub-chronic dosing. Di(2-ethylhexyl) phthalate (99.5% pure) was administered as a single oral dose (10 g/kg bw) or by 12 weeks of feeding at a concentration of 1.2% in the diet followed by various known promotion regimens, such as phenobarbital treatment or partial hepatectomy. There was no increase in number or mean volume of foci in liver sections examined using multiple histological markers and no tumours were identified, indicating that di(2-ethylhexyl) phthalate had no initiating activity (Garvey *et al*., 1987).

Groups of 18–20 male Fischer rats (weighing 160 g) were given a single intraperitoneal injection of 200 mg/kg bw NDEA. Two weeks later, they were fed a diet containing 3000 ppm di(2-ethylhexyl) phthalate [purity unspecified] for six weeks. At week 3, they were subjected to a partial hepatectomy. All rats were killed at week 8. Di(2-ethylhexyl) phthalate-treated rats had no increase in foci staining positively for glutathione *S*-transferase placental form (8.5 per cm^2 versus 11.6 for NDEA alone) (Ito *et al*., 1988).

Male Fischer 344 rats were fed 200 ppm 2-acetylaminofluorene (AAF) for seven weeks to induce hepatocellular altered foci, and were subsequently fed 0 or 12 000 ppm di(2-ethylhexyl) phthalate (98% pure) in the diet. No evidence of induction of hepatocellular altered foci or hepatic neoplasms was found when di(2-ethylhexyl) phthalate was given alone for 24 weeks. Di(2-ethylhexyl) phthalate fed for 24 weeks increased basophilic foci, but showed no promoting effect on iron-excluding altered hepatic foci induced by AAF, and produced no significant enhancement of the occurrence of AAF-induced liver neoplasms (3/6 compared with 3/12) (Williams *et al*., 1987).

Di(2-ethylhexyl) phthalate exerted weak promoting activity in weanling female Sprague-Dawley rats after doses of 200 or 500 mg/kg bw, given three times per week by gavage for 11 consecutive weeks after initiation with a single oral dose of 8 mg/kg bw NDEA. Lower doses were ineffective. The incidence of ATPase-deficient foci was enhanced about two-fold compared with rats treated with NDEA alone. The incidence of foci with expression of γ-GT was not affected by di(2-ethylhexyl) phthalate treatment (Oesterle & Deml, 1988).

Male Fischer 344 rats were fed diets containing 200 ppm AAF for seven weeks to induce hepatocellular altered foci, and were then fed diets containing either 0 or 12 000 ppm di(2-ethylhexyl) phthalate (purity, 98%) for 24 weeks. In foci that were induced by AAF, di(2-ethylhexyl) phthalate reduced the activity of γ-GT, as detected histochemically, but did not increase the number, mean volume or volume percentage of foci detected by deficiencies in iron storage, glucose-6-phosphatase, adenosine triphosphatase or fibronectin. Although the numbers of haematoxylin/eosin-stained foci were increased in di(2-ethylhexyl) phthalate-treated rats, the volume percentage was not

increased and no difference in the numbers of iron storage foci was seen (Maruyama *et al.*, 1990).

Urinary system: Groups of 20 male Fischer 344 rats were given 0.05% *N*-ethyl-*N*-hydroxyethylnitrosamine (EHEN) for two weeks in the diet followed by di(2-ethylhexyl) phthalate [purity unspecified] at a concentration of 0 or 1.2% in the diet for 24 weeks. Rats were killed at 27 weeks. Di(2-ethylhexyl) phthalate increased the numbers of rats with renal (tubular) cell tumours (EHEN + di(2-ethylhexyl) phthalate 65% versus 20% for EHEN alone; $p < 0.01$) and the mean number of tumours per kidney (EHEN + di(2-ethylhexyl) phthalate 1.1 versus EHEN alone 0.2, $p < 0.01$) (Kurokawa *et al.*, 1988).

The modifying potential of di(2-ethylhexyl) phthalate on second-stage *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN)-initiated urinary bladder carcinogenesis was investigated in male Fischer 344 rats, using a uracil-accelerated transitional-cell proliferation model. Six-week-old animals received 0.05% BBN in their drinking-water for four weeks followed by di(2-ethylhexyl) phthalate [purity unspecified] (0, 0.3, 0.6 or 1.2% in the diet) for experimental weeks 5–8 and weeks 12–20. Uracil was administered during weeks 9–11 at a dietary level of 3.0%. Surviving animals were killed at the end of week 20 of the experiment. Di(2-ethylhexyl) phthalate did not promote hyperplastic lesions (papillary or nodular) of the urinary bladder or papillomas induced by BBN (Hagiwara *et al.*, 1990).

3.4.3 *Hamster*

Groups of 50 male and 50 female Syrian golden hamsters, six weeks old, were given intraperitoneal injections of 3 g/kg bw di(2-ethylhexyl) phthalate (> 99% pure) either once, or once per week for two or four weeks. *N*-Nitrosodimethylamine (NDMA) was given orally at 1.67 mg/kg bw once [exact week of dosing for both chemicals not given]. Hamsters were maintained for their natural lifespan. Survival was reduced among hamsters receiving NDMA. Di(2-ethylhexyl) phthalate did not affect tumour yield (liver tumours: 16/50 and 9/50 in di(2-ethylhexyl) phthalate + NDMA and NDMA males; and 6/50 and 6/50 in di(2-ethylhexyl) phthalate + NDMA and NDMA females) [statistical analysis not given] (Schmezer *et al.*, 1988).

3.5 Carcinogenicity of the metabolite 2-ethylhexanol

3.5.1 *Mouse*

Groups of 50 male and 50 female B6C3F_1 mice, seven weeks of age, were given 2-ethylhexanol by gavage five times weekly at doses of 0, 50, 200 and 250 mg/kg bw for 18 months. Body weight gain was reduced by 24–26% in the high-dose group and mortality was dose-related. In females, liver carcinomas occurred in 0/50 control, 1/50 low-dose, 3/50 mid-dose and 5/50 ($p < 0.05$ Fisher's exact test) high-dose mice (Astill *et al.*, 1996).

3.5.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were given 2-ethylhexanol by gavage, five times weekly, at doses of 0, 50, 150 or 500 mg/kg bw for 104 weeks. A dose-related depression of body weight gain in male and female rats and increased mortality in high-dose female rats were observed. There was no increase in the incidence of tumours in any treated group (Astill *et al.*, 1996).

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

4.1 Absorption, distribution, metabolism and excretion

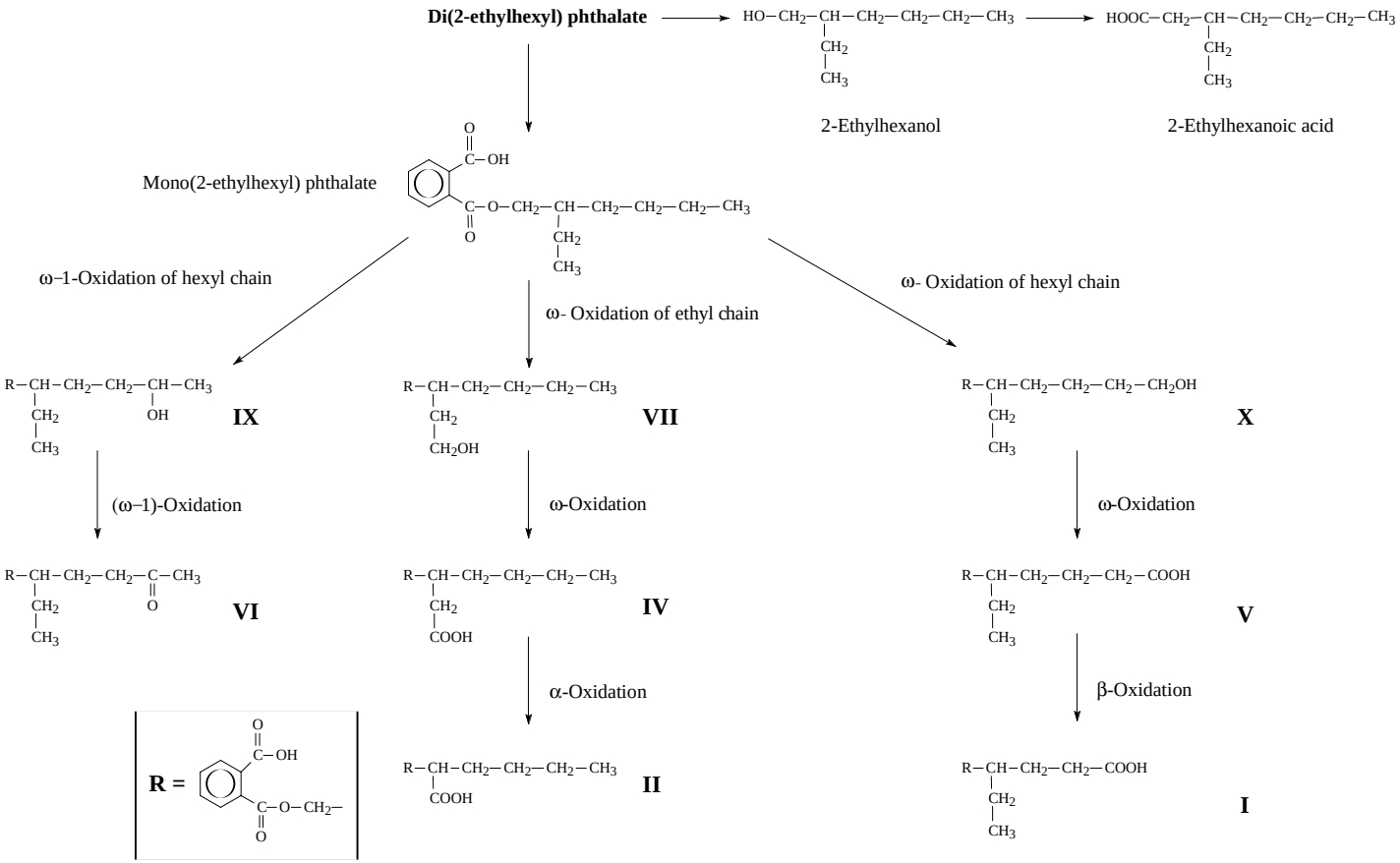
4.1.1 *Humans*

Human exposure to di(2-ethylhexyl) phthalate can occur via the dermal, inhalation, oral and intravenous routes. The high level of exposure has prompted many studies on the absorption, distribution, metabolism and excretion of di(2-ethylhexyl) phthalate in humans (Lawrence & Tuell, 1979; Thomas & Thomas, 1984; Burg, 1988; Albro & Lavenhar, 1989; Kamrin & Mayor, 1991; Huber *et al*., 1996; Doull *et al*., 1999).

In a very early study, Shaffer *et al*. (1945) administered single oral doses of 5 and 10 g di(2-ethylhexyl) phthalate to two human subjects and reported that approximately 4.5% of the dose was excreted in the urine within 24 h. Schmid and Schlatter (1985) also administered di(2-ethylhexyl) phthalate orally to two human subjects, but at the much lower dose of 30 mg per person. These authors reported that 11–15% of the dose was excreted in the urine and a urinary elimination half-life of about 12 h can be estimated from the data. In the same study, the two volunteers also received 10 mg di(2-ethylhexyl) phthalate daily for four days, there being no evidence of accumulation, with 11 and 33 % of the dose recovered each day in the urine. In contrast, Rubin and Schiffer (1976) reported data from two patients receiving platelet transfusions from bags containing di(2-ethylhexyl) phthalate, who excreted between 60 and 90% of the infused dose in the urine collected for 24 h after transfusion.

Figure 1 summarizes the metabolic pathways responsible for the metabolism of di-(2-ethylhexyl) phthalate in humans and in animals, using the widely accepted metabolite nomenclature of Albro and Lavenhar (1989). The quantitative urinary metabolic profile of di(2-ethylhexyl) phthalate reported by Schmid and Schlatter (1985) is quite similar to that found by Albro *et al*. (1982), who determined the urinary metabolites of di(2-ethylhexyl) phthalate in leukaemia patients who received platelet transfusions from bags containing di(2-ethylhexyl) phthalate. These data are also in good agreement with the results of Dirven *et al*. (1993a), who studied the excretion of di(2-ethylhexyl) phthalate metabolites in five workers occupationally exposed to di(2-ethylhexyl) phthalate. The major phase I metabolites of di(2-ethylhexyl) phthalate in human urine are mono(2-

Figure 1. Major pathways involved in the metabolism of di(2-ethylhexyl) phthalate



The numbering of the metabolites is based on the nomenclature of Albro and Lavenhar (1989). This chart is based on the work of Huber *et al.* (1996) and Astill (1989). I, phthalic acid, mono(4-carboxy-2-ethylhexyl) ester; II, phthalic acid, mono(2-carboxyhexyl) ester; IV, phthalic acid, mono(2-(carboxymethyl)hexyl) ester; V, phthalic acid, mono(5-carboxy-2-ethylpentyl) ester; VI, phthalic acid, mono(2-ethyl-5-oxohexyl) ester; VII, phthalic acid, mono(2-(2-hydroxyethyl)hexyl) ester; IX, phthalic acid, mono(2-ethyl-5-hydroxyhexyl) ester; X, phthalic acid, mono(2-ethyl-6-hydroxyhexyl) ester

ethylhexyl) phthalate, and compounds V, VI and IX (Figure 1; Albro *et al*., 1982; Schmid & Schlatter, 1985, Dirven *et al*., 1993b). Dirven *et al*. (1993b) found that the urinary concentration of metabolites VI and IX was 1.7 times higher than the concentration of metabolite V, and concluded that metabolism via (ω–1)-hydroxylation is favoured over ω-hydroxylation in humans. Metabolites VI and IX were reported by Dirven *et al*. (1993b) to be almost completely conjugated, while only 32–45% of metabolite V was present in the conjugated form. The same authors found a marked interindividual variation in the conjugation of mono(2-ethylhexyl) phthalate. In two of the five subjects studied, 77–100% of mono(2-ethylhexyl) phthalate was in the free form, whereas in urine samples from the other three subjects, only 20–38% of mono(2-ethylhexyl) phthalate was not conjugated. Both Albro *et al*. (1982) and Schmidt and Schlatter (1985) reported that up to 80% of the urinary metabolites of di(2-ethylhexyl) phthalate in humans were excreted as glucuronide conjugates. Conjugates of di(2-ethylhexyl) phthalate metabolites with sulfate, taurine and glycine have not been found in any species studied to date.

Rubin and Schiffer (1976) reported peak blood plasma levels of di(2-ethylhexyl) phthalate in adult patients transfused with platelets ranging from 3 to 8 mg/L and described the kinetics of plasma disappearance as mono-exponential, with a half-life of 28 min. The plasma levels ranged from 0.3 to 1.2 mg/kg bw di(2-ethylhexyl) phthalate. This is a low level compared with di(2-ethylhexyl) phthalate levels in whole blood stored in blood bags for up to 21 days (Jaeger & Rubin, 1972; Rock *et al*., 1978; Peck *et al*., 1979), from which a transfusion of 2.5 L of blood to a 70-kg person would give an exposure of 1.3–2.6 mg/kg bw di(2-ethylhexyl) phthalate. Similar levels of exposure (1.7 to 4.2 mg/kg bw) have been reported in newborn infants during single exchange transfusions (Sjöberg *et al*., 1985a).

Lewis *et al*. (1978) determined the disappearance of di(2-ethylhexyl) phthalate in seven adult patients following dialysis. The mean serum levels were 606 μg/L immediately after dialysis, 323 μg/L at 30 min, 167 μg/L at 1 h and 145 μg/L at 3 h after completion of dialysis. They concluded that most of the di(2-ethylhexyl) phthalate present in serum at the completion of dialysis is likely to disappear within 5–7 h. Sjöberg *et al*. (1985b) and Plonait *et al*. (1993) also described a rapid decline in serum di(2-ethylhexyl) phthalate levels in term newborns similar to that seen in adults. Sjöberg *et al*. (1985b) reported that plasma levels of di(2-ethylhexyl) phthalate declined biexponentially after transfusions in newborn infants, with the half-life of the terminal phase being approximately 10 h. The terminal half-life of the active metabolite of di(2-ethylhexyl) phthalate, mono(2-ethylhexyl) phthalate, in newborn infants was similar (Sjöberg *et al*., 1985b). However, in preterm infants, di(2-ethylhexyl) phthalate can persist in serum for a much longer time and it is postulated that either there are different kinetics resulting from immature metabolism or there is a biphasic disappearance pattern, as Stern *et al*. (1977) and Sjöberg *et al*. (1985c,d) observed in rats (Plonait *et al*., 1993). In adult haemodialysis patients, circulating levels of mono(2-ethylhexyl) phthalate are similar to

those of di(2-ethylhexyl) phthalate, showing that this metabolite is formed after systemic intake in humans (Pollack *et al*., 1985). Further, di(2-ethylhexyl) phthalate can be hydrolysed by plasma proteins in blood products to mono(2-ethylhexyl) phthalate. Hence, patients undergoing transfusion may be exposed to mono(2-ethylhexyl) phthalate at doses of up to one tenth those of di(2-ethylhexyl) phthalate (Rock *et al*., 1978).

Absorption of di(2-ethylhexyl) phthalate via the lungs is considered to be the major route of occupational exposure, as dermal absorption through preparations of human skin *in vitro* is low (Scott *et al*., 1987; Dirven *et al*., 1993a; Huber *et al*., 1996). Occupational exposure to di(2-ethylhexyl) phthalate based on personal air sampling data in the range 9–1266 μg/m^3 has been reported (Dirven *et al*., 1993a). Furthermore, Dirven *et al*. (1993a) evaluated the inhalation exposure to di(2-ethylhexyl) phthalate in a group of nine volunteers in a boot factory in an attempt to evaluate the absorption and disposition of the plasticizer. They reported that the median values of three major di(2-ethylhexyl) phthalate metabolites studied (VI, IX and V; see Figure 1) were significantly increased (1.2–2.3-fold) in urine samples collected at the end of the workday compared with urine samples collected at the start of the workday. However, a similar comparison in six cable factory workers detected no statistically significant increase in post-shift urinary di(2-ethylhexyl) phthalate metabolite concentrations compared with pre-shift values, although the airborne exposure levels were within a similar range. As pointed out by the authors, a meaningful interpretation of these data is impeded by the lack of understanding of the toxicokinetics of di(2-ethylhexyl) phthalate metabolites in humans.

4.1.2 *Experimental systems*

The absorption and disposition of di(2-ethylhexyl) phthalate has been extensively investigated in laboratory animals (Thomas & Thomas, 1984; Albro & Lavenhar, 1989; Astill, 1989; Huber *et al*., 1996; Doull *et al*., 1999). The first step in the metabolism of di(2-ethylhexyl) phthalate in all species is the hydrolysis of one of the two ethylhexyl side-chains to yield mono(2-ethylhexyl) phthalate and 2-ethylhexanol. After oral administration of di(2-ethylhexyl) phthalate, this pathway is primarily catalysed by pancreatic lipase. The level of pancreatic lipase in the intestine of laboratory animals has been shown to exhibit marked species and strain differences (Albro & Thomas, 1973). Lower levels of di(2-ethylhexyl) phthalate-hydrolysing enzyme activity are expressed in the liver, blood and other tissues (Albro, 1986; Huber *et al*., 1996). It is generally accepted that in all mammalian species the majority of di(2-ethylhexyl) phthalate reaching the intestine is absorbed as hydrolysis products rather than as the intact diester (Albro & Lavenhar, 1989). For example, Teirlynck and Belpaire (1985) studied the disposition of both di(2-ethylhexyl) phthalate and mono(2-ethylhexyl) phthalate in immature male Wistar rats and reported that, after a single oral dose of di(2-ethylhexyl) phthalate (2.8 g/kg), plasma concentrations of 8.8 ± 1.7 μg/mL di(2-ethylhexyl) phthalate and 63.2 ± 8.7 μg/mL mono(2-ethylhexyl) phthalate were reached

after 3 h. The plasma concentration of mono(2-ethylhexyl) phthalate declined with a half-life of 5.2 h, whereas the corresponding value for di(2-ethylhexyl) phthalate could not be estimated due to very low levels of the parent diester.

The efficiency of di(2-ethylhexyl) phthalate elimination from the gastrointestinal tract differs between species. Ikeda *et al*. (1980) fed 50 mg/kg bw di(2-ethylhexyl) phthalate per day to male Sprague-Dawley rats, male pure-bred beagles and male immature swine of the Hormel strain for 21–28 days and then administered a single dose of ^{14}C-labelled di(2-ethylhexyl) phthalate at 50 mg/kg bw on the last day. In rats, 37.5 and 53.2% of the administered radioactivity were excreted in the urine and faeces, respectively, within four days. In dogs, the figures showed that the faecal route of excretion dominated (75%), with the urinary route accounting for 20.7% of the radioactivity. The opposite was the case in pigs, with the faecal and urinary routes accounting for 25.7% and 79.4%, respectively. In male Wistar rats given a single 2.9 mg/kg bw oral dose of [^{14}C]di(2-ethylhexyl) phthalate, Daniel and Bratt (1974) found 42% and 57% of the dose in the urine and faeces within seven days. In the same study, in rats fed 2.6 mg/kg bw [^{14}C]di(2-ethylhexyl) phthalate, 14% of the dose was excreted in the bile.

Rhodes *et al*. (1986) found that the absorption of di(2-ethylhexyl) phthalate was considerably lower in marmosets than in rats. These authors reported that 45% and 7% of di(2-ethylhexyl) phthalate were absorbed by marmosets at doses of 100 mg/kg bw and 2000 mg/kg bw, respectively. However, Astill (1989) observed that, after a single 100 mg/kg bw gavage dose of di(2-ethylhexyl) [*carbonyl*-^{14}C]phthalate, faecal elimination accounted for approximately 50% of the dose in cynomolgus monkeys, male Fischer 344 rats and male B6C3F$_1$ mice. Urinary excretion levels were also similar across the three species, ranging from 28% in monkeys, 33% in rats to 37% in mice over approximately the first 96 h. According to this study, the excretion of di(2-ethylhexyl) phthalate in rats varied depending on whether it was administered by gavage or in the diet. In African green monkeys, di(2-ethylhexyl) phthalate was rapidly and extensively metabolized, with urinary excretion being 80% in the form of glucuronide conjugates (Albro *et al*., 1981, 1982). Peck and Albro (1982) also showed that di(2-ethylhexyl) phthalate was rapidly and extensively metabolized in both African green monkeys and humans. Di(2-ethylhexyl) phthalate is excreted largely in the urine by both African green monkeys and humans (> 60% in 24 h) as glucuronide conjugates (~80%) of the oxidation products of mono(2-ethylhexyl) phthalate. The predominant hydrolysable urinary metabolites were metabolite IX (26.1%) and mono-(2-ethylhexyl) phthalate (19.6%) and faecal excretion in monkeys accounted for approximately 8% of the administered di(2-ethylhexyl) phthalate 48 h after infusion. In contrast to the extent of conjugation of urinary metabolites in African green monkeys, only 16% of the urinary metabolites of di(2-ethylhexyl) phthalate in cynomolgus monkeys were in the form of hydrolysable products (Astill, 1989). Di(2-ethylhexyl) phthalate excretion in urine and faeces and the subsequent metabolic patterns of di(2-ethylhexyl) phthalate were stated to be quantitatively similar in cynomolgus monkeys, rats and mice.

In making species comparisons, careful interpretation of data is required, as the physical state of the di(2-ethylhexyl) phthalate used can affect the outcome (Thomas & Thomas, 1984; Albro & Lavenhar, 1989; Astill, 1989). For example, the disposition half-life in the rat of [^{14}C]di(2-ethylhexyl) phthalate solubilized in aqueous polysorbate emulsion, in plasma containing polysorbate emulsion, in plasma containing ethanol or in the plasticizer leached from the plastic into the plasma was 263, 83, 181 and 31 min, respectively (Miripol *et al*., 1975, cited in Thomas & Thomas, 1984). Albro and Lavenhar (1989) reported that intact di(2-ethylhexyl) phthalate was not absorbed until the dose reached 500 mg/kg bw in CD-1 mice and 450 mg/kg bw in Fischer 344 rats. In contrast, no threshold was seen in B6C3F$_1$ mice: the amount of di(2-ethylhexyl) phthalate absorbed was directly proportional to the dose down to a dose of 20 mg/kg bw. Further, Astill (1989) reported that prolonged feeding or the use of high doses of up to 1000 mg/kg bw did not influence the absorption of di(2-ethylhexyl) phthalate from the gastrointestinal tract of male Fischer 344 rats. While most of a dose of di(2-ethylhexyl) phthalate is absorbed as mono(2-ethylhexyl) phthalate, more of the parent diester reaches the circulation at high doses (Albro *et al*., 1982).

Because distribution studies have monitored total radioactivity, our understanding of the distribution of intact di(2-ethylhexyl) phthalate is limited. Chu *et al*. (1978) studied the metabolism and distribution of mono(2-ethylhexyl) phthalate in rats after oral dosing and found that the intestine contained the highest tissues levels after 24 h. The liver, heart, lung and muscle each contained approximately half the level in the intestine. They also reported that 80% of the ^{14}C-dose of mono(2-ethylhexyl) phthalate was eliminated 24 h after oral administration, 72% in the urine and 8% in the faeces. Twenty minutes after the administration of an intravenous dose of [^{14}C]mono(2-ethylhexyl) phthalate, Chu *et al*. (1978) found comparable levels in the liver, kidney and bladder, with other organs containing approximately 10–25% of the level of the liver.

Species differences in the metabolism of di(2-ethylhexyl) phthalate have been reported and attempts have been made to explain the susceptibility of animals to di(2-ethylhexyl) phthalate-induced hepatic peroxisome proliferation based on their metabolic profiles (Doull *et al*., 1999). As mentioned above, the bulk of a di(2-ethylhexyl) phthalate dose is absorbed as the mono-ester, mono(2-ethylhexyl) phthalate, and following absorption this metabolite is subjected to extensive oxidative metabolism mediated by cytochrome P450 enzymes (Albro & Lavenhar, 1989; Astill, 1989; Huber *et al*., 1996; Doull *et al*., 1999). The metabolism of mono(2-ethylhexyl) phthalate has been summarized by Doull *et al*. (1999) as follows (see Figure 1):

1. hydroxylation of the terminal carbon atoms (ω-oxidation) of both the hexyl and ethyl side-chains and the penultimate carbon (ω–1-oxidation) of the hexyl chain;
2. conversion of these hydroxyl groups to either a carboxylic acid (ω-oxidation) or a ketone (ω–1-oxidation); and
3. further metabolism (shortening of the carbon chains) of the dicarboxylic acid products of ω-oxidation by α- or β-oxidation.

Mono(2-ethylhexyl) phthalate and metabolites VI and IX are considered to be primarily responsible for the peroxisome-proliferating activity of di(2-ethylhexyl) phthalate (Mitchell *et al*., 1985; Elcombe & Mitchell, 1986; Cornu *et al*., 1992; Keith *et al*., 1992; Elcombe *et al*., 1996; Doull *et al*., 1999). These are the keto and hydroxyl metabolites of mono(2-ethylhexyl) phthalate that are produced via ω–1-oxidation. Mono(2-ethylhexyl) phthalate undergoes extensive metabolism in rats, with approximately 75% of all hydrolysable urinary metabolites consisting of dicarboxylic acids, mainly metabolited V, resulting from two oxidative steps, which accounts for 51.3% of the urinary metabolites of di(2-ethylhexyl) phthalate in rats (Albro *et al*., 1981). African green monkeys, guinea-pigs and humans excrete approximately 5% of a dose of di(2-ethylhexyl) phthalate in the urine as metabolite V, while mice and hamsters excrete 1.1% and 14%, respectively (Albro *et al*., 1981, 1982). Interestingly, two other studies have reported that this metabolite comprises approximately 20–30% of urinary metabolites in humans (Schmid & Schlatter, 1985; Dirven *et al*., 1993b). In another comparative study, Astill (1989) reported that 5.7%, 8.4% and 0.3% of a 100-mg/kg bw single gavage dose of di(2-ethylhexyl) phthalate was excreted in the urine in the first 24 h as metabolite V by cynomolgus monkeys, rats and mice, respectively. In mice, 18.6% of hydrolysable metabolites of di(2-ethylhexyl) phthalate was excreted as mono(2-ethylhexyl) phthalate and this metabolite ranged from 71.2% in guinea-pigs, 28.9% in African green monkeys, 18.3% in humans to 4.5% in hamsters (Albro *et al*., 1981, 1982). In contrast, mono(2-ethylhexyl) phthalate was present only in trace amounts in rat urine. In rats and mice, metabolite IX accounted for 13.3% and 12.3% of hydrolysable urinary metabolites, whereas in African green monkeys, hamsters and humans, this metabolite could account for as much as 30% of urinary metabolites (Albro *et al*., 1982). In contrast, Astill (1989) reported that similar amounts of metabolite IX were excreted in the urine of cynomolgus monkeys, rats and mice. It should be emphasized that the above data for African green monkeys and humans in the studies by Albro and coworkers (Albro *et al*., 1981, 1982) were collected following intravenous administration of di(2-ethylhexyl) phthalate. The study by Dirven *et al*. (1993b) involved occupational exposure of humans via inhalation, whereas all the remaining studies reported findings following a single oral dose. Therefore, the possibility that the route of administration affected the metabolic profile cannot be ruled out.

Dermal absorption of di(2-ethylhexyl) phthalate is slow in rats (Melnick *et al*., 1987; Scott *et al*., 1987; Albro & Lavenhar, 1989; Chu *et al*., 1996; Deisinger *et al*., 1998). Fetal exposure to [^{14}C]di(2-ethylhexyl) phthalate or its metabolites occurs following intraperitoneal administration to pregnant rats (Singh *et al*., 1975). Liver microsomes from fetuses (21 days of gestation) and neonates (one- and five-day-old) *in vitro* are capable of metabolizing mono(2-ethylhexyl) phthalate. Further, liver microsomes from mature animals metabolize mono(2-ethylhexyl) phthalate at a rate similar to those from five-day-old rats, demonstrating rapid postnatal development of mono(2-ethylhexyl) phthalate (ω–1)-hydroxylase activity in rats (Sjöberg *et al*., 1988). In rats given high oral doses of di(2-ethylhexyl) phthalate (2000 mg/kg bw)

during lactation, large amounts of di(2-ethylhexyl) phthalate (e.g., 216 µg/mL 6 h after dosing) and smaller, yet significant amounts of mono(2-ethylhexyl) phthalate (e.g., 25 µg/mL 6 h after dosing) were transported into the milk (Dostal *et al*., 1987a). While occupational exposure via inhalation is considered to be a major route of human exposure to di(2-ethylhexyl) phthalate, there is no information available on the toxicokinetics or metabolic disposition of this compound in animals exposed via this route.

Placental transfer of di(2-ethylhexyl) phthalate has been observed following intraperitoneal administration of di(2-ethylhexyl) [*carbonyl*-^{14}C]phthalate on gestational day 5 or 10 in rats (Singh *et al*., 1975). The dams were killed at 24-h intervals starting on days 8 and 11 until day 20 of gestation. Radioactivity was detected in fetal tissues, amniotic fluid and placenta at all time points. The radioactivity peaked at 48 h and declined rapidly thereafter. The concentration was less than that in maternal blood and less than 1% of the administered dose.

Radiolabelled di(2-ethylhexyl) phthalate (10 mL/kg) was administered by oral gavage to ddY-SLC mice on gestation day 8. Three and 12 h after exposure, levels of di(2-ethylhexyl) phthalate in the fetuses were 522 µg/g and 426 µg/g, respectively. The level of mono(2-ethylhexyl) phthalate in the fetus was approximately 1% that of di(2-ethylhexyl) phthalate (Tomita *et al.*, 1986).

Sjöberg *et al*. (1985d) also studied the kinetics of orally administered di(2-ethylhexyl) phthalate and its excretion in groups of 10 rats 25, 40 or 60 days old. The AUC (area under the plasma curve) for 0–30 h was significantly higher (about two-fold) in the 25-day-old rats than in the older rats; the half-life was, however, not significantly higher in the 25-day-old rats (3.9 h compared with 3.1 h and 2.8 h at 40 and 60 days, respectively). The amount of di(2-ethylhexyl) phthalate-derived products excreted in the urine was twice as high in 25- as in 60-day-old rats. It was concluded that the toxicokinetic differences may in part explain the age-dependent effect of di(2-ethylhexyl) phthalate on the testes (see Section 4.3.2).

4.2 Toxic effects

4.2.1 *Humans*

Dermally applied di(2-ethylhexyl) phthalate was considered to be moderately irritating, but only slightly or non-sensitizing to human skin (Shaffer *et al.*, 1945; Mallette & von Haam, 1952). Two adults given single oral doses of either 5 or 10 g di(2-ethylhexyl) phthalate exhibited no untoward effects apart from mild gastric disturbances and moderate diarrhoea at the higher dose (Shaffer *et al*., 1945). There are few data on effects of occupational exposure specifically to di(2-ethylhexyl) phthalate (WHO, 1992). In a study involving workers at a Swedish PVC-processing factory, peripheral nervous system symptoms and signs were investigated in 54 workers exposed to di(2-ethylhexyl) phthalate and other phthalate diesters. Some workers showed various peripheral nervous system symptoms and signs, but these

were not related to the level of exposure to phthalate diesters. None of the workers reported symptoms indicating work-related obstructive lung disease and conventional lung function tests showed no association with exposure to phthalate diesters (Nielsen *et al.*, 1985). One case of occupational exposure to di(2-ethylhexyl) phthalate was associated with asthma in a worker at a PVC-processing plant (WHO, 1992).

Di(2-ethylhexyl) phthalate is leached in significant amounts from the PVC tubing used in transfusion and dialysis. Dialysis patients were studied for evidence of liver peroxisome proliferation in biopsy samples (Ganning *et al*., 1984, 1987). Based on subjective ultrastructural evaluation of one subject, no effect was seen after one month of dialysis. However, in a liver biopsy from another subject after 12 months of dialysis, an increased number of peroxisomes was reported to be present. Others have suggested that more cautious evaluation, including objective measurements, increased numbers of biopsy intervals, and appropriate controls, would be needed to determine conclusively whether peroxisome proliferation due to di(2-ethylhexyl) phthalate occurs in dialysis patients (Huber *et al*., 1996). [The Working Group noted that biopsy study of Ganning *et al.* (1984, 1987) is not adequate for evaluation.]

The toxicity of di(2-ethylhexyl) phthalate was evaluated in 28 term infants with respiratory failure, 18 of whom received extracorporeal membrane oxygenation (ECMO) and were compared with 10 untreated infants. Various clinical parameters of liver, pulmonary and cardiac dysfunction were found to be unaffected in treated infants, even though the rate of administration ranged up to 2 mg/kg bw di(2-ethylhexyl) phthalate over 3–10 days (mean peak plasma concentration, 8 μg/mL). ECMO is considered to be the clinical intervention that results in the highest intravenous dose of di(2-ethylhexyl) phthalate (Karle *et al*., 1997).

Mettang *et al*. (1996b) investigated the relationship between di(2-ethylhexyl) phthalate exposure and uraemic pruritus in dialysis patients. There was no relationship between severity of pruritus and post-dialysis serum concentrations of di(2-ethylhexyl) phthalate, mono(2-ethylhexyl) phthalate, phthalic acid or 2-ethylhexanol. Furthermore, serum concentrations of di(2-ethylhexyl) phthalate and these related compounds were not significantly different between patients with or without uraemic pruritus.

4.2.2 *Experimental systems*

In-vivo studies of general toxic effects

Acute oral LD_{50} values for di(2-ethylhexyl) phthalate ranging from 26.3 to 33.9 g/kg bw in rats, mice, guinea-pigs and rabbits have been reported (Shaffer *et al*., 1945; Lawrence *et al*., 1975; IARC, 1982). LD_{50} values after intraperitoneal administration were 30.7 g/kg bw in rats and 14.2 and 37.8 g/kg bw in mice (Shaffer *et al*., 1945; Calley *et al*., 1966; Lawrence *et al*., 1975).

Nair *et al*. (1998) evaluated the systemic toxicity of di(2-ethylhexyl) phthalate (0–7.5 mg/kg bw given up to six times by intraperitoneal injection on alternate days) in male Wistar rats (approximately 150 g bw). Animals were evaluated by organ

weight (testis and liver), light microscopy (liver, heart, brain and testis) and plasma clinical chemistry (γ-GT, lactic dehydrogenase, alanine aminotransferase, alkaline phosphatase). No evidence of toxicity was observed.

In rats, age may be an important factor in sensitivity to the lethal effects of oral di(2-ethylhexyl) phthalate. Dostal *et al*. (1987b) evaluated the effects of di(2-ethylhexyl) phthalate (five daily oral doses of 0, 10, 100, 1000 or 2000 mg/kg bw) in male Sprague-Dawley rats, beginning at 6, 14, 16, 21, 42 or 86 days of age. Suckling rats (starting at 6–21 days of age) suffered severe growth retardation at doses of 1000 mg/kg bw and death at 2000 mg/kg bw, while older rats showed only decreased weight gain at 2000 mg/kg bw. Lethality was observed at doses of 1000 mg/kg bw starting at 14 days of age but not at six days or ≥ 16 days.

A number of other studies have examined the toxic effects of di(2-ethylhexyl) phthalate in rodents and other species following oral administration. Young, post-weanling male and female Sprague-Dawley (CD) rats were fed diets containing 0.2, 1.0 and 2.0% di(2-ethylhexyl) phthalate for 17 weeks, resulting in mean di(2-ethylhexyl) phthalate intakes of 143, 737 or 1440 mg/kg bw per day in males and 154, 797 or 1414 mg/kg bw per day in females, respectively (Gray *et al*., 1977). Significant dose-dependent increases in relative liver weight to 116–204% of control values were observed in both males and females at all levels of di(2-ethylhexyl) phthalate treatment. Di(2-ethylhexyl) phthalate levels of 1.0 and 2.0% resulted in reduced testis weights in male rats, associated with marked seminiferous tubule atrophy. Apart from the liver, no relative organ weights were affected in male and female rats given 0.2% di(2-ethylhexyl) phthalate in the diet.

In another study, young male and female Sprague-Dawley rats (10 per sex per group) were fed diets containing 5, 50, 500 or 5000 ppm di(2-ethylhexyl) phthalate for 13 weeks (Poon *et al.*, 1997). Mean di(2-ethylhexyl) phthalate intakes were 0.4, 3.7, 38 and 375 mg/kg bw per day in males and 0.4, 4.2, 42 and 419 mg/kg bw per day in females, respectively. No clinical signs of toxicity were observed, and body weight gain and food consumption were not affected. Significant increases in relative liver weight, to 146 and 124% of control values in male and female rats, respectively, were observed only in animals given 5000 ppm di(2-ethylhexyl) phthalate. Relative testis weight was reduced in male rats fed 5000 ppm di(2-ethylhexyl) phthalate. Morphological examination revealed minimal to mild centrilobular hypertrophy in the liver and mild to moderate seminiferous tubule atrophy in the testis in male rats fed 5000 ppm di(2-ethylhexyl) phthalate and Sertoli cell vacuolation of minimal nature in male rats fed 500 ppm di(2-ethylhexyl) phthalate.

In-vivo studies of hepatic peroxisome proliferation in rats and mice

Many studies have examined the hepatic biochemical and morphological effects of di(2-ethylhexyl) phthalate (reviewed in Huber *et al*., 1996). Such effects in rodents include peroxisome proliferation, which is characterized by increases in the number of

hepatocellular peroxisomes, the induction of peroxisomal and microsomal fatty acid-oxidizing enzymes and hepatocellular hyperplasia.

Young male Wistar rats were given 2000 mg/kg bw di(2-ethylhexyl) phthalate per day by gavage for periods of 3–21 days (Lake *et al*., 1975). Treatment caused increases in relative liver weight and in microsomal cytochrome P450 content. Ultrastructural examination revealed marked peroxisome proliferation and a dilation of the smooth and rough endoplasmic reticulum. Rats were also treated with mono(2-ethylhexyl) phthalate, 2-ethylhexanol and phthalic acid at doses equimolar to 2000 mg/kg bw per day di(2-ethylhexyl) phthalate for seven days. While phthalic acid had no effect, both mono(2-ethylhexyl) phthalate and 2-ethylhexanol increased relative liver weight and produced hepatic peroxisome proliferation.

Male Fischer 344 rats (body weight, 100–150 g) were fed 0.5–4% di(2-ethylhexyl) phthalate in the diet for one or four weeks and male Swiss Webster mice (20–30 g) were fed 2 or 4% di(2-ethylhexyl) phthalate in the diet for one or four weeks (Reddy *et al*., 1976). Di(2-ethylhexyl) phthalate increased relative liver weights and markedly induced hepatic carnitine acetyltransferase activity in both species (up to 25-fold in rats and 10-fold in mice). Some increase in hepatic catalase activity (approximately two-fold) was observed and subjective (non-morphometric) ultrastructural examination revealed marked peroxisome proliferation. This study also demonstrated that di(2-ethylhexyl) phthalate was a hypolipidaemic agent, as serum triglyceride levels were reduced to one seventh of control values in rats and one third of control values in mice.

Male and female Fischer 344 rats and B6C3F_1 mice were fed di(2-ethylhexyl) phthalate for up to 13 weeks (David *et al*., 1999). In rats fed 12 500 ppm di(2-ethylhexyl) phthalate, there was an increase in hepatocyte replicative DNA synthesis (measured after continuous bromodeoxyuridine administration (osmotic pump) for three days before sampling) after one week (but not after two or 13 weeks) and an increase in hepatic peroxisomal β-oxidation activity after one, two and 13 weeks' administration. In mice fed 10 000 and 17 500 ppm di(2-ethylhexyl) phthalate, there was no increase in hepatocyte replicative DNA synthesis (measured after continuous bromodeoxyuridine three days before sampling) after one, two or 13 weeks of administration, but there was an increase in hepatic peroxisomal β-oxidation activity after one, two and 13 weeks' administration. In mice fed 1000 ppm di(2-ethylhexyl) phthalate, there was no statistically significant increase in hepatic peroxisomal β-oxidation activity after one, two or 13 weeks' administration (bromodeoxyuridine labelling was not evaluated at this lower dietary concentration of di(2-ethylhexyl) phthalate).

Treatment of five-week-old male Sprague-Dawley rats with 1000 mg/kg bw di(2-ethylhexyl) phthalate per day by gavage for 14 days caused increased relative liver weight and hepatic peroxisome proliferation (Lake *et al*., 1984). In addition, di(2-ethylhexyl) phthalate treatment induced cyanide-insensitive palmitoyl-coenzyme A (CoA) oxidation activity and microsomal lauric acid 12-hydroxylase activity in the

liver. While the former is considered to be a specific marker of the peroxisomal fatty acid β-oxidation cycle, the latter reflects induction of cytochrome P450 isoforms in the CYP4A subfamily. CYP4A induction is generally associated with peroxisome proliferation (Huber *et al.*, 1996). Induction of these two enzymatic markers has also been observed in other studies. Di(2-ethylhexyl) phthalate at dietary levels of 0.01–2.5% to young male and female Fischer 344 rats for 21 days produced dose-related increases in relative liver weight and in cyanide-insensitive palmitoyl-CoA oxidation and lauric acid 12-hydroxylase activities (Barber *et al.*, 1987). Reddy *et al.* (1986) fed diets containing 0.25–2.0% di(2-ethylhexyl) phthalate to young male Fischer 344 rats for 30 days. Dose-related increases in relative liver weight, cyanide-insensitive palmitoyl-CoA oxidation activity and peroxisome volume density were observed. In this study, an excellent correlation between the enzymatic marker of the peroxisomal fatty acid β-oxidation cycle and changes in peroxisome morphometry was observed, demonstrating that peroxisomal cyanide-insensitive palmitoyl-CoA oxidation is a good marker for peroxisome proliferation in rodent liver.

Di(2-ethylhexyl) phthalate-induced hepatic peroxisome proliferation in rodents is not due to the parent diester *per se*, but rather to its metabolites. With mono(2-ethylhexyl) phthalate, the two 'proximate' peroxisome proliferators were found to be the (ω–1)-hydroxy (IX) and keto (VI) metabolites, while for 2-ethylhexanol the 'proximate' peroxisome proliferator was 2-ethylhexanoic acid (Mitchell *et al.*, 1985; Elcombe & Mitchell, 1986; Cornu *et al.*, 1992; Lewis & Lake, 1993).

Studies of peroxisome proliferator-activated receptor α (PPARα)

It has been established that the peroxisome proliferation and hepatocellular proliferation effects of di(2-ethylhexyl) phthalate and other peroxisome proliferators in rodent liver are mediated through activation of the peroxisome proliferator-activated receptor α (PPARα). This nuclear receptor is a member of the steroid hormone receptor superfamily. Its role in mediating the hepatic effects of peroxisome proliferators was demonstrated with knock-out mice that do not express the PPARα receptor (Lee *et al.*, 1995; Peters *et al.*, 1997). Knock-out mice fed di(2-ethylhexyl) phthalate at 1.2% in the diet for 24 weeks showed none of the hepatic effects (liver weight increase, induction of mRNA expression for peroxisomal acyl CoA oxidase and microsomal CYP4A) that were observed in SV129 strain control mice (Ward *et al.*, 1998). This relatively high intake of di(2-ethylhexyl) phthalate induced histological evidence of testicular and renal toxicity in both strains of mice, although earlier onset and greater severity were seen in the SV129 strain control mice than in the knock-out mice. These results establish an absolute requirement for PPARα in the manifestation of hepatic effects of di(2-ethylhexyl) phthalate, and indicate a contributory role of the receptor in mediating extrahepatic toxicity.

In non-hepatocytic systems *in vitro*, addition of mono(2-ethylhexyl) phthalate results in activation of PPARα (Issemann & Green, 1990; Isseman *et al.*, 1993). Such

systems were also used to study the ability of di(2-ethylhexyl) phthalate and its metabolites to activate nuclear receptors PPARα from mouse and human (Maloney & Waxman, 1999). In COS-1 cells transfected to express high levels of mouse or human PPARα, mono(2-ethylhexyl) phthalate and 2-ethylhexanoic acid, but not di(2-ethylhexyl) phthalate, caused transactivation of a responsive reporter gene construct.

Other hepatic studies in vivo

Other hepatic effects of di(2-ethylhexyl) phthalate in rodent liver include effects on replicative DNA synthesis and oxidative stress. Male Fischer 344 rats (seven to nine weeks old) were fed di(2-ethylhexyl) phthalate in the diet for various periods up to 365 days (Marsman *et al*., 1988; Conway *et al*., 1989). Replicative DNA synthesis was studied employing subcutaneously implanted osmotic pumps to continuously infuse [^{3}H]thymidine over seven-day periods (Marsman *et al*., 1988). Liver sections were processed for autoradiography and subsequent determination of the hepatocyte labelling index. Di(2-ethylhexyl) phthalate produced a burst of replicative DNA synthesis during treatment days 1–8, whereas at later time points, rates of replicative DNA synthesis were similar to those in control animals. Over the 365-day treatment period, di(2-ethylhexyl) phthalate produced a sustained stimulation of peroxisome proliferation, as demonstrated by cyanide-insensitive palmitoyl-CoA oxidation activity and peroxisome morphometry. Di(2-ethylhexyl) phthalate was found to increase levels of lipofuscin, as a marker of oxidative stress, three-fold after 39 days of treatment, this level being maintained throughout the rest of the treatment period (Conway *et al*., 1989). In another study, male Sprague-Dawley rats were fed a diet containing 2% di(2-ethylhexyl) phthalate for two years (Lake *et al*., 1987). Levels of conjugated dienes were increased in liver homogenates and morphological examination of liver sections revealed increased lipofuscin deposition in non-nodular/tumorous areas of the liver. Takagi *et al*. (1990a,b) investigated the relationship between hepatic peroxisome proliferation and levels of 8-hydroxydeoxyguanosine in hepatic DNA. Male Fischer 344 rats (six weeks old) were fed 1.2% di(2-ethylhexyl) phthalate in the diet for periods of 1–12 months. Treatment with di(2-ethylhexyl) phthalate resulted in sustained stimulation of cyanide-insensitive palmitoyl-CoA activity and produced up to a twofold increase in levels of 8-hydroxydeoxyguanosine in hepatic DNA.

Two studies have demonstrated that oral administration of di(2-ethylhexyl) phthalate to mice results in increased replicative DNA synthesis in hepatocytes. In the first study, administration of oral di(2-ethylhexyl) phthalate (1150 mg/kg bw per day) to male B6C3F$_1$ mice for two days resulted in a 2.4-fold increase in replicative DNA synthesis (James *et al*., 1998). In the second study, feeding a diet containing 6000 ppm di(2-ethylhexyl) phthalate to male B6C3F$_1$ mice for seven days resulted in a sevenfold increase in replicative DNA synthesis in hepatocytes, while no increase was observed when the animals were fed for 14 or 28 days (Smith-Oliver & Butterworth, 1987).

Other hepatic effects have also been attributed to di(2-ethylhexyl) phthalate. Increases of about 30% in hepatic phosphatidylcholine and phosphatidylethanolamine were observed in male Wistar rats fed 2% di(2-ethylhexyl) phthalate in the diet for seven days (Mizuguchi *et al*., 1999).

Interspecies comparisons in vivo

Many studies have demonstrated marked species differences in hepatic peroxisome proliferation. Male Wistar rats (250–350 g), male and female Sprague-Dawley rats (140–170 g), male mice (20–30 g) and male guinea-pigs (320–340 g) were fed 2% di(2-ethylhexyl) phthalate in the diet for two weeks (Osumi & Hashimoto, 1978). Hepatic cyanide-insensitive palmitoyl-CoA oxidation activity was significantly increased in both rat strains, with a more marked effect in male than in female Sprague-Dawley rats. Marked induction of enzyme activity was also observed in the mice, but none in guinea-pigs. In another study, male Fischer 344 rats and male Dunkin-Hartley guinea-pigs were given 950 mg/kg bw per day by gavage for four days (Hasmall *et al*., 2000). Significant increases in liver weight, hepatic β-oxidation activity and hepatocyte DNA replication, and decreases in hepatocyte apoptosis were observed in the rats but not the guinea-pigs. In another study, male Sprague-Dawley rats (five weeks old) and male Syrian hamsters (five weeks old) were given di(2-ethylhexyl) phthalate at doses of 25–1000 mg/kg bw per day by gavage for 14 days (Lake *et al*., 1984). In rats, treatment with di(2-ethylhexyl) phthalate produced dose-related increases in relative liver weight and hepatic cyanide-insensitive palmitoyl-CoA oxidation and carnitine acetyltransferase activities. While some effect was also observed in Syrian hamsters, this species was clearly less responsive to di(2-ethylhexyl) phthalate-induced hepatic peroxisome proliferation than rats (liver weight: up to 176% increase in rats versus 122% increase in hamsters; cyanide-insensitive palmitoyl-CoA oxidation: up to 1400% increase in rats versus 200% increase in hamsters; carnitine acetyltransferase: up to 1000% increase in rats versus 180% increase in hamsters). In the same study, rats and Syrian hamsters were also treated with 500 mg/kg bw mono(2-ethylhexyl) phthalate per day. As with di(2-ethylhexyl) phthalate, mono(2-ethylhexyl) phthalate produced a greater increase in relative liver weight and a greater stimulation of enzyme activities in rats than in Syrian hamsters (liver weight: 65% increase in rats versus 10% increase in hamsters; cyanide-insensitive palmitoyl-CoA oxidation: 1380% increase in rats versus 171% increase in hamsters; carnitine acetyltransferase: 2100% increase in rats versus 162% increase in hamsters).

A number of studies have compared the ability of di(2-ethylhexyl) phthalate to induce hepatic peroxisome proliferation in rats and primates. Male and female Wistar-derived rats (six to eight weeks old) and male and female marmosets (12–18 months old) were given 2000 mg/kg bw per day di(2-ethylhexyl) phthalate by gavage for 14 days (Rhodes *et al*., 1986). Male and female marmosets (24 months old) were also given 1000 mg/kg bw per day di(2-ethylhexyl) phthalate by daily intraperitoneal

injection for 14 days. In rats, treatment with di(2-ethylhexyl) phthalate increased relative liver weight and produced hepatic peroxisome proliferation, as demonstrated by ultrastructural examination and increased cyanide-insensitive palmitoyl-CoA oxidation and lauric acid hydroxylation. No such effects were observed in marmosets given di(2-ethylhexyl) phthalate by either oral or intraperitoneal administration. Groups of four male and four female marmosets (final body weight, around 360 g) were given 100, 500 or 2500 mg/kg bw di(2-ethylhexyl) phthalate per day by gavage for 13 weeks (Kurata *et al*., 1998). A preliminary dose-setting study indicated that 2500 mg/kg bw di(2-ethylhexyl) phthalate per day was close to the maximum tolerated dose in this species. Significant suppression of body weight gain was observed in male marmosets given 2500 mg/kg di(2-ethylhexyl) phthalate per day. Treatment with di(2-ethylhexyl) phthalate did not affect relative liver weight or hepatic cyanide-insensitive palmitoyl-CoA oxidation and carnitine acetyltransferase activities. Ultrastructural examination did not reveal significant changes in peroxisome numbers per hepatocyte or in peroxisome volume density, although small increases in mean peroxisomal volume were noted in male marmosets given 500 or 2500 mg/kg bw di(2-ethylhexyl) phthalate per day. As peroxisomal volume density is considered the most accurate morphometric measurement of peroxisome proliferation by ultrastructural evaluation (IARC, 1995), the results are considered not to have demonstrated peroxisome proliferation. Furthermore, di(2-ethylhexyl) phthalate did not produce morphological changes in the livers of male and female marmosets or in the testes of the males. Thus, in addition to demonstrating a lack of hepatic peroxisome proliferation, these results indicate that di(2-ethylhexyl) phthalate treatment did not produce testicular damage in adult marmosets.

Male Fischer 344 rats were fed diets containing 100–25 000 ppm di(2-ethylhexyl) phthalate for 21 days, while male cynomolgus monkeys were given 100 or 500 mg/kg bw di(2-ethylhexyl) phthalate per day by gavage for 25 days (Short *et al*., 1987). In rats, di(2-ethylhexyl) phthalate treatment produced a dose-related increase in relative liver weight and in enzymatic markers and ultrastructural evidence (subjective evaluation) of hepatic peroxisome proliferation. Treatment with di(2-ethylhexyl) phthalate did not affect relative liver weight or activities of cyanide-insensitive palmitoyl-CoA oxidation, carnitine acetyltransferase and lauric acid 12-hydroxylase in male cynomolgus monkeys. In addition, no treatment-related changes were observed by light or electron microscopic examination of liver sections.

Interspecies comparisons with hepatocytes in vitro

Peroxisome proliferation may also be demonstrated *in vitro* in cultured rat and mouse hepatocytes. Many of the known characteristics of peroxisome proliferation *in vivo*, including increased number and size of peroxisomes, differential induction of peroxisomal enzyme activities and stimulation of replicative DNA synthesis, have been demonstrated in cultured rat and mouse hepatocytes (IARC, 1995).

Hepatocytes isolated from male Wistar rats (180–250 g) were treated with 0.2 mM mono(2-ethylhexyl) phthalate or 1 mM 2-ethylhexanol for 48 h (Gray *et al*., 1982). Both di(2-ethylhexyl) phthalate metabolites increased carnitine acetyltransferase activity about nine-fold. In studies with hepatocytes from male Sprague-Dawley rats (180–220 g), treatment with 0.2 mM mono(2-ethylhexyl) phthalate and 1.0 mM 2-ethylhexanol for 48 h resulted in induction of carnitine acetyltransferase activity about 15-fold and six-fold, respectively (Gray *et al*., 1983). Mono(2-ethylhexyl) phthalate was also shown to induce cyanide-insensitive palmitoyl-CoA oxidation and, by ultrastructural examination, to increase numbers of peroxisomes. Hepatocytes were isolated from Wistar-derived rats (180–220 g) and treated for 72 h with 0–0.5 mM mono(2-ethylhexyl) phthalate and some mono(2-ethylhexyl) phthalate metabolites (Mitchell *et al*., 1985). Treatment with mono(2-ethylhexyl) phthalate and metabolites VI and IX (see Figure 1) resulted in a concentration-dependent induction of cyanide-insensitive palmitoyl-CoA oxidation. In addition, 0–0.5 mM mono(2-ethylhexyl) phthalate and 0–1.0 mM metabolite VI produced concentration-dependent increases in lauric acid hydroxylation. Treatment with metabolites I and V resulted in only small effects on the enzymatic markers of peroxisome proliferation. In another study with hepatocytes from Wistar-derived rats (180–220 g), metabolite VI was shown by subjective ultrastructural examination to cause proliferation of peroxisomes (Elcombe & Mitchell, 1986).

Primary hepatocyte cultures may also be employed to study species differences in hepatic peroxisome proliferation (IARC, 1995; Doull *et al*., 1999). Hepatocytes were isolated from male Sprague–Dawley rats (180–220 g), male Syrian hamsters (70–80 g) and male Dunkin-Hartley guinea-pigs (400–450 g). Treatment with 20–200 μM mono(2-ethylhexyl) phthalate for 70 h caused strong induction of cyanide-insensitive palmitoyl-CoA oxidation activity in rat hepatocytes (up to 600% of control levels), while no marked effect was observed in Syrian hamster (up to 120% of control) or guinea-pig (down to 80% of control) hepatocytes (Lake *et al*., 1986).

Hepatocytes were isolated from male Wistar-derived rats (180–220 g) and male Alderley Park guinea-pigs (400–500 g) and treated with 0–0.5 mM mono(2-ethylhexyl) phthalate or metabolite IX for 72 h (Mitchell *et al*., 1985).While both caused concentration-dependent induction of cyanide-insensitive palmitoyl-CoA oxidation in rat hepatocytes, no such effect was observed in guinea-pig hepatocytes.

Species comparisons of hepatic peroxisomal proliferation have also included studies of human and non-human primate primary hepatocyte cultures. Hepatocytes isolated from Wistar-derived rats (180–220 g), male Alderley Park guinea-pigs (400–500 g), male marmosets (350–500 g) and three human liver samples (renal transplant donors) were treated with 0–0.5 mM mono(2-ethylhexyl) phthalate for 72 h (Elcombe & Mitchell, 1986). While there was a concentration-dependent induction of cyanide-insensitive palmitoyl-CoA oxidation in rat hepatocytes, no induction was observed in guinea-pig or human hepatocytes and only small non-concentration-dependent effects were observed in marmoset hepatocytes. Metabolite VI induced cyanide-insensitive palmitoyl-CoA oxidation and lauric acid hydroxylation in cultured

rat hepatocytes. In contrast, treatment of marmoset hepatocytes with 0–1.0 mM metabolite VI and guinea-pig and human hepatocytes with 0–2.0 mM metabolite VI resulted in no induction of cyanide-insensitive palmitoyl-CoA oxidation activity. Similarly, lauric acid hydroxylation activity was not induced in marmoset or human hepatocytes treated with 0–2.0 mM metabolite VI.

Hepatocytes were isolated from male Wistar rats (200 g), male Dunkin-Hartley guinea-pigs (350 g), male New Zealand rabbits (2500 g) and cynomolgus monkeys (two to three years old) and treated with mono(2-ethylhexyl) phthalate or with metabolites VI or V for 72 h (Dirven *et al*., 1993c). The treatment of rat hepatocytes with 0–300 μM of mono(2-ethylhexyl) phthalate or metabolite VI resulted in a concentration-dependent induction of cyanide-insensitive palmitoyl-CoA oxidase and lauric acid 12-hydroxylase activities; metabolite V had very little effect. In guinea-pig, rabbit and cynomolgus monkey hepatocytes, neither mono(2-ethylhexyl) phthalate nor metabolite VI had any effect on cyanide-insensitive palmitoyl-CoA oxidase activity at concentrations of up to 300 μM. Small increases in cyanide-insensitive palmitoyl-CoA oxidase activity were observed at mono(2-ethylhexyl) phthalate and metabolite VI concentrations of 600 μM, whereas treament with 0–600 μM of either compound had no effect on lauric acid 12-hydroxylase activity in guinea-pig, rabbit or cynomolgus monkey hepatocyte cultures.

Hepatocytes were isolated from male Wistar-derived rats, male Alderley Park guinea-pigs and three human liver samples (liver transplant donors). Treatment with up to 1100 μM metabolite VI for 72 h caused a concentration-dependent induction of cyanide-insensitive palmitoyl-CoA oxidation in rat hepatocytes, but had no significant effect in either guinea-pig or human hepatocytes. Ultrastructural examination revealed an increase in the numbers of peroxisomes in rat hepatocytes, but no such effect was observed in cultured human hepatocytes (Elcombe *et al*., 1996).

Hepatocytes were isolated from male Fischer 344 rats and from two human liver samples (liver surgery patients). Treatment with 200 μM mono(2-ethylhexyl) phthalate for either 48 or 72 h induced carnitine acetyltransferase activity in cultured rat but not human hepatocytes (Butterworth *et al.*, 1989).

Hepatocytes were isolated from male Fischer 344 rats and from three human liver (liver transplantation donors). Treatment with mono(2-ethylhexyl) phthalate induced β-oxidation activity, replicative DNA synthesis and inhibited apoptosis induced by transforming growth factor β (TGFβ) in cultured rat but not human hepatocytes (Hasmall *et al*., 1999).

Hepatocytes were isolated from male Wistar rats, two dogs (age, breed and sex not stated) and two human subjects (69–71 years of age, sex not stated) (Hildebrand *et al*., 1999). In collagen sandwich cultures, the rat hepatocytes responded to di(2-ethylhexyl) phthalate in the culture medium with slightly increased carnitine acetyltransferase activity, while dog and human hepatocytes did not respond.

Hepatocytes were isolated from male Alderley Park (Wistar-derived) rats, male Alderley Park (Swiss) mice, male Alderley Park (Dunkin-Hartley) guinea-pigs and

male captive bred (ICI, Alderley Park) marmosets. Treatment with either 2-ethylhexanol or 2-ethylhexanoic acid for 72 h produced a concentration-dependent induction of cyanide-insensitive palmitoyl-CoA oxidation in rat and mouse but not guinea-pig or marmoset hepatocytes (Cornu *et al.*, 1992).

Peroxisome proliferators have also been shown to induce replicative DNA synthesis in cultured rodent hepatocytes (IARC, 1995). In contrast, several peroxisome proliferators have failed to induce replicative DNA synthesis in human hepatocyte cultures (Doull *et al.*, 1999). Hepatocytes were isolated from male Wistar-derived rats and from three human liver samples (liver transplantation donors) and treated with 2-ethylhexanoic acid and some other peroxisome proliferators for 72 h (Elcombe *et al.*, 1996). While 2-ethylhexanoic acid induced replicative DNA synthesis in cultured rat hepatocytes, no effect was observed in human hepatocytes. Hepatocytes were isolated from male Fischer 344 rats and three humans and treated in culture with 250–2000 μM mono(2-ethylhexyl) phthalate (Hasmall *et al.*, 1999). Increased peroxisomal ω-oxidation (at 250–750 μM), replicative DNA synthesis (at 500–1000 μM), and inhibition of apoptosis (at 250–1000 μM) were observed in rat hepatocytes. None of these parameters was affected by mono(2-ethylhexyl) phthalate in human hepatocytes.

Other effects

The effect of di(2-ethylhexyl) phthalate in diet (2% for 21 days) on lipoprotein metabolism in male Wistar rats was evaluated (Mocchiutti & Bernal, 1997). The observed reduction in plasma triglyceride levels was associated with (and attributed to) increased activity of extrahepatic lipoprotein lipase.

An effect of di(2-ethylhexyl) phthalate on estrogen metabolism has been reported (Eagon *et al.*, 1994). Male Fischer 344 rats fed diets containing 1.2% di(2-ethylhexyl) phthalate for four, eight or 16 weeks had significantly increased serum estradiol levels. This was explained by the observation that these rats showed significant loss of hepatic activity of a major male estrogen-metabolizing enzyme, estrogen 2-hydroxylase, and a male-specific estrogen-sequestering protein.

The testicular toxicity of di(2-ethylhexyl) phthalate is described in Section 4.3.

Female Fischer 344 rats treated with a single oral dose (up to 5000 mg/kg bw) or with repeated doses (up to 1500 mg/kg bw per day for 14 days) of di(2-ethylhexyl) phthalate showed no neurobehavioural effects, as evaluated by functional observational battery and motor activity testing (Moser *et al.*, 1995).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

The teratogenicity and reproductive toxicity of di(2-ethylhexyl) phthalate have been reviewed (Huber *et al.*, 1996). It was noted that di(2-ethylhexyl) phthalate impairs the fertility in both sexes of adult rats at doses above 100 mg/kg bw per day, and that several studies indicate that di(2-ethylhexyl) phthalate is embryotoxic and teratogenic in rodents. Di(2-ethylhexyl) phthalate was associated with a reduction in relative testis weight, decreases in sperm production and a depletion of testicular zinc. The testicular response appeared to be species-specific, but was not consistent with the species sensitivity to peroxisome proliferation (e.g., guinea-pigs were more sensitive to the testicular effects than were Syrian hamsters). The metabolite mono(2-ethylhexyl) phthalate was judged to be more potent in causing both teratogenicity and reproductive toxicity. Both the embryotoxic and testicular effects in adults were considered to be observed at doses above those at which peroxisome proliferation was recorded, but no mechanism for either response was identified. Effects on testicular development in rats following exposure to di(2-ethylhexyl) phthalate prenatally and during suckling or during adolescence at dose levels below those associated with peroxisome proliferation have been reported (Poon *et al.*, 1997; Arcadi *et al.*, 1998).

(*a*) *Developmental toxicity studies*

(i) *Rats*

Sprague-Dawley rats were administered intraperitoneal injections of 5 or 10 mL/kg bw [4930 or 9860 mg/kg] di(2-ethylhexyl) phthalate on gestation days 5, 10 and 15. Fetal body weight was reduced in both treated groups, resorptions increased from about 8% in controls and the low-dose group to 27% in the high-dose group and an increase in the incidence of abnormalities (e.g., haemangiomas of the legs) but not skeletal abnormalities was also evident in the high-dose group (Singh *et al.*, 1972).

Groups of 25 Wistar rats were exposed by head–nose inhalation to di(2-ethylhexyl) phthalate (> 99% pure) at aerosol concentrations (mass median aerodynamic diameter (MADD) 50% of < 1.2 μm) of 0, 0.1, 0.05 and 0.3 mg/L [a range-finding study noted a dose-related trend for hepatic peroxisome proliferation at levels of 0.2, 0.5 and 1.0 mg/L (the maximum concentration that could be achieved)] for 6 h per day on gestation days 6–15. Twenty females were killed on gestation day 20 and their fetuses examined for viability, growth and malformations. The only dose-related effect noted was an increase in the extent of retarded development (renal pelvic dilatations) in the highest-exposure group. The remaining five females per group were allowed to litter and their offspring were examined for postnatal growth and viability. No dose-related effects were noted. The only maternal effect seen was a reduction in body weight on post-partum day 21 in the high-dose group (Merkle *et al.*, 1988).

Groups of 22–25 Fischer 344 rats were fed diets containing 0, 0.5, 1.0, 1.5 or 2.0% di(2-ethylhexyl) phthalate (> 99% pure) on days 0–20 of gestation (Tyl *et al.*, 1988). Fetuses were examined on gestation day 20 for growth, viability, external, visceral and

skeletal malformations and variations. Maternal food intake was decreased and water consumption and liver weights were increased at all dose levels. Maternal body weight gain effects and reduced fetal weights were evident at levels of 1.0% and above. Fetal viability was reduced at the highest exposure level. No significant increase in the incidence of variations or malformations was observed.

The effects on testicular development in the offspring of rats exposed to di(2-ethylhexyl) phthalate *in utero* were studied by Tandon *et al*. (1991). Groups of six Wistar rats were exposed to 0 or 1000 mg/kg di(2-ethylhexyl) phthalate by gavage for the entire period of gestation and spermatogenesis was assessed in the offspring at 31, 61 and 91 days of age. Testicular weight was significantly reduced in the offspring only at 31 days of age. Alterations in the activity of testicular γ-GT, sorbitol dehydrogenase, lactate dehydrogenase and β-glucuronidase were noted, especially at the youngest age, suggesting alterations in Sertoli cell function and germ cell maturation. Epididymal sperm counts were reduced by about 22% at 91 days of age.

In another study, Fischer 344 rats were exposed to 0, 0.25, 0.5 or 1% di(2-ethylhexyl) phthalate in the diet during gestation and offspring were evaluated for reproductive function and fertility. In the summary of the study, it was reported that the high-dose level reduced the body weight of the parental generation by 25%, while reductions in food consumption were seen at the mid- and high-dose level. The only sign of reproductive toxicity was an 8% decrease in pup birth weight (National Toxicology Program, 1997a).

In a screening assay for developmental toxicity, groups of 16–21 Fischer 344 rats were exposed to di(2-ethylhexyl) phthalate (98% pure) by oral gavage at doses of 0, 1125 or 1500 mg/kg bw per day on gestation days 6–19 (Narotsky & Kavlock, 1995). The litters were followed postnatally for growth, viability and malformations. Exposure to either level resulted in decreased maternal weight gain on gestation days 6–8 and a significant increase in females with fully resorbed litters. No treated pups survived to postnatal day 6 and malformations (e.g., microphthalmia, missing innominate artery and cleft palate) were present in a few pups that were found dead. In a follow-up study, groups of 10–12 Fischer 344 rats were exposed to 0, 333, 500, 750 or 1125 mg/kg bw di(2-ethylhexyl) phthalate per day by gavage on gestation days 6–15. There were no effects upon maternal body weight or clinical findings and the only developmental effects were a delay in parturition and micro/anophthalmia at 750 and 1125 mg/kg bw. These effects were in contrast to the earlier study, so a comparison study was made using dose levels of 1125 and 1500 mg/kg bw administered on gestational days 6–15 or 6–19. Detailed results were not presented, but the authors stated that in the 6–15-day groups, there were significant maternal weight loss, increased prenatal mortality, delay in parturition and eye malformations (Narotsky *et al*., 1995).

Di(2-ethylhexyl) phthalate was one of several phthalate esters evaluated for prenatal toxicity in Wistar rats (Hellwig *et al*., 1997). Groups of 10 females received di(2-ethylhexyl) phthalate at doses of 0, 40, 200 and 1000 mg/kg bw per day by oral

gavage on gestation days 6–15. The highest dose level decreased food consumption and body weight gains, and increased relative liver and kidney weights at term; two dams in this group showed vaginal haemorrhage during treatment. Also in the high-dose group, there were pronounced postimplantation loss, reduced numbers of live fetuses and an increase in malformations, predominantly of the tail, brain, urinary tract, gonads, vertebral column and sternum.

There are reports indicating major organ toxicity in the offspring including irreversible testicular effects following exposure to di(2-ethylhexyl) phthalate prenatally and during suckling (Arcadi *et al.*, 1998). Groups of 12 female Long-Evans rats were exposed daily to drinking water containing 32.5 or 325 μL/L di(2-ethylhexyl) phthalate from day 1 of pregnancy to day 21 after the delivery. Based on estimated water intake, the exposure was roughly calculated to correspond to 3.0–3.5 and 30–35 mg/kg di(2-ethylhexyl) phthalate per day during pregnancy; during suckling this value increased by at least 30%, which was assumed to be due to increased water intake. At different times after delivery (21, 28, 35, 42, 56 days), eight pups per group were killed. Pup body weight gain and kidney, liver and testis weights were measured. Histopathology of the kidneys, liver and testes was also studied. Female pups were used for behavioural assessment 30 days after birth in the 'beam walking' test, designed to assess the locomotor activity by employing a learned avoidance test. Pregnancy rate, body weight gain and gross appearance in the dams were not affected by the treatment. Perinatal exposure produced no significant changes in body weight gain in the pups. A reduction in kidney weight (absolute and relative) was observed at both dose levels, accompanied by histopathological findings (shrinkage of renal glomeruli with signs of glomerulonephritis, dilation of renal tubuli and light fibrosis) between weeks 0 and 4 of age. The alterations were less pronounced at week 8. The increase in liver weight was not dose-related. A dose-dependent reduction in testicular weight (absolute and relative) was observed and did not appear to return to normal with growth. The perinatal exposure caused severe histological damage to the testes. At 21 and 28 days of age, there was a gross disorganization of the seminiferous tubular structure, detachment of the spermatogonial cells from basal membrane and absence of spermatocytes in both exposure groups. At the end of the observation period, at 56 days, there were still severe histopathological changes in the testes of pups. Low-dose rats exhibited only a few elongated spermatids in tubules showing a pervious lumen. In high-dose animals, the histological findings included a generalized disorganization of the tubular epithelium, with spermatogonia detached from the basal membrane, absence of elongated spermatids and spermatozoa and tubular lumen filled with cellular deposits. Four adult male rats exposed to the same levels via the drinking-water for 42 days did not show any significant change in either kidney, liver or testis relative weights, and only minor histological damage of the testes. Female pups exposed perinatally to 325 μL/L di(2-ethylhexyl) phthalate showed a significantly increased time necessary to perform the beam walking test, indicating a behavioural effect expressed as reduced locomotor activity.

(ii) *Postnatal studies in rats*

Parmar *et al*. (1985) exposed groups of five dams (albino rats) to 0 or 2000 mg/kg bw di(2-ethylhexyl) phthalate. Litters of seven pups were dosed with di(2-ethylhexyl) phthalate through mothers' milk throughout the lactation period (from parturition to day 21). Pup body weights were recorded at five-day intervals and on day 21 when the pups were killed. Pooled liver homogenates were prepared for an assay of the activities of arylhydrocarbon hydroxylase, aniline hydroxylase and ethylmorphine *N*-demethylase, and concentration of cytochrome P450. The body weight of the di(2-ethylhexyl) phthalate-treated pups was lower than that of the control group throughout the whole period. Absolute liver weight was significantly decreased in the di(2-ethylhexyl) phthalate-treated pups; relative liver weight was similar in the two groups. All four biochemical parameters showed significant decreases in the di(2-ethylhexyl) phthalate-treated pups relative to control. In the livers of the pups (5), a concentration of 25.7 ± 0.3 μg/g di(2-ethylhexyl) phthalate was found.

Female Sprague-Dawley rats were given five oral doses of 2000 mg/kg bw per day di(2-ethylhexyl) phthalate (> 99% pure) in corn oil by gavage on days 2–6, 6–10 or 14–18 of lactation (Dostal *et al*., 1987a). The rats were killed 24 h after the last dose. The body weights of lactating rats and of their suckling pups were significantly reduced at all treatment intervals. Food consumption was reduced in the dams dosed on days 14–18. Relative liver weights were increased in the lactating dams at all three stages of lactation but not in the suckling pups. The hepatic peroxisomal enzyme activities (cyanide-insensitive palmitoyl-CoA oxidase and carnitine acetyltransferase) were increased five- to eight-fold in treated dams at all three stages of lactation. Twofold increases in these enzyme activities were also observed in pups suckling the treated dams. Hypolipidaemia was observed in treated lactating rats at all three stages of lactation. Plasma cholesterol and triglyceride concentrations were decreased by 30–50%.

The transfer of di(2-ethylhexyl) phthalate into the milk of lactating rats was shown in groups of female Sprague-Dawley rats given three oral doses of 2000 mg/kg bw per day di(2-ethylhexyl) phthalate in corn oil by gavage on days 15–17 of lactation. Two hours after dosing on day 17, 10 pups per litter were removed from the dams to allow milk to accumulate. Six hours after the last dose, the dams were killed and milk and mammary glands were collected. Two pups from each litter were killed 3–4 h after the third dose. Increased activities of cyanide-insensitive palmitoyl-CoA oxidase and carnitine acetyltransferase in dams and pups were observed. In treated rats, mammary gland weights, both absolute and relative, were significantly reduced, and total milk solids, lipid, and protein were increased relative to control rats, whereas milk lactose was significantly decreased. Milk collected 6 h after the third dose contained 216 μg/mL di(2-ethylhexyl) phthalate and 25 μg/mL mono(2-ethylhexyl) phthalate. In contrast, plasma contained virtually no di(2-ethylhexyl) phthalate (< 0.5 μg/mL) but substantial amounts of mono(2-ethylhexyl) phthalate (76 μg/mL), resulting in a high milk:plasma ratio for di(2-ethylhexyl) phthalate and a low milk:plasma ratio for

mono(2-ethylhexyl) phthalate. Di(2-ethylhexyl) phthalate and mono(2-ethylhexyl) phthalate were not detected in the plasma of the pups (Dostal *et al.*, 1987a).

Groups of 10 male Sprague-Dawley rats were given 0, 10, 100, 1000 or 2000 mg/kg bw per day di(2-ethylhexyl) phthalate (> 99% pure) in corn oil by gavage for five days, beginning at the age of six (one week old), 14–16 (two weeks old), 21 (three weeks old), 42 (six weeks old) or 86 (12 weeks old) days (Dostal *et al*., 1987b). The control group was given the vehicle. After two doses of 2000 mg/kg bw per day, virtually all pups in the three youngest age groups died, whereas six- and 12-week-old rats showed significantly decreased body weight but no fatalities. Five daily doses of 1000 mg/kg bw per day caused significant decreases in body weight gain in one-, two- and three-week-old rats. Absolute and relative liver weights were significantly increased at 100 mg/kg bw per day in all age groups (except for one-week-old rats) and in all age groups at higher dose levels. Absolute kidney weight was reduced in some cases, whereas relative kidney weight was increased at doses of 1000 mg/kg bw per day or more in rats three or more weeks old. Morphological examination revealed increased peroxisome proliferation in neonatal as well as in adult rats. The activities of cyanide-insensitive palmitoyl-CoA oxidase and carnitine acetyltransferase were increased in a dose-dependent manner in all age groups. The activities of these enzymes were similar in control rats of all ages. Plasma cholesterol concentrations were higher in suckling control rats (one- and two-week-old) than in weanling (three-week-old) and adult controls. In di(2-ethylhexyl) phthalate-treated rats, plasma cholesterol concentrations were significantly reduced in weanling and adult rats given doses of 1000 mg/kg bw per day or more. In suckling rats, plasma cholesterol levels were increased at 1000 mg/kg bw per day. Plasma triglyceride levels in the control group were similar at all ages, whereas significant decreases in plasma triglycerides were observed in weanling and adult rats; in suckling rats only small decreases (not significant) occurred.

Tandon *et al*. (1990) performed a study on testis development in male rats after exposure during the suckling period. Groups of four female rats were given vehicle (groundnut oil) or 2000 mg/kg bw per day di(2-ethylhexyl) phthalate orally for 21 days from parturition. The pups were killed at the age of 31, 61 or 91 days when testes, epididymis, prostate and seminal vesicles were examined. The offspring of di(2-ethylhexyl) phthalate-treated mothers showed a significant increase in the activity of testicular γ-GT, lactate dehydrogenase and β-glucuronidase, and a significant decrease in the activity of acid phosphatase and sorbitol dehydrogenase at 31 and 61 days of age compared with controls. No effect on these testicular enzymes was seen in 91-day-old rats. The authors concluded that exposure to di(2-ethylhexyl) phthalate during early life through mother's milk causes biochemical alterations which may affect the functional development of the testis.

In a neonatal rat model used to assess di(2-ethylhexyl) phthalate toxicity following intravenous administration, groups of 12 neonates, two to four days of age, were injected intravenously with 30.8, 91.7 or 164.8 mg/kg bw di(2-ethylhexyl)

phthalate in 4% bovine serum albumin (BSA) solution for 18 consecutive days (Greener *et al*., 1987). Control neonates were injected with a solution of 4% BSA or saline, or were untreated. Neonates were examined for signs of toxicity immediately after treatment and again 1–3 h later. Animals were killed 24 h after the last treatment (postnatal days 20–23) and a complete necropsy was performed and selected tissues (brain, heart, lungs, liver, spleen, kidneys, injection site, eyes, stomach, duodenum and caecum) were prepared for histopathological evaluation. Body weight gains and average weight gain per day were significantly and dose-dependently decreased from days 4 to 21 of the treatment period. Absolute and relative liver weights were significantly increased in a dose-related manner. No conclusive histopathological alterations were detected in the tissues.

(iii) *Mice*

In a study designed to identify the gestational days when mice are particularly sensitive to di(2-ethylhexyl) phthalate, groups of three to eight female ddY-Slc(SPF) mice, seven to eight weeks of age, were given 0, 1.0, 2.5, 5, 7.5, 10 or 30 mL/kg bw di(2-ethylhexyl) phthalate (> 99% pure; corresponding to 1/30, 1/12, 1/6, 1/4, 1/3 or 1/1 of the acute oral LD_{50}) by gavage on day 6, 7, 8, 9 or 10 of gestation (Yagi *et al*., 1980). The average body weights of the fetuses were decreased at all dose levels, regardless of the day of maternal exposure. The number of resorptions was increased largely depending on the dose and particularly on the day of dosing. A high and dose-related increase was observed in animals dosed on days 7 and 8 of gestation at all dose levels tested. Doses of 5 or 10 mL/kg bw given on day 7 led to 100% fatality of the fetuses. The incidences of fetal deaths were 8% and 5%, respectively, when 10 mL/kg bw di(2-ethylhexyl) phthalate was administered on day 9 or 10 of gestation. Dose levels of 2.5 or 7.5 mL/kg di(2-ethylhexyl) phthalate given to mice on day 7 or 8 of gestation induced a high incidence of gross and skeletal abnormalities including encephaly, open eyelid and club foot. There was no information on maternal toxicity.

Groups of 6–19 ddY-Slc × CBA mice were administered 0.05–30 mL/kg di(2-ethylhexyl) phthalate on gestational day 6, 7, 8, 9 or 10 (Tomita *et al*., 1982a). Dose- and stage-dependent effects on fetal development were observed. The numbers of live fetuses were greatly reduced by treatment on gestational days 7–9, but particularly by 2.5 mL/kg bw on day 7. High rates of external (e.g., exencephaly, open eyelid, club foot and bent tail) and skeletal anomalies (e.g., abnormal vertebrae and ribs) were noted in groups receiving 2.5 or 7.5 mL/kg bw on day 7 or 8 of gestation.

Di(2-ethylhexyl) phthalate was administered in the diet to groups of 7–24 ICR mice at levels of 0, 0.05, 0.1, 0.2, 0.4 or 1.0% by weight throughout gestation and effects on the developing fetus were assessed (Shiota & Nishimura, 1982). Maternal body weight at the end of pregnancy was reduced at dose levels of 0.2% and higher. Dose levels of 0.1% and above significantly reduced embryonic viability. In the 0.2% di(2-ethylhexyl) phthalate group, malformations such as exencephaly, myeloschisis and tail anomalies were observed.

Groups of 9–11 Slc-ICR mice were exposed to di(2-ethylhexyl) phthalate by oral gavage at concentrations of 0, 250, 500, 1000 or 2000 mg/kg bw or by intraperitoneal injection (3–9 per group) at doses of 0, 500, 1000, 2000, 4000 or 8000 mg/kg bw on days 7–9 of gestation (Shiota & Mima, 1985). Controls received the olive oil vehicle. When given by the oral route, di(2-ethylhexyl) phthalate exposure caused resorptions, reduced fetal weight and an increased incidence of malformations (exencephaly, anencephaly, open eyelids, tail anomalies) at doses of 1000 mg/kg and above. One of 11 females died in the highest-dose group. No effects on the fetuses were observed following intraperitoneal exposure, although two of three females exposed to the high dose died. Using a similar experimental design, these authors reported that mono(2-ethylhexyl) phthalate was not toxic to development in the mouse.

Groups of 24–30 CD-1 mice were fed diets containing 0, 0.025, 0.05, 0.10 or 0.15% di(2-ethylhexyl) phthalate (> 99% pure) on days 0–17 of gestation (Tyl *et al.*, 1988). Fetuses were examined on gestational day 17 for growth, viability, external, visceral and skeletal malformations and variations. Maternal body weight gain was reduced and liver weights were increased at 0.10% and above. Fetal body weight was reduced in females at 0.10%, whereas this effect was not observed until the 0.15% level in male offspring. Concentrations of 0.10% and above reduced fetal viability and, beginning at the 0.05% level, increased the incidence of malformations (affecting the eye, central nervous system, heart, axial skeleton and tail).

In another study, CD-1 mice were exposed via the diet to 0. 0.01, 0.025 or 0.05% di(2-ethylhexyl) phthalate in the diet on days 0–17 of gestation, and the offspring were evaluated for acquisition of developmental landmarks, spontaneous locomotor activity and fertility. The summary report of the study indicates that no effects were observed on reproductive toxicity in either generation (National Toxicology Program, 1997b).

(*b*) *Mechanistically oriented developmental toxicity studies*

Groups of 7–10 Wistar rats were dosed by gavage with undiluted di(2-ethylhexyl) phthalate (12.5 or 25 mmol/kg), 2-ethylhexanol (6.25 or 12.5 mmol/kg) or 2-ethylhexanoic acid (6.25 or 12.5 mmol/kg) on day 12 of gestation and fetuses were examined for viability, growth and morphology on day 20 of gestation (Ritter *et al.*, 1987). Control females were untreated [no maternal effects were reported]. All three chemicals induced malformations (hydronephrosis, laevocardia, septal defects, short and kinky tails, ectrodactyly, misplaced digits and bowed radius), with the order of potency suggesting that 2-ethylhexanoic acid may be the proximate teratogen [malformation data not shown].

Fetal body weights were reduced by about 23% and relative liver weights increased by about 22% in rats exposed by oral gavage to 1000 mg/kg bw di(2-ethylhexyl) phthalate on gestational days 6–15. This dose also reduced maternal weight gain during pregnancy and decreased the activity of several enzymes (dehydrogenase, malate dehydrogenase, adenosine triphosphatase and cytochrome c oxidase) in fetal livers, although no malformations were observed (Srivastava *et al.*, 1989).

Di(2-ethylhexyl) phthalate (0, 0.5, 1, 2.5 or 5 g/kg per day) was administered to Fischer 344 rats by oral gavage from birth through lactation day 21 and the activity of several peroxisomal enzymes was determined in the livers, kidneys and brains of the females and their offspring (Cimini *et al*., 1994). No pups survived exposure to doses of 2.5 g/kg per day. Pup growth was impaired at the two lowest doses. In the liver, cyanide-insensitive palmitoyl-CoA oxidase activity showed similar increases in pups and adult females.

Groups of 10–13 pregnant female mice, either homozygous wild type (+/+) or PPARα null (–/–), were administered di(2-ethylhexyl) phthalate by gavage at 0 or 1000 mg/kg on days 8–9 of gestation. Offspring were evaluated on gestational days 10 and 18. Similar developmental toxicicity (resorptions, growth retardation, incidence of malformations) was seen in mice of both genotypes, suggesting that the developmental effects are not PPARα-mediated. Additional analysis showed that di(2-ethylhexyl) phthalate induced maternal hepatic CYP4A1 mRNA in the wild-type females only, and both genotypes showed induced MT-1 and zinc levels in the livers and reduced serum and fetal concentrations after exposure to di(2-ethylhexyl) phthalate (Peters *et al*., 1997a,b).

The reproductive effects of 10 known or suspected anti-androgens on sexual differentiation were investigated in male rats. Groups of 10 Long-Evans hooded rats were administered 0 or 750 mg/kg di(2-ethylhexyl) phthalate by gavage from day 14 of pregnancy to day 3 of lactation. In the offspring, di(2-ethylhexyl) phthalate induced lower body weight, decreased anogenital distance, retained nipples and high levels of testicular and epididymal abnormalities, including atrophy and agenesis. In eight-day-old pups, several males from different litters displayed haemorrhagic testes that were visible by gross examination of the inguinal region. Testicular lesions of this nature have not been observed with known anti-androgens, which indicates that di(2-ethylhexyl) phthalate affects the developing male reproductive system by a mechanism that is distinct from that of previously described reproductive toxicants. The profile of the effects induced by di(2-ethylhexyl) phthalate was different from that induced by some known androgen-receptor antagonists (i.e., vinclozolin, procymidone, *p,p′*-DDE (1,1-dichloro-2,2-bis(*para*-chlorophenyl)ethylene)) (Gray *et al*., 1999).

(*c*) *Reproductive toxicity studies*

(i) *Rats*

Sprague-Dawley rats received daily intraperitoneal injections of 50 or 100 mg/kg bw di(2-ethylhexyl) phthalate on alternate days for 20 days. Prostatic and testicular zinc levels were reduced by this treatment (Curto & Thomas, 1982).

Groups of five or 15 male and female Sprague-Dawley rats were exposed to di(2-ethylhexyl) phthalate in the diet at concentrations of 0, 0.2, 1.0, or 2.0% (0, 143, 737 or 1440 mg/kg bw per day in males) for two, six or 17 weeks. The absolute and relative testicular weights of rats in the mid- and high-dose groups were lower than

those of control rats. Histological examination revealed severe seminiferous tubular atrophy and cessation of spermatogenesis related to the dietary level of di(2-ethylhexyl) phthalate. These changes were observed after exposure for two weeks (Gray *et al*., 1977).

Testicular effects were also investigated after oral administration of 2000 mg/kg bw di(2-ethylhexyl) phthalate for seven consecutive days to 13-week-old male Wistar rats (Saxena *et al*., 1985). Degeneration was observed in about 40% of the seminiferous tubules. Loss of succinic dehydrogenase, NADH-diaphorase and acid phosphatase activity and increases in adenosine triphosphatase, glucose-6-phosphate dehydrogenase and alkaline phosphatase activity were observed in treated rats.

The reversibility of testicular effects was studied after oral administration of 2000 mg/kg bw per day di(2-ethylhexyl) phthalate for 14 days (Oishi, 1985). One day after the last administration, 10 treated animals were killed and compared with 10 rats fed control diet for an additional 45 days without further administration of di(2-ethylhexyl) phthalate. Testicular morphology was characterized by a marked shrinkage of the seminiferous tubules, the germinal epithelium consisting of Sertoli cells, very few spermatogonia and several multinucleated cells. The interstitial tissue and Leydig cells appeared normal. After a recovery period of 45 days after termination of di(2-ethylhexyl) phthalate administration, the majority of tubules showed a lack of spermatogenesis, but some tubules had intact epithelium. The percentage of spermatogenic tubules in a representative cross-section was 0 (total atrophy) or 12.8%.

Oral administration of di(2-ethylhexyl) phthalate at levels of 0, 250, 500 or 1000 mg/kg bw to adult albino rats for 15 days resulted in a significant decrease in sperm count in the epididymus and increased activity of β-glucuronidase, γ-GT and lactate dehydrogenase and a decrease in the activity of acid phosphatase in the testes. The authors interpreted these findings as indicating germ-cell depletion and deterioration of the germinal epithelium in the testes (Parmar *et al*., 1986).

Dietary exposure of groups of 24 adult male Fischer 344 rats to 0, 320, 1250, 5000 or 20 000 ppm di(2-ethylhexyl) phthalate (> 99% pure) for 60 days resulted in dose-dependent reductions in body weight and testis, epididymis and prostate weights beginning at 5000 ppm. Histopathologically, the testes showed severe atrophy of the seminiferous epithelium and loss of spermatogonia at the highest dose. At this dose level, there were reductions in the sperm density in the epididymus and percentage of motile sperm, while abnormal sperm shapes, testicular zinc and serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were elevated. The size of the litters at birth was significantly reduced at the highest dose (Agarwal *et al*., 1986).

In a 102-week study, adult male Sprague-Dawley rats were exposed to di(2-ethylhexyl) phthalate in the diet at dose levels of 0, 0.02, 0.2 or 2% (0, 7, 70, or 700 mg/kg bw per day) (Ganning *et al*., 1991). In all dose groups, di(2-ethylhexyl) phthalate inhibited spermatogenesis and general seminiferous tubular atrophy at the end of the study was reported. [The Working Group noted that the study was designed to study the effects of phthalates on the liver and there was little information on testicular effects].

Male Sprague-Dawley rats, aged one, two, three, six or 12 weeks of age, received five daily doses ranging from 10 to 2000 mg/kg bw per day di(2-ethylhexyl) phthalate by oral gavage and testicular effects were examined 24 h after the last dose (Dostal *et al*., 1988). Relative testicular weight was reduced at doses of 1000 mg/kg bw per day in one-, two-, three- and six-week-old rats, while 2000 mg/kg was required for this effect when the animals were 12 weeks of age. Sertoli cell number was transiently reduced in rats exposed to 1000 mg/kg bw per day at one week of age. Offspring from this treatment showed normal fertility at six weeks of age, although there was histological evidence of decreased maturation of spermatids at doses as low as 500 mg/kg bw per day. Losses of spermatocytes were evident in rats exposed to 1000 or 2000 mg/kg bw per day at ages of two weeks or older. Decreased testicular zinc levels did not appear to be related to the effect on spermatogenesis.

Groups of 10 male Wistar rats, nine weeks of age, were exposed by inhalation to 0, 0.01, 0.05 or 1.0 mg/L di(2-ethylhexyl) phthalate for 6 h per day on five days per week for 28 days and had normal fertility when mated with untreated females two and six weeks later (Klimisch *et al*., 1992). At the end of the exposure, relative liver weights were increased in the high-dose males, but there was no evidence of testicular histopathological effects.

Adult male albino rats received 0, 500 or 1000 mg/kg di(2-ethylhexyl) phthalate by gavage for 15 days and were killed 24 h later (Siddiqui & Srivastava, 1992). Relative testis weight and epididymal sperm counts were reduced at the highest dose, as was the activity of several enzymes relevant to spermatogenesis (aldose reductase and sorbitol dehydrogenase). The activity of testicular lactate dehydrogenase was increased at this dose.

Effects on female reproductive function were evaluated in groups of six to nine Sprague-Dawley rats exposed by gavage to 2 g/kg di(2-ethylhexyl) phthalate (purity, > 99%) by gavage daily for 1–12 days (Davis *et al*., 1994). Di(2-ethylhexyl) phthalate-exposed females had prolonged estrous cycles, suppressed or delayed ovulations and smaller preovulatory follicles. Endocrinologically, the treated females had reduced preovulatory granulosa cell estrogen production, with secondary increases in FSH and insufficient LH surge for ovulation.

In a 90-day study, groups of 10 young male Sprague-Dawley rats (105–130 g at initiation of dosing) were given 0, 5, 50, 500 or 5000 ppm (0, 0.4, 3.7, 37.6 or 375.2 mg/kg bw) di(2-ethylhexyl) phthalate per day in the diet for 13 weeks. No clinical signs of toxicity were observed. Feed consumption and body weight gain were not affected. At 5000 ppm, rats had significantly increased absolute and relative liver weights and relative kidney weight. In the 500-ppm dose group, a high incidence of minimal to mild Sertoli cell vacuolation was observed in 7/10 rats. No other effects were noted at this dose level. At 5000 ppm, the absolute and relative testis weights were significantly reduced. Microscopic examination revealed mild to moderate, bilateral, multifocal or complete atrophy of the seminiferous tubules with complete loss of spermatogenesis and cytoplasmic vacuolation of the Sertoli cells lining the tubules in 9/10 rats (Poon *et al*., 1997).

(ii) *Mice*

Adult Swiss Webster mice received daily intraperitoneal injections of 50 or 100 mg/kg bw di(2-ethylhexyl) phthalate for five days or alternate daily intraperitoneal injections of 50 or 100 mg/kg bw di(2-ethylhexyl) phthalate for 20 days. Prostatic and testicular zinc levels were not affected by treatment with di(2-ethylhexyl) phthalate (Curto & Thomas, 1982).

In a fertility assessment by continuous breeding, groups of 20 male and 20 female CD-1 mice were fed di(2-ethylhexyl) phthalate (> 99% pure) at dietary levels of 0.01, 0.1 or 0.3% (equivalent to 20, 200 or 600 mg/kg bw per day, respectively) and a control group of 40 male and 40 female mice received basal diet (Lamb *et al*., 1987). Both male and female mice were exposed during a seven-day premating period and were then randomly grouped as mating pairs. Treatment continued for the 98-day cohabitation period and for 21 days thereafter. Reproductive function was evaluated by measuring the number of litters per breeding pair, number of pups per litter, proportion of pups born alive and mean pup weight. Dietary levels of 0.1 and 0.3% di(2-ethylhexyl) phthalate produced dose-dependent and significant decreases in fertility and in the number and proportion of pups born alive. In males, 0.3% di(2-ethylhexyl) phthalate caused significantly reduced weights of the testes, epididymis, prostate and seminal vesicles. All high-dose males but one showed some degree of bilateral atrophy of the seminiferous tubules. Sperm analysis showed a significant decrease in the percentage of motile sperm and a significantly decreased sperm concentration in cauda epididymis. Exposure to 0.3% di(2-ethylhexyl) phthalate also caused an increased incidence of abnormal sperm forms. Di(2-ethylhexyl) phthalate did not significantly decrease body-weight gain in the high-dose group. A cross-over mating trial conducted with F_0 mice showed a decrease in fertility for both treated males and treated females. Only four litters out of 20 were born to treated males mated with control females and the proportion of pups born alive was decreased. No pups were born when treated females were mated with control males.

A two-generation study in CD-1 mice was performed by feeding 0.01, 0.025 or 0.05% di(2-ethylhexyl) phthalate (> 99% pure) in the diet (NTIS, 1988). The doses were equivalent to about 19, 48 or 95 mg/kg bw per day. The study was carried out to examine the effect of prenatally administered di(2-ethylhexyl) phthalate on the growth, development and reproductive performance of the F_1 generation. The F_1 generation was mated within dose groups at sexual maturity and F_2 offspring were evaluated for viability and growth at postnatal day 4. For F_1 litters, the percentage of prenatal mortality was increased at the high dose from 9% to 26.4%. During the neonatal period, the percentage of viable pups was significantly decreased at the dose of 0.05% di(2-ethylhexyl) phthalate. No other effects of di(2-ethylhexyl) phthalate upon growth, viability, age of acquisition for developmental landmarks (incisor eruption, wire grasping, eye opening, testis descent, or vaginal opening or spontaneous locomotor activity) were observed on postnatal days 14, 21 or 50.

Groups of male and female ICR mice received doses of di(2-ethylhexyl) phthalate ranging from 1 to 100 mL/kg bw by subcutaneous injections on experimental days 1, 5 and 10 and were evaluated on day 21 (Agarwal *et al.*, 1989). Doses of 10 mL/kg or greater reduced the fertility of both males and females. At 15 mL/kg, relative testis weights were reduced, while changes in ovarian and testicular ATPase and lysosomal enzymes occurred at 10 mL/kg. Histopathological changes were observed in the testes from 10 mL/kg and in the ovaries from 1 mL/kg.

Thirty mice of an inbred colony were used to study the effect of di(2-ethylhexyl) phthalate on reproductive function (Jain & Joshi, 1991). Fifteen mice were dosed orally with 1000 mg/kg bw di(2-ethylhexyl) phthalate [purity not specified] in 0.1 mL olive oil for one week. The fertility (evaluated by the ability of the motile spermatozoa to fertilize normal cycling females) was reduced from 90 to 75%. Sperm density and sperm motility were also significantly reduced.

(iii) *Marmosets*

In a 13-week study, groups of four mature male marmosets were given daily doses of 0, 100, 500 or 2500 mg/kg bw di(2-ethylhexyl) phthalate. Body-weight gain was significantly depressed at 2500 mg/kg bw. No significant changes were observed in testis weights or histopathology of the testis, epididymis, seminal vesicles or prostate (Kurata *et al.*, 1998).

(*d*) *Mechanistic-based reproductive toxicity studies*

The Sertoli cell appears to be the primary site of phthalate toxicity in the testes, and theories have been proposed related to (1) reduced testicular zinc levels, (2) altered hormonal status, (3) altered metabolic function and (4) altered FSH reactivity. None of these alone appears to account for the observed testicular effects (reviewed in Boekelheide, 1993). Younger rats tend to be more sensitive to the testicular effects of phthalates, although this difference appears to be related to changes in absorption, metabolism and distribution rather than to changes in tissue sensitivity (Heindel & Powell, 1992). Administration of 2 g/kg mono(2-ethylhexyl) phthalate by oral gavage to 20-day-old Fischer 344 rats resulted in a collapse of Sertoli cell vimentin filaments within 3 h of exposure. This response was accompanied at first by a decrease, and then several hours later by an increase, in germ-cell apoptosis. The authors suggested that these events may be linked via signal transduction events (Richburg & Boekelheide, 1996). Subsequently, it has been demonstrated that expression of Fas, a trans-membrane receptor protein, and of the Fas ligand (FasL), which occur in the germ and Sertoli cells, respectively, is up-regulated in the Fischer 344 rat testes following exposure to 2 g/kg mono(2-ethylhexyl) phthalate. Expression of Fas in the germ cell is related to apoptosis, and terminal deoxynucleotide transferase (TdT)-mediated dUTP-biotin nick-end labelling (TUNEL)-positive cells (i.e., those showing the staining reaction indicative of apoptosis) were present in same regions as the heightened Fas expression. This suggests that damage to Sertoli cells increases the

output of FasL, leading to increased germ-cell loss in an effort to maintain testicular homeostasis (Lee *et al*., 1997).

The estrogenic activities of phthalates were investigated in competitive ligand-binding assays, yeast and mammalian gene expression assays and in a uterotrophic assay. Di(2-ethylhexyl) phthalate did not compete for estrogen receptors, induce luciferase activity in transfected MCF-7 cells or stably transfected HeLa cells, support estrogen-inducible growth in yeast cells or demonstrate any estrogenic activity in mammalian assays *in vivo* (Zacharewski *et al*., 1998).

The involvement of testosterone in the testicular atrophy caused by di(2-ethylhexyl) phthalate was examined by co-administration of testosterone (1 mg/kg bw) subcutaneously with 2000 mg/kg bw di(2-ethylhexyl) phthalate [purity not specified] in groundnut oil to adult male Wistar rats for 15 days (Parmar *et al*., 1987). Administration of di(2-ethylhexyl) phthalate reduced the sperm count and also significantly increased the activity of γ-GT, lactate dehydrogenase and β-glucuronidase and decreased the activity of sorbitol dehydrogenase and acid phosphatase. Co-administration of testosterone seemed to normalize the sperm count and the activity of testicular enzymes. The role of testosterone in the testicular toxicity of di(2-ethylhexyl) phthalate has not been fully elucidated. Several reports refer to increased or decreased testosterone levels in plasma and testicular tissue.

A study of the influence of a low-protein diet on the testicular toxicity of di(2-ethylhexyl) phthalate was performed in adult male Wistar rats (12 weeks old) (Tandon *et al*., 1992). One group received a synthetic diet containing 20% casein and the other a diet containing 8% starch. After 15 days of consumption, half of the rats in each group received 1000 mg/kg bw di(2-ethylhexyl) phthalate [purity not stated] in 0.2 mL groundnut oil orally for 15 days. The other half served as a control group. The group on the low-protein diet had a more severe response to di(2-ethylhexyl) phthalate in terms of sperm count, and of increased activity of β-glucuronidase and γ-GT.

Gray and Butterworth (1980) found an age-dependent induction of testicular atrophy in rats, with younger rats being more sensitive than older ones. Four-, 10- and 15-week-old Wistar rats were administered 2800 mg/kg bw di(2-ethylhexyl) phthalate [purity not specified] dissolved in corn oil by gavage for 10 days. In some experiments, testosterone propionate (200 µg/kg per day in corn oil) or FSH was given subcutaneously. In four-week-old rats, di(2-ethylhexyl) phthalate produced uniform seminiferous tubular atrophy, comprising loss of spermatids and spermatocytes. In 10-week old rats, 5–50% of the tubules were atrophic; the testicular weight was not affected. A marked decrease in testicular weight (% of control) was seen in four-week-old rats. The weights of the seminal vesicles and ventral prostate were reduced in the four- and 10-week-old males. In four-week-old rats fed a diet containing 2.0% di(2-ethylhexyl) phthalate, the testicular effects were reversible within 12 weeks when treatment was stopped before puberty, but recovery was slower when treatment continued throughout puberty. Simultaneous administration of testosterone or FSH with di(2-ethylhexyl) phthalate did not affect the development of testicular atrophy,

but prevented the reduction of accessory gland weights. The interstitial tissue appeared to be unaffected in all dosed animals. No effects were observed in 15-week-old rats.

Groups of eight male Sprague-Dawley rats (25, 40 or 60 days old) were dosed with 0 or 1000 mg/kg bw di(2-ethylhexyl) phthalate in corn oil by gavage for 14 days (Sjöberg *et al*., 1985d). The liver weight was increased in all three age groups. In the 25-day-old rats, the absolute testicular weight was decreased and histopathological examination showed severe testicular damage, whereas the older animals were unaffected. In the youngest age group, there were a marked reduction in the number of germ cells, a high occurrence of degenerating cells and a reduction in the tubular diameter. There was also a marked reduction in the number of spermatogonia. The authors suggested that this indicated that the damage was not completely reversible. In some animals that were allowed to survive for 100 days, the majority of tubules were totally devoid of germ cells.

To determine which compound or compounds were responsible for the testicular damage after oral administration of di(2-ethylhexyl) phthalate, Sjöberg *et al*. (1986a) administered di(2-ethylhexyl) phthalate and five of its major metabolites (mono(2-ethylhexyl) phthalate, 2-ethylhexanol and three identified metabolites (V, VI, or IX) of mono(2-ethylhexyl) phthalate) for five days. Groups of six male Sprague-Dawley rats (35 days old at the start of the experiment) were given 2.7 mmol/kg bw di(2-ethylhexyl) phthalate (1055 mg/kg bw) or one of the metabolites. Counting of degenerated cells per tubular cross-section was carried out. No testicular damage was observed following oral doses of di(2-ethylhexyl) phthalate or 2-ethylhexanol. The number of degenerated spermatocytes and spermatids was increased in animals receiving mono-(2-ethylhexyl) phthalate; no such effects were seen in animals given the mono(2-ethylhexyl) phthalate-derived metabolites.

In another study, Sjöberg *et al*. (1986b) examined the age-dependent testicular toxicity of di(2-ethylhexyl) phthalate (1000 and 1700 mg/kg bw in the diet for 14 days) in rats at 25, 40 and 60 days of age. Body weight gain was retarded in all treated groups. Testicular weight was markedly reduced and severe testicular damage occurred in the 25- and 40-day-old rats given doses of 1700 mg/kg bw. No changes were found in the 60-day-old rats. Only a few seminiferous tubules (1–10%) were affected in the 1000 mg/kg bw group at any age of exposure. The authors concluded that the difference in response to di(2-ethylhexyl) phthalate of male rats of different ages may be due to higher absorption of the metabolite mono(2-ethylhexyl) phthalate in younger animals.

Groups of eight male Sprague-Dawley rats (four, 10 or 15 weeks of age) were used to study the age-dependence of effects on male reproductive organs (Gray & Gangolli, 1986). The rats were given 2800 mg/kg bw di(2-ethylhexyl) phthalate orally for 10 days. Administration to four-week-old rats produced a marked reduction in absolute weights of the testes, seminal vesicles and prostate. There was only a slight reduction in testis weight in 10-week-old rats, but the seminal vesicle and prostate weights were significantly reduced. Di(2-ethylhexyl) phthalate had no effect in 15-week-old rats. Histologically, the testes of the four-week-old rats showed severe atrophy affecting

virtually all the tubules, which were populated only by Sertoli cells, spermatogonia and occasional primary spermatocytes. In the 10-week-old rats, these histological changes were evident in 5–50% of the tubules, the remainder appearing to be essentially normal. No histological abnormalities were seen in testes from the 15-week-old rats. In the same study, the effects of mono(2-ethylhexyl) phthalate on Sertoli cell function in immature rats were studied by measuring the secretion of seminiferous tubule fluid and androgen-binding protein. A single dose of 1000 mg/kg bw mono(2-ethylhexyl) phthalate reduced fluid and protein production to around 50% of the concurrent control group and to 25% after three repeated doses.

To elucidate further the mechanisms responsible for the enhanced sensitivity of the testes of developing animals to di(2-ethylhexyl) phthalate, the activities of the testicular enzymes associated with spermatogenesis including lactate dehydrogenase, γ-GT, sorbitol dehydrogenase, β-glucuronidase and acid phosphatase were studied in 25-day-old male Wistar rats (Parmar *et al.*, 1995). Doses of 0, 50, 100, 250 or 500 mg/kg bw di(2-ethylhexyl) phthalate in groundnut oil were given for 30 consecutive days to groups of six male rats. There was an exposure-related and significant decrease of absolute and relative testicular weight at all dose levels. From 50 mg/kg bw, a dose-dependent and significant increase in the activities of lactate dehydrogenase and γ-GT was noted, while activity of sorbitol dehydrogenase decreased. β-Glucuronidase activity was elevated at 250 or 500 mg/kg bw, while acid phosphatase decreased at the same dose levels. The administration also resulted in marked destructive changes in the advanced germ cell layers and marked degrees of vacuolar degeneration in the testes at 250 and 500 mg/kg bw. The significant alterations in the activities of sorbitol dehydrogenase, lactate dehydrogenase and γ-GT occurred thus at much lower levels of di(2-ethylhexyl) phthalate and before the histopathological changes. The Leydig cells and the fibroblasts appeared to be normal.

4.4 Genetic and related effects

Di(2-ethylhexyl) phthalate was one of the compounds used in the IPCS evaluation of short-term tests for carcinogenicity (IPCS, 1985). The data from this and other evaluations of the genetic and related effects of di(2-ethylhexyl) phthalate have been reviewed (Huber *et al.*, 1996).

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 7 for references)

Di(2-ethylhexyl) phthalate was not mutagenic to *Salmonella typhimurium* strains TA100, TA102, TA1535, TA1537, TA1538, TA98, TA97 or the TA7000 series in the

Table 7. Genetic and related effects of di(2-ethylhexyl) phthalate

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Bacillus subtilis rec, differential toxicity	–	NT	500 µg/disc	Tomita *et al*. (1982b)
Salmonella typhimurium, forward mutation	–	–	500	Liber (1985)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	9860	Kirby *et al*. (1983)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	4000	Robertson *et al*. (1983)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	2000	Yoshikawa *et al*. (1983)
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	10 000	Baker & Bonin (1985)
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	5000	Matsushima *et al*. (1985)
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	10 000	Nohmi *et al*. (1985)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	5000	Rexroat & Probst (1985)
Salmonella typhimurium TA100, TA1535, TA98, TA97, reverse mutation	–	–	10 000	Zeiger & Haworth (1985)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	10 000	Zeiger *et al*. (1985)
Salmonella typhimurium TA1537, TA98, TA7001, TA7002, TA7003, TA7004, TA7005, TA7006, reverse mutation	–	–	1000	Gee *et al*. (1998)
Escherichia coli WP2 uvrA, reverse mutation	–	–	2000	Yoshikawa *et al*. (1983)
Saccharomyces cerevisiae, gene conversion	(+)	(+)	5000	Arni (1985)
Saccharomyces cerevisiae, gene conversion	–	–	2000	Brooks *et al*. (1985)
Saccharomyces cerevisiae, gene conversion	–	–	1000	Inge-Vechtomov *et al*. (1985)

Table 7 (contd)

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Saccharomyces cerevisiae, gene conversion	+	+	1500	Mehta & von Borstel (1985)
Saccharomyces cerevisiae D6, aneuploidy	+	+	5000	Parry & Eckardt (1985)
Saccharomyces cerevisiae, gene conversion	–	–	5000	Parry & Eckardt (1985)
Saccharomyces cerevisiae, homozygosis	–	–	5000	Arni (1985)
Saccharomyces cerevisiae, homozygosis	–	–	1000	Inge-Vechtomov *et al*. (1985)
Aspergillus nidulans haploid, mutation	–	NT	9900	Carere *et al.* (1985)
Aspergillus nidulans non-disjunction	–	NT	9900	Carere *et al.* (1985)
Aspergillus nidulans, mitotic crossing-over	–	NT	9900	Carere *et al*. (1985)
Saccharomyces cerevisiae, forward mutation	–	–	1000	Inge-Vechtomov *et al*. (1985)
Saccharomyces cerevisiae, reverse mutation	–	–	5000	Arni (1985)
Saccharomyces cerevisiae, reverse mutation	–	–	1000	Inge-Vechtomov *et al*. (1985)
Saccharomyces cerevisiae, reverse mutation	+	+	1500	Mehta & von Borstel (1985)
Saccharomyces cerevisiae, reverse mutation	–	–	5000	Parry & Eckardt (1985)
Saccharomyces pombe, forward mutation	?	–	5900	Loprieno *et al*. (1985)
Saccharomyces cerevisiae DEL assay, and ICR recombination	–	–	200 000	Carls & Schiestl (1994)
Drosophila melanogaster, crossing-over/recombination	–		39 000 µg/g food	Würgler *et al*. (1985)
Drosophila melanogaster, somatic mutation	(+)		6930 µg/cm^2 food surface	Fujikawa *et al*. (1985)
Drosophila melanogaster, somatic mutation	(+)		780 µg/g food	Vogel (1985)
Drosophila melanogaster, somatic mutation	–		39 000 µg/g food	Würgler *et al*. (1985)

Table 7 (contd)

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Drosophila melanogaster, sex-linked recessive lethal mutation	–		20 inj.	Yoon *et al*. (1985)
Drosophila melanogaster, sex-linked recessive lethal mutation	–		18 600 µg/g food	Zimmering *et al.* (1989)
Drosophila melanogaster, DNA double strand breakage *in vivo*	–		7540 µg/g food	Kawai (1998)
Drosophila melanogaster, DNA repair test *in vivo*	–		7540 µg/g food	Kawai (1998)
Drosophila melanogaster, wing spot test, mutation *in vivo*	–		7540 µg/g food	Kawai (1998)
DNA single-strand breaks, rat hepatocytes *in vitro*	–	NT	3900	Bradley (1985)
DNA strand breaks, Chinese hamster ovary cells *in vitro*	–	–	39 000	Douglas *et al*. (1985)
DNA single-strand breaks, rat or Syrian hamster hepatocytes *in vitro*	–	NT	9750	Schmezer *et al*. (1988)
Unscheduled DNA synthesis, rat primary hepatocytes	–	NT	3900	Butterworth *et al*. (1984)
Unscheduled DNA synthesis, rat primary hepatocytes	–	NT	3900	Kornbrust *et al*. (1984)
Unscheduled DNA synthesis, rat primary hepatocytes	–	NT	3900	Probst & Hill (1985)
URP, Unscheduled DNA synthesis, rat primary hepatocytes	–	NT	10 000	Williams *et al*. (1985)
URP, Unscheduled DNA synthesis, rat primary hepatocytes	–	NT	1000	Astill *et al*. (1986)
UIA, Unscheduled DNA synthesis, B6C3F_1 mouse primary hepatocytes *in vitro*	–	NT	390	Smith-Oliver & Butterworth (1987)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	(+)	–	980	Kirby *et al*. (1983)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	?	–	2500	Amacher & Turner (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	4900	Myhr *et al*. (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	(+)	(+)	7.5	Oberly *et al*. (1985)

Table 7 (contd)

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	9800	Styles *et al*. (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	250	Astill *et al*. (1986)
Gene mutation, mouse lymphoma L5178Y cells, ouabain resistance *in vitro*	–	–	200	Garner & Campbell (1985)
Gene mutation, mouse lymphoma L5178Y cells, ouabain resistance *in vitro*	–	–	9800	Styles *et al*. (1985)
Gene mutation, BALB/c-3T3 mouse cells, ouabain resistance *in vitro*	NT	–	1960	Matthews *et al*. (1985)
Sister chromatid exchange, Chinese hamster Don cells *in vitro*	–	NT	3900	Abe & Sasaki (1977)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	–	3900	Douglas *et al*. (1985)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	(+)	–	5000	Gulati *et al*. (1985)
Sister chromatid exchange, rat liver RL4 cells *in vitro*	–	NT	1000	Priston & Dean (1985)
Micronucleus formation, Chinese hamster ovary cells *in vitro*	–	–	3900	Douglas *et al*. (1985)
Micronucleus formation, rat hepatocytes *in vitro*	–	NT	3900	Müller-Tegethoff *et al*. (1995)
Micronucleus formation, Syrian hamster embryo cells, *in vitro*	+	NT	NG	Fritzenschaf *et al.* (1993)
Chromosomal aberrations, Chinese hamster Don cells *in vitro*	–	NT	3900	Abe & Sasaki (1977)
Chromosomal aberrations, Chinese hamster lung cells *in vitro*	–	NT	160	Ishidate & Odashima (1977)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	NT	781	Phillips *et al*. (1982)
Chromosomal aberrations, Chinese hamster liver cells *in vitro*	–	NT	50	Danford (1985)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	–	5000	Gulati *et al*. (1985)
Chromosomal aberrations, Chinese hamster lung cells *in vitro*	–	–	4130	Ishidate & Sofuni (1985)

Table 7 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, rat liver RL4 cells *in vitro*	–	NT	1000	Priston & Dean (1985)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	–	+	39	Tsutsui *et al*. (1993)
Aneuploidy, Chinese hamster liver cells *in vitro*	(+)	NT	50	Danford (1985)
Mitotic aberrations, Chinese hamster primary liver cells *in vitro*	(+)	NT	50	Parry (1985)
Aneuploidy, rat liver RL4 cells *in vitro*	–	NT	1000	Priston & Dean (1985)
Cell transformation, BALB/3T3 mouse cells	–	–	25 000	Matthews *et al*. (1985)
Cell transformation, BALB/3T3 mouse cells	–	–	20	Astill *et al*. (1986)
Cell transformation, C3H10T½ mouse cells	(+)	(+)	40	Lawrence & McGregor (1985)
Cell transformation, C3H10T½ mouse cells	–	NT	3.9	Sanchez *et al*. (1987)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	1	Barrett & Lamb (1985)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	4	Sanner & Rivedal (1985)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	10	Mikalsen *et al*. (1990)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	30	Mikalsen & Sanner (1993)
Cell transformation, Syrian hamster embryo cells, clonal assay	(+)	+	1.2	Tsutsui *et al*. (1993)
Ornithine decarboxylase superinduction, Syrian hamster embryo cells[d]	–	NT	39	Dhalluin *et al.* (1998)
Cell transformation, RLV/Fischer rat	+	NT	1000	Suk & Humphreys (1985)
Cell transformation, SA7/Syrian hamster embryo cells	+	NT	500	Hatch & Anderson (1985)
Cell transformation, Syrian hamster embryo cells	+	NT	39	Dhalluin *et al.* (1998)
Unscheduled DNA synthesis, human hepatocytes *in vitro*	–	NT	3900	Butterworth *et al*. (1984)
Gene mutation, human lymphocytes, *TK* and *HPRT* loci *in vitro*	–	–	1000	Crespi *et al*. (1985)
Sister chromatid exchange, human lymphocytes *in vitro*	–	–	1000	Obe *et al*. (1985)
Sister chromatid exchange, human lymphocytes (metabolic activation by co-cultured with rat liver cells) *in vitro*	–	(+)	39	Lindahl-Kiessling *et al.* (1989)
Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	75	Turner *et al*. (1974)

Table 7 (contd)

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	60	Stenchever *et al*. (1976)
Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	160	Tsuchiya & Hattori (1976)
Aneuploidy, human fetal lung cells *in vitro*	–	NT	6	Stenchever *et al*. (1976)
Comet assay on human blood *in vitro*	+	–	156	Anderson *et al.* (1999)
Body fluids, Sprague Dawley rat urine, microbial mutagenicity	–	–	2000 × 15 po	DiVincenzo *et al*. (1985)
DNA strand breaks, Wistar rat liver *in vivo*	–		2000 × 28 po	Elliott & Elcombe (1987)
DNA oxidative damage, Fischer 344 rat liver *in vivo*	+ [c]		12 000 mg/kg diet 1 y	Takagi *et al*. (1990a)
DNA oxidative damage, Fischer 344 rat liver *in vivo*	+ [c]		12 000 mg/kg diet, 1–2 w	Takagi *et al*. (1990b)
DNA single-strand breaks, Fischer 344 rat liver *in vivo*	–		20 000 mg/kg diet 78w	Tamura *et al*. (1991)
DNA oxidative damage, Fischer 344 rat liver *in vivo*	–		12 000 mg/kg diet 22w	Cattley & Glover (1993)
Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	–		500 × 1 po, 150 × 14 po or 12 000 ppm diet, 30 days + 500 × 1 po	Butterworth *et al*. (1984)
Unscheduled DNA synthesis, Sprague-Dawley rat hepatocytes *in vivo*	–		5000 × 1 po	Kornbrust *et al*. (1984)
Unscheduled DNA synthesis, Fischer 344 rat hepatocytes *in vivo*	–		12 000 mg/kg diet 28 d	Cattley *et al*. (1988)
Unscheduled DNA synthesis, B6C3F$_1$ mouse hepatocytes *in vivo*	–		6000 mg/kg diet 28 d	Smith-Oliver & Butterworth (1987)

Table 7 (contd)

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, *lac*I transgenic C57BL/6 mouse liver *in vivo*	–		6000 mg/kg diet 120 d	Gunz *et al*. (1993)
Micronucleus formation, mice *in vivo*	–		5000 × l po	Astill *et al*. (1986)
Micronucleus formation, B6C3F$_1$ mouse erythrocytes *in vivo*	–		6000 × 5 ip	Douglas *et al*. (1986)
Chromosomal aberrations, Fischer 344 rat bone marrow *in vivo*	–		4900 × 5 po	Putman *et al*. (1983)
Chromosomal aberrations, Syrian hamster embryos *in vivo*	+		7500 × 1 po	Tomita *et al*. (1982b)
Dominant lethal test, ICR Swiss mice *in vivo*	+		12 780 × 1 ip	Singh *et al*. (1974)
Dominant lethal test, mice *in vivo* [strain not specified]	+		980 × 3 sc	Autian (1982)
Dominant lethal test, ICR Swiss mice *in vivo*	+		980 × 3 sc	Agarwal *et al*. (1985)
Aneuploidy, Fischer 344 rat hepatocytes *in vivo*	–		12 000 mg/kg diet 7 d	Hasmall & Roberts (1997)
Cell transformation, Syrian hamster embryos *in vivo/in vitro*	+		7500 po	Tomita *et al*. (1982b)
Binding (covalent) to Fischer 344 rat hepatocyte DNA *in vitro*	–	NT	390	Gupta *et al*. (1985)
Binding (covalent) to Fischer 344 rat liver DNA *in vivo*	–		10 000 mg/kg diet 11 d	Albro *et al*. (1982)
Binding (covalent) to Fischer 344 rat liver DNA *in vivo*	–		10 000 mg/kg diet 4 w	Däniken *et al*. (1984)
Binding (covalent) to Fischer 344 rat liver DNA *in vivo*	–		2000 × 3 po	Gupta *et al*. (1985)
Binding (covalent) to Fischer 344 rat liver DNA *in vivo*	–		500 × 1 po	Lutz (1986)
Inhibition of gap-junctional intercellular communication, cynomolgus monkey liver cells *in vivo*	–		500 × 14 po	Pugh *et al.* (1999)
Inhibition of gap-junctional intercellular communication, Chinese hamster V79 cells *in vitro*	+	NT	3	Malcolm *et al*. (1983)
Inhibition of gap-junctional intercellular communication, Chinese hamster V79 cells *in vitro*	–	NT	0.1	Kornbrust *et al*. (1984)

Table 7 (contd)

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Inhibition of gap-junctional intercellular communication, Chinese hamster V79 cells *in vitro*	+	NT	5	Elmore *et al*. (1985)
Inhibition of gap-junctional intercellular communication, Chinese hamster V79 cells *in vitro*	+	NT	10	Malcolm & Mills (1989)
Inhibition of gap-junctional intercellular communication, Syrian hamster embryo cells *in vitro*	+	NT	30	Mikalsen & Sanner (1993)
Inhibition of gap-junctional intercellular communication, Chinese hamster V79 cells *in vitro*	+	NT	78	Vang *et al*. (1993)
Inhibition of gap-junctional intercellular communication, Chinese hamster V79 cells and Syrian hamster embryo cells *in vitro*	+	NT	10	Cruciani *et al*. (1997)
Sperm morphology, B6C3F_1 mice *in vivo*	–		6000 × 5 ip	Douglas *et al*. (1986)
Sperm morphology, Sprague Dawley rats *in vivo*	–		5200 × 5 ip	Douglas *et al*. (1986)
Metabolites				
Mono(2-ethylhexyl) phthalate (MEHP)				
Bacillus subtilis rec, differential toxicity	+	NT	400 µg/disc	Tomita *et al*. (1982b)
Salmonella typhimurium TA100, reverse mutation	–	–	1250 µg/plate	Tomita *et al*. (1982b)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	0.2 µL/plate	Kirby *et al*. (1983)
Salmonella typhimurium TA100, TA102, TA 98, TA 97, reverse mutation	–	–	1000 µg/plate	Dirven *et al*. (1991)
Unscheduled DNA synthesis, B6C3F_1 mouse primary hepatocytes *in vitro*	–	NT	139	Smith-Oliver & Butterworth (1987)

Table 7 (contd)

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	0.3 µL/mL	Kirby *et al.* (1983)
Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	+	NT	25	Tomita *et al.* (1982b)
Chromosomal aberrations in Syrian hamster embryo cells *in vitro*	–	+	2.8	Tsutsui *et al.* (1993)
Cellular transformation in Syrian hamster embryo cells *in vitro*	–	(+)	56	Tsutsui *et al.* (1993)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	23	Mikalsen *et al.* (1990)
Cell transformation, C3H10T½ mouse cells	–	NT	417	Sanchez *et al.* (1987)
Inhibition of gap-junctional intercellular communication, Chinese hamster V79 cells and Syrian hamster embryo cells *in vitro*	+	NT	28	Cruciani *et al.* (1997)
Unscheduled DNA synthesis, human primary hepatocytes *in vitro*	–	NT	139	Butterworth *et al.* (1984)
DNA strand breaks, Wistar rat liver *in vivo*	–	NT	500 po × 14	Elliot & Elcombe (1987)
Mono(2-ethyl 5-hydroxyhexyl) phthalate (IX)				
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 µg/plate	Dirven *et al.* (1991)
Mono(2-ethyl 5-oxohexyl) phthalate (VI)				
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 µg/plate	Dirven *et al.* (1991)
Mono(5-carboxyl 2-ethylpentyl) phthalate (V)				
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 µg/plate	Dirven *et al.* (1991)

Table 7 (contd)

Test system	Result [a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2-Ethylhexanol				
Bacillus subtilis rec, differential toxicity	–	NT	500	Tomita *et al.* (1982b)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	1 µL/plate	Kirby *et al.* (1983)
Gene mutation, mouse lymphoma L5178 cells, *Tk* locus *in vitro*	–	–	0.3 µL/mL	Kirby *et al.* (1983)
Phthalic acid				
Bacillus subtilis rec, differential toxicity	–	NT	500	Tomita *et al.* (1982b)

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

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; po, oral; ip, intraperitoneal; d, day; w, week; y, year; sc, subcutaneous

[c] No oxidative damage in kidney DNA

[d] Positive if followed by 5 h 0.16 µM 12-*O*-tetradecanoylphorbol 13-acetate

presence or absence of exogenous metabolic activation. It was also not mutagenic to *Escherichia coli* WP2 *uvr*A in the presence or absence of exogenous metabolic activation. It did not induce gene mutations in *Saccharomyces cerevisiae* strain D7 in four of five studies. It induced aneuploidy in a single study. Mitotic gene conversion was induced in two out of five studies with *S. cerevisiae*. In a single study, mitotic recombination was not induced in *S. cerevisiae* in the presence or absence of exogenous metabolic activation. Neither mutation nor genetic crossing-over was induced in *Aspergillus nidulans* cultured with di(2-ethylhexyl) phthalate in the absence of exogenous metabolic activation in one study.

A small increase in somatic mutation frequency was reported in the eye-colour spot test with *Drosophila melanogaster* exposed to di(2-ethylhexyl) phthalate in the feed but no effect was observed in two independent wing-spot tests. In a single study, mitotic recombination was not induced by di(2-ethylhexyl) phthalate; neither was sex-linked recessive lethal mutation induced in two studies in *D. melanogaster* treated with di(2-ethylhexyl) phthalate in the feed or by injection. When administered to *D. melanogaster*, di(2-ethylhexyl) phthalate and *N*-nitrosodiethylamine induced DNA double-strand breakage and DNA repair, although neither compound was active when administered alone.

DNA single-strand breaks were not induced by di(2-ethylhexyl) phthalate in primary cultures of rat or Syrian hamster hepatocytes or in Chinese hamster ovary (CHO) cells. Unscheduled DNA synthesis was not induced in rat or mouse primary hepatocytes exposed to di(2-ethylhexyl) phthalate. Only one of six studies reported induction of gene mutations at the *Tk* locus in mouse lymphoma L5178Y cells exposed to di(2-ethylhexyl) phthalate in the absence of an exogenous metabolic activation system. Ouabain-resistant mutants were not induced in mouse lymphoma or BALB/c-3T3 cells.

In one of two studies, di(2-ethylhexyl) phthalate induced a small increase in sister chromatid exchange frequencies in Chinese hamster ovary cells cultured without but not with exogenous metabolic activation. In other studies conducted only without metabolic activation, it caused no increase in sister chromatid exchanges in either Chinese hamster Don cells or rat liver RL4 cells. Di(2-ethylhexyl) phthalate did not induce micronuclei in Chinese hamster ovary cells or in cultured rat hepatocytes, whereas the induction of micronuclei by di(2-ethylhexyl) phthalate in Syrian hamster embryo cells has been reported.

Chromosomal aberrations were not induced by di(2-ethylhexyl) phthalate in any of eight studies in various types of cultured cells in the absence of metabolic activation. Only three of these studies for chromosomal aberrations included an exogenous metabolic activation system. Of these, one, using Syrian hamster embryo cells, found an increase in aberration frequency. Weak effects were detected for the induction of aneuploidy and mitotic division aberrations in Chinese hamster lung cells.

Di(2-ethylhexyl) phthalate induced cell transformation in the Syrian hamster embryo clonal assay, the virally enhanced SA7/Syrian hamster embryo assay and

RLV/Fischer rat assay without the addition of an exogenous metabolic activation system. In a single study, mouse JB6 epidermal cells were transformed by di(2-ethylhexyl) phthalate without activation and in one of two studies a weak response was reported in the C3H10T½ cell transformation assay with di(2-ethylhexyl) phthalate in either the absence or presence of exogenous metabolic activation. BALB/c-3T3 cells were not transformed by di(2-ethylhexyl) phthalate with or without metabolic activation. Di(2-ethylhexyl) phthalate inhibited gap-junctional intercellular communication in Chinese hamster V79 cells in six of seven studies, but not in one study of liver cells of cynomolgus monkeys *in vivo*. Di(2-ethylhexyl) phthalate treatment of Syrian hamster embryo cells in a two-stage exposure with 12-*O*-tetradecanoylphorbol 13-acetate resulted in superinduction of ornithine decarboxylase, an early event in morphological transformation; no effect was seen after a one-stage treatment with di(2-ethylhexyl) phthalate alone.

Di(2-ethylhexyl) phthalate did not increase the frequency of chromosomal aberrations in human lymphocytes in the absence of exogenous metabolic activation *in vitro*. In single studies with human lymphocytes *in vitro*, di(2-ethylhexyl) phthalate did not induce unscheduled DNA synthesis or gene mutations at the *TK* or *HPRT* loci without exogenous metabolic activation, or aneuploidy in human fetal lung cells cultured without metabolic activation. One single study using human lymphocytes found that di(2-ethylhexyl) phthalate did not induce sister chromatid exchange, while another study found weak induction when the lymphocytes were co-cultured with rat liver cells. A single study investigating the effect of di(2-ethylhexyl) phthalate on human blood cells by the Comet assay reported induction of DNA damage but none in the presence of exogenous metabolic activation.

Urine samples from rats treated by gavage with 15 daily doses of di(2-ethylhexyl) phthalate were not mutagenic to *S. typhimurium* strains TA100, TA1535, TA1537, TA1538 or TA98. In one study, the formation of 8-hydroxydeoxyguanosine (8-OH-dG) was measured as an indicator of DNA oxidative damage in liver and kidney of rats exposed to di(2-ethylhexyl) phthalate in the diet for one to two weeks: 44–64% increased levels of 8-OH-dG were observed in liver but not kidney DNA. Similar results were reported in a follow-up study in which rats received di(2-ethylhexyl) phthalate in the diet for one year. A significant increase in 8-OH-dG was measured in liver DNA at the one- and 12-month sampling times but not at three, six or nine months. Di(2-ethylhexyl) phthalate did not induce DNA strand breaks in rat liver or unscheduled DNA synthesis in rat or mouse liver following single or multiple oral treatments, nor did it give rise to covalent DNA binding in rat hepatocytes *in vitro* or rat liver *in vivo*.

Di(2-ethylhexyl)phthalate did not increase the mutation frequency in the *lac*I gene of liver DNA isolated from female *lac*I transgenic mice given di(2-ethylhexyl) phthalate in the feed for 120 days. Micronuclei were not induced in mice fed with di(2-ethylhexyl) phthalate. Di(2-ethylhexyl)phthalate did not induce chromosomal aberrations in rat bone marrow sampled 6 h after the last of five daily treatments

administered by gavage and it did not induce aneuploidy in hepatocytes of rats fed a diet containing di(2-ethylhexyl) phthalate for seven days. One study reported that di(2-ethylhexyl) phthalate was antimutagenic and co-recombinogenic in combination with *N*-ethyl-*N*-nitrosourea in the mouse spot test (Fahrig & Steinkamp-Zucht, 1996). Chromosomal aberrations and cell transformations were induced in Syrian golden hamster embryos exposed transplacentally to di(2-ethylhexyl) phthalate for 24 h after pregnant females were treated on day 11 of gestation. Di(2-ethylhexyl) phthalate was reported to induce dominant lethal mutations in mice in three studies. Two re-evaluations (Adler & Ashby, 1989; Ashby & Clapp, 1995) of these studies considered that cytotoxicity can interfere with the recognition of a dominant lethal effect, so that this reported mutagenic activity of di(2-ethylhexyl) phthalate is questionable. Di(2-ethylhexyl) phthalate did not induce changes in sperm morphology in mice or rats.

Metabolites

Five metabolites of di(2-ethylhexyl) phthalate, mono(2-ethylhexyl) phthalate, mono(2-ethyl-5-hydroxyhexyl) phthalate (IX), mono(2-ethyl-5-oxohexyl) phthalate (VI), mono(5-carboxy-2-ethylpentyl) phthalate (V) and 2-ethylhexanol, were tested in genotoxicity assays. None induced mutations in *S. typhimurium*.

Negative results were also obtained in the *Bacillus subtilis* rec assay with mono(2-ethylhexyl) phthalate, 2-ethylhexanol and phthalic acid.

Unscheduled DNA synthesis was not induced in either mouse or human primary hepatocyte cultures with mono(2-ethylhexyl) phthalate, and neither this metabolite nor 2-ethylhexanol induced mutations in mouse lymphoma cells *in vitro*. Mono(2-ethylhexyl) phthalate induced sister-chromatid exchange in Chinese hamster V79 cells and chromosomal aberrations in Syrian hamster embryo cells. It also induced transformation in Syrian hamster embryo cells, but not in mouse C3H10T½ cells. Gap-junctional intercellular communication was inhibited by mono(2-ethylhexyl) phthalate in Syrian hamster embryo cells and in Chinese hamster V79 cells. As reported in an abstract (Baker *et al.*, 1996), this function was also inhibited in rat and mouse hepatocytes, but not in Syrian hamster or human hepatocytes.

In the single study with mono(2-ethylhexyl) phthalate, DNA strand breaks were not induced in rat liver *in vivo*.

4.5 Mechanistic considerations

Significant species differences have been observed in the absorption and disposition of di(2-ethylhexyl) phthalate. The peroxisome-proliferating effect of di(2-ethylhexyl) phthalate has been related most specifically in susceptible species to metabolites VI, IX and mono(2-ethylhexyl) phthalate; however, analysis of the disposition data does not provide an explanation for the observed species differences in di(2-ethylhexyl) phthalate-induced hepatic peroxisome proliferation.

The weight of evidence for di(2-ethylhexyl) phthalate and for other rodent peroxisome proliferators in general demonstrates that they do not act as direct DNA-damaging agents.

Chronic administration of peroxisome proliferators to rodents results in sustained oxidative stress due to overproduction of peroxisomal hydrogen peroxide. This can theoretically generate reactive oxygen species which can damage DNA and other intracellular targets. The induction of peroxisomal fatty acid β-oxidation and increases in peroxisomal volume density by di(2-ethylhexyl) phthalate *in vivo* under bioassay conditions (Marsman *et al*., 1988; Lake *et al*., 1987) supports this hypothesis. Supporting data on induction of oxidative stress (lipofuscin accumulation, 8-OH-dG in DNA) in rat liver by di(2-ethylhexyl) phthalate are available (Conway *et al*., 1989; Takagi *et al*., 1990a); however, there are no data for mouse liver.

Similarly, modulation of hepatocellular proliferation by peroxisome proliferators has been implicated in the mechanism of carcinogenesis. This can theoretically result in increased levels of mutation by increasing the frequency of replicative DNA synthesis as well as increasing the number of hepatocytes at risk. Furthermore, hepatocellular proliferation is probably involved in the promotion of growth of preneoplastic hepatocytes. There is clear evidence that di(2-ethylhexyl) phthalate causes acute and sustained hepatocellular proliferation under bioassay conditions which resulted in liver tumours in rats (Marsman *et al*., 1988).

Marked species differences in hepatic peroxisome proliferation have been reported (Ashby *et al*., 1994; IARC, 1995; Lake, 1995a,b; Cattley *et al*., 1998). In biopsies from humans receiving hypolipidaemic drugs, there was no effect or changes were much smaller than those that would be produced in rodent hepatocytes at equivalent dose levels (Lake, 1995a,b; Cattley *et al*., 1998). While peroxisome proliferation may be readily demonstrated in cultured rat and mouse hepatocytes, such effects are not observed in hepatocytes from non-responsive species including guinea-pigs, primates and humans. No study has yet compared the responsiveness to di(2-ethylhexyl) phthalate of human versus rodent livers *in vivo*. Evidence from humans, rats and mice indicates that differences in disposition, including formation of active metabolites, are of a quantitative, not qualitative nature. Taken together, these disposition data do not provide an explanation for the species differences in response to di(2-ethylhexyl) phthalate. However, several studies using human hepatocytes, unlike those with hepatocytes from mice and rats, have demonstrated that administration of di(2-ethylhexyl) phthalate metabolites does not lead to peroxisome proliferation *in vitro*. A growing body of evidence concerning the molecular basis of peroxisome proliferation, summarized below, indicates why human livers and hepatocytes would be expected to be refractory to induction of peroxisome proliferation and carcinogenesis by di(2-ethylhexyl) phthalate.

Several lines of investigation have enhanced our understanding of the molecular basis of peroxisome proliferation, with important implications for cancer risk and species differences. In rodent liver, increased organ weight, peroxisome proliferation, increased replicative DNA synthesis and induction of peroxisomal and microsomal fatty

acid-oxidizing enzymes require the expression of functional PPARα. This nuclear receptor is a member of the steroid hormone receptor superfamily and binds to DNA as a heterodimer with the retinoid X receptor (RXR). Peroxisome proliferator response elements (PPREs) have been found in genes for both peroxisomal and microsomal fatty acid-oxidizing enzymes (Lake, 1995b; Cattley *et al*., 1998). Studies with PPARα knock-out mice have demonstrated that all the above effects are lost in these mice (Lee *et al*., 1995; Peters *et al*., 1997a; Aoyama *et al*., 1998; Ward *et al*., 1998). Moreover, unlike wild-type mice, the potent peroxisome proliferator Wy-14,643 did not produce liver tumours in PPARα-deficient mice (Peters *et al*., 1997a). In this study, WY-14,643 at 0.1% in the diet or control diet was fed for 11 months to PPARα-deficient and wild-type mice and the livers were removed and examined. Of nine PPARα-deficient mice fed WY-14,643, three died and were lost to evaluation and one was euthanized and examined when moribund before 11 months. WY-14,643 produced multiple hepatocellular tumours in 6/6 wild-type mice; in three of these mice, some of these tumours were malignant. In contrast, the same treatment produced no tumours in PPARα-deficient mice (0/9), and no preneoplastic lesions were found by microscopy. No gross tumours or microscopic evidence of preneoplasia were seen in either wild-type mice or PPARα-deficient mice on the control diet (incidence, 0/9 and 0/9, respectively). Taken together, these results demonstrate that peroxisome proliferation, related hepatic effects and carcinogenesis induced by peroxisome proliferators are all absolutely dependent on functional PPARα.

Studies of PPARα activation *in vitro* have shown that di(2-ethylhexyl) phthalate metabolites are capable of activating PPARα. A study of PPARα knock-out mice treated with di(2-ethylhexyl) phthalate *in vivo* demonstrated that the increased liver weights and induction of peroxisomal and microsomal enzymes are absolutely dependent on PPARα (Ward *et al*., 1998).

The species differences, particularly with respect to humans compared to rats and mice, can be potentially attributed to several aspects of PPARα-mediated regulation of gene expression. These include the level of expression and functional capability of PPARα, the presence or absence of active PPREs in the promoter region of specific genes, and other aspects of interaction with transcriptional regulatory proteins.

Recent evidence confirms that species differences can involve more than one aspect of PPARα-mediated regulation of gene expression. The insensitivity of human liver to rodent peroxisome proliferators is associated with low levels of expression of PPARα in human liver. Marked species differences in the expression of PPARα mRNA have been demonstrated between rodent and human liver, with the latter expressing 1–10% of the levels found in mouse or rat liver (Palmer *et al*., 1994; Tugwood *et al*., 1996; Palmer *et al*., 1998). Using a sensitive and specific immuno/DNA binding assay, Palmer *et al*. (1998) have shown that active PPARα protein is expressed at variable concentrations in human livers. The study compared 20 different human livers and found that those with the highest levels of PPARα protein expression contained less than 10% of the level in mice. Most of the samples (13/20) contained no detectable PPARα activity, but did

exhibit PPRE-binding activity that did not correspond to PPARα. All six subjects aged 10 years or less lacked detectable PPARα activity. Such data support a 'threshold through competition' concept. In most human samples studied, it was found that PPREs are mainly bound by other competing proteins that may block peroxisome proliferator responsiveness. In addition, the low levels of PPARα protein detected in human liver were lower than those estimated from RNA analysis and this was explained by the finding that a significant fraction of PPARα mRNA is mis-spliced in human liver. It is possible that the low level of PPARα in human liver may explain, at least in part, the apparent lack of responsiveness of human hepatocytes to peroxisome proliferators. In support of this hypothesis, Palmer *et al.* (1998) have shown that transcriptional responses to peroxisome proliferators are not evident in the Huh cell line, which contains similar levels of PPARα and RXR to those found in intact human liver. However, increasing PPARα levels by heterologous expression in the Huh cell line conferred responsiveness to peroxisome proliferators, suggesting that the low level of endogenous PPARα is the major factor in the non-responsiveness of these cells.

The truncated human PPARα resulting from mRNA mis-splicing, identified by Palmer *et al.* (1998), has been further characterized by Gervois *et al.* (1999). They found that the truncated PPARα mRNA accounted for 25–50% of total PPARα mRNA in 10 human liver samples, while no truncated PPARα mRNA was found in livers of rats and mice. The truncated human PPARα mRNA was expressed *in vitro*, where it was shown to (*a*) fail to bind to PPRE, a necessary step for gene activation and (*b*) interfere with gene activation by expressed full-length human PPARα, in part due to titration of coactivator CREB-binding protein, an additional element of transcriptional regulation. Taken together, these results demonstrate that a certain fraction of human PPARα is probably truncated and inactive and may interfere with the function of any full-length human PPARα present in human hepatocytes.

Peroxisome proliferator responsiveness is unlikely to be a linear function of PPARα expression levels. This may be due to competition for binding to PPREs by other transcription factors such as HNF4, ARP-1 and TR (Palmer *et al*., 1994; Miyamoto *et al*., 1997), binding of PPARα to other transcription factors (e.g., LXRα) leading to formation of non-functional heterodimers (Miyata *et al*., 1996), competition of PPARα with other hormone receptors (e.g., TR) for dimerization with RXR (Juge-Aubry *et al*., 1995) or lack of co-activating proteins such as steroid receptor co-activator-1 (SRC-1) and PPAR-binding protein (PBP) (Zhu *et al*., 1997). Other human PPARs may compete with PPARα; for example PPAR* (hNUC1) can repress the activation of hPPARα (Jow & Mukherjee, 1995).

In addition to the role of receptor expression and other transcriptional regulators, differential species sensitivity to peroxisome proliferators could depend on gene-specific factors. In the case of peroxisomal acyl-coenzyme A oxidase, the promoter regions containing PPRE responsible for transcriptional activation of the rodent gene are not present in the promoter region of the human gene (Lambe *et al.*, 1999; Woodyatt *et al*., 1999). It is possible that similar differences in promoter regions of

other genes could also help to explain differences between rodent and human responses to peroxisome proliferators.

The insensitivity towards peroxisome proliferators exhibited by human hepatocytes is reflected in guinea-pigs. Guinea-pigs are also refractory to the hepatic effects of rodent peroxisome proliferators (reviewed in Doull *et al*., 1999), including di(2-ethylhexyl) phthalate metabolites (Elcombe & Mitchell, 1986; Dirven *et al*., 1993c; Elcombe *et al*., 1996) and, like humans, express similar low levels of PPARα (Bell *et al*., 1998; Tugwood *et al*., 1998). Significant responses (peroxisome proliferation, induction of fatty acid-oxidizing enzymes and stimulation of replicative DNA synthesis) believed to be associated with hepatocarcinogenesis in rodents are not observed in humans or guinea-pigs. However, these species do exhibit hypolipidaemic responses when exposed to some rodent peroxisome proliferators (Lake, 1995a; Bell *et al*., 1998; Cattley *et al*., 1998). The hypolipidaemic response, which is not associated with any hypertrophic or hyperplastic response, has been attributed to PPARα-mediated regulation of genes encoding lipoprotein lipase and various lipoproteins. The existence of such a response, in spite of low levels of PPARα, may be explained by differences in the mechanism of action of PPARα in relation to these hypolipidaemic genes compared with those that regulate hypertrophic or hyperplastic responses: the former may have a lower threshold of activation and may require lower concentrations of receptors due to different binding affinities for different PPREs. Differences in the activation properties for different PPRE-containing promoters have been demonstrated (Hsu *et al*., 1995).

In addition to results from guinea-pigs, recent studies with rabbits suggest a further potential model for the refractory nature of human liver to peroxisome proliferation (Staels & Auwerx, 1998). In humans, fibrates increase plasma levels of high-density lipoprotein (HDL) via induction of human *apo A-I* gene expression. The fibrate effect on human *apo A-I* is mediated by PPARα that interacts with a PPRE in its promoter. In normal rabbits, plasma lipoprotein levels are not changed by fibrate treatment. However, fibrate treatment of transgenic apo A-I rabbits results in increased plasma HDL and human apo A-I concentrations due to induction of the human apoA-I transgene expression in the rabbit liver. Significantly, this induction of lipoprotein gene expression occurs without affecting liver weight or peroxisomal acyl-coenzyme A oxidase activity. This demonstrates that PPARα-mediated effects of fibrates on human lipoprotein gene expression can occur in the absence of peroxisome proliferation.

In summary:

1. The weight of evidence for di(2-ethylhexyl) phthalate and its metabolic products demonstrates that they do not act as direct DNA-damaging agents.
2. Di(2-ethylhexyl) phthalate produces liver tumours in rats and mice.
3. Under conditions of the bioassays, di(2-ethylhexyl) phthalate induces peroxisome proliferation and cell replication in liver that are characteristic of a peroxisome proliferator in mice and rats.
4. Rodent peroxisome proliferators exercise their pleiotropic effects in liver due to activation of PPARα. This process is essential for liver hypertrophy and

hyperplasia and eventual hepatocarcinogenesis in response to peroxisome proliferators.

5. Hepatic peroxisome proliferation has not been adequately evaluated in studies of human livers following exposure to di(2-ethylhexyl) phthalate *in vivo*; however, the effect of treatment of human and mouse hepatocytes with di(2-ethylhexyl) phthalate metabolites which are active in rat hepatocytes, as well as other peroxisome proliferators, indicate that humans can reasonably be predicted to be refractory to induction of peroxisome proliferation and hepatocellular proliferation by di(2-ethylhexyl) phthalate. The evidence indicates that the mechanism of peroxisome proliferation induced by di(2-ethylhexyl) phthalate in rat hepatocytes does not operate in humans.
6. The absence of a significant response of human liver to induction of peroxisome proliferation and hepatocellular proliferation is explained by several aspects of PPARα-mediated regulation of gene expression.
7. Overall, these findings indicate that the increased incidence of liver tumours in mice and rats treated with di(2-ethylhexyl) phthalate results from a mechanism that does not operate in humans.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Di(2-ethylhexyl) phthalate is a liquid of low volatility, widely used as a plasticizer in flexible poly(vinyl chloride) products at concentrations of up to 40%, as well as in a number of other minor applications. Occupational exposure occurs mainly by inhalation as an aerosol during its manufacture and its use as a plasticizer in poly(vinyl chloride) product manufacturing plants, at concentrations usually below 1 mg/m^3.

Di(2-ethylhexyl) phthalate is ubiquitous in the general environment as a result of its widespread use in poly(vinyl chloride) products. It is found in ambient air at levels usually below 100 ng/m^3. The highest levels of di(2-ethylhexyl) phthalate in foods are found in milk products, meat and fish and in other products with a high fat content, where concentrations up to 10 mg/kg have been reported. The leaching of di(2-ethylhexyl) phthalate from flexible plastics used in medical devices, such as during dialysis and transfusion, can result in large direct exposures.

5.2 Human carcinogenicity data

One small study of workers in a di(2-ethylhexyl) phthalate production plant did not show any excess of cancer mortality. However, this study did not have adequate power to detect a potential excess risk.

5.3 Animal carcinogenicity data

Di(2-ethylhexyl) phthalate was tested for carcinogenicity by oral administration in the diet in two experiments in mice and six experiments in rats. Hepatocellular tumours were produced consistently in both species.

In a number of initiation/promotion studies in strains of mice susceptible to liver carcinogenesis, administration of di(2-ethylhexyl) phthalate following administration with known carcinogens enhanced the incidences of hepatocellular preneoplastic foci, adenomas and carcinomas. In a number of similar studies in rats and in one study in hamsters, in general, no promoting activity of di(2-ethylhexyl) phthalate was demonstrated. No initiating activity of di(2-ethylhexyl) phthalate was found in the liver of mice or rats. In two *N*-nitrosamine-initiation target organ models in rats, one showed enhancement of renal tubule tumours by di(2-ethylhexyl) phthalate, whereas the other showed no promotion of urinary bladder tumours.

5.4 Other relevant data

The absorption and disposition of di(2-ethylhexyl) phthalate has been investigated extensively in humans and laboratory animals. In all species studied, the compound underwent rapid metabolism, with the urine and faeces being the major routes of excretion. Following oral administration, the bulk of a di(2-ethylhexyl) phthalate dose was absorbed as the monoester, mono(2-ethylhexyl) phthalate. This ester is also formed by esterases in the body following intravenous administration and is subject to extensive oxidative metabolism by the cytochrome P450 system.

The peroxisome-proliferating effects of di(2-ethylhexyl) phthalate in susceptible species (e.g., rats and mice) have primarily been related to mono(2-ethylhexyl) phthalate and two other specific metabolites. However, while species differences have been observed in the absorption and disposition of di(2-ethylhexyl) phthalate, they do not provide an explanation for the species differences in hepatic peroxisome-proliferating activity.

The literature on potential toxic effects of di(2-ethylhexyl) phthalate following human exposure is limited. Taken together, the data indicate that di(2-ethylhexyl) phthalate does not cause observable toxicity following oral and intravenous exposure, but do not contribute information relevant to the evaluation of human carcinogenicity.

A considerable amount of information on the hepatic effects of orally administered di(2-ethylhexyl) phthalate indicates that it causes hepatic peroxisome proliferation (ultrastructural effects and enzyme induction), hepatomegaly and increased replicative DNA synthesis in rats and mice. At a lower magnitude in Syrian hamsters, enzyme induction and hepatomegaly have been observed (ultrastructural effects and replicative DNA synthesis have not been evaluated). Guinea-pigs, marmosets and cynomolgus monkeys evaluated under the same or similar experimental conditions did not exhibit peroxisome proliferation responses. Studies of di(2-ethylhexyl) phthalate

metabolites in primary rat, mouse and, to a lesser extent, Syrian hamster hepatocyte cultures *in vitro* elicited markers of peroxisome proliferation, while the same or similar experimental conditions did not elicit markers of peroxisome proliferation in primary cultures of either guinea-pig, rabbit, dog, cynomolgus monkey, marmoset or, most notably, human hepatocytes.

Hepatic peroxisome proliferation depends on a nuclear receptor, PPARα, to mediate these responses in mice, based on lack of response to peroxisome proliferators in PPARα-deficient mice. In one study with another peroxisome proliferator, WY-14,643, carcinogenesis was shown to be dependent on the same receptor. Oral administration of di(2-ethylhexyl) phthalate failed to elicit markers of peroxisome proliferation in PPARα-deficient mice, while the same treatment elicited this response in normal mice. Metabolites of di(2-ethylhexyl) phthalate caused activation of PPARα-mediated gene expression in mammalian cell co-transfection assays. Differences between responsive rodents and humans in various aspects of PPARα-mediated regulation of gene expression are consistent with the lack of activity of di(2-ethylhexyl) phthalate metabolites in hepatocyte cultures from 12 people studied to date.

No data on reproductive and developmental effects in humans were available.

Oral exposure of rats and mice to di(2-ethylhexyl) phthalate during organogenesis caused malformations and fetal death. A study in knock-out mice suggested that the developmental effects are not PPARα-mediated.

Irreversible testicular damage has been observed in male rat pups exposed prenatally and during suckling via maternal exposure to drinking water containing the compound.

Oral exposure of adult rats and mice caused effects on fertility in males and females and serious effects on the testicles. Young animals were much more sensitive to gonadal effects than adults and in some cases, the onset of occurrence of the testicular effects was earlier in young animals. Dose-dependent testicular effects were seen in young rats exposed to di(2-ethylhexyl) phthalate in the diet.

In one study using small groups of adult marmosets, oral exposure did not cause testicular toxicity at doses higher than those producing testicular effects in adult rats.

The Sertoli cells in the testes appear to be the main target of the testicular toxicity. Proposed mechanistic hypotheses relate to reduced testicular zinc levels, altered hormonal status, altered metabolic function and altered follicle-stimulating hormone reactivity.

Di(2-ethylhexyl) phthalate has been studied extensively for its genotoxic effects in a wide range of test systems, both *in vitro* and *in vivo*. The majority of these studies did not reveal any activity. No mutagenic activity was observed in bacteria. In fungi, all but two studies failed to show any evidence of recombinational events or mutation. A single study in yeast for aneuploidy was positive. Low levels of mutation were induced in *Drosophila melanogaster* in somatic cells in some studies, but no germ-cell mutations or DNA damage were induced in these insects. In cultured mammalian cells, no primary DNA damage, mutation, sister chromatid exchange or chromosomal

aberrations were induced (except in a single study for DNA strand breakage), whereas transformation of cells was induced in a number of different systems.

In vivo, neither covalent binding to DNA nor DNA strand breakage was induced in several studies on rat liver, and unscheduled DNA synthesis was not induced in the liver of either rats or mice. Gene mutations were not induced in the liver of dosed mice in a single study and there was no evidence for induction of chromosomal aberrations in mice or rats. Aberrations were induced, however, in the embryos of dosed pregnant Syrian hamsters. Dominant lethal effects were reported to be induced in male mice, but re-evaluation of these data did not confirm this conclusion.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of di(2-ethylhexyl) phthalate.

There is *sufficient evidence* in experimental animals for the carcinogenicity of di-(2-ethylhexyl) phthalate.

Overall evaluation

Di(2-ethylhexyl) phthalate is *not classifiable as to its carcinogenicity to humans (Group 3)*.

In making its overall evaluation of the carcinogenicity to humans of di(2-ethylhexyl) phthalate, the Working Group took into consideration that (*a*) di(2-ethylhexyl) phthalate produces liver tumours in rats and mice by a non-DNA-reactive mechanism involving peroxisome proliferation; (*b*) peroxisome proliferation and hepatocellular proliferation have been demonstrated under the conditions of the carcinogenicity studies of di(2-ethylhexyl) phthalate in rats and mice; and (*c*) peroxisome proliferation has not been documented in human hepatocyte cultures exposed to di(2-ethylhexyl) phthalate nor in the liver of exposed non-human primates. Therefore, the mechanism by which di(2-ethylhexyl) phthalate increases the incidence of hepatocellular tumours in rats and mice is not relevant to humans.

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DI(2-ETHYLHEXYL) ADIPATE

This substance was considered by previous working groups in October 1981 (IARC, 1982) and March 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated in the monograph and taken into consideration in the evaluation.

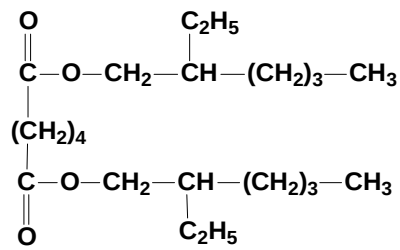
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 103-23-1
Deleted CAS Reg. Nos: 39393-67-4; 63637-48-9; 70147-21-6
Chem. Abstr. Name: Hexanedioic acid, bis(2-ethylhexyl) ester
IUPAC Systematic Names: Adipic acid bis(2-ethylhexyl) ester; bis(2-ethylhexyl) adipate
Synonyms: BEHA; DEHA; dioctyl adipate; DOA; hexanedioic acid, dioctyl ester; octyl adipate

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{22}H_{42}O_4$ Relative molecular mass: 370.58

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Light-coloured, oily liquid (Verschueren, 1996)
(*b*) *Boiling-point*: 417 °C (Lewis, 1993)
(*c*) *Melting-point*: –67.8 °C (Lide & Milne, 1996)
(*d*) *Density*: 0.922 g/cm^3 at 20 °C (Lide & Milne, 1996)
(*e*) *Spectroscopy data*: Infrared (grating [8003]), nuclear magnetic resonance [943C] and mass spectral data have been reported (Lide & Milne, 1996; Aldrich Chemical Co., 1998; National Institute for Standards and Technology, 1998)
(*f*) *Solubility*: Very slightly soluble in water (< 200 mg/L at 20 °C) (Verschueren, 1996); very soluble in acetone, diethyl ether and ethanol (Lide & Milne, 1996)
(*g*) *Volatility*: Vapour pressure, 346 Pa at 200 °C (Lewis, 1993; Verschueren, 1996); flash-point, 196 °C (Lewis, 1993)
(*h*) *Octanol/water partition coefficient (P)*: log P, 8.1 (Verschueren, 1996)
(*i*) *Conversion factor*[1]: mg/m^3 = 15.16 × ppm

1.1.4 *Technical products and impurities*

Di(2-ethylhexyl) adipate is commercially available with the following specifications: purity, 99–99.9%; acidity, 0.25 meq/100 g max.; moisture, 0.05–0.10% max. (C.P. Hall Co., undated; Solutia, Inc., 1995; Velsicol Chemical Corp., 1997; Aldrich Chemical Co., 1998; Eastman Chemical Co., 2000).

Trade names for di(2-ethylhexyl) adipate include Adimoll DO; Adipol 2EH; ADO; ADO (lubricating oil); Arlamol DOA; Bisoflex DOA; Crodamol DOA; Diacizer DOA; Eastman DOA Plasticizer; Effomoll DA; Effomoll DOA; Ergoplast AdDO; Flexol A 26; Hatcol 2908; Kodaflex DOA; Lankroflex DOA; Monoplex DOA; Plasthall DOA; Plastomoll DOA; Reomol DOA; Sansocizer DOA; Sicol 250; Truflex DOA; Vestinol OA; Wickenol 158; Witamol 320.

1.1.5 *Analysis*

Di(2-ethylhexyl) adipate can be extracted from a water sample by passing this water through a cartridge or disk containing a solid inorganic matrix coated with a chemically bonded C18 organic phase (liquid–solid extraction). Organic material eluted from the liquid–solid extraction cartridge or disk with dichloromethane is analysed for di(2-ethylhexyl) adipate by gas chromatography/mass spectrometry (Environmental Protection Agency, 1995).

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[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

1.2 Production

Di(2-ethylhexyl) adipate can be prepared by the reaction of adipic acid and 2-ethylhexanol in the presence of an esterification catalyst such as sulfuric acid or *para*-toluenesulfonic acid (National Library of Medicine, 1999).

Information available in 1999 indicated that di(2-ethylhexyl) adipate was manufactured by eleven companies in Japan, eight companies in the United States, five companies each in India and Taiwan, four companies each in Canada, France and Germany, three companies each in Brazil, Mexico and Spain, two companies each in Argentina, Australia, Chile, China, Italy and the United Kingdom and one company each in Belgium, Colombia, the Czech Republic, the Republic of Korea, the Netherlands, Peru, Romania, Russia, South Africa, Turkey and Venezuela (Chemical Information Services, 1999).

1.3 Use

Di(2-ethylhexyl) adipate is used primarily as a plasticizer in the flexible vinyl industry and is widely used in flexible poly(vinyl chloride) (PVC) food film (cling film). It is commonly blended with di(2-ethylhexyl) phthalate (see monograph in this volume) and di(isooctyl) phthalate in PVC and other polymers. It is used as a solvent and as a component of aircraft lubricants. It is important in the processing of nitrocellulose and synthetic rubber, in plasticizing polyvinyl butyral, cellulose acetate butyrate, polystyrene and dammar wax and in cosmetics (cellulose-based liquid lipsticks) (Cadogan & Howick, 1992, 1996; Verschueren, 1996; National Toxicology Program, 1999).

1.4 Occurrence

1.4.1 *Natural occurrence*

Di(2-ethylhexyl) adipate is not known to occur as a natural product.

1.4.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey, as many as 15 600 workers in the United States were potentially exposed to di(2-ethylhexyl) adipate (NOES, 1999). Occupational exposure may occur through inhalation, mainly as an aerosol, during its manufacture and its use, particularly as a plasticizer of PVC films and in other materials used in food packaging such as adhesives, cellophane and hydroxyethyl cellulose films. Exposure may also occur during the manufacture of rubber products, nonferrous wire, cosmetics, lubricants and hydraulic fluids (Opresko, 1984). No measurements of di(2-ethylhexyl) adipate exposure in manufacturing and processing industries are available.

Workers wrapping meat are potentially exposed to particulate di(2-ethylhexyl) adipate while cutting PVC films by drawing them across a heated cutter (hot wire or cool rod process) (Smith *et al.*, 1983). Exposure concentrations of 0.25 mg/m^3 and 0.14 mg/m^3 just above the hot wire of a PVC film cutting machine have been reported in tests simulating normal operating conditions when the wire was operated at 182 °C and 104 °C, respectively (Van Houten *et al.*, 1974). Cook (1980) estimated from test emission data that maximum di(2-ethylhexyl) adipate concentrations of 0.2 mg/m^3 in workroom air could be reached in hot wire operations. In the United States, the National Institute for Occupational Safety and Health reported non-detectable levels of di(2-ethylhexyl) adipate (less than 0.08 mg/m^3) near a cool rod machine (operating temperature of 190 °C) used to cut PVC film in a meat cutting and wrapping department of a grocery store (Daniels *et al.*, 1985).

1.4.3 *Environmental occurrence*

Di(2-ethylhexyl) adipate may be released into the environment during its manufacture and distribution, during PVC blending operations and cutting of PVC film, and from consumer use and disposal of finished products (IARC, 1982; Environmental Protection Agency, 1998).

(*a*) *Air*

According to the Toxics Release Inventory (Environmental Protection Agency, 1996), air emissions of di(2-ethylhexyl) adipate from 148 industrial facilities amounted to approximately 315 000 kg in 1994 in the United States.

(*b*) *Water*

Di(2-ethylhexyl) adipate has been detected infrequently in fresh water, generally at < 1 μg/L (Sheldon & Hites, 1979; IARC, 1982; Felder *et al.*, 1986; WHO, 1996). Di(2-ethylhexyl) adipate is relatively insoluble in water and is likely to partition to sediment and biota in the aquatic environment. A survey of 23 natural surface water sites in 12 states showed that 7% of 82 samples contained di(2-ethylhexyl) adipate at levels ranging from 0.25 to 1.0 μg/L with an average of 0.46 μg/L (Felder *et al.*, 1986).

Surface water discharges of di(2-ethylhexyl) adipate from 148 industrial facilities in the United States in 1994 amounted to 560 kg, as reported in the Toxics Release Inventory (Environmental Protection Agency, 1996).

Di(2-ethylhexyl) adipate was found at microgram-per-litre levels in two of five samples of finished water from a water-treatment plant in the United States (WHO, 1996). It was detected in 'finished' drinking-water in New Orleans, Louisiana, at an average concentration of 0.10 μg/L but not in drinking-water in two smaller nearby cities (IARC, 1982). Di(2-ethylhexyl) adipate was detected in the Delaware River at levels of 0.08–0.3 μg/L (Sheldon & Hites, 1979). It has also been identified in Europe

as a trace level contaminant of the River Rhine (WHO, 1996) and in the Great Lakes of North America at levels of 0.01–7.0 µg/L (Hrudey *et al*., 1976).

Di(2-ethylhexyl) adipate was found at levels of 2000 µg/L near a chemical plant source near the Delaware River, north of Philadelphia in 1977 and at levels of 90 and 10 µg/L at sampling sites at influent and effluent waste-treatment sites, respectively (Sheldon & Hites, 1979).

(*c*) *Soil*

Releases of di(2-ethylhexyl) adipate to land from 148 industrial facilities in the United States in 1994 amounted to 67 000 kg, as reported in the Toxic Release Inventory (Environmental Protection Agency, 1996).

(*d*) *Biodegradation and bioconcentration*

Model experiments with acclimated activated sludge systems have shown essentially complete biodegradation of relatively high concentrations (~ 20 mg/L) of di(2-ethylhexyl) adipate to carbon dioxide and water in 35 days (Saeger *et al*., 1976; Felder *et al*., 1986).

A bioconcentration study with bluegill showed that di(2-ethylhexyl) adipate is not an accumulative or persistent chemical in this species of fish (Felder *et al*., 1986).

(*e*) *Food*

Food is the major source of exposure of the general population to di(2-ethylhexyl) adipate because of its migration, particularly to fatty foods such as cheese and meat, from PVC films used for packaging that have been plasticized with di(2-ethylhexyl) adipate (IARC, 1982; Castle *et al*., 1987; Startin *et al*., 1987; Page & Lacroix, 1995; WHO, 1996).

Di(2-ethylhexyl) adipate has been found at generally low levels in a broad variety of foods including milk, cheese, margarine, butter, meat, cereals, poultry, baked goods and sandwiches, fruits and vegetables (Castle *et al*., 1987; Startin *et al*., 1987; Mercer *et al*., 1990; Gilbert *et al*., 1994; Page & Lacroix, 1995; Petersen *et al*., 1995).

A United Kingdom survey of di(2-ethylhexyl) adipate levels in 83 retail samples wrapped in plasticized PVC films was reported by Castle *et al*. (1987). Foodstuffs analysed (from both retail and take-away outlets) included fresh meat and poultry, ready-cooked poultry, cheese, fruit, vegetables and baked goods (cakes, bread rolls and sandwiches). Ranges of di(2-ethylhexyl) adipate levels were 1.0–72.8 mg/kg in uncooked meat and poultry, 9.4–48.6 mg/kg in cooked chicken portions, 27.8–135.0 mg/kg in cheese, 11.0–212 mg/kg in baked goods and sandwiches, and < 2.0 mg/kg in fruits and vegetables. The level of di(2-ethylhexyl) adipate in meat exposed to plasticized film was not reduced significantly by volatilization or chemical transformation on subsequent cooking by grilling or frying.

The highest levels of migration of di(2-ethylhexyl) adipate from PVC films during home-use and microwave cooking in the United Kingdom were observed for cheese, cooked meats, cakes and microwave-cooked foods, whilst lower levels were found for wrapping of unfilled buttered sandwiches, fruit and vegetables (except avocado). Levels of migration of di(2-ethylhexyl) adipate into purchased ready-cooked meats, rewrapped in the home in PVC film and kept for seven days at 5 °C or 30 days at –18 °C were: chicken, 75 and 29 mg/kg, respectively; salami, 181 and 109 mg/kg, respectively; ham, 107 and 25 mg/kg, respectively; and beef (minced), 78 and 23 mg/kg, respectively. Overall, migration of di(2-ethylhexyl) adipate increased with both the length of contact time and temperature of exposure, with the highest levels found where there was direct contact between the film and food and where the latter had a high fat content in the contact surface (Startin *et al.*, 1987).

A survey of di(2-ethylhexyl) adipate in Canadian packaging and food sampled during the period 1985–89 was reported by Page and Lacroix (1995). Selected foods (260 samples) packaged in materials with potential to contribute plasticizers to the food and available food composites (98 samples) obtained from the Canadian Health Protection Branch Total Diet Program were analysed for plasticizers including di(2-ethylhexyl) adipate and di(2-ethylhexyl) phthalate. Di(2-ethylhexyl) adipate was found in food-contacting PVC film and as a migrant in store-wrapped meat, poultry, fish, cheese and ready-to-eat foods at levels as high as 310 mg/kg (cheese). Di(2-ethylhexyl) adipate levels in unheated film-wrapped ready-to-eat foods were increased by heating. Di(2-ethylhexyl) adipate residues found in fresh fruits and vegetables were typically < 4 mg/kg.

In a study in New South Wales, Australia, of 184 samples of food packaged in a range of plastics, only samples in contact with PVC film were found to contain a detectable amount of di(2-ethylhexyl) adipate. Of the 98 samples wrapped in PVC films, 44 (45%) showed levels of migration of di(2-ethylhexyl) adipate exceeding 30 mg/kg. Significant quantities of di(2-ethylhexyl) adipate were found in cheeses which had been wrapped at the point of sale. Di(2-ethylhexyl) adipate was detected in 36 of 38 samples of cheese wrapped in PVC film, at levels ranging from 31 to 429 mg/kg. Five out of 42 samples (12%) of fresh meat packaged in PVC film gave positive results, with levels ranging from 49 to 151 mg/kg. Migration of di(2-ethylhexyl) adipate at levels of 64 and 325 mg/kg was also found in other foods such as sandwiches wrapped in PVC (Kozyrod & Ziaziaris, 1989).

Badeka and Kontominas (1996) reported the effect of microwave heating on the migration of di(2-ethylhexyl) adipate from food-grade PVC into olive oil and water. Migration was dependent on heating time, microwave power setting, the nature of the food simulant and the initial concentration of the plasticizer in the film.

Petersen *et al.* (1997) reported that, compared with a specific migration limit of 3 mg di(2-ethylhexyl) adipate/dm^2 from PVC cling films used in Denmark, 77% of the films used for fatty foodstuffs sampled from importers, wholesalers and retail shops

were found to be unacceptable. The migration of di(2-ethylhexyl) adipate to non-fatty foods defined as the food simulant water was ≤ 0.1 mg/dm^2 for all PVC films.

The maximum daily intake of di(2-ethylhexyl) adipate through the diet in the United Kingdom was estimated in 1987 to be 16 mg (Anon., 1991; Loftus *et al*., 1993, 1994; WHO, 1996). Reformulation of PVC film reflecting the use of less di(2-ethylhexyl) adipate necessitated a more recent evaluation which suggested that the maximum daily intake of di(2-ethylhexyl) adipate in the United Kingdom was 8.2 mg (Loftus *et al*., 1993, 1994).

The major urinary metabolite of di(2-ethylhexyl) adipate, 2-ethylhexanoic acid, has been shown to be an appropriate marker for biological monitoring of dietary di(2-ethylhexyl) adipate intake (Loftus *et al*., 1993, 1994). A limited population study in the United Kingdom was undertaken to estimate the daily intake of di(2-ethylhexyl) adipate following intake of a mean dose of 5.4 mg di(2-ethylhexyl) adipate presented with food. The study involved the determination of the urinary metabolite, 2-ethylhexanoic acid (24-h urine sample) in 112 individuals from five geographical locations. A skewed distribution with a median value for the daily intake of 2.7 mg was determined (Loftus *et al*., 1994). This value is about one third of the indirectly estimated maximum intake of 8.2 mg per day. The probability of a daily intake in excess of 8.2 mg in the limited population (112 individuals) was calculated to be 3% (Loftus *et al*., 1994).

1.5 Regulations and guidelines

The World Health Organization has established an international drinking water guideline for di(2-ethylhexyl) adipate of 80 µg/L (WHO, 1996). The United States Environmental Protection Agency (1998) has set a maximum contaminant level (MCL) for di(2-ethylhexyl) adipate in drinking water of 0.4 mg/L.

In the United States, the Food and Drug Administration (1999) permits the use of di(2-ethylhexyl) adipate as a component of adhesives used in food packaging, as a component of cellophane, as a plasticizer in polymeric substances used in the manufacture of articles for the food industry, as a component of paper and paper-board in contact with aqueous and fatty foods, and as a component of closures with sealing gaskets for food containers.

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 B6C3F_1 mice, six weeks of age, were fed diets containing 0, 12 000 or 25 000 mg/kg diet (ppm) di(2-ethyhexyl) adipate (> 98% pure) for 103 weeks and were killed 105–107 weeks after the beginning of treatment. Mean body weights of treated mice of each sex were lower than those of the corresponding controls and the decrease in weight gain was dose-related. Survival in males was 36/50 (72%), 32/50 (64%) and 41/50 (82%) in control, low-dose and high-dose animals, respectively, and that in females was 42/50 (84%), 39/50 (78%) and 36/49 (73%) in the control, low-dose and high-dose animals, respectively. Di(2-ethylhexyl) adipate increased the incidence of hepatocellular adenomas in both males (6/50 control, 8/49 low-dose and 15/49 high-dose ($p < 0.025$) and females (2/50, 5/50 and 6/49 in control, low-dose and high-dose animals). Hepatocellular carcinomas were observed in 7/50 control, 12/49 low-dose and 12/49 high-dose males and 1/50 control, 14/50 low-dose ($p < 0.001$) and 12/49 high-dose ($p = 0.001$) females. The incidences of hepatocellular adenomas and carcinomas combined were also increased in males (control, 13/50; low-dose, 20/49 and high-dose, 27/49, $p = 0.003$, pairwise comparison; $p = 0.002$, trend test) and in females (control, 3/50, low-dose, 19/50 $p < 0.001$; and high-dose, 18/49 $p < 0.001$, pairwise comparisons). [The Working Group noted that negative trends were reported for certain tumour types (lymphomas, lung and subcutaneous tumours in males and pituitary adenomas in females)] (National Toxicology Program, 1982; Kluwe *et al*., 1985).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, five weeks of age, were fed diets containing 0, 12 000 or 25 000 ppm di(2-ethylhexyl) adipate (purity, > 98%) for 103 weeks and were killed 105–107 weeks after the beginning of treatment. Mean body weights of high-dose rats of each sex were lower than those of the controls throughout the study. Survival in males was 34/50 (68%) in the control and low-dose groups and 40/50 (80%) in the high-dose group and in females was 29/50 (58%), 39/50 (78%), and 44/50 (88%) in the control, low-dose and high-dose groups, respectively. There was no treatment-related increase in tumours. Neoplastic nodules or hepatocellular carcinomas were found in 2/49 control, 2/50 low-dose and 2/50 high-dose males and in 0/49, 3/50, and 1/50 females, respectively (National Toxicology Program, 1982; Kluwe *et al*., 1985).

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

In six male volunteers given 46 mg deuterium-labelled di(2-ethylhexyl) adipate [approx. 0.5 mg/kg bw] in corn oil, 2-ethylhexanoic acid was the only metabolite that could be determined in the plasma. It had an elimination half-life of 1.65 h. In urine, the following metabolites were identified (percentage fraction of administered deuterium label): 2-ethylhexanoic acid (8.6%), 2-ethyl-5-hydroxyhexanoic acid (2.6%), 2-ethyl-1,6-hexanedioic acid (0.7%), 2-ethyl-5-ketohexanoic acid (0.2%) and 2-ethylhexanol (0.1%). The half-life for elimination of all metabolites excreted in the urine averaged 1.5 h, and none of the metabolites could be detected after 36 h (Loftus *et al.*, 1993).

4.1.2 *Experimental systems*

Di(2-ethylhexyl) adipate is rapidly and completely absorbed from the gastrointestinal tract of experimental animals. In rats, there is evidence for cleavage of the parent compound and subsequent absorption of the monoester and the acid (Takahashi *et al.*, 1981), whereas in cynomolgus monkeys unchanged di(2-ethylhexyl) adipate is also absorbed (BUA, 1996). Radiolabel from di(2-ethylhexyl) [*carbonyl*-^{14}C]adipate is distributed to a number of tissues, with maximum levels being reached after 6–12 h. While most radioactivity was found in the gastrointestinal tract, muscle, liver, fat, blood and kidney had relatively high levels of di(2-ethylhexyl) adipate-associated radiolabel (Takahashi *et al*., 1981; Bergman & Albanus, 1987; BUA, 1996).

A half-life of 6 min for metabolism of di(2-ethylhexyl) adipate has been determined in rat small intestinal mucous membrane homogenates. The dominant urinary metabolite of di(2-ethylhexyl) adipate (500 mg/kg bw) in male Wistar rats is adipic acid, which accounts for 20–30% of the administered oral dose. The other major metabolite which was found only in the stomach is mono(2-ethylhexyl) adipate (Takahashi *et al.*, 1981). In cynomolgus monkeys, the glucuronide of mono(2-ethylhexyl) adipate and traces of unchanged di(2-ethylhexyl) adipate were found in the urine (BUA, 1996).

Di(2-ethylhexyl) adipate is rapidly eliminated, with most of the dose appearing in the urine after oral administration to Fischer 344 rats, B6C3F$_1$ mice and cynomolgus monkeys (rats, 34–78% of the dose after 24 h; mice, 75–92%; monkeys, 47–57%). In rats, total radioactivity in the body after 96 h was approximately 0.5%. Some of the biliary-secreted radioactivity (approximately 3% in rats) flows into the enterohepatic circulation. Passage of di(2-ethylhexyl) adipate (84.3 µg per animal) through the placenta of pregnant NMRI mice has been described (Bergman & Albanus, 1987; BUA, 1996).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

The acute oral LD_{50} values for di(2-ethylhexyl) adipate in Fischer 344 rats were estimated to be 45 (males) and 25 (females) g/kg bw by gavage and in B6C3F_1 mice were estimated to be 15 (males) and 25 (females) g/kg bw by gavage (National Toxicology Program, 1982). Consumption of 2.5% di(2-ethylhexyl) adipate in the diet [5 g/kg bw per day] by female B6C3F_1 mice and 4.0% di(2-ethylhexyl) adipate in the diet [3 g/kg bw per day] by female Fischer 344 rats was not associated with lethality, although body weight gain was diminished (Lake *et al.*, 1997). Similar dietary administration of di(2-ethylhexyl) adipate (25 000 ppm [2.5%] in diet) did not affect survival in studies of two years' duration in mice and rats (National Toxicology Program, 1982).

The effects of di(2-ethylhexyl) adipate (1% of diet) on plasma lipids were evaluated in male Upjohn:TUC (SD) rats (Bell, 1984). After two weeks and four weeks (but not seven weeks) of feeding, plasma cholesterol levels were significantly decreased. After four weeks (but not two or seven weeks) of feeding, plasma triglyceride levels were significantly decreased. Hepatic cholesterol synthesis was diminished by di(2-ethylhexyl) adipate consumption (Bell, 1983, 1984).

A variety of studies have evaluated the effects of oral administration of di(2-ethylhexyl) adipate on rodent liver. In male and female Fischer 344 rats and B6C3F_1 mice, administration of di(2-ethylhexyl) adipate by gavage in corn oil at levels of 0.5–2.5 mg/kg bw per day for 14 days increased peroxisomal cyanide-insensitive palmitoyl-coenzyme A (CoA) oxidation activity from approximately twofold up to 15-fold in male rats and female mice, up to fourfold in female rats and ninefold in male mice. These effects were accompanied by slight but statistically significant increases in catalase activity in mice but not rats. Light microscopic evaluation of liver sections from rats revealed a di(2-ethylhexyl) adipate-dependent loss of glycogen in hepatocytes that progressed from centrilobular to panlobular with increasing dose. In both rats and mice, there was dose-related hypertrophy and increased eosinophilia of hepatocytes. No evidence of hepatotoxicity was observed by light microscopy. Morphometric analysis of liver ultrastructure demonstrated increases in peroxisomal volume density, as summarized in Table 1 (Keith *et al.*, 1992). Similar increases in peroxisomal volume density (by morphometric analysis) were reported following dietary administration of di(2-ethylhexyl) adipate (1.0 or 2.0 but not ≤ 0.5% of diet) to Fischer 344 rats [sex not specified] for 30 days (Reddy *et al.*, 1986).

The hepatic effects of di(2-ethylhexyl) adipate were evaluated in female B6C3F_1 mice and Fischer 344 rats fed diets containing 0–4.0% (up to 3140 mg/kg bw per day

Table 1. The effect of di(2-ethylhexyl) adipate administered daily by gavage for 14 days on peroxisomal volume density in Fischer 344 rats and B6C3F$_1$ mice

DEHA (mg/kg bw per day)		0.0	0.5	1.0	1.5	2.5
Species	Sex	Peroxisomal volume density (% of cytoplasmic volume)				
Fischer 344 rats	Male	1.4	2.8	4.0	6.2	10.4
	Female	1.4	2.5	7.1	7.2	Not measured
B6C3F$_1$ mice	Male	1.4	1.5	2.9	4.3	6.3
	Female	1.4	2.0	3.8	5.0	7.1

From Keith *et al*. (1992)

in rats and 5330 mg/kg bw per day in mice) di(2-ethylhexyl) adipate for one, four and 13 weeks (Lake *et al*., 1997). In mice, di(2-ethylhexyl) adipate at ≥ 0.6% (1495 mg/kg bw per day) induced dose-dependent increases in relative liver weight and hepatic peroxisome proliferation, as demonstrated by the induction of peroxisomal cyanide-insensitive palmitoyl-CoA oxidation (increases were statistically significant for at least one treatment interval). Microsomal lauric acid 11- and 12-hydroxylase activities (CYP4A) were similarly increased at the same or the next lowest dietary concentration (0.3%; 282 mg/kg bw per day in rats and 808 mg/kg bw per day in mice). Hepatocellular replication (measured as nuclear 5-bromo-2′-deoxyuridine [BrdU] labelling) was increased during week 1 of di(2-ethylhexyl) adipate treatment of mice at ≥ 0.6% (1495 mg/kg bw per day) and was still elevated at weeks 4 and 13 at doses of ≥ 1.2% (3075 mg/kg bw per day). In contrast to mice, rats fed di(2-ethylhexyl) adipate had much smaller increases in relative liver weight and peroxisomal palmitoyl-CoA oxidation at doses matching those used in a bioassay (National Toxicology Program, 1981), although at even higher dietary concentrations, rats were similarly responsive. This apparent difference in magnitude of response between mice and rats could in part be accounted for by the different rates of di(2-ethylhexyl) adipate intake (see Table 2). Therefore, the apparent difference in the results of carcinogenicity testing between mice and rats could be related to differences in intake of di(2-ethylhexyl) adipate and the resulting peroxisome proliferation and related responses in liver. In rats, there was similar induction of microsomal lauric acid 11- and 12-hydroxylase activity. While hepatocellular replication (measured as nuclear BrdU labelling) was increased during week 1 of administration in rats, this response was not sustained during weeks 4 or 13, although the magnitude of response during week 1 was similar to that in mice.

Increased liver weights (absolute, 32%; relative, 36% over respective control values) were observed in male Fischer 344 rats fed di(2-ethylhexyl) adipate (2.5% of

Table 2. Comparison of responses in liver of female mice and rats following four weeks of di(2-ethylhexyl) adipate treatment

Diet (%)	Intake (mg/kg bw per day)		Relative liver weight (% increase over controls)		Peroxisomal palmitoyl-CoA oxidation (increase over control)		Microsomal lauric acid hydroxylase (increase over control)			
							Mouse		Rat	
	Mouse	Rat	Mouse	Rat	Mouse	Rat	11-position	12-position	11-position	12-position
0.15	343	144	NC	NC	NC	NE	NC	NC	NC	NC
0.30	808	282	NC	NC	NC	NC	NC	2-fold	NC	NC
0.60	1495	577	10	NC	2-fold	NC	2-fold	4-fold	NC	NC
1.20[a]	3075	1135	50	10	7-fold	< 2-fold	3-fold	8-fold	NC	NC
2.50[a]	5330	2095	60	30	13-fold	8-fold	5-fold	16-fold	2-fold	2-fold
4.00	NE	3140	NE	80	NE	17-fold	NE	NE	3-fold	8-fold

Adapted from Lake *et al*. (1997)

[a] Dietary levels administered to mice and rats in a carcinogenesis bioassay, resulting in an increase in tumour incidence in mice but not in rats (National Toxicology Program, 1981).

NC, not different from controls; NE, not evaluated

diet) for one week. Slight but statistically significant increases in 8-hydroxydeoxyguanosine (8-OH-dG), an indicator of oxidative DNA damage, in liver but not kidney DNA were reported, and also after two weeks' administration (Takagi *et al.*, 1990).

Several studies have evaluated the effects of oral di(2-ethylhexyl) adipate on various aspects of hepatic lipid metabolism. Feeding di(2-ethylhexyl) adipate (2% of diet) to male Wistar rats for seven days resulted in increased hepatic fatty acid-binding protein as well as in increased microsomal stearoyl-CoA desaturation activity (Kawashima *et al*., 1983a,b). Feeding the compound at this dose for 14 days resulted in increased levels of hepatic phospholipids and a decline in phosphatidylcholine:phosphatidylethanolamine ratio (Yanagita *et al*., 1987). Feeding di(2-ethylhexyl) adipate (2% of diet) to male NZB mice for five days resulted in induction of fatty acid translocase, fatty acid transporter protein and fatty acid binding protein in the liver (Motojima *et al*., 1998).

Primary hepatocyte cultures may be employed to study species differences in hepatic peroxisome proliferation (IARC, 1995). The effects of di(2-ethylhexyl) adipate and its metabolites in cultured hepatocytes from rats, mice, guinea-pigs and marmosets have been studied (Cornu *et al*., 1992). In hepatocytes from each species, the parent compound di(2-ethylhexyl) adipate had no effect on peroxisomal cyanide-insensitive palmitoyl-CoA oxidation activity. However, in rat and mouse hepatocytes, the metabolites mono(2-ethylhexyl) adipate, 2-ethylhexanol, 2-ethylhexanoic acid and 2-ethyl-5-hydroxyhexanoic acid at concentrations ≤ 1 mM induced peroxisomal palmitoyl-CoA oxidation. No induction of peroxisomal palmitoyl-CoA oxidation was seen at concentrations ≤ 1 mM for mono(2-ethylhexyl) adipate or ≤ 2 mM for 2-ethylhexanol, 2-ethylhexanoic acid and 2-ethyl-5-hydroxyhexanoic acid in guinea-pig or marmoset hepatocytes (2-ethylhexanol was evaluated only at ≤ 1 mM in marmoset hepatocytes).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

(*a*) *Developmental toxicity studies*

Groups of five Sprague-Dawley rats were given intraperitoneal injections of 1, 5 or 10 mL/kg bw di(2-ethylhexyl) adipate on days 5, 10 and 15 of pregnancy. Fetal weight in the two high-dose groups showed a dose-dependent reduction. The incidence of externally visible malformations [no further details supplied] was significantly higher in the high-dose group. The rate of skeletal malformations lay in the same range as in the control groups; the rate of visceral malformations was reported

to be higher in the middle- and high-dose groups than in the control animals. Correlations with maternally toxic effects were not described (Singh *et al.*, 1973).

Groups of 24 Alpk:APF50 rats were given approximately 28, 170 and 1080 mg/kg bw via the feed from days 1 to 22 of gestation. In the high-dose group, there was a slight reduction of maternal body weight gain. The incidence of unilateral ureter kinking was slightly but significantly higher in the middle- and high-dose groups. In addition, in the high-dose group, there was a significantly higher incidence of skeletal variations/retardations (Hodge, 1991, cited in BUA, 1996).

(*b*) *Mechanistically oriented developmental toxicity studies*

No effects on three-day-old chick embryos were found after exposure by injection of 17 μmol di(2-ethylhexyl) adipate per egg into the air chamber of the egg (Korhonen *et al.*, 1983a,b).

A single intraperitoneal injection of di(2-ethylhexyl) adipate (12.5 mL/kg bw) on day 15 of gestation increased the cytochrome P450 content in hepatic microsomes in pregnant and non-pregnant NP C57BL/6J mice, but increased the aminopyrine-*N*-demethylase activity only in pregnant mice. In the pregnant mice, di(2-ethylhexyl) adipate decreased levels of P450-gest, an isoenzyme induced in mouse pregnancy, but increased other P450 isoenzymes (Lamber *et al.*, 1987, cited in BUA, 1996).

Radioactivity was observed in fetal liver, intestine and bone marrow during the first 24 h after intravenous or intragastric administration of di(2-ethylhexyl) [*carbonyl*-^{14}C]adipate to pregnant NMRI mice on gestational day 17 (Bergman & Albanus, 1987). When [*ethylhexyl*-^{14}C]di(2-ethylhexyl) adipate was administered, there was very little accumulation of radiolabel but some was found in the urinary bladder, liver and intestinal contents of the fetus as well as in the amniotic fluid. A remarkably strong uptake of radioactivity was observed in the corpora lutea of the ovary in the pregnant mice.

(*c*) *Reproductive toxicity studies*

In a dominant lethal study, a reduced percentage of pregnancies and an increased number of fetal deaths were observed in Harlan/ICR albino Swiss mice after a single intraperitoneal dose of 10 mL/kg bw was given to male mice before an eight-week mating period (Singh *et al.*, 1975).

Groups of 30 female and 15 male Alpk:APF50 rats were fed diets containing 300, 1800 or 12 000 ppm di(2-ethylhexyl) adipate for a period of 10 weeks before mating and during the gestation and lactation periods. In the high-dose group, there was a significant reduction in body weight gain of the females during the last part of the gestation period. There was no effect on male and female fertility, the number of live births or the survival rate of the pups up to day 22 of life. In the high-dose group, the body weight gain of the pups was significantly reduced throughout the postnatal follow-up period (up to day 36 of life) (Tinston, 1988, cited in BUA, 1996).

In a multigeneration study, rats were given 100 mg/kg di(2-ethylhexyl) adipate per day via the feed. For four successive generations, no substance-specific influence on reproduction rate, lactation or growth was reported [no further details supplied] (Le Breton, 1962, cited in BUA, 1996).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 3 for references)

Di(2-ethylhexyl) adipate was not mutagenic to *Salmonella typhimurium* strains TA100, TA1535,TA1537, TA1538 or TA98 in the presence or absence of exogenous metabolic activation in three studies. It was also not mutagenic to *Photobacterium phosphoreum* in a single study. A single study found no induction of sex-linked recessive lethal mutations after administration of di(2-ethylhexyl) adipate to adult *Drosophila melanogaster* by feeding or injection.

One study employing the mouse lymphoma gene mutation assay found no induction of mutations at the *Tk* locus in L5178Y mouse lymphoma cells following exposure to di(2-ethylhexyl) adipate in the absence of exogenous metabolic activity. With exogenous metabolic activation, one experiment similarly showed no induction of mutation, while a second experiment showed an effect but only at a concentration at which precipitation occurred (above 1000 μg/mL). A single in-vitro study using rat hepatocytes found no induction of sister chromatid exchanges, chromosomal aberrations or micronuclei after treatment with di(2-ethylhexyl) adipate for either 3 or 51 h. In bone marrow of mice treated *in vivo* with di(2-ethylhexyl) adipate, chromosomal aberrations were not induced in one study and no effect was seen in a single micronucleus assay.

A weak positive result has been reported in a dominant lethal assay in male mice.

Urine samples from rats treated by gavage with 15 daily doses of di(2-ethylhexyl) adipate were not mutagenic to *S. typhimurium* strains TA100, TA1535, TA1537, TA1538 or TA98. In one study, formation of 8-OH-dG was measured as an indicator of oxidative DNA damage in liver and kidney of rats exposed to di(2-ethylhexyl) adipate in the diet for two weeks. Increased levels of 8-OH-dG were found in the liver but not in the kidney. A separate study found no evidence of covalent binding of di(2-ethylhexyl) adipate to mouse liver DNA.

Three putative metabolites of di(2-ethylhexyl) adipate—(mono(2-ethylhexyl) adipate, mono(2-ethyl-5-hydroxyhexyl) adipate and mono(2-ethyl-5-oxohexyl) adipate)—were not mutagenic to *S. typhimurium* strains TA100, TA102, TA98 or TA97 in the presence or absence of exogenous metabolic activation.

Table 3. Genetic and related effects of di(2-ethylhexyl) adipate and some derivatives

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	5000 µg/plate	Simmon *et al.* (1977)
Salmonella typhimurium TA100, reverse mutation	–	–	NR	Seed *et al.* (1982)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	10 000 µg/plate	Zeiger *et al.* (1985)
Photobacterium phosphoreum, bioluminescence assay Mutatox	–	NT	NR	Elmore & Fitzgerald (1990)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		20 000 ppm in feed	Woodruff *et al.* (1985)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		1.15 µg/animal; inj.	Woodruff *et al.* (1985)
Gene mutation, L5178Y cells, *Tk* locus *in vitro*, forward mutation	–	–	1000[c]	McGregor *et al.* (1988)
Sister chromatid exchange, primary female Fischer 344 rat hepatocytes *in vitro*	–	NT	74 (3 and 51 h incubation)	Reisenbichler & Eckl (1993)
Chromosomal aberrations, primary female Fischer 344 rat hepatocytes *in vitro*	–	NT	74 (3 and 51 h incubation)	Reisenbichler & Eckl (1993)
Micronucleus assay, primary female Fischer 344 rat hepatocytes *in vitro*	–	NT	74 (3 and 51 h incubation)	Reisenbichler & Eckl (1993)
Chromosomal aberrations and micronucleus formation, male $B6C3F_1$ mouse bone marrow *in vivo*	–		NR	Shelby & Witt (1995)
Micronucleus test, male $B6C3F_1$ mouse bone marrow *in vivo*	–		2000 ip × 3	Shelby *et al.* (1993)
Rat (Sprague-Dawley) urine/*Salmonella typhimurium* TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	2000 po × 15	DiVincenzo *et al.* (1985)
Binding (covalent) to DNA, female NMR1 mouse liver *in vivo*	–		1100–1440 po × 1[d]	Däniken *et al.* (1984)
Oxidative DNA damage (8-OH-dG), male Fischer 344 rat liver DNA *in vivo*	+[e]		2.5% diet for 1 and 2 w	Takagi *et al.* (1990)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Dominant lethal assay, male Harlan/ICR albino Swiss mice *in vivo*	(+)		9220 ip × 1	Singh *et al.* (1975)
Mono(2-ethylhexyl) adipate				
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 µg/plate	Dirven *et al.* (1991)
Mono(2-ethyl-5-hydroxyhexyl) adipate				
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 µg/plate	Dirven *et al.* (1991)
Mono(2-ethyl-5-oxohexyl) adipate				
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 µg/plate	Dirven *et al.* (1991)

[a] +, positive; –, negative; NT, not tested; NR, not reported
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; po, oral; d, day; w, week
[c] Precipitate formed at doses ≥ 1000 µg/mL
[d] There was no effect of pre-treatment with 10 000 mg/kg in the diet for four weeks.
[e] Oxidative damage was not found in rat kidney DNA; 8-OH-dG, 8-hydroxydeoxyguanosine

4.5 Mechanistic considerations

Some general considerations about the role of peroxisome proliferation as a mechanism of carcinogenicity are presented in the General Remarks section of this volume. Studies of this mechanism are reviewed fully in Section 4.5 of the monograph on di(2-ethylhexyl) phthalate in this volume.

The weight of evidence for di(2-ethylhexyl) adipate, and for other rodent peroxisome proliferators in general, demonstrates that they do not act as direct DNA-damaging agents.

Chronic administration of peroxisome proliferators to rodents results in sustained oxidative stress due to overproduction of peroxisomal hydrogen peroxide. This can theoretically generate reactive oxygen species which can damage DNA and other intracellular targets. The induction of peroxisomal fatty acid β-oxidation by di(2-ethylhexyl) adipate *in vivo* under carcinogenicity testing conditions in rats and mice (Lake *et al*., 1997) supports this hypothesis. Limited supporting data on induction of oxidative stress (formation of 8-OH-dG in DNA) in rat liver by di(2-ethylhexyl) adipate are available (Takagi *et al*., 1990); however, there are no data for mouse liver.

Similarly, the modulation of hepatocellular proliferation by peroxisome proliferators has been implicated in the mechanism of carcinogenesis. This can theoretically result in increased levels of mutation by increasing the frequency of replicative DNA synthesis as well as increasing the number of hepatocytes at risk. Furthermore, hepatocellular proliferation is likely to be involved in the promotion of growth of preneoplastic hepatocytes. There is clear evidence that di(2-ethylhexyl) adipate causes acute and sustained hepatocellular proliferation under bioassay conditions which resulted in liver tumours in mice. Interestingly, the duration of hepatocellular proliferation was limited in rats, which did not respond with liver tumours in the bioassay as did the mice (Lake *et al*., 1997).

Marked species differences in hepatic peroxisome proliferation have been reported (Ashby *et al*., 1994; IARC, 1995; Lake, 1995a,b; Cattley *et al*., 1998). In biopsies from humans receiving hypolipidaemic drugs, there was no effect or changes were much smaller than those that would be produced in rodent hepatocytes at equivalent dose levels (Lake, 1995a,b; Cattley *et al*., 1998). While peroxisome proliferation may be readily demonstrated in cultured rat and mouse hepatocytes, such effects are not observed in hepatocytes from non-responsive species including guinea-pigs, primates and humans. No study has yet compared the responsiveness of human versus rodent livers *in vivo* or hepatocytes *in vitro* to di(2-ethylhexyl) adipate; however, a growing body of evidence concerning the molecular basis of peroxisome proliferation, summarized below, indicates that human livers and hepatocytes would be refractory to induction of peroxisome proliferation by di(2-ethylhexyl) adipate.

Studies of PPARα activation *in vitro* or in PPARα knock-out mice *in vivo* have not yet been conducted with di(2-ethylhexyl) adipate; however, given that the receptor mediates the same response for a variety of other peroxisome proliferators, it is likely

to mediate the hepatic effects of di(2-ethylhexyl) adipate. Stated another way, induction of peroxisome proliferation by a PPARα-independent mechanism would be unprecedented.

Cultured hepatocytes from non-human primates (marmosets and macaques) and humans have been similarly unresponsive to a variety of peroxisome proliferators (reviewed in Doull *et al*., 1999). No evaluation of peroxisome proliferation in human hepatocytes treated with di(2-ethylhexyl) adipate metabolites *in vitro* has been published. The lack of peroxisome proliferation in hepatocytes from marmosets suggests that human hepatocytes also would be unresponsive (Cornu *et al*., 1992). These negative results were significant in that the same metabolites induced typical induction of peroxisomal (cyanide-insensitive) palmitoyl-CoA oxidation activity in rat and mouse hepatocytes.

The insensitivity of human hepatocytes towards peroxisome proliferators is reflected in the guinea-pig. The guinea-pig is also refractory to the hepatic effects of rodent peroxisome proliferators (reviewed in Doull *et al*., 1999), including di(2-ethylhexyl) adipate metabolites (Cornu *et al*., 1992), and like humans expresses similar low levels of PPARα (Bell *et al*., 1998; Tugwood *et al*., 1998). Significant responses (peroxisome proliferation, induction of fatty acid oxidizing enzymes and the stimulation of replicative DNA synthesis) believed to be associated with hepatocarcinogenesis in rodents are not observed in humans and guinea-pigs. However, these species do exhibit hypolipidaemic responses when exposed to some rodent peroxisome proliferators (Lake, 1995a ; Bell *et al*., 1998; Cattley *et al*., 1998). This hypolipidaemic response, which is not associated with any hypertrophic or hyperplastic response, has been attributed to PPARα-mediated regulation of genes encoding lipoprotein lipase and various lipoproteins. The existence of such a response, in spite of low levels of PPARα, may be explained by differences in the mechanism of action of PPARα in relation to these hypolipidaemic genes compared to those that regulate hypertrophic and hyperplastic responses: the former may have a lower threshold of activation and may require lower concentrations of receptor due to different binding affinities for different PPREs. Differences in the activation properties for different PPRE-containing promoters have been demonstrated (Hsu *et al*., 1995).

In summary:

1. Di(2-ethylhexyl) adipate does not show evidence of genotoxicity.
2. Di(2-ethylhexyl) adipate produces liver tumours in mice.
3. Under conditions of the bioassays, di(2-ethylhexyl) adipate induces peroxisome proliferation and cell replication in liver that are characteristic of a peroxisome proliferator in mice and, to a limited extent, in rats.
4. Rodent peroxisome proliferators exercise their pleiotropic effects due to activation of PPARα. This process is essential for liver hypertrophy and hyperplasia and eventual hepatocarcinogenesis in response to peroxisome proliferators.

5. The absence of significant response of human liver to induction of peroxisome proliferation and hepatocellular proliferation is explained by several aspects of PPARα-mediated regulation of gene expression.
6. Hepatic peroxisome proliferation has not been evaluated in studies of human subjects or systems treated with di(2-ethylhexyl) adipate. However, interspecies comparisons with other peroxisome proliferators, along with the role of PPARα in this response, indicate that humans can reasonably be predicted to be refractory to induction of peroxisome proliferation and hepatocellular proliferation by di(2-ethylhexyl) adipate.
7. Overall, these findings suggest that the increased incidence of liver tumours in mice treated with di(2-ethylhexyl) adipate results from a mechanism that does not operate in humans. However, studies of di(2-ethylhexyl) adipate or its metabolites regarding peroxisome proliferation in human cells are not available.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Di(2-ethylhexyl) adipate is a liquid of low volatility, widely used as a plasticizer in flexible poly(vinyl chloride) products, notably food films, as well as in other plastics and in a number of other minor applications, such as lubricants and cosmetics. Occupational exposure may occur by inhalation of di(2-ethylhexyl) adipate as an aerosol during its manufacture and its use. Meat-wrapping workers may be exposed while cutting poly(vinyl chloride) film across a heated cutter. Food is the major source of exposure of the general population to di(2-ethylhexyl) adipate because of migration from poly(vinyl chloride) packaging, particularly into fatty foods such as cheese and meat.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Di(2-ethylhexyl) adipate was tested for carcinogenicity by oral administration in one experiment in mice and one experiment in rats. In mice, liver adenomas and carcinomas were produced in both males and females. No treatment-related tumours were observed in rats.

5.4 Other relevant data

Di(2-ethylhexyl) adipate is rapidly and completely absorbed after oral administration, rapidly and extensively metabolized and rapidly excreted in humans and experimental animals. It is hydrolysed in the gastrointestinal tract before absorption.

No data on the toxic effects of di(2-ethylhexyl) adipate in humans were available to the Working Group.

In mice and rats, di(2-ethylhexyl) adipate induced hepatic markers of peroxisome proliferation (ultrastructural and biochemical) as well as hepatomegaly and increased replicative DNA synthesis. The species differences in carcinogenicity assays of di(2-ethylhexyl) adipate (increased hepatocellular tumours in mice, not rats) are consistent with a higher intake of di(2-ethylhexyl) adipate and a greater extent of peroxisome proliferation and associated responses in livers of mice compared with rats fed the same dietary doses.

In hepatocytes isolated from rats and mice, treatment of primary cultures with metabolites of di(2-ethylhexyl) adipate increased peroxisomal palmitoyl-coenzyme A oxidation activity. The same treatment of primary cultures of hepatocytes from guinea-pigs and marmosets failed to cause any similar increase in activity.

No data on reproductive and developmental effects in humans were available to the Working Group.

Exposure of rats to di(2-ethylhexyl) adipate during organogenesis caused an increased frequency of variations and retardations in the fetuses at doses below the maternally toxic range.

No effects on male or female fertility were found in rats given di(2-ethylhexyl) adipate in the feed. The body weight gain of the pups at the highest dose was reduced throughout the postnatal period. In mice, a single high intraperitoneal dose given to males before mating was associated with a reduced percentage of pregnancies and increased number of fetal deaths.

No data on the genetic and related effects of di(2-ethylhexyl) adipate in humans or human cells were available to the Working Group.

Di(2-ethylhexyl) adipate did not bind covalently to mouse liver DNA *in vivo*. One report showed evidence of oxidative damage in rat liver DNA *in vivo* but not in rat kidney DNA. A weak dominant lethal effect has been reported in male mice. Analyses of mouse bone marrow after treatment with di(2-ethylhexyl) adipate *in vivo* found no induction of micronuclei in one study and no induction of chromosomal aberrations in one study. Urine from rats treated with di(2-ethylhexyl) adipate by gavage was not mutagenic to *Salmonella typhimurium*.

Di(2-ethylhexyl) adipate did not induce gene mutations, sister chromatid exchanges, chromosomal aberrations or micronuclei in rodent cells *in vitro*. It did not induce sex-linked recessive lethal mutations in *Drosophila* when administered either by diet or injection. Di(2-ethylhexyl) adipate was not mutagenic to either *Photo-*

bacterium phosphoreum or *Salmonella typhimurium* in the presence or absence of exogenous metabolic activation.

These data indicate that di(2-ethylhexyl) adipate is not genotoxic.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of di(2-ethylhexyl) adipate were available.

There is *limited evidence* in experimental animals for the carcinogenicity of di(2-ethylhexyl) adipate.

Overall evaluation

Di(2-ethylhexyl) adipate is *not classifiable as to its carcinogenicity to humans (Group 3)*.

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CINNAMYL ANTHRANILATE

This substance was considered by previous working groups, in June 1977 (IARC, 1978), October 1982 (IARC, 1983) and March 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated in the monograph and taken into consideration in the evaluation.

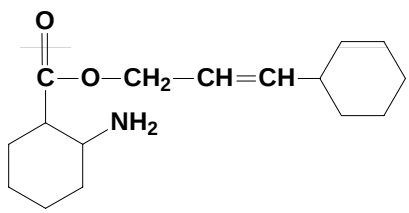
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 87-29-6
Chem. Abstr. Name: 3-Phenyl-2-propen-1-ol, 2-aminobenzoate
IUPAC Systematic Name: Anthranilic acid, cinnamyl ester
Synonyms: Cinnamyl alcohol anthranilate; 3-phenyl-2-propenyl 2-aminobenzoate; 3-phenyl-2-propenyl anthranilate

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{16}H_{15}NO_2$ Relative molecular mass: 253.30

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Crystalline solid (Budavari, 1996); brownish powder with a balsamic, fruity odour (Burdock, 1995)

(*b*) *Boiling-point*: 332 °C (National Toxicology Program, 1991)
(*c*) *Melting-point*: 61–61.5 °C (Budavari, 1996)
(*d*) *Density*: 1.18 g/cm^3 at 15.5 °C (Burdock, 1995)
(*e*) *Solubility*: Very slightly soluble in water (< 1 mg/mL at 17 °C); very soluble in acetone and dimethyl sulfoxide; soluble in chloroform, diethyl ether and ethanol (National Toxicology Program, 1991)
(*f*) *Stability*: Sensitive to oxidation in air, photooxidation and hydrolysis (National Toxicology Program, 1991)
(*g*) *Conversion factor*[1]: mg/m^3 = 10.36 × ppm

1.1.4 *Technical products and impurities*

Cinnamyl anthranilate is not known to be currently commercially available.

1.1.5 *Analysis*

Cinnamyl anthranilate can be assayed by a method based on ester hydrolysis. Bulk samples of food-grade cinnamyl anthranilate have been analysed for purity by thin-layer chromatography and high-performance liquid chromatography. A method has been described for determining the content of this compound in food products by steam distillation followed by paper chromatography and examination under ultra-violet light; it has a limit of detection of 1 μg (IARC, 1983).

1.2 Production

Cinnamyl anthranilate can be synthesized by esterification of anthranilic acid with cinnamyl alcohol (Burdock, 1995). Annual production in the United States in the 1970 was in the range of a few hundred kg (IARC, 1983). It has not been commercially available, except for research purposes, since 1985 (Lucas *et al.*, 1999; Food and Drug Administration, 1999).

1.3 Use

Cinnamyl anthranilate was used for nearly 50 years at very low levels as a synthetic flavouring and fragrance agent. It was used as a flavouring agent to impart a grape or cherry flavour in non-alcoholic beverages, ice cream and ices, sweets, baked goods, gelatins and puddings and chewing gum. It has been used as a fragrance ingredient in various cosmetic products including soaps, detergents, creams and lotions and as a perfume ingredient in orange blossom, neroli, cologne and other blends (Opdyke, 1975; IARC, 1983; National Toxicology Program, 1991; Budavari, 1996).

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[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

1.4 Occurrence

1.4.1 *Natural occurrence*

Cinnamyl anthranilate is not known to occur as a natural product.

1.4.2 *Occupational exposure*

No data were available to the Working Group.

1.4.3 *Environmental occurrence*

No data were available to the Working Group.

1.5 Regulations and guidelines

Although cinnamyl anthranilate was classified in the past as a Generally Recognized As Safe (GRAS) substance by the Food and Drug Administration, its use in human food has been prohibited in the United States since 1985. The Food and Agriculture Organization and the World Health Organization have recommended that cinnamyl anthranilate not be used in food (FAO/WHO, 1981; National Toxicology Program, 1991; Food and Drug Administration, 1999).

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 B6C3F_1 mice, six weeks of age, were fed diets containing 0, 15 or 30 g/kg cinnamyl anthranilate (96% pure; at least five unidentified impurities were found by thin-layer chromatography and two by high-performance liquid chromatography) for 103 weeks. The doses were selected on the basis of a subchronic experiment. Survival rates at the end of the study (105–107 weeks) in the control, low-dose and high-dose groups were 88, 82 and 80% in males and 78, 82 and 74% in females. Dose-related reductions in mean body weight were noted in both males and females. A significant dose-related increase ($p < 0.001$, Cochran-Armitage

trend test) in the incidence of hepatocellular carcinomas was found in both males and females: females—1/50, 8/49 ($p = 0.014$) and 14/49 ($p < 0.001$) in the control, low- and high-dose groups, respectively; males—6/48, 7/50 and 12/47 [$p = 0.047$]. There was a significant dose-related increase in the incidence of liver-cell carcinomas and adenomas combined in males and females ($p < 0.001$, Cochran-Armitage test for trend) (males—14/48 controls, 30/50 low-dose, 37/47 high-dose; females—3/50 controls, 20/49 low-dose, 33/49 high-dose). A few metastases were seen in the lungs of high-dose females (National Cancer Institute, 1980).

3.1.2 *Rat*

Two groups of 50 male and 50 female Fischer 344 rats, seven weeks of age, were fed diets containing 0, 15 or 30 g/kg cinnamyl anthranilate (same sample as used above) for 103 weeks. The dietary levels were selected on the basis of a subchronic study. Surviving animals were killed at 105–107 weeks, at which time 64, 80 and 80% of the males and 78, 88 and 92% of the females were still alive in the control, low- and high-dose groups, respectively. Dose-related reductions in mean body weight were noted in both males and females. There was an increased incidence of mineralization and inflammation of the kidneys of treated rats. A non-statistically significant increase in the incidence of renal tubule tumours was observed in high-dose male rats (4/49; two adenocarcinomas and two adenomas); there was also a non-statistically significant increase in the incidence of acinar-cell pancreatic tumours (3/45: one carcinoma and two adenomas). No such tumour was observed in the matched controls. [The Working Group noted that the historical control incidence in male rats for renal tubule tumours was 0.37% and that that for acinar-cell pancreatic tumours was 0.28%] (National Cancer Institute, 1980).

3.2 Intraperitoneal administration

Mouse: Cinnamyl anthranilate was tested in a lung adenoma screening assay. In the first series, groups of 15 male and 15 female A/He mice, six to eight weeks of age, were given thrice weekly intraperitoneal injections of cinnamyl anthranilate [purity unspecified] dissolved in tricaprylin, as 24 doses of 500 mg/kg bw (the maximal tolerated dose, as found in subchronic experiments) or 100 mg/kg bw (total doses, 12 and 2.4 g/kg bw, respectively). A control group of 25 females (but no control males) received intraperitoneal injections of the vehicle according to the same schedule. All animals were killed 24 weeks after the first injection and lung tumours were found in 10/15 high-dose females, 11/13 high-dose males, 7/15 low-dose females, 10/15 low-dose males and 10/22 control females. The numbers of tumours per mouse were 0.59 ± 0.13 in control females, 1.50 ± 0.37 in low-dose females, 2.14 ± 0.70 ($p < 0.05$) in high-dose females, 1.13 ± 0.29 in low dose males and 2.69 ± 0.75 ($p < 0.05$) in high-dose males (Stoner *et al*., 1973).

In the second mouse lung adenoma assay, cinnamyl anthranilate was administered to 15 females and 15 males under similar experimental conditions, except that redistilled tricaprylin was used as the vehicle. The control group consisted of 80 females and 80 males. The numbers of tumours per mouse were increased at the high dose, being 0.85 ± 0.23 ($p < 0.01$) in high-dose females, 1.40 ± 0.36 ($p < 0.001$) in high-dose males, 0.54 ± 0.15 ($p < 0.05$) in low-dose females and 0.47 ± 0.12 in low-dose males compared with 0.20 0.02 in control females and 0.24 ± 0.03 in control males (Stoner *et al*., 1973).

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Five human volunteers took a single oral dose of 250 mg cinnamyl anthranilate in water and no unchanged compound was detected in the 0–24-h urine, using analytical methods able to detect 0.04% of the dose (Keyhanfar & Caldwell, 1996).

4.1.2 *Experimental systems*

Bronaugh *et al*. (1985) determined the percutaneous absorption of cinnamyl anthranilate *in vivo* in rhesus monkeys and *in vitro* through human skin. Absorption through the shaved abdominal skin of four adult female rhesus monkeys after a 24-h application of 4 μg/cm^2 was 26.1 ± 2.3% of the dose, which rose to 39.0 ± 2.8% when the site of application was covered with an occlusive dressing. Penetration through excised full-thickness human abdominal skin was determined using diffusion cells with a non-ionic surfactant (6% oleth 20, a polyethylene glycol 20 ether) as the receptor fluid. After application of 4 μg/cm^2 cinnamyl anthranilate, 24.0 ± 5.1% ($n = 8$) of the dose was absorbed within 48 h. When the surface of the skin was occluded, the penetration rose to 53.3 ± 6.7% of the dose ($n = 7$).

The metabolism of cinnamyl anthranilate in rats and mice has been studied (Keyhanfar & Caldwell, 1996). Male Fischer 344 rats and male CD-1 mice were given 250 mg/kg bw [3-^{14}C] cinnamyl anthranilate by intraperitoneal injection. The majority of the administered ^{14}C was excreted in the 0–24-h urine (70% of the dose in rats and 78% in mice). A further 10% (rat) and 6% (mouse) was recovered in the 24–72-h urine with 10% (rat) and 7% (mouse) in the 0–72-h faeces. In the rat, the major urinary metabolite was hippuric acid (95% of urinary ^{14}C), together with much smaller amounts of benzoic acid. However, in mice, the urine contained relatively less hippuric acid (~ 80%) and more benzoic acid (16% of urinary ^{14}C), together with 2.2% of the dose as unchanged cinnamyl anthranilate.

In further studies, the effect of intraperitoneal dose size upon the fate of cinnamyl anthranilate in mice was examined over a range of 5–250 mg/kg bw. No intact ester was found in the urine after 5 mg/kg bw, but at 50 mg/kg bw, 3.1% of the dose was excreted as cinnamyl anthranilate and, at 250 mg/kg, the percentage was 2.2% (Keyhanfar & Caldwell, 1996).

In an earlier study, Caldwell *et al*. (1985) gave a single oral dose of 500 mg/kg bw to B6C3F$_1$ mice and found 0.3–0.4% of the dose as unchanged cinnamyl anthranilate in the 0–24-h urine, accompanied by 17% as anthranilic acid and 35% as hippuric acid. Cinnamyl anthranilate was detected in the plasma, rapidly declining from peak levels seen at 0.5 h after dosing. The peak levels were some 3.5 times higher in males than in females.

The influence of dose size was also examined in male and female B6C3F$_1$ mice given 0, 10, 100, 1000, 5000, 15 000 or 30 000 ppm (mg/kg diet) cinnamyl anthranilate in the diet for four days (Caldwell *et al*., 1985). The urinary excretion of cinnamyl anthranilate, hippuric acid and anthranilic acid within 24-h after removal of the test diet rose with increasing cinnamyl anthranilate dose. Cinnamyl anthranilate was detected in increasing quantities in the urine of male mice at 1000 ppm and above. In females, it was only seen at 5000 ppm and above and the levels were two- to nine-fold lower.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Dietary consumption (30 g/kg diet) of 11 g/kg bw per day by female B6C3F$_1$ mice and 3 g/kg bw per day by female Fischer 344 rats for up to 13 weeks was not associated with lethality, although body weight gain was diminished (Lake *et al*., 1997). Similar dietary administration (15 or 30 g/kg diet) did not affect survival in studies of eight weeks' and two years' duration (National Toxicology Program, 1980).

Cinnamyl anthranilate was shown to be a hepatic enzyme inducer in mice by Caldwell *et al.* (1985). Male and female B6C3F$_1$ mice were fed diets containing 0, 10, 100, 1000, 5000, 15 000 or 30 000 ppm (mg/kg diet) cinnamyl anthranilate for 19 days. The relative liver weight (% of body weight) and hepatic microsomal cytochrome P450 content increased in a dose-dependent fashion, significant above 1000 ppm: this was more evident in males than females, but the maximum response (approximately twofold) was the same in both sexes. Although the P450 marker activities, aniline hydroxylase and *para*-nitroanisole *O*-demethylase, in the 9000 × *g* supernatant were unaltered by cinnamyl anthranilate administration, SDS-PAGE examination of the liver microsomes showed marked induction of a P450 isozyme of 53.1 kDa. The dose threshold for increased liver weight and microsomal enzyme induction was the same, and correlated

with that for excretion of unchanged cinnamyl anthranilate noted in the shorter (four-day) experiment (Section 4.1.2).

The hepatic effects of cinnamyl anthranilate were evaluated in male CD1 mice and male Fischer 344 rats treated by intraperitoneal injection for three consecutive days (Viswalingam & Caldwell, 1997). At doses of 100 and 1000 mg/kg bw per day, relative liver weights of mice increased by 22% and 50%, respectively, 24 h after the final dose and peroxisomal (cyanide-insensitive) palmitoyl-coenzyme A (CoA) oxidation activity increased fivefold at both levels. Microsomal lauric acid 11- and 12-hydroxylase activity (CYP4A) was increased 15-fold at 100 mg/kg bw per day and 17-fold at 1000 mg/kg bw per day. Limited evaluation indicated that cinnamyl anthrnilate increased the size and number of peroxisomes in electron micrographs of hepatocytes of treated mice. In rats, relative liver weights and peroxisomal palmitoyl-CoA oxidation activity were significantly increased only at 1000 mg/kg bw per day (22% and twofold, respectively).

In a separate experiment, groups of male CD1 mice were given intraperitoneal injections of 0–200 mg/kg bw cinnamyl anthranilate daily for three days. At doses of 20 mg/kg bw and above, there were dose-dependent increases in relative liver weight, total cytochrome P450, and cyanide-insensitive palmitoyl-CoA oxidation. The hepatic effects of cinnamyl anthranilate are apparently due to the intact ester, since neither its expected metabolites alone nor an equimolar mixture of the hydrolysis products, cinnamyl alcohol and anthranilic acid, had a significant effect on the weight or marker enzyme content of mouse liver (Viswalingam & Caldwell, 1997).

Lake *et al.* (1997) have confirmed and extended these findings. Female B6C3F_1 mice and female Fischer 344 rats were fed diets containing 0–30 mg/kg diet (11 030 mg/kg bw per day in mice, 2700 mg/kg bw per day in rats) cinnamyl anthranilate for one, four or 13 weeks. In mice, feeding cinnamyl anthranilate at ≥ 0.15% (439 mg/kg bw per day) resulted in dose-dependent increases in relative liver weight and hepatic peroxisome proliferation, as demonstrated by the induction of peroxisomal (cyanide-insensitive) palmitoyl-CoA oxidation (see Table 1). Microsomal lauric acid 12-hydroxylase activity (CYP4A) was similarly increased. Hepatocellular replication (measured as nuclear 5-bromo-2′-deoxyuridine [BrdU] labelling) was increased during week 1 of cinnamyl anthranilate treatment at ≥ 0.15% in the diet (439 mg/kg bw per day) and continued to be elevated during weeks 4 and 13 at ≥ 0.30% (883 mg/kg bw per day). In contrast to mice, rats fed cinnamyl anthranilate had much smaller increases in relative liver weight and peroxisomal palmitoyl-CoA oxidation, even at the higher dietary concentrations. This apparent difference in magnitude of response was not accounted for by rate of intake (see Table 1). In rats, there was no induction of microsomal lauric acid 12-hydroxylase activity. While BrdU labelling was increased during week 1 of administration in rats, this response was not sustained during weeks 4 or 13, and the magnitude of response during week 1 was again lower in rats.

Table 1. Comparison of responses in liver of female mice and rats following four weeks of cinnamyl anthranilate treatment

Diet (%)	Intake (mg/kg bw per day)		Relative liver weight (% increase over controls)		Peroxisomal palmitoyl-CoA oxidation (increase over controls)		Microsomal lauric acid hydroxylase (increase over controls)			
	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse		Rat	
							11-position	12-position	11-position	12-position
0.03	87	NE	NC	NE	NC	NE	NC	NE	NE	NE
0.15	439	159	30	NC	3-fold	NC	3-fold	NE	NC	NC
0.30	883	321	40	NC	4-fold	NC	4-fold	6-fold	NC	NC
0.75	2140	763	60	10	7-fold	2-fold	8-fold	8-fold	2-fold	NC
1.50[a]	4640	1370	80	15	10-fold	3-fold	12-fold	11-fold	3-fold	NC
3.00[a]	11 030	2700	100	25	15-fold	6-fold	16-fold	13-fold	6-fold	NC

Adapted from Lake *et al*. (1997)

[a] Dietary levels administered to mice and rats in a carcinogenesis bioassay and associated with increased incidence of liver tumours in mice, but not in rats (National Toxicology Program, 1980).

NC, not different from controls; NE, not evaluated

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 2 for references)

Cinnamyl anthranilate was not mutagenic to *Salmonella typhimurium* when tested in the presence or absence of rat liver S9 fraction. It has been reported to give positive results for mutagenic activity in a bioluminescence assay in *Photobacterium phosphoreum* with exogenous activation. It was not active in mutation experiments with *Drosophila* and did not cause sister chromatid exchange or chromosomal aberrations in Chinese hamster ovary cells. It gave negative or equivocal results for the induction of sister chromatid exchanges in Syrian hamster embryo cells. It did not induce unscheduled DNA synthesis in rat hepatocytes in an in-vivo/in-vitro assay. It has been shown to enhance cell transformation of Syrian hamster embryo cells by SA7 virus. Cinnamyl anthranilate is reported to be active in the mouse lymphoma mutation assay.

4.5 Mechanistic considerations

There is a marked species difference in the hepatic effects of cinnamyl anthranilate between mice (both CD-1 and B6C3F_1) and Fischer 344 rats. In mice, this compound has the characteristic biochemical and morphological effects of a potent peroxisome proliferator, increasing liver weight, fatty acid oxidation, CYP4A isozymes and replicative DNA synthesis (Caldwell *et al*., 1985; Lake *et al*., 1997; Viswalingam & Caldwell, 1997). In rats, these effects are much less evident. The species-specificity of peroxisome proliferation has been attributed to differences in the metabolism of cinnamyl anthranilate between rats and mice (Caldwell, 1992; Keyhanfar & Caldwell, 1996; Viswalingam & Caldwell, 1997). Hepatic effects were seen in mice only (*a*) after administration of the intact ester but not an equimolar mixture of its hydrolysis products and (*b*) at dose levels of cinnamyl anthranilate at which intact cinnamyl anthranilate is excreted in the urine. In contrast, rats, which are relatively resistant to its peroxisome-proliferating effect, do not excrete cinnamyl anthranilate in the urine at any dose level.

Some general considerations about the role of peroxisome proliferation as a mechanism of carcinogenicity are presented in the General Remarks section of this volume. Studies of this mechanism are reviewed fully in Section 4.5 of the monograph on di(2-ethylhexyl) phthalate in this volume.

Table 2. Genetic and related effects of cinnamyl anthranilate

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	3600 µg/plate	Wild *et al.* (1983)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	3333 µg/plate	Tennant *et al.* (1987)
Photobacterium phosphoreum, bioluminescence assay, reverse mutation	–	+	NR	Elmore & Fitzgerald (1990)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		1267 in feed	Wild *et al.* (1983)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		2000 ppm, inj[c]; 5000 in feed	Foureman *et al.* (1994)
Mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	NT ?	+	10	Tennant *et al.* (1987)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	–	30 µg/mL	Gulati *et al.* (1989)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	–	40 µg/mL	Gulati *et al.* (1989)
Cell transformation, Syrian hamster embryo cells *in vitro*	?	NT	50 µg/mL	Tu *et al.* (1986)
Cell transformation, C3H/10T½ mouse cells	–	NT	40 µg/mL	Dunkel *et al.* (1988)
Cell transformation, SA7 virus/Syrian hamster embryo cells	+	NT	20.3	Hatch *et al.* (1986)
Unscheduled DNA synthesis, male Fischer 344 rats *in vivo*	–		1000 po × 1	Mirsalis *et al.* (1989)
Micronucleus formation, male B6C3F$_1$ mouse bone-marrow cells *in vivo*	?		1000 ip × 3	Shelby *et al.* (1993)
Micronucleus formation, male and female NMRI mouse bone-marrow cells *in vivo*	–		2533 ip × 1	Wild *et al.* (1983)

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

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

[c] Injection volume not reported

The weight of evidence for cinnamyl anthranilate and for other rodent peroxisome proliferators in general, demonstrates that they do not act as direct DNA-damaging agents.

Chronic administration of peroxisome proliferators to rodents results in sustained oxidative stress due to overproduction of peroxisomal hydrogen peroxide. The induction of peroxisomal fatty acid β-oxidation by cinnamyl anthranilate *in vivo* under bioassay conditions (Lake *et al.*, 1997) supports this hypothesis. Other data on the induction of oxidative stress are not available for cinnamyl anthranilate.

Similarly, the modulation of hepatocellular proliferation by peroxisome proliferators has been implicated in the mechanism of carcinogenesis. This can theoretically result in increased levels of mutation by increasing the frequency of replicative DNA synthesis as well as increasing the number of hepatocytes at risk. Furthermore, hepatocellular proliferation is likely to be involved in the promotion of growth of preneoplastic hepatocytes. There is clear evidence that cinnamyl anthranilate causes acute and sustained levels of hepatocellular proliferation under bioassay conditions which resulted in liver tumours in mice. Interestingly, the magnitude and duration of hepatocellular proliferation were limited in rats, which did not respond with liver tumours in the bioassay (Lake *et al*., 1997).

Marked species differences in hepatic peroxisome proliferation have been reported (Ashby *et al*., 1994; IARC, 1995; Lake, 1995a,b; Cattley *et al*., 1998). No study has yet compared the responsiveness of human versus rodent livers *in vivo* or hepatocytes *in vitro* to cinnamyl anthranilate; however, a growing body of evidence concerning the molecular basis of peroxisome proliferation indicates that human livers and hepatocytes would be refractory to induction of peroxisome proliferation by cinnamyl anthranilate (Doull *et al*., 1999).

In summary:

1. The only standard genotoxicity assay in which cinnamyl anthranilate is active is the mouse lymphoma mutation assay.
2. Cinnamyl anthranilate produces liver tumours in mice.
3. Under conditions of the bioassays, cinnamyl anthranilate induced peroxisome proliferation and cell replication in the liver that are characteristic of a peroxisome proliferator in mice and, to a limited extent, in rats.
4. The species difference in peroxisome proliferation in response to cinnamyl anthranilate is associated with a species difference in its metabolism: the compound is completely hydrolysed by rats but not mice.
5. Rodent peroxisome proliferators exercise their pleiotropic effects due to activation of PPARα. This process is essential for liver hypertrophy and hyperplasia and eventual hepatocarcinogenesis in response to peroxisome proliferators.
6. The absence of a significant response of human liver to induction of peroxisome proliferation and hepatocellular proliferation is explained by several aspects of PPARα-mediated regulation of gene expression.

7. Hepatic peroxisome proliferation has not been evaluated in studies of human subjects or systems treated with cinnamyl anthranilate. However, interspecies comparisons with other peroxisome proliferators, along with the role of PPARα in this response, indicate that humans can reasonably be predicted to be refractory to induction of peroxisome proliferation and hepatocellular proliferation by cinnamyl anthranilate. This conclusion is further supported by the failure to detect intact cinnamyl anthranilate in the urine of human volunteers given large single doses.
8. Overall, these findings suggest that the increased incidence of liver tumours in mice treated with cinnamyl anthranilate results from a mechanism that is not expected to operate in humans, although studies of human systems have not been performed with this compound.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Cinnamyl anthranilate was used as a synthetic flavouring and fragrance agent. It has not been commercially available since 1985. No information was available on its occurrence in the workplace or in the environment.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Cinnamyl anthranilate was tested for carcinogenicity in one experiment in mice and in one experiment in rats by oral administration in the diet. In mice, a dose-related increase in the incidence of hepatocellular tumours was found, but there was no increased incidence of tumours in rats. In a mouse lung tumour bioassay, an increased multiplicity of lung tumours was found.

5.4 Other relevant data

Cinnamyl anthranilate is metabolized by hydrolysis to anthranilic acid and cinnamyl alcohol, which is oxidized to benzoic acid. In mice, but not in rats or humans, the hydrolysis is saturated at high doses, leading to excretion of unchanged cinnamyl anthranilate in the urine.

Cinnamyl anthranilate has the characteristic effects of a peroxisome proliferator on mouse liver, increasing the activity of peroxisomal fatty acid-metabolizing enzymes and microsomal CYP4A and increasing hepatocellular proliferation. These effects are mediated by the intact ester, and were not seen after administration of the hydrolysis products, cinnamyl alcohol and anthranilic acid. The corresponding effects on rat liver were very much weaker. No relevant data from humans were available.

The only standard genotoxicity assay in which cinnamyl anthranilate was active was the mouse lymphoma mutation assay.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of cinnamyl anthranilate were available.

There is *limited evidence* in experimental animals for the carcinogenicity of cinnamyl anthranilate.

Overall evaluation

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

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COUMARIN

This substance was considered by previous Working Groups, in October 1975 (IARC, 1976) 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.

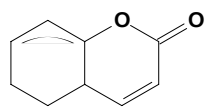
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 91-64-5
Chem. Abstr. Name: 2*H*-1-Benzopyran-2-one
IUPAC Systematic Name: Coumarin
Synonyms: 1,2-Benzopyrone; 5,6-benzo-2-pyrone; benzo-α-pyrone; *cis-ortho*-coumarinic acid lactone; coumarinic anhydride; *ortho*-hydroxycinnamic acid lactone

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_9H_6O_2$ Relative molecular mass: 146.15

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Orthorhombic, rectangular plates; pleasant, fragrant odour resembling that of vanilla beans; burning taste (Budavari, 1998)
(*b*) *Boiling-point*: 301.7 °C (Lide, 1999)
(*c*) *Melting-point*: 71 °C (Lide, 1999)
(*d*) *Density*: 0.935 g/cm^3 at 20 °C (Lide, 1999)

(*e*) *Spectroscopy*: Infrared (prism [1691]; grating [270]), ultraviolet [492], nuclear magnetic resonance (proton, [10407, V-225]; C-13 [242]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980; Lide & Milne, 1996)
(*f*) *Solubility*: Slightly soluble in water (100 mg/L at 25 °C) and ethanol; very soluble in chloroform, diethyl ether and pyridine (Lide & Milne, 1996; Verschueren, 1996; Budavari, 1998)
(*g*) *Volatility*: Vapour pressure, 0.13 kPa at 106 °C (Verschueren, 1996)
(*h*) *Octanol/water partition coefficient (P)*: log P, 1.39 (Verschueren, 1996).
(*i*) *Conversion factor*[1]: $mg/m^3 = 5.98 \times ppm$

1.1.4 *Technical products and impurities*

Coumarin is commercially available with a minimum purity of 99% (Rhodia, undated). Coumarin is usually sold in the form of colourless shiny leaflets or rhombic crystals (Boisde & Meuly, 1993).

1.1.5 *Analysis*

Coumarin can be determined in vanilla extract by a photometric method, reading the absorbance or transmittance at 490 nm, and comparing against a standard (AOAC International, 1998).

1.2 Production

Until the late 1890s, coumarin was obtained commercially only from natural sources by extraction from tonka beans. Synthetic methods of preparation and industrial manufacturing processes were developed starting principally from *ortho*-cresol (Raschig process), phenol (Pechmann reaction) and salicylaldehyde (Perkin reaction). Various methods can be used to obtain coumarin from each of these starting materials. In order to be suitable for perfumery uses, synthetic coumarin must be highly pure (Bauer *et al.*, 1988; Boisde & Meuly, 1993).

Information available in 1999 indicated that coumarin was manufactured by five companies in China, three companies each in Japan and the United States, two companies in France and one company each in Germany, Hong Kong and India (Chemical Information Services, 1999).

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[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

1.3 Use

Coumarin is widely distributed in the plant kingdom, but for commercial use has been mostly produced synthetically for many years. In addition to its use in the perfumery, cosmetic and related industries, coumarin has several other industrial applications. Formerly, large quantities of coumarin were used in the food industry, mostly associated with vanillin, for flavouring chocolates, baked goods, and in cream soda-flavoured beverages (Perone, 1972), but since 1954 its use as a direct food additive has been suspended in the United States (Boisde & Meuly, 1993; Lake, 1999).

Because of its unique sweet note and stability, coumarin has long been recognized as an important raw material in the fragrance industry. It is widely used in hand soaps, detergents, lotions and perfumes at concentrations usually extending from 0.01 to 0.8%. It is normally associated in perfumery with herbaceous odours and enters into the formulation of fern and Chypre-type fragrances. It is used as an odour-enhancer to achieve a long-lasting effect when combined with natural essential oils such as lavender, citrus, rosemary and oak moss. Coumarin is used in tobacco to enhance its natural aroma. It is also applied in large quantities to give pleasant aromas to household materials and industrial products or to mask unpleasant odours (Egan *et al*., 1990; Boisde & Meuly, 1993).

Coumarin has also found use in toothpastes, antiperspirant deodorants, bath products, body lotions, face creams, fragrance creams, hair sprays, shampoos, shower gels and toilet soaps (Cohen, 1979; Lake, 1999). It has been used in detergents as a brightener or bleaching agent (Perone, 1972).

Coumarin possesses both immunomodulatory and direct antitumour activity (Marshall *et al*., 1994).

Coumarin has been recommended for treatment of a number of clinical conditions, including high protein oedema and brucellosis. It is currently undergoing clinical trials for treatment of lymphoedema following breast cancer treatment and in treatment of lung and kidney cancer and of melanoma alone or in combination with cimetidine (Marshall *et al*., 1987a,b, 1989; Thornes *et al*., 1989; Dexeus *et al*., 1990; Marshall *et al*., 1990; Casley-Smith *et al*., 1993; Lake, 1999). It has also been used for prevention of dental caries (Perone, 1972). Coumarin and some of its derivatives have been tested for treatment of schizophrenia, microcirculation disorders and angiopathic ulcers, and also for treatment of high protein oedemas in animals (Boisde & Meuly, 1993).

In industry, it is used in rubber and plastic materials and in paints and sprays to neutralize unpleasant odours (Lake, 1999). In other fields, coumarin has a significant use in the electroplating industry, mostly in the automotive area, to provide high polished quality to chrome-plated steel, but this use is declining (Egan *et al*., 1990; Boisde & Meuly, 1993).

1.4 Occurrence

1.4.1 *Natural occurrence*

Coumarin was first isolated by Vogel in 1820 by extraction from tonka beans (*Dipteryx odorata*). It was subsequently identified in a large number of plants belonging to many different families. Its better known occurrences are in sweet clover (*Melilotus alba* and *M. officinalis*), sweet woodruff (*Asperula odorata*), vanilla leaf (*Trilisa odoratissima*), vanilla beans *(Vanilla planifolia)*, cassia (*Cinnamorum cassia*), lavender (*Lavendula officinalis*) and balsam of Peru (*Myroxylon pereirae*) (Perone, 1972; Marles *et al.*, 1987*;* Boisde & Meuly, 1993; Budavari, 1998).

Coumarin has been isolated from legumes, orchids, grasses and citrus fruits (Perone, 1972). It is found at particularly high levels in some essential oils, such as cinnamon leaf and bark oil, cassia leaf oil and lavender oil (Lake, 1999).

A broad spectrum of coumarin derivatives (present both in the free state and as glucosides) have also been found in many plants; to date at least 1300 have been identified, principally as secondary metabolites in green plants (Hoult & Payd, 1996).

1.4.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1999), approximately 240 000 workers in the United States were potentially exposed to coumarin. National estimates of workers potentially exposed were not available from other countries. Occupational exposure to coumarin may occur in its production and in its use in the manufacture and formulation of many products.

No other data on occupational exposures were available to the Working Group.

1.4.3 *Environmental occurrence*

Reports of the release of coumarin to the environment through various waste streams are scant. The maximum total daily human exposure to coumarin has been estimated to be 0.06 mg/kg bw, comprising 0.02 mg/kg bw per day from dietary exposure, and 0.04 mg/kg bw per day from fragrance use in cosmetic products (Lake, 1999).

(*a*) *Foods and fragrances*

Coumarin is a natural product found at high levels in some essential oils, particularly cinnamon leaf oil (40 600 ppm (mg/kg)), cinnamon bark oil (7000 ppm), other types of cinnamon (900 ppm), cassia leaf oil (17 000–87 300 ppm), peppermint oil (20 ppm), lavender oil, woodruff and sweet clover as well as in green tea (0.2–1.7 ppm), fruits such as bilberry and cloudberry and other foods such as chicory root (Boisde & Meuly, 1993; TNO, 1996; Lake, 1999). It is also found in Mexican vanilla extracts (Sullivan, 1981; Marles *et al.*, 1987).

(*b*) *Medical uses*

As a consequence of its medical use, many individuals have been exposed to therapeutic doses of coumarin ranging from 100 to 7000 mg per day for periods ranging from two weeks to over two years (Marshall *et al*., 1994).

(*c*) *Personal care products*

In personal care products, usual (and maximum) concentrations were found to be for soap, 0.03% (0.2%), detergent, 0.003% (0.02%), creams and lotions, 0.015% (0.1%) and perfumes, 0.3% (0.8%) (Cohen, 1979). More recently, coumarin was found in 11 of 22 perfumes at concentrations (w/v) of 0.046–6.043% (Rastogi *et al.*, 1996) and 40 of 73 deodorants on the European market at concentrations ranging from 1 to 1411 ppm [0.0001–0.14%] (Rastogi *et al*., 1998).

Coumarin penetrates human skin rapidly and efficiently (see Section 4.1).

1.5 Regulations and guidelines

No occupational exposure limit for coumarin in workplace air has been reported.

The Council of Europe has listed coumarin as an ‘active principle’ and the maximum permitted concentrations of coumarin in foodstuffs are given in Annex II of European directive (88/388/EEC) (European Commission, 1988). The general limit for coumarin in food and non-alcoholic beverages is 2 mg/kg, while in alcoholic beverages and certain caramel confectionary products, the permitted limit is 10 mg/kg and in chewing gum it is 50 mg/kg (Lake, 1999).

Food containing any added coumarin as such or as a constituent of tonka beans or tonka extract is deemed to be ‘adulterated under the act’, based upon an order published in the Federal Register of 5 March 1954 (19 FR 1239) (Food and Drug Administration, 1999).

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 50–51 male and 50–51 female B6C3F_1 mice, six to seven weeks, were administered coumarin (purity, > 97%) in corn oil by gavage at doses of 0, 50, 100 and

200 mg/kg bw daily for 103 weeks. Survival of all dosed groups was similar to that of the controls. Body weight gain was reduced in high-dose females. As shown in Table 1, there was significantly increased incidence of alveolar/bronchiolar tumours (adenomas and carcinomas) at the 200 mg/kg bw dose in both males and females and increased incidence of hepatocellular adenomas in females at the low and medium doses, but not at the highest dose. There were marginal increases in the incidence of squamous-cell papillomas and carcinomas of the forestomach in the low-dose males and females (National Toxicology Program, 1993).

Groups of 52 male and 52 female Swiss CD-1 mice, four weeks of age, were administered coumarin (purity, > 98%) in the diet at concentrations of 0, 300, 1000 or 3000 mg/kg of diet (ppm) for either 101 weeks (males) or 109 weeks (females). The achieved intakes of coumarin for the three doses were 26, 86 and 280 mg/kg bw per day, respectively, for males and 28, 91 and 271 mg/kg bw per day, respectively, for females. Survival did not differ between groups, although exact rates were not recorded. Body weight gain was significantly decreased by 18% in the high-dose males and by 10% in the mid-dose males, relative to the controls. The authors reported that there was no significant increase in the incidence of pulmonary adenocarcinomas in males (11/52 control, 17/52 low-dose, 10/52 mid-dose and 20/52 high-dose) and that the incidences were within the historical control range. Although there was an increased incidence of hepatocellular tumours (adenomas and carcinomas combined) in low-dose females (0/50 in controls, 8/52 in low-dose, 4/52 in mid-dose and 3/52 in high-dose females), the authors discounted these increases as being within the historical control range for that laboratory (Carlton *et al*., 1996). [The Working Group noted that no information was provided on statistical evaluation in this paper. However, the Working Group was aware of an unpublished company report in which statistical analyses had been applied to mortality-adjusted tumour rates. The Fisher's exact test for differences between treatment groups and Mantel's test for dose-related trends showed no treatment-related effect for any tumour type.]

3.1.2 *Rat*

Coumarin was tested by oral administration in two early studies in rats (Hagan *et al.*, 1967; Bär & Griepentrog, 1967; Griepentrog, 1973) and found to produce bile duct carcinomas at high levels of exposure in the latter study (IARC, 1976). However, in a re-evaluation by other pathologists of the slides from the Bär and Griepentrog (1967) study, these lesions were deemed to be non-neoplastic cholangiofibrosis and not bile duct carcinomas (Cohen, 1979; Evans *et al*., 1989).

Groups of 50 male and 50 female Fischer 344/N rats, six to seven weeks of age, were administered coumarin (purity, > 97%) in corn oil by oral gavage at doses of 0, 25, 50 and 100 mg/kg bw daily for 103 weeks. Survival of low-, mid- and high-dose males (9/50, 2/50 and 0/50 respectively) was significantly lower ($p < 0.001$) than that of controls (28/50), as was body weight gain. In males, increased mortality was attributed

Table 1. Incidence of primary tumours in B6C3F$_1$ mice exposed to coumarin

Tumour site	Animals with tumours							
	Males				Females			
	Control	50 mg/kg bw	100 mg/kg bw	200 mg/kg bw	Control	50 mg/kg bw	100 mg/kg bw	200 mg/kg bw
Lung								
Alveolar/bronchiolar adenomas	14/50	8/50	14/50	24/51*	2/51	5/49	7/49	20/51**
Alveolar/bronchiolar carcinomas	1/50	1/50	2/50	1/51	0/51	0/49	0/49	7/51**
Liver[a]	26/50	29/50	29/50	27/51	8/50	26/49**	29/51**	12/50
Forestomach[b]	2/50	9/50*	4/50	0/51	1/52	6/50	3/51	2/51

From National Toxicology Program (1993)
[a] Hepatocellular adenomas
[b] Squamous-cell papillomas and carcinomas
* $p < 0.05$, logistic regression test
** $p < 0.01$, logistic regression test

to increased severity of age-related spontaneous chronic progressive nephropathy. As shown in Table 2, there was no increased incidence of renal tubule adenomas after conventional single-section evaluation, but the incidence was increased based on the results of step-sectioning of kidney tissue, although there was no dose–response relationship. There was an increased severity of bile duct hyperplasia and renal tubule hyperplasia in both sexes. An accompanying stop-exposure study was carried out in which groups of 20 male rats received 100 mg/kg bw per day of coumarin by gavage in corn oil for nine or 15 months followed by corn oil gavage only until the end of the study at 103 weeks. Survival was 9/20 and 2/20 in the two groups, respectively. Whereas hepatic lesions including bile duct hyperplasia were reversible and the incidence of renal tubule adenomas, based on single sections, was not significantly increased, there was an increase in the nine-month 100 mg/kg bw dose group after step-sectioning of the kidney (National Toxicology Program, 1993). [The Working Group noted that the incidence of renal tubule tumours was not increased after conventional examination of the kidney.]

Groups of 50 male and 50 female Sprague-Dawley rats were administered coumarin (purity, > 98%) in the diet at concentrations of 0, 333, 1000, 2000, 3000 and 5000 mg/kg of diet (ppm) for 104 weeks (males) or 110 weeks (females). Groups receiving 3000 and 5000 ppm were 21–28 days of age at the beginning of the study, while the other treated groups were exposed to coumarin diet *in utero* and throughout the postnatal and chronic periods. The achieved intakes of coumarin for the five doses were 13, 42, 87, 130 and 234 mg/kg bw per day, respectively, for males and 16, 50, 107, 156 and 283 mg/kg bw per day, respectively, for females. Survival was less than 50% in all groups (including controls) except the groups receiving the two highest doses, in which it was between 50 and 70%. Dose-related decreases in body weight gain in excess of 10–15% occurred in the 2000-, 3000- and 5000-ppm dose groups. Significantly increased incidences of cholangiocarcinomas, some of which were reported as metastasizing, were found in the highest-dose males and females (males: 0/65, 0/65, 0/65, 0/65, 1/65 and 37/65; females: 0/65, 0/65, 0/65, 0/65, 0/65 and 29/65, in the control, 333-, 1000-, 2000-, 3000- and 5000-ppm dose groups respectively). Hepatocellular tumours were also found in high-dose males: 2/65, 2/65, 1/65, 1/65, 6/65 and 29/65 and females: 0/65, 0/65, 0/65, 0/65, 1/65 and 12/65 (Carlton *et al*., 1996). [The Working Group noted the unusually sharp increase in tumour incidence in the liver at only the highest of five doses and the lack of adequate histopathological description of both tumour types. Given the issue related to misdiagnosis of bile duct tumours in an earlier study, the Working Group was concerned that no descriptive information or illustrations were provided to confirm the diagnosis of cholangiocarcinoma, nor a discussion of the pathology.]

3.1.3 *Hamster*

Groups of 11 or 12 male and 10–13 female Syrian golden hamsters, eight weeks old, were administered coumarin (purity, 99%) in the diet at concentrations of 0, 0.1 and 0.5% for up to two years. Survival was poor in all groups receiving coumarin, and

Table 2. Incidence of primary tumours in Fischer 344/N rats exposed to coumarin

Tumour site	Animals with tumours							
	Males				Females			
	Control	25 mg/kg bw	50 mg/kg bw	100 mg/kg bw	Control	25 mg/kg bw	50 mg/kg bw	100 mg/kg bw
Kidney[a], single section	1/49	2/50	2/51	1/50	0/49	0/50	0/50	2/49
step sections	0/49	4/50	5/51*	4/50	0/49	0/50	1/50	1/49

From National Toxicology Program (1993)
[a] Renal tubule adenoma
* $p < 0.05$, logistic regression test

in the control females. There was no evidence of increased bile duct hyperplasia, cholangiofibrosis or cholangiocarcinoma due to coumarin exposure or tumours at other sites (Ueno & Hirono, 1981). [The Working Group noted the small number of animals and the poor survival, making the study inadequate for evaluation.]

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

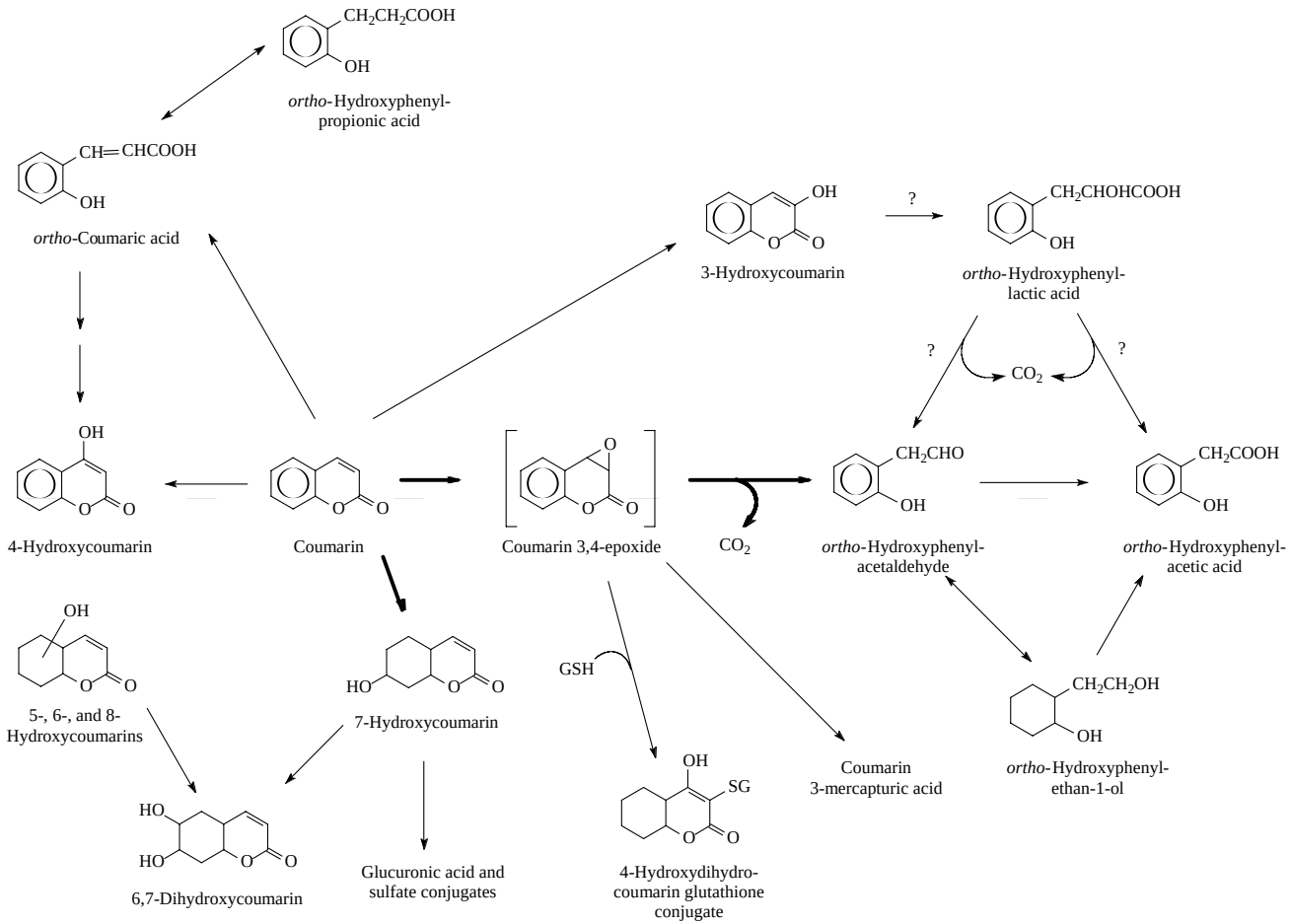
The absorption, distribution, metabolism and excretion of coumarin in humans have been reviewed (Cohen, 1979; Fentem & Fry, 1993; Pelkonen *et al*., 1997; Lake, 1999).

Toxicokinetic studies in humans have demonstrated that coumarin is rapidly absorbed from the gastrointestinal tract after oral administration and extensively metabolized by the liver in the first pass, with only 2–6% reaching the systemic circulation intact (Ritschel *et al*., 1977, 1979; Ritschel & Hoffmann, 1981).The elimination of coumarin from the systemic circulation is rapid, the half-lives following intravenous doses of 0.125, 0.2 and 0.25 mg/kg bw being 1.82, 1.46 and 1.49 h [109, 88 and 89 min], respectively (Ritschel *et al.*,1976). Coumarin is also extensively absorbed after dermal application. In one study with human subjects, some 60% of a 2.0-mg dose applied for 6 h was absorbed (reviewed in Lake, 1999). The percutaneous absorption of coumarin has also been demonstrated *in vitro* with human skin (Beckley-Kartey *et al.*, 1997; Yourick & Bronaugh, 1997).

The rapid excretion of coumarin, primarily as 7-hydroxycoumarin conjugates, in the urine of human subjects given coumarin orally suggests that there is little or no biliary excretion of coumarin metabolites in humans (Shilling *et al*., 1969; Cholerton *et al*., 1992; Egan & O'Kennedy, 1992; Rautio *et al*., 1992; Iscan *et al*., 1994).

Coumarin exhibits marked species differences in its metabolism (Cohen, 1979; Fentem & Fry, 1993; Lake, 1999). The major primary pathways of coumarin metabolism are 7-hydroxylation or metabolism of the lactone ring by ring opening and cleavage at carbon atom 2 to yield carbon dioxide. The first step in the latter pathway is the formation of the unstable coumarin 3,4-epoxide which degrades spontaneously to form *ortho*-hydroxyphenylacetaldehyde and may be subsequently converted to *ortho*-hydroxyphenylethanol and *ortho*-hydroxyphenylacetic acid. Coumarin may also be metabolized by hydroxylation to yield 3-, 4-, 5-, 6- or 8-hydroxycoumarin and 6,7-dihydroxycoumarin and, by opening of the lactone ring, to yield *ortho*-coumaric acid (*ortho*- hydroxyphenylcinnamic acid) and *ortho*-hydroxyphenylpropionic acid (Norman & Wood, 1984; Fentem *et al.*, 1991; Lake *et al*., 1992a,b; Born *et al*., 1997). The pathways of coumarin metabolism are shown in Figure 1.

Figure 1. Metabolic pathways of coumarin in animals and humans



Adapted from Lake (1999)

The major pathway of coumarin metabolism in most human subjects is 7-hydroxylation to form 7-hydroxycoumarin, which is excreted in the urine as both glucuronic acid and sulfate conjugates. Coumarin 7-hydroxylation activity exhibits a Gaussian distribution in Caucasian populations (Cholerton *et al.*, 1992; Rautio *et al.*, 1992), but some individuals are deficient in this activity.

Hadidi *et al.* (1997) gave members of a family 2 mg coumarin orally and collected their urine for 8 h. One subject excreted < 0.03% of the dose as 7-hydroxycoumarin and 50% as *ortho*-hydroxyphenylacetic acid, but three others excreted mainly 7-hydroxycoumarin (> 41% of dose) and 4–10% as *ortho*-hydroxyphenylacetic acid. Oscarson *et al.* (1998) refer to two individuals (among a population of two hundred) who were totally deficient in 7-hydroxycoumarin excretion after an oral dose of 5 mg coumarin.

CYP2A6 (cytochrome P450 2A6) has been purified from human liver and CYP2A6 cDNA expression systems are available. Many studies have demonstrated marked interindividual variation in the levels of hepatic CYP2A6 protein, mRNA and associated microsomal coumarin 7-hydroxylase activity (reviewed in Pelkonen *et al.*, 1997; Lake, 1999). The role of CYP2A6 in the metabolism of coumarin by human liver microsomes has been confirmed by Sai *et al.* (1999), who found that a monoclonal antibody to CYP2A6 inhibited coumarin 7-hydroxylation by more than 94%.

The marked interindividual variation in coumarin metabolism to 7-hydroxycoumarin has led to studies to evaluate whether a genetic polymorphism exists in human CYP2A6.

The occurrence of variant alleles in the human *CYP2A6* gene was shown by Fernandez-Salguero *et al.* (1995); these were designated *CYP2A6*1* (wild type), *CYP2A6*2* and *CYP2A6*3*. *CYP2A6*2* has a point mutation in codon 160 and the resulting protein product is unable to 7-hydroxylate coumarin (Fernandez-Salguero *et al.*, 1995; Gullstén *et al.*, 1997; Hadidi *et al.*, 1997). The functional significance of the rare *CYP2A6*3* allele is uncertain. The population frequency of these mutant alleles is uncertain at present; initial claims that the incidence of the *CYP2A6*2* allele is 4–17% of European populations (Fernandez-Salguero *et al.*, 1995; Gullstén *et al.*, 1997) have been challenged by Oscarson *et al.* (1998), who found the incidence to be 1–3%. These authors highlighted methodological uncertainties in polymerase chain reaction-based genotyping procedures. Establishment of the significance of the genetic polymorphism in *CYP2A6* must await definitive genotyping and phenotyping procedures.

While 7-hydroxylation is the major metabolic pathway of coumarin in most subjects, humans also convert coumarin to *ortho*-hydroxyphenylacetic acid.

Shilling *et al.* (1969) reported that after an oral dose of 200 mg coumarin per subject, while 7-hydroxycoumarin accounted for 79% of the excreted dose (range, 68–92%), a further 4% of the dose (range, 1–6%) was present in the first 24-h urine as *ortho*-hydroxyphenylacetic acid.

Ten human subjects received coumarin orally (1 g and 2 g) and intravenously (250 mg) in a randomized cross-over study and their 0–48 h urines were assayed for *ortho*-hydroxyphenylacetic acid. After intravenous administration, 1.5% (range, 0.3–3.6%)

of the dose was recovered as *ortho*-hydroxyphenylacetic acid, while after oral administration the recoveries were 3.5% (1.8–7.0%) and 5.1% (1.1–13.5%) after the 1-g and 2-g doses, respectively (Meineke *et al.*, 1998).

4.1.2 *Experimental systems*

The toxicokinetics of coumarin have been studied in a number of species including rats (intraperitoneal, intravenous, oral and topical administration) (Hardt & Ritschel, 1983; Ritschel & Hussain, 1988), dogs (intravenous and oral) (Ritschel & Grummich, 1981), gerbils (intraperitoneal) (Ritschel & Hardt, 1983) and rhesus monkeys (intravenous and oral) (Ritschel *et al.*, 1988). Generally, the half-life for the elimination of coumarin is similar in all species examined, being around 1–4 h (Lake, 1999). In rats, the toxicokinetics of coumarin are non-linear at intraperitoneal doses greater than 10 mg/kg bw (Hardt & Ritschel, 1983).

Following oral administration, coumarin is rapidly absorbed from the gastrointestinal tract and is distributed throughout the body (Cohen, 1979; Fentem & Fry, 1993; Pelkonen *et al.*, 1997; Lake, 1999). The compound is extensively metabolized in all species, with little excretion of unchanged coumarin. No significant tissue accumulation of coumarin and/or coumarin metabolites occurred after oral administration to rats and rabbits (Kaighen & Williams, 1961) or intraperitoneal administration to rats (van Sumere & Teuchy, 1971).

There are important quantitative differences between species in the routes of elimination of coumarin metabolites. In rats, biliary excretion occurs with an appreciable proportion of the dose excreted in the faeces (Cohen, 1979; Lake, 1999). For example, after a 50-mg/kg bw oral or intraperitoneal dose of coumarin to rats, some 50% was excreted in the bile as unknown metabolites within 24 h (Williams *et al.*, 1965). The urine appears to be the major route of coumarin excretion in Syrian hamsters, rabbits and baboons, but not in marmosets (Kaighen & Williams, 1961; Waller & Chasseaud, 1981; Lake *et al.*, 1989a, 1990; Lake, 1999).

Unlike in humans, the major metabolic pathway of coumarin in rats is the 3,4-epoxidation pathway. After a 100-mg/kg bw oral dose of [3-^{14}C]coumarin, urinary 3-hydroxycoumarin, 7-hydroxycoumarin, *ortho*-hydroxyphenyllactic acid and *ortho*-hydroxyphenylacetic acid accounted for 1.8, 0.4, 0.8 and 20% of the dose, respectively. Various metabolites including *ortho*-hydroxyphenylacetic acid were detected in the faeces (Kaighen & Williams, 1961). Other studies *in vivo* have confirmed that rats are poor 7-hydroxylators of coumarin, with urinary 7-hydroxycoumarin accounting for < 1% of the dose (van Sumere & Teuchy, 1971; Lake *et al.*, 1989a).

Because of the relative ease of measurement of 7-hydroxycoumarin, many studies have examined this pathway of coumarin metabolism after oral administration. Overall, several species including rats, most mouse strains, Syrian hamsters, guinea-pigs, dogs, marmosets and squirrel monkeys are poor 7-hydroxylators, excreting ≤ 5% of the administered dose as urinary 7-hydroxycoumarin (Cohen, 1979; Lake, 1999).

Certain mouse strains, such as DBA/2 and 129/Rr strains, excrete up to 26% of an intraperitoneally administered dose of coumarin as 7-hydroxycoumarin (Lush & Andrews, 1978). Species such as rabbits, cats and pigs have been reported to excrete 12–19% of the dose as urinary 7-hydroxycoumarin (Kaighen & Williams, 1961; Gangolli *et al*., 1974; Lake, 1999). In contrast, baboons, like humans, are extensive 7-hydroxylators of coumarin, excreting 60–66% of a dose of coumarin as urinary 7-hydroxycoumarin (Gangolli *et al.*, 1974; Waller & Chasseaud, 1981).

Apart from glucuronic acid and sulfate conjugation of hydroxycoumarins, other phase II pathways of coumarin metabolism have been identified. For example, *ortho*-coumaric acid may be conjugated with glycine (Lake, 1999), and a coumarin mercapturic acid conjugate has also been reported (Huwer *et al*., 1991). Coumarin may also be metabolized by the gastrointestinal microflora to 3,4-dihydrocoumarin and *ortho*-hydroxyphenylpropionic acid under anaerobic conditions (Scheline, 1968).

Coumarin metabolism has been studied *in vitro* in liver microsomes, hepatocytes and liver slices. Metabolites of coumarin detected in such systems include all six hydroxycoumarins, *ortho*-hydroxyphenylacetaldehyde, *ortho*-hydroxyphenylethanol, *ortho*-hydroxyphenylacetic acid, *ortho*-hydroxyphenyllactic acid, *ortho*-hydroxyphenylpropionic acid, *ortho*-coumaric acid and 6,7-dihydroxycoumarin (Lake, 1999). Studies with radiolabelled coumarin have demonstrated that coumarin can be converted to metabolite(s) that can bind covalently to proteins (Lake, 1984). In keeping with in-vivo studies, 7-hydroxycoumarin formation is only a minor pathway of metabolism in most species. Coumarin metabolism was examined in hepatic microsomes from eight animal species and humans. The overall rate of metabolism varied from 6.7 to 0.11 nmol/mg protein/min, in the rank order hamster>rat>mouse, cynomolgus monkey, human, rabbit>guinea-pig, dog>>cat. The contribution of 7-hydroxylation to the metabolism varied even more, by 225-fold (from 8 to 1800 pmol/mg protein/min for cat and monkey microsomes, respectively, accounting for 84% of total metabolism in human and 73% in monkey, but only 7% in cat, 3% in mouse and 0.3% in rat (Pearce *et al.*, 1992). Depending on the substrate concentration employed, *ortho*-hydroxyphenylacetaldehyde can be a major metabolite of coumarin in liver microsomes from rats, mice, Syrian hamsters and gerbils (Fentem & Fry, 1992; Lake *et al.*, 1992a,b). In mice, marked strain differences have been observed, with high coumarin 7-hydroxylase activity in female mice from strains such as DBA/2 and 129/J (Negishi *et al.*, 1989; Lovell *et al.*, 1999). However, the major pathway of coumarin metabolism in mouse remains 3,4-epoxidation, even in strains with high 7-hydroxylase activity (Lovell *et al*., 1999). The CYP isoform responsible for coumarin 7-hydroxylation in mouse liver is CYP2A5 and both baboons and cynomolgus monkeys possess hepatic CYP2A isoforms with properties similar to those of human CYP2A6 (Pelkonen *et al*., 1997; Lake, 1999).

It is of interest that studies with cloned human cytochromes P450 expressed in insect SF9 cells have shown that only CYP2A6 catalysed 7-hydroxylation, while the formation of *ortho*-hydroxyphenylacetaldehyde was supported by CYP1A1, 1A2, 2B6, 2E1 and 3A4 (Zhuo *et al.*, 1999).

4.2 Toxic effects

4.2.1 *Humans*

Recommended doses of coumarin in clinical medicine range from 8 mg per day for the treatment of venous constriction to 7000 mg per day in antineoplastic therapies (Marshall *et al*., 1994; Lake, 1999). While various mild side-effects have been reported following coumarin treatment, alterations in liver function have been noted in only a small proportion of patients. Reports of overt toxicity are rare (Cox *et al*., 1989).

Casley-Smith and Casley-Smith (1995) reported two cases of hepatotoxicity in five trials involving 1106 lymphoedema patients taking 400 mg coumarin daily for a mean duration of 14.6 months. This incidence is similar to that reported by Cox *et al*. (1989) in trials with 2173 patients with cancer or chronic infections. The majority of these patients received 100 mg coumarin per day for one month followed by 50 mg per day for two years. Only eight patients (0.37%) developed elevated serum aminotransferase levels (peak levels of 360–696 I.U./L) after total coumarin doses between 1 and 15 g. No evidence of liver toxicity was observed in 45 renal-cell carcinoma patients given 100 mg coumarin daily in combination with cimetidine (Marshall *et al*., 1987a). In other studies with similar dosing regimens, no evidence of liver toxicity was found in groups of 17 (Nolte *et al*., 1987), 22 (Marshall *et al*., 1989), 24 (Marshall *et al*., 1987b) and 50 (Dexeus *et al*., 1990) cancer patients. No evidence of liver toxicity was reported in extensive trials with lymphoedema patients given 400 mg coumarin per day in combination with diethylcarbamazine (Jamal *et al*., 1989).

Some cases of hepatotoxicity have been reported to be associated with exposure to coumarin. One possible case was reported by Beinssen (1994) and six by Loprinzi *et al*. (1997). Marshall *et al*. (1994) reported one case in which elevated serum aminotransferase levels were measured in a patient given 5 g coumarin per day. In two lymphoedema patients given 90 mg coumarin per day for five months, Koch *et al*. (1997) reported elevated serum alanine aminotransferase activity. Faurschou (1982) reported a case of toxic hepatitis in a patient given coumarin daily for eight weeks, which was characterized by hepatomegaly and elevated serum enzyme levels. All signs of liver toxicity returned to normal on cessation of treatment.

Coumarin was not toxic at concentrations up to 100 μg/mL to human peripheral blood mononuclear cells *in vitro*. Coumarin and its metabolite, 7-hydroxycoumarin, produced significant suppression of human bone marrow progenitor stem cell activity at concentrations greater than 200 μg/mL, whereas coumarin concentrations of 25 μg/mL and above produced suppression of murine bone marrow progenitor stem cell activity (Gallicchio *et al*., 1989).

In various human tumour cell lines (lymphoblastic cell line, CCRF CEM; gastric carcinoma cell line, St 23132; hepatoma-derived cell line, HepG2; colon-carcinoma cell line, Caco-2) coumarin [IC_{50} values ranging from 232 to 522 μg/mL] and its major metabolite 7-hydroxycoumarin [IC_{50} values ranging from 110 to 436 μg/mL] inhibited

cell proliferation after 48 h incubation. The glucuronide of 7-hydroxycoumarin (tested up to 2315 μg/mL) did not produce this response (Weber *et al*., 1998).

4.2.2 *Experimental systems*

(*a*) *Single-dose studies*

The acute oral LD_{50} of coumarin has been reported to be 420 mg/kg bw in C3H/HeJ mice and 780 mg/kg bw in DBA/2J mice (Endell & Seidel, 1978). In three mouse strains, the oral LD_{50} of coumarin has been reported to range from 196 to 780 mg/kg bw (reviewed in Cohen, 1979; Egan *et al*., 1990). The oral LD_{50} for coumarin in various strains of rats is reported to be 292–680 mg/kg bw (Hazleton *et al*., 1956).

In male Sprague-Dawley or Wistar rats, single oral or intraperitoneal doses of coumarin ranging from 125 to 500 mg/kg bw resulted in rapid depletion of hepatic non-protein sulfhydryl groups, primarily glutathione. After 24 h, centrilobular hepatic necrosis was observed together with dose-dependent elevation in plasma transaminase activities (Lake, 1984; Lake *et al*., 1989b; Fentem *et al*., 1992; Lake & Evans, 1993; Lake *et al*., 1994). Studies with various modulators of cytochrome P450 activity and with depletors of glutathione levels demonstrated that coumarin-induced hepatotoxicity in the rat is likely to be mediated via one or more reactive metabolites generated by cytochrome P450-dependent enzymes and that glutathione conjugation constitutes a detoxification pathway (Lake, 1984, 1999). Lake *et al*. (1989b) showed that dihydrocoumarin, which lacks the 3,4-double bond, produced little hepatotoxicity in male Sprague-Dawley rats *in vivo* (127 and 254 mg/kg bw intraperitoneally). Subsequently, Lake *et al*. (1994) demonstrated that coumarin (intraperitoneally, 0.86 and 1.71 mmol/kg bw), but none of the coumarin methyl derivatives examined (intraperitoneally, 1.71 and/or 2.57 mmol/kg bw), produced dose-related hepatic necrosis in male Sprague-Dawley rats.

In male Wistar rats, coumarin, 3-methylcoumarin or 4-methylcoumarin was administered intraperitoneally at a single dose of 1.03 mmol/kg bw and rats were killed 24 h later. Coumarin produced histological evidence of centrilobular necrosis, while the methyl analogues were much less toxic at equivalent doses. In the same study, these compounds had the same order of cytotoxicity in isolated hepatocytes as that observed *in vivo* (Fernyhough *et al*., 1994).

Cottrell *et al.* (1996) reported that a single oral dose of coumarin produced liver necrosis in mice; 200 mg/kg bw coumarin was hepatotoxic to both C3H/He and DBA/2 mice. Hepatotoxicity was characterized by an increase in plasma aminotransferase activity, mild subcapsular linear hepatocyte necrosis and, in some C3H/He mice, centrilobular necrosis. Mice were pretreated with β-naphthoflavone (80 mg/kg bw), Aroclor 1254 (54, 125 or 162 mg/kg bw) or vehicle alone by intraperitoneal injection for three consecutive days. Twenty-four hours later, a single dose of coumarin (200 mg/kg bw) or vehicle was administered by gavage. Pretreatment with

β-naphthoflavone or Aroclor 1254 did not significantly alter coumarin hepatotoxicity in C3H/He mice, while hepatic microsome metabolism of [^{14}C]coumarin doubled following administration of either inducer. In DBA/2 mice, pretreatment with either β-naphthoflavone or Aroclor 1254 did not affect coumarin-induced hepatotoxicity.

The toxicity of coumarin has also been assessed in other species. A single intraperitoneal injection of coumarin (125 mg/kg bw) caused centrilobular necrosis in Wistar rats but not in Mongolian gerbils (Fentem *et al.*, 1992). Coumarin has been reported to be hepatotoxic and nephrotoxic in dogs at an oral dose of 100 mg/kg bw given for periods of 8–22 days (Hazleton *et al.*, 1956; Hagan *et al.*, 1967). Hepatotoxic effects were also noted in dogs given 25 or 50 mg/kg bw coumarin for longer periods, but not in dogs given 10 mg/kg bw coumarin for up to 350 days (Hagan *et al.*, 1967).

A significant reduction in CYP2A and CYP2G levels in the olfactory mucosa of Wistar rats and C57BL/6 mice and suppression of nasal CYP2A in rats occurred within 48 h following a single intraperitoneal injection of coumarin (50 mg/kg bw). The decrease in cytochrome P450 levels was accompanied by necrosis, cell loss and basal cell metaplasia in the olfactory mucosa. Neither 7-hydroxycoumarin nor 3,4-dihydrocoumarin at a dose of 50 mg/kg bw depleted nasal cytochromes P450 (Gu *et al.*, 1997).

Coumarin produces pulmonary toxicity in mice. Male and female B6C3F$_1$ mice were dosed orally by gavage with 0, 10, 20, 50, 100, 150 or 200 mg/kg bw coumarin and lungs were evaluated histologically 24 h later. At doses of 150 mg/kg bw or greater, coumarin caused selective injury to the Clara cells in the distal bronchiolar epithelium. The time course of this injury and recovery of the cells was studied up to seven days following a single dose of 200 mg/kg bw coumarin. At 24–48 h, Clara cells were observed sloughed into the lumen of the terminal bronchioles along with thinning of the epithelium and flattening of the remaining ciliated cells. By seven days after dosing, the Clara cells had regenerated and the bronchiolar epithelium appeared normal. In male Fischer 344 rats dosed by gavage (200 mg/kg bw; single dose), Clara cell toxicity was not observed in the distal bronchioles. However, coumarin caused generalized epithelial necrosis in the upper airways of rats involving both ciliated and non-ciliated cells. Oral administration of coumarin (200 mg/kg bw; single dose) to female mice and male rats resulted in no evidence of hepatocellular necrosis in mice, whereas marked centrilobular necrosis was observed in the rat liver. Clinical markers of hepatic injury (aminotransferase and succinate dehydrogenase activity) were increased nearly 100-fold in rats and 2- to 3-fold in mice 24 h after treatment (Born *et al.*, 1998).

(*b*) *Multiple-dose studies*

A number of studies have examined the hepatic biochemical and morphological changes in rats produced by coumarin administration for periods ranging from one week to two years (reviewed in Lake, 1999). Coumarin administration results in increased liver weight and in a variety of morphological changes in treated rats that are dependent

on the magnitude of the dose and the duration of treatment. Such changes include necrosis, apoptosis, vacuolation, fatty change and bile duct hyperplasia. While centrilobular hepatic necrosis may be observed in rats treated with coumarin for at least four weeks, this lesion tends to regress with prolonged treatment in favour of the development of bile duct hyperplasia and cholangiofibrosis in the periportal area of the liver lobule (Grasso *et al*., 1974; Evans *et al*., 1989; Lake & Grasso, 1996). Kidney lesions were not observed in studies with Sprague-Dawley and Osborne-Mendel rats chronically fed coumarin at dietary levels of up to 0.5% (Hagan *et al*., 1967; Carlton *et al*., 1996).

Over a 16-day period, coumarin was administered by gavage to groups of five male and five female Fischer 344 rats and B6C3F_1 mice at doses of 0, 25, 50, 100, 200 or 400 mg/kg bw or 0, 40, 75, 150, 300, or 600 mg/kg bw, respectively, on five days per week for a total of 12 treatment days. All female and four male rats receiving 400 mg/kg bw coumarin died. There were no clinical signs of organ-specific toxicity in rats. Clinical findings in mice included inactivity, excessive lacrimation, piloerection, bradypnoea, ptosis or ataxia. All the mice receiving 600 mg/kg bw coumarin died, together with one male and one female in the 300-mg/kg bw groups and one male in the 75-mg/kg bw group. No histopathological examination was performed in this study (National Toxicology Program, 1993).

When B6C3F_1 mice were treated orally for five days with 200 mg/kg bw coumarin, mice became tolerant to the coumarin-induced swelling of Clara cells and necrosis in the terminal bronchioles which is observed after a single dose of coumarin (200 mg/kg bw). Although after five days of coumarin treatment, there were still areas of bronchiolar epithelial flattening and hyperplasia, by 10 days mouse lungs appeared to be normal. Together with Clara cell necrosis (following a single dose), the levels of cytochromes P450 such as CYP2A4/5, CYP2B10, CYP2C29, CYP2E1 and CYP2F2 were decreased. In tolerant mice, the levels of these enzymes returned to normal (Born *et al.*, 1999).

Coumarin administration in the diet (0.25–0.5%) of male Wistar rats and female ICR/Ha mice for two weeks induced glutathione peroxidase activity in the stomach (1.7-fold) and glutathione *S*-transferase in the liver (5.3-fold), respectively (Sparnins *et al.*, 1982; van Lieshout *et al.*, 1998).

In a 13-week study, groups of ten Fischer 344 rats and ten B6C3F_1 mice were administered coumarin by gavage at doses of 0, 19, 38, 75, 150 or 300 mg/kg bw. Three male and three female rats and two male mice receiving 300 mg/kg bw coumarin died. Absolute and relative liver weights increased in male and female rats and mice treated with 150 or 300 mg/kg bw coumarin. At the same doses, centrilobular hepatocellular degeneration and necrosis were observed in rats along with chronic active inflammation and bile duct hyperplasia. A re-evaluation of the kidneys revealed minimal nephropathy with tubular casts in the high-dose male and female rats and minimal to marked focal necrosis of proximal convoluted tubule epithelium was also observed. In coumarin-treated mice (300 mg/kg bw), centrilobular hepatocellular hypertrophy was observed (National Toxicology Program, 1993).

In one-, four- and 13-week studies, the effects of coumarin treatment were compared in male Sprague-Dawley rats, CD-1 mice and Syrian hamsters. Rats were fed 0–0.75% coumarin for one and four weeks and 0.5% coumarin for 13 weeks. Mice and hamsters were fed 0–0.5 and 0–1.0% coumarin, respectively, for one, four or 13 weeks. In the rat, coumarin caused dose-related hepatotoxic effects which included vacuolar degeneration, apoptosis and bile duct proliferation and increases in serum bilirubin content and both serum and hepatic γ-glutamyltranspeptidase activity. A sustained stimulation of hepatocyte replicative DNA synthesis was observed in rats treated for four and 13 weeks. Levels of total hepatic glutathione were increased approximately twofold, and there were statistically significant decreases in microsomal cytochrome P450 content and ethylmorphine *N*-demethylase activity. These effects were reduced or not observed in mice and hamsters (Lake & Grasso, 1996).

In a two-year study in which male and female Syrian hamsters were fed coumarin at dietary levels of 0.1 and 0.5%, no evidence of any coumarin-induced liver lesion was observed (Ueno & Hirono, 1981).

Coumarin was administered in the diet for two years to Sprague-Dawley rats and CD-1 mice in a study by Carlton *et al.* (1996) which is described more fully in Section 3.1.2. Histopathological evidence of hepatotoxicity was observed in rats, with males affected more severely than females. No dose-related clinical signs were observed in the CD-1 mice nor were there any changes in clinical pathology, haematology or microscopic pathology (Carlton *et al.*, 1996).

Coumarin was administered by oral gavage for two years to male and female Fischer 344 rats and B6C3F_1 mice, at doses of 0, 25, 50, and 100 mg/kg bw and 0, 50, 100, and 200 mg/kg bw, respectively (see Section 3.1.2). Hepatic lesions consisted of hepatocellular necrosis, fibrosis, cytological alteration and increased severity of bile duct hyperplasia. The incidence of forestomach ulcers was significantly greater in treated rats than in controls. Administration of coumarin to mice was associated with centrilobular hypertrophy, syncytial alteration and eosinophilic foci in the liver. No non-neoplastic lesions of the lungs of dosed mice were considered to be chemical-related (National Toxicology Program, 1993).

Administration of coumarin to baboons at dose levels of 0, 2.5, 7.5, 22.5 or 67.5 mg/kg bw per day in the diet for 16–24 months resulted in increased relative liver weight only at the highest dose level. While light microscopic examination of liver sections revealed no abnormalities, ultrastructural examination revealed a dilatation of the endoplasmic reticulum in three of four baboons given 67.5 mg/kg per day coumarin (Evans *et al.*, 1979).

(*c*) *In-vitro studies and species comparisons*

Lake *et al.* (1989b) compared the toxicity of coumarin and selected coumarin metabolites, including 7-hydroxycoumarin, 3-hydroxycoumarin and *ortho*-hydroxyphenylacetic acid, in metabolically-competent rat hepatocytes. Coumarin was more toxic than

any of its metabolites. The IC_{50} value for inhibition of protein synthesis was approximately 0.5 mmol/L for coumarin, whereas the 7-hydroxy and 3-hydroxy metabolites had IC_{50} values greater than 1 mmol/L, with *ortho*-hydroxyphenylacetic acid being essentially non-toxic.

Ratanasavanh *et al*. (1996) prepared hepatocytes from male Sprague-Dawley rats, DBA/2J mice, Fauve de Bourgogne rabbits and humans. The hepatocytes were incubated with 0.1, 0.25 or 0.5 mmol/L coumarin (and 1 mmol/L for human hepatocytes). Coumarin was hepatotoxic in cells prepared from rats, mice and rabbits, as judged by cell morphology and by lactate dehydrogenase release. Human hepatocytes were sensitive to coumarin toxicity only at a concentration of 1 mmol/L.

Price *et al*. (1996) compared the toxicity of 0.5, 1 and 2 mmol/L coumarin in 24 h cultured precision-cut male Sprague-Dawley rat, Dunkin-Hartley guinea-pig, cynomolgus monkey and human liver slices. Coumarin toxicity, based on liver protein synthesis and potassium content, was concentration-dependent in rat and guinea-pig liver, whereas monkey and human liver were relatively resistant.

Born *et al*. (2000) demonstrated that *ortho*-hydroxyphenylacetaldehyde (4 mmol/L) was much more cytotoxic than coumarin (4 mmol/L) to Chinese hamster ovary cells K_1, a cell line that does not contain cytochromes P450. When both of these compounds were investigated in metabolically active hepatocytes isolated from male Sprague-Dawley rats, *ortho*-hydroxyphenylacetaldehyde (0.8 mmol/L) caused a greater cytotoxic response compared with coumarin (0.8 mmol/L). 3-Hydroxycoumarin (0.8 mmol/L), not a product of coumarin epoxidation, did not cause a change in cell viability or an increase in lactate dehydrogenase activity.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Groups of 26–30 female NMRI mice were fed diets containing 0, 0.05, 0.1 and 0.25% coumarin on days 6–17 of gestation. There was no effect on the total number of implantations or on the proportions that were resorptions or fetal deaths, nor any reduction in fetal weight (Roll & Bär, 1967).

No embryotoxic or fetotoxic effects were seen in either rats, rabbits or miniature pigs given 10–100-fold the human therapeutic dose of coumarin plus rutin (Grote & Günther, 1971; Grote & Weinmann, 1973; Grote *et al.*, 1977).

Coumarin was one of a series of chemicals used in an assessment of the predictability of two in-vitro assays for mammalian teratogenesis. The assays were the human epithelial palatal mesenchymal (HEPM) cell assay, which evaluates effects on proliferative potential, and the mouse ovarian tumour (MOT) cell assay, which evaluates

the effect on attachment of ascites tumour cells to concanavalin-coated beads. The IC_{50} values for coumarin in both assays were in excess of 1 mmol/L, and the authors considered the results to be negative (Steele *et al*., 1988).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 3 for references)

Genotoxicity data on coumarin have been recently reviewed (Lake, 1999).

Coumarin induced gene mutations in *Salmonella typhimurium* strain TA100 with metabolic activation. A positive result was also reported in *Salmonella* strain TA7002, which responds specifically to reversions at a site where the change is T:A to A:T. However, coumarin failed to induce gene mutations in other strains of this type. Coumarin induced sister chromatid exchanges in Chinese hamster cells *in vitro* only without exogenous metabolic activation, and chromosomal aberrations only in the presence of exogenous metabolic activation. It did not induce micronuclei in rat primary hepatocytes, although micronuclei were found in established human hepatoma cells treated with coumarin. The two positive chromosome responses (aberrations, micronuclei) occurred only at the highest dose tested, and there was no clear dose–response relationship. Coumarin failed to induce gene mutations at the *Hprt* locus in Chinese hamster ovary cells. It did not induce unscheduled DNA synthesis in human liver slices *in vitro*.

Sex-linked recessive lethal mutations were not induced in *Drosophila melanogaster*. Coumarin did not induce either micronuclei in mice or unscheduled DNA synthesis in rats *in vivo*.

Coumarin modulates the mutagenic effects of other chemicals such as aflatoxin B_1 and a range of heterocyclic amines, and of physical agents such as ultraviolet light, and has been generally considered to be an antimutagen (Ohta *et al*., 1983; Sanyal *et al*., 1997; Goeger *et al.*, 1998; Simic *et al*., 1998). However, there are reports that it also acts as a co-mutagen in some assays (Goeger *et al*., 1999).

4.5 Mechanistic considerations

Marked inter-species differences have been observed in the metabolism and toxicity of coumarin. The metabolism of coumarin involves two primary pathways, 7-hydroxylation and ring-opening to *ortho*-hydroxyphenylacetaldehyde. Coumarin is hepatotoxic in rat, mouse and dog, species in which ring-opening predominates. In contrast, humans and baboons, in which 7-hydroxylation is most evident, rarely show

Table 3. Genetic and related effects of coumarin

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, reverse mutation	–	+[c]	1000 µg/plate	Haworth *et al.* (1983)
Salmonella typhimurium TA1535, TA1537, TA98, reverse mutation	–	–	3333 µg/plate[d]	Haworth *et al.* (1983)
Salmonella typhimurium TA1537, TA98, TA7001, TA7002, TA7003, TA7004, TA7005, TA7006, Mix[e], reverse mutation	–	–	1000 µg/mL	Gee *et al*. (1998)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		500 µg/mL; inj	Yoon *et al.* (1985)
Gene mutation, K_1BH_4 Chinese hamster ovary cells *Hprt* locus *in vitro*	–	–	500 µM	Goeger *et al*. (1999)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	–	100	National Toxicology Program (1993)
Micronucleus formation, primary rat hepatocytes *in vitro*	–	NT	73	Müller-Tegethoff *et al*. (1995)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	(+)	1600	National Toxicology Program (1993)
Micronucleus formation, human Hep-G2 cells *in vitro*	(+)	NT	500	Sanyal *et al*. (1997)
Unscheduled DNA synthesis, human liver slices *in vitro*	–	NT	730	Beamand *et al.* (1998)
Unscheduled DNA synthesis, rat hepatocytes *in vivo*	–		320 po × 1	Edwards *et al.* (2000)
Micronucleus formation, $B6C3F_1$ mice *in vivo*	–		300 po; 13 w	National Toxicology Program (1993)

Table 3 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, ICR mice *in vivo*	–		130 po × 6[f]	Morris & Ward (1992)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro test, µg/mL; in-vivo test, mg/kg bw/day; inj, injection; po, oral; w, week
[c] Only in the presence of hamster S9
[d] Slightly toxic dose
[e] Mix of strains TA7001-7006
[f] Treatments on days 1–3 and 5–7 of one week

hepatotoxicity. Susceptibility to liver toxicity, in the rat at least, is also associated with extensive biliary excretion.

Coumarin is toxic in the liver of rats and the liver and lung of mice.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Coumarin is a natural product occurring in the essential oils of a large number of plants, such as cinnamon, cassia, lavender and woodruff. It is used for its fragrance in many personal care products (perfumes, deodorants, soaps) and in tobacco, in household and industrial products to mask unpleasant odours and, in some countries, as a flavouring agent in food and beverages. It has also been used to treat several medical conditions. Exposure to coumarin may occur from its production, its natural presence in many plants and essential oils, and its several industrial, medical and consumer uses.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Coumarin has been adequately tested by oral administration in two experiments in mice and in one experiment in rats. In mice of one strain, it produced increases in lung tumours (adenomas and carcinomas) in both males and females and in hepatocellular adenomas in females. There was no increase in tumour incidences in another strain of mouse. In one study in rats, coumarin produced a low incidence of renal tubule adenomas in males, seen only after step-sectioning of the kidney. Three other studies in rats could not be evaluated.

5.4 Other relevant data

Coumarin is rapidly and extensively absorbed after topical or oral administration to human subjects. It undergoes very extensive metabolism along two major pathways, 7-hydroxylation and ring-opening to *ortho*-hydroxyphenylacetaldehyde. There are numerous minor metabolites, many of which are secondary products from the primary metabolites. The relative extent of these two major pathways is highly variable between species. Ring-opening predominates in rodents, while 7-hydroxylation is particularly evident in humans.

In humans exposed to coumarin for treatment of various clinical conditions, a few cases of hepatotoxicity have been reported. However, a clear relationship between the

dose of coumarin and the hepatotoxic responses observed has not been established. The target organs for coumarin toxicity are primarily the liver in rats and the liver and lung in mice. There are marked species differences in these responses, with the mouse being particularly susceptible to coumarin-induced Clara cell injury. Coumarin is hepatotoxic in rats and mice. Hamsters and gerbils are resistant to acute coumarin-induced hepatotoxicity. *In vitro*, coumarin is toxic in either hepatocytes or liver slices from rats, mice, rabbits and guinea-pigs, whereas monkey and human cells and/or slices appear to be resistant.

No data on reproductive and developmental effects in humans were available. No signs of teratogenicity were observed in mice, rats, rabbits or miniature pigs.

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

Coumarin did not induce micronuclei in mice *in vivo* and was not mutagenic in *Drosophila melanogaster*. It was weakly positive in induction of micronuclei in human cells *in vitro*, but failed to induce unscheduled DNA synthesis in human liver cells *in vitro*. Coumarin induced sister chromatid exchanges without metabolic activation and chromosomal aberrations with metabolic activation, but not micronuclei or gene mutations in mammalian cells *in vitro*. It was mutagenic in only two out of 11 *Salmonella typhimurium* strains tested, with metabolic activation.

Coumarin was antimutagenic in various assays, but also had co-mutagenic properties.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of coumarin were available.

There is *limited evidence* in experimental animals for the carcinogenicity of coumarin.

Overall evaluation

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

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ETHYLBENZENE

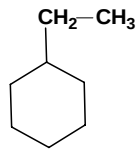
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 100-41-4
Chem. Abstr. Name: Ethylbenzene
IUPAC Systematic Name: Ethylbenzene
Synonyms: EB; ethylbenzol; α-methyltoluene; phenylethane

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_8H_{10} Relative molecular mass: 106.17

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid with an aromatic odour (Coty *et al*., 1987)
(*b*) *Boiling-point*: 136.1 °C (Lide & Milne, 1996)
(*c*) *Melting-point*: –94.9 °C (Lide & Milne, 1996)
(*d*) *Density*: 0.8670 g/cm^3 at 20 °C (Lide & Milne, 1996)
(*e*) *Spectroscopy data*: Infrared, ultraviolet [97], nuclear magnetic resonance and mass spectral data have been reported (Lide & Milne, 1996)
(*f*) *Solubility*: Slightly soluble in water (152 mg/L at 20 °C) (ECETOC, 1986) and chloroform; miscible with diethyl ether and ethanol (Lide & Milne, 1996)
(*g*) *Volatility*: Vapour pressure, 1.28 kPa at 25 °C (Lide & Milne, 1996); relative vapour density (air = 1), 3.7 (Verschueren, 1996); flash-point (closed-cup), 15 °C (Coty *et al*., 1987)

(*h*) *Octanol/water partition coefficient (P)*[1]: log P, 3.15 (Verschueren, 1996)
(*i*) *Conversion factor*[2]: mg/m^3 = 4.34 × ppm

1.1.4 *Technical products and impurities*

Because ethylbenzene is used almost exclusively to produce styrene, the product specification on ethylbenzene is set to provide a satisfactory feedstock for styrene production. Levels of cumene, *n*-propylbenzene, ethyltoluenes and xylenes in ethylbenzene are controlled to meet the required styrene purity specification. A typical sales specification is as follows: purity, 99.5 wt% min.; benzene, 0.1–0.3 wt%; toluene, 0.1–0.3 wt%; *ortho*-xylene + cumene, 0.02 wt% max.; *meta*-xylene + *para*-xylene, 0.2 wt% max.; allylbenzene + *n*-propylbenzene + ethyltoluene, 0.2 wt% max.; diethylbenzene, 20 mg/kg max.; total chlorides (as chlorine), 1–3 mg/kg max.; and total organic sulfur, 4 mg/kg max. (Coty *et al*., 1987).

1.1.5 *Analysis*

Selected methods for the analysis of ethylbenzene in various matrices are given in Table 1. Ethylbenzene can be determined in biological material (blood, subcutaneous fat, plant foliage, fish samples) using head-space gas chromatography (GC), GC with mass spectrometry, and GC with flame ionization detection (WHO, 1996a).

Determination of mandelic acid in urine has been recommended as a biomarker of exposure to ethylbenzene. Several methods can be used to determine mandelic acid in urine samples. These include derivatization of the acid and GC analysis (detection limit, 1.0 mg/L); isotachophoresis (detection limit, 0.04 mmol/L); and high-performance liquid chromatography (detection limit, 0.01 mmol/L) (WHO, 1996a).

1.2 Production

Ethylbenzene was first produced on a commercial scale in the 1930s in Germany and the United States. The ethylbenzene–styrene industry remained relatively insignificant until the Second World War, when the demand for synthetic styrene–butadiene rubber prompted accelerated technology improvements and tremendous capacity expansion (Coty *et al*., 1987).

Almost all ethylbenzene is produced commercially by alkylating benzene with ethylene, either in the liquid phase with aluminium chloride catalyst or in the vapour phase with a synthetic zeolite or Lewis acid catalyst (Coty *et al*., 1987; Cannella, 1998).

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[1] Partition coefficients of ethylbenzene for other media (water/air, blood/air, oil/air, oil/water and oil/blood) have also been measured (Sato & Nakajima, 1979)

[2] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

Table 1. Selected methods for the analysis of ethylbenzene

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Adsorb (charcoal); desorb (carbon disulfide)	GC/FID	1–10 µg/sample	Department of Health and Human Services (1994) [NIOSH Method 1501]
Drinking, ground, and surface water	Purge (inert gas); trap (Chromosorb W); desorb into capillary GC column	GC/MS	0.03–0.06 µg/L	Environmental Protection Agency (1995a) [Method 524.2]
Drinking water and raw source water	Purge (inert gas); trap (Chromosorb W); desorb into capillary GC column	GC/PID	0.01–0.05 µg/L	Environmental Protection Agency (1995b) [Method 502.2]
Wastewater, municipal and industrial	Purge (inert gas); trap (Tenax or OV-1 on Chromosorb W); thermally desorb into GC column	GC/PID	0.2 µg/L	Environmental Protection Agency (1999a) [Method 602]
	Purge (inert gas); trap (Tenax or OV-1 on Chromosorb W or silica gel); thermally desorb into GC column	GC/MS	7.2 µg/L	Environmental Protection Agency (1999b) [Method 624]
	Add isotope-labelled analogue; purge (inert gas); trap (Tenax or OV-1 on Chromosorb W or silica gel); thermally desorb into GC column	GC/MS	10 µg/L	Environmental Protection Agency (1999c) [Method 1624B]
Solid waste matrices[b]	Purge (inert gas); trap (Tenax or Chromosorb W) *or* thermally desorb *or* headspace sampling *or* direct injection into capillary GC column	GC/PID	0.005 µg/L	Environmental Protection Agency (1996a) [Method 8021B]

Table 1 (contd)

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
	Purge (inert gas); trap (Tenax or Chromosorb W) *or* thermally desorb *or* headspace sampling *or* direct injection into capillary GC column	GC/MS	0.03–0.06 µg/L	Environmental Protection Agency (1996b) [Method 8260B]

[a] Abbreviations: GC/FID, gas chromatography/flame ionization detection; GC/MS, gas chromatography/mass spectrometry; GC/PID, gas chromatography/photoionization detection
[b] Includes: groundwater, sludges, caustic and acid liquors, waste solvents, oily wastes, mousses, tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils, and sediments

Worldwide capacities for the production of ethylbenzene in 1985 and 1995 are presented in Table 2.

Information available in 1999 indicated that ethylbenzene was manufactured by nine companies in the United States, eight in Japan, seven in China, four in the Republic of Korea, two each in Brazil, Canada, France, Germany, Poland, Romania, the Russian Federation and the United Kingdom and one each in Australia, Belgium, Bulgaria, the Czech Republic, Iran, Italy, Mexico, the Netherlands, Singapore, Slovakia, Spain, Taiwan and the Ukraine (Chemical Information Services, 1999).

Table 2. Worldwide capacity for production of ethylbenzene in 1985 and 1995 (thousand tonnes)

Location	Capacity	
	1985	1995
North America	5900	7048
South America	380	NR
Western Europe	3820	5157
Eastern Europe	1630	1841
Japan	1470	3587
Asia, Oceania and Far East (except Japan)	640	2644
Other (including the Middle East and Africa)	390	1158
Total	14 230	21 435

From Coty *et al.* (1987); Cannella (1998)
NR, not reported

1.3 Use

Ethylbenzene is almost exclusively (> 99%) used as an intermediate for the manufacture of styrene monomer. Styrene production, which uses ethylbenzene as a starting material, consumes approximately 50% of the world's benzene production. Less than 1% of the ethylbenzene produced is used as a paint solvent or as an intermediate for the production of diethylbenzene and acetophenone. The ethylbenzene present in recovered mixed xylenes is largely converted to xylenes or benzene (Coty *et al*., 1987; Cannella, 1998).

Ethylbenzene has been added to motor and aviation fuels because of its anti-knock properties. Estimates of ethylbenzene in gasoline have ranged from < 1–2.7% (Fishbein, 1985; IARC, 1989; Backer *et al*., 1997).

Ethylbenzene is also used as a negative photoresist solvent in the semiconductor industry (Ladou & Rohm, 1998) and as a general solvent and diluent (Angerer *et al*., 1994).

Ethylbenzene is also present in commercial mixed xylenes at levels up to 25%, and, as such, is present in many paints and lacquers, printing inks, insecticides and solvents in the rubber and chemical manufacturing industries (Fishbein, 1985; ECETOC, 1986).

1.4 Occurrence

1.4.1 *Natural occurrence*

Ethylbenzene may occur naturally, as it has been found in orange peel, parsley leaves (Górna-Binjul *et al.*, 1996), dried legumes (WHO, 1996a) and other foodstuffs.

1.4.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1999), as many as 200 000 workers in the United States were potentially exposed to ethylbenzene (see General Remarks). National estimates of workers potentially exposed in other countries were not available.

Measured airborne and blood concentrations of ethylbenzene in several occupational settings are presented in Tables 3 and 4, respectively. Most occupational exposures to ethylbenzene result from use of products containing technical grades of mixed xylenes. No ethylbenzene was found from off-gassing of cured paint in a hyperbaric pressure chamber under normal atmospheric pressure, but under higher pressures, levels of 0.4–4.5 ppm [1.7–19.5 mg/m^3] were measured (Lillo *et al*., 1990). Silk screen operations were found to entail exposure levels of less than 4 mg/m^3 (Verhoeff *et al*., 1988). Ethylbenzene may also be present in low-grade toluene preparations (Inoue *et al.*, 1995).

Ethylbenzene exposure occurs in the chemical processing industries, including the rubber and plastics industries. Ethylbenzene emissions during the production of mixed

Table 3. Levels of occupational exposure to ethylbenzene

Source	Type of sample	Concentration[a] (mg/m^3)	Range[b] (mg/m^3)	No.[c]	Reference
Dip painting	Personal	AM, 10 ± 4.7 GM, 9.0 ± 6.6	Max., 22	30	Kawai *et al.* (1992)
Dip painting	Personal	GM, 3.9 ± 14	Max., 49	121	Kawai *et al.* (1991)
Varnishing and priming vehicles and metal pieces	Personal	AM, 4.0 ± 39	–	35	Angerer & Wulf (1985)
Varnishing and priming vehicles and metal pieces	Area	AM, 7.1 ± 16.4	–	192	Angerer & Wulf (1985)
Spray-painting aircraft	Personal	AM, 48.5	7.8–89.6	23	Vincent *et al.* (1994)
Paint or plastic-coated wire production or printing or painting workshops	Personal	GM, 7.8 ± 14.8	Max., 191	360	Inoue *et al.* (1995)
Oil refinery, outside (the control room) operators	Personal	AM, 0.08 ± 0.17	L&UCL, < 0.03–0.12	30	Rappaport *et al.* (1987)
Gasoline transport drivers	Personal	AM, 0.08 ± 0.10	L&UCL, 0.05–0.11	44	Rappaport *et al.* (1987)
Gasoline service station attendants	Personal	AM, 0.06 ± 0.06	L&UCL, 0.04–0.08	38	Rappaport *et al.* (1987)
Jet mechanics	Personal	GM, 0.02 ± 3.8	Max., 1.3	92[d]	Holm *et al.* (1987)
	Personal	GM, 0.07 ± 9.7	Max., 8.0	46[e]	
Coal-tar-free paving	Personal	AM, 0.09	< 0.03–1.27	77	Norseth *et al.* (1991)
Solvent used in histology laboratory	Area	AM, 178 ± 78 Med., 182	–	16[f]	Angerer & Lehnert (1979)
	Area	AM, 148 ± 61 Med., 161	–	16[f]	

[a] All 8-h time-weighted averages reported except as noted. AM, arithmetic mean ± arithmetic standard deviation; GM, geometric ± geometric standard deviation; Med., median
[b] Max., maximum level found; L&UCL, lower and upper confidence limits
[c] No., number of measurements
[d] 4-h TWA measurements
[e] 5-min TWA measurements
[f] 30-min TWA measurements taken consecutively during one day

Table 4. Ethylbenzene concentrations in the blood of occupationally exposed workers

Source	Concentration (μg/L)[a]	Range (μg/L)	No.[b]	Reference
Varnishing and priming vehicles and metal pieces	AM, 61.4 (SD, 62.3)	–	35	Angerer & Wulf (1985)
Before pumping regular gasoline	Med., 0.10	0.02–0.73	26	Backer *et al*. (1997)
After pumping regular gasoline	Med., 0.16	0.06–1.40	26	Backer *et al*. (1997)
Before pumping ethanol-blended gasoline	Med., 0.11	0.04–0.55	22	Backer *et al*. (1997)
After pumping ethanol-blended gasoline	Med., 0.16	0.06–0.64	22	Backer *et al*. (1997)
Solvent use in histology laboratory	AM, 69	50–80	4	Angerer & Lehnert (1979)
Nonsmoking firefighters in Kuwaiti oil fields	Med., 0.53	0.082–2.8	25	Etzel & Ashley (1994)

[a] AM, arithmetic mean; SD, standard deviation; Med., median
[b] No., number of measurements

xylenes occur from the reactor, distillation and crystallization vents, from storage units, from loading and handling of ethylbenzene and from leaks, so that xylene production workers are likely to have exposure. The total emissions of ethylbenzene from the catalytic reformate production of xylene in the United States in 1978 were estimated to have been 970 000 kg. Occupational exposure during the production of styrene was considered to be minimal or unlikely due to the enclosed nature of the styrene production process. Unfractionated crude oil contains 1–2.5% by weight of C_6–C_8 aromatics, mainly toluene, the xylenes and ethylbenzene, and oil refining therefore is also likely to result in exposures (Fishbein, 1985). Ethylbenzene has been detected in bitumen fumes during road paving (Norseth *et al.*, 1991).

Another source of occupational exposure to ethylbenzene is the production and handling of gasoline and other fuels in which it is a component (Rappaport *et al.*, 1987; Backer *et al.*, 1997).

A source of ethylbenzene exposure to physicians and nurses in the operating room may be electrocautery smoke generated from dissection of tissues and cauterization of blood vessels (36 μg/m^3 maximum detected in an area sample) (Sagar *et al*., 1996). Exposures to ethylbenzene from solvent use also occurred in a histology laboratory (Angerer & Lehnert, 1979).

1.4.3 *Environmental occurrence*

Ethylbenzene is widely distributed in the environment (principally due to its use as a solvent alone and as a component of mixed xylenes, and as a fuel additive), generally at very low levels in both ambient and indoor air, water, sediment soil and biota. Large quantities have been emitted during its production, use and disposal. The highest levels of ethylbenzene found in the environment are often associated with industrial operations, and it is one of the most commonly found substances at hazardous waste sites. Ethylbenzene is also found in motor vehicle emissions, some food samples, cigarette smoke and consumer products and has been detected in human and animal tissues (Fishbein, 1985; ECETOC, 1986; Lawryk *et al.*, 1995; WHO, 1996a; Agency for Toxic Substances and Disease Registry, 1997a,b; Environmental Protection Agency, 1999d).

Human exposure to ethylbenzene occurs mainly via inhalation of vapour and/or mist and, to a smaller extent, by dermal contact or ingestion. Ethylbenzene is produced by the incomplete combustion of natural materials, making it a component of smoke from forest fires and cigarettes. It is also a constituent of asphalt and naphtha (Agency for Toxic Substances and Disease Registry, 1997a; WHO, 1996a).

(*a*) *Air*

Ethylbenzene is ubiquitous in urban and rural atmospheres, resulting primarily from vehicle, petroleum and industrial emissions (ECETOC, 1986; Shah & Heyerdahl, 1988). Because of its high vapour pressure and low solubility, released ethylbenzene will disperse into the atmosphere. More than 99% of ethylbenzene can be expected in the air compartment (ECETOC, 1986; WHO, 1996a). Ethylbenzene undergoes atmospheric transformations with photolytically generated hydroxyl radicals, and an atmospheric half-life of about 65 hours has been estimated in the United States (Agency for Toxic Substances and Disease Registry, 1997a).

Air levels of ethylbenzene have been measured in Austria, Finland, Germany, Italy, the Netherlands, Sweden and the United States and were reported to range from < 2 µg/m^3 to > 100 µg/m^3 in urban areas (Table 5). Measurements in rural areas were lower (< 2 µg/m^3) (WHO, 1996a). The range of measured indoor air levels overlaps with those measured outdoors, but when outdoor and indoor levels are compared for a specific house, higher levels of ethylbenzene are usually found indoors (De Bortoli *et al.*, 1986; Minoia *et al.*, 1996; Wallace *et al.*, 1989). Other sources of emissions are consumer products such as solvents, adhesives, fabric and leather treatments, liquid process photocopiers and pesticides (Hodgson *et al.*, 1991; Sack *et al.*, 1992; Wallace *et al.*, 1987a,b; 1989; Kostiainen, 1995; WHO, 1996a; Agency for Toxic Substances and Disease Registry, 1997a).

Tobacco smoke is a major contributor to indoor air concentrations of ethylbenzene (Wallace & Pellizzari, 1986; Wallace *et al.*, 1987a,c). In an Environmental Protection Agency Total Exposure Assessment Methodology (TEAM) study carried out between

Table 5. Occurrence of ethylbenzene in air

Source	Arithmetic mean concentration (μg/m^3)	No.[a]	Location	Reference
Urban air	1.3–6.5 (median)	724	USA	Edgerton *et al.* (1989)
	1.1 (median)	8723	USA	Kelly *et al.* (1994)
	0.9–2.8	~1050	Netherlands	Guicherit & Schulting (1985)
	6.0–22.0 (annual means)	12	Germany	Bruckmann *et al.* (1988)
	7.4	15	Italy	De Bortoli *et al.* (1986)
	< 2	54	Austria	Lanzerstorfer & Puxbaum (1990)
	13	5	Germany	Seifert & Abraham (1982)
	6.2–100	260	Sweden	Jonsson *et al.* (1985)
Rural sites	< 2	NR	NR	WHO (1996a)
Indoor air (homes)	13	30	Germany	Seifert & Abraham (1982)
	3.2	50	Finland	
	2.41 (median)			
	10 (range, 1.5–161)	230	Germany	Gold *et al.* (1993)
	27	15	Italy	De Bortoli *et al.* (1986)
	6.46 (winter)	754	Canada	Fellin & Otson (1994)
	8.15 (spring)			
	4.35 (summer)			
	13.98 (autumn)			
	4.34	95	USA	Shah & Heyerdahl (1988)
	3.5–8.3	783	USA	Wallace & Pellizzari (1986)
	4–6	346	Italy	Minoia *et al.* (1996)
	0.8 nonsmokers (breath samples)	322	USA	Wallace & Pellizzari (1986)
	2.6 smokers (breath samples)	198	USA	Wallace & Pellizzari (1986)
	6.3 (median) (personal air monitoring)	347	USA	Wallace *et al.* (1986)
Indoor air (office buildings)	0.5 (geometric mean)	384	USA	Daisey *et al.* (1994)
	7.0–19	4	USA	Hodgson *et al.* (1991)

NR, not reported
[a] No., number of measurements

1980 and 1984 in the United States measuring the personal air exposures and exhaled breath concentrations for 198 smokers and 322 non-smokers, smokers showed significantly higher breath concentrations of ethylbenzene, compared to nonsmokers. The indoor air concentrations (geometric means) of ethylbenzene in homes with smokers ($n = 345$) were 8.3 μg/m^3 in the fall and winter, significantly higher ($p < 0.05$) than those

in homes without smokers (n = 164), 5.1 μg/m^3. The levels of ethylbenzene during the spring and summer in homes with smokers (n = 169) and homes without smokers (n = 105) were the same, 3.5 μg/m^3 (Wallace & Pellizzari, 1986). Nonsmokers exposed at work had significantly higher levels of ethylbenzene than unexposed nonsmokers (Wallace *et al.*, 1987c).

Ethylbenzene concentrations averaged over eight parallel commuter trips in an automobile and train in Gothenborg, Sweden were 17.9 and 2.1 μg/m^3, respectively (Löfgren *et al.*, 1991)

Ethylbenzene has been shown to be released from volatile organic chemical-laden wastewater in municipal sewer systems in the United States (Quigley & Corsi, 1995).

According to the Environmental Protection Agency (1999d) Toxics Release Inventory (TRI) in 1997, air emissions of ethylbenzene from 1005 industrial facilities were approximately 4 000 000 kg in the United States, which accounted for about 92% of the total environmental releases of ethylbenzene.

(*b*) *Water*

Ethylbenzene, usually at < 1 μg/L, is found only infrequently in drinking water from ground or surface sources (Table 6).

The levels of ethylbenzene in surface water are generally less than 0.1 μg/L in non-industrial areas. In industrial and urban areas, concentrations of up to 15 μg/L ethylbenzene have been reported (WHO, 1996a,b). The Commission of the European Communities also reported in 1976 that ethylbenzene levels in water were, in most cases, less than 1 μg/L (ECETOC, 1986).

Releases of ethylbenzene to water come from a variety of sources including: industrial discharges (Snider & Manning, 1982), fuel spillages (Tester & Harker, 1981), leaking petroleum pipelines or leaking underground storage tanks (Cotruvo, 1985), landfill leachate (Barker, 1987; Hallbourg *et al*., 1992; Chen & Zoltek, 1995; Beavers *et al*., 1996) and inappropriate disposal of wastes containing ethylbenzene (Eiceman *et al*., 1986).

Ethylbenzene was listed as one of the 58 most frequently detected chemicals associated with groundwater contamination in the United States. It was detected in over 4% of the surface water samples and 11% of the groundwater samples analysed at the 1177 National Priority List (NPL) sites (Agency for Toxic Substances and Disease Registry, 1997a).

Surface water discharges of ethylbenzene from 1005 industrial facilities in 1997 in the United States amounted to 2600 kg, which accounted for about 0.06% of the total environmental releases (4 280 000 kg), according to the Environmental Protection Agency (1999d) Toxics Release Inventory.

(*c*) *Soil and sediments*

Ethylbenzene can be released to soils from a variety of sources (Fishbein, 1985; ECETOC, 1986; WHO, 1996a; Agency for Toxic Substances and Disease Registry,

Table 6. Occurrence of ethylbenzene in water

Source	Arithmetic mean concentration of positive samples	Frequency of positive samples	Location	Reference
Drinking-water	0.8 µg/L	3/466	USA	Cotruvo (1985)
	0.94 µg/L (median)	2/280	USA	Westrick *et al.* (1984)
	0.87 µg/L (median)	3/321	USA	Westrick *et al.* (1984)
	0.74 µg/L (median)	1/186	USA	Westrick *et al.* (1984)
	< 1 µg/L	NR	Canada	Otson *et al.* (1982)
Seawater	1.8–22 ng/L	NR	USA	Gschwend *et al.* (1982)
	0.4–4.5 ng/L	8/8	USA	Sauer *et al.* (1978)
	46.3 ng/L	1/48	United Kingdom	Dawes & Waldock (1994)
Ambient surface water	< 5.0 µg/L (median)	110/1101	USA	Staples *et al.* (1985)
Polluted surface water	0.005–15 µg/L	2/2	Spain	Gomez-Belinchon *et al.* (1991)
	10–26 µg/L	NR	USA	Storage and Retrieval of Water Quality Information (1986)
Polluted groundwater	12–74 µg/L	NR	Canada	Barker (1987)
	7.5–1110 µg/L	4/4	United Kingdom	Tester & Harker (1981)
	30–300 µg/L	NR	Netherlands	Van Duijvenboden & Kooper (1981)
	92–450 µg/L	NR	USA	Stuermer *et al.* (1982)
Industrial effluents	10–100 µg/L	4/25	USA	Perry *et al.* (1979)
	> 100 µg/L	2/25	USA	Perry *et al.* (1979)
	< 3.0 µg /L (median)	101/1368	USA	Staples *et al.* (1985)
	1–2 µg /L	59/1475	USA	Cole *et al.* (1984)
	~35 µg/kg	1/1	USA	Snider & Manning (1982)

NR, not reported

1997a), including spillage of gasoline and other fuels (Tester & Harker, 1981; Sauer & Tyler, 1995), leaking underground storage tanks (Cotruvo, 1985), leaching from landfill sites (Barker, 1987) and disposal of solvents and household products such as paint, cleaning and degreasing solvents, varnishes and pesticides (Agency for Toxic Substances and Disease Registry, 1997a).

The median concentration of ethylbenzene found in sediment samples was 5.0 µg/kg dry weight, with ethylbenzene being detected in 11% of the 350 samples collected between 1980 and 1982 in the United States Environmental Protection Agency's STORET water quality database (Staples *et al*., 1985).

Releases of ethylbenzene in 1997 to land from 1005 industrial facilities in the United States amounted to 24 590 kg, which accounted for about 0.4% of the total

environmental releases according to the Environmental Protection Agency (1999d) Toxics Release Inventory. An additional estimated 253 500 kg of ethylbenzene or 5.9% of total environmental releases were released via underground injection.

(*d*) *Food*

There are few data on concentrations of ethylbenzene in foodstuffs. It has been identified as a trace component in the volatiles from honey, jasmine, papaya, olive oil and cheese flavour and in the neutral component of roast beef flavour isolate (Min *et al*., 1979; Fishbein, 1985). Trace quantities of ethylbenzene have been detected in split peas (13 μg/kg), lentils (5 μg/kg) and beans (mean, 5 μg /kg; maximum 11 μg /kg (Lovegren *et al*., 1979). Concentrations of ethylbenzene in orange peel (23.6 ng/g dry weight) and in parsley leaves (0.257 μg/g dry weight) have been reported (Górna-Binjul *et al*., 1996).

Mean concentrations of ethylbenzene in freshwater fish samples in the Canadian Arctic in 1985 and 1986 ranged from 2.45 to 49.6 μg/kg in muscle tissue and from 1.81 to 46.3 μg/kg in liver tissue from turbot; in white fish muscle tissue samples, levels ranged from 7.46 to 104 μg/kg (Lockart *et al*., 1992). Ethylbenzene was detected in 43 of 138 fish samples at 16 of 42 sites in Japan in 1986, with concentrations ranging from 1.0 to 9.8 μg/kg wet weight (detection limit, 1 μg/kg wet weight) (WHO, 1996a).

Migration of ethylbenzene from polystyrene into various foods has been reported. The following ethylbenzene levels were found: sour milk beverages, < 2.5–6 μg/L; noodle soup, 15–21 μg/L; noodle curry, 89–153 μg/kg and wantan soup 9–28 μg/L (ECETOC, 1986). Migration of ethylbenzene from polystyrene containers into dairy products resulted in concentrations of ethylbenzene ranging from 2 to 4 μg/kg in yoghurt and 4 μg/kg for chocolate dessert (Ehret-Henry *et al*., 1994).

Ethylbenzene has been shown to migrate at levels of < 6–34 μg/kg from samples of thermoset polyester (containing up to 25 mg/kg ethylbenzene) into pork during cooking (Gramshaw & Vandenburg, 1995).

Concentrations of ethylbenzene were determined in olives and olive oils exposed to gasoline vapours from gasoline-powered engines, either on the tree or during storage. The concentrations of ethylbenzene in the air of storage sites ranged from 7 to 88 μg/m^3. Three days after storage, levels in oil in olives ranged from 15 to 55 μg/kg and in pressed oil from 6 to 60 μg/kg (Biedermann *et al*., 1996).

(*e*) *Consumer products*

In a survey of volatile organic chemicals in 1159 household items including household cleaners and polishes, paint-related products, fabric and leather treatments, cleaners for electronic equipment, oils, greases and lubricants, adhesive-related products, automotive products and miscellaneous products, ethylbenzene was identified in 157 of 658 (24%) of the products tested. The highest mean concentrations and percentage of products in each category in which ethylbenzene was found were as follows: 7.2% w/w in 7.5% of automotive products, 2.4% w/w in 47.8% of paint-related

products and 1.0% w/w in 11.8% of fabric and leather treatment products (Sack *et al*., 1992; Agency for Toxic Substances and Disease Registry, 1997a).

(*f*) *Tobacco smoke*

In a 1962 study, levels of ethylbenzene in mainstream cigarette smoke from cigarettes of Argentina, Russia and the United Kingdom were found to range from 7 to 20 μg/g cigarette weight (typical cigarette weight is 1 g) (Johnstone *et al*., 1962). In the United States, the amount of ethylbenzene in mainstream smoke from a single cigarette containing 16 mg of tar and nicotine was 8 μg (Wallace *et al*., 1987c).

Hodgson *et al*. (1996) determined the concentration of several volatile organic chemicals related to the environmental tobacco smoke in smoking environments. The average emission factor for ethylbenzene for six brands of cigarettes was 101 μg per cigarette (range, 83–142 μg per cigarette). The average concentration of ethylbenzene in five smoking areas ranged from 1.3 to 8.7 μg/m^3.

Conkle *et al*. (1975) measured trace quantities of ethylbenzene in the expired air of eight male subjects ranging in age from 23 to 47 years. Ethylbenzene was detected in five of the eight subjects, (range, 0.78–14 μg/h), with smokers having the highest levels.

(*g*) *Human tissues*

Ethylbenzene was measured in the blood of 631 non-occupationally exposed people in the United States (as a subset of the Third National Health and Nutrition Examination Survey, at mean and median levels of 0.11 and 0.06 μg/L, respectively (Ashley *et al*., 1994). In an earlier study, the authors reported a mean ethylbenzene concentration of 0.12 μg/L in 13 blood samples (Ashley *et al*., 1992).

In venous blood samples collected from 13 non-smokers and 14 cigarette smokers, the concentrations of ethylbenzene tended to be higher in smokers. The median and mean levels of ethylbenzene in non-smokers were 431 and 651 ng/L, respectively (range, 175–2284 ng/L), compared with smokers with median and mean levels of 535 and 837 ng/L, respectively (range, 378–2697 ng/L) (Hajimiragha *et al*., 1989).

Ethylbenzene was detected (no concentrations reported) in human milk samples from eight of 17 lactating women in three urban areas in the United States (Pellizzari *et al*., 1982).

Ethylbenzene was measured in 96% of the 46 composite samples analysed for volatile organic chemicals in the 1982 National Adipose Tissue Survey conducted by the Environmental Protection Agency in the United States. Although a wet tissue concentration range of not detected (detection limit, 2 ng/g) to 280 ng/g was cited, the average concentration was not reported (Agency for Toxic Substances and Disease Registry, 1997a).

1.5 Regulations and guidelines

Occupational exposure limits for ethylbenzene are given in Table 7.

The determination of mandelic acid in urine is recommended as a biological exposure test for ethylbenzene. The Biological Exposure Index (BEI) Committee of the American Conference of Governmental Industrial Hygienists recommends a mandelic acid concentration in urine of 1.5 g/g creatinine [about 10 mmol/L] as a BEI for ethylbenzene exposure. BEIs represent the levels of the determinants that are most likely to be observed in biological samples collected from healthy workers exposed by inhalation to air concentrations at the level of the TLV. Urine specimens must be collected during

Table 7. Occupational exposure limits and guidelines for ethylbenzene

Country	Year	Concentration (mg/m^3)	Interpretation[a]
Australia	1993	435	TWA
		545	STEL
Belgium	1993	434	TWA
		543	STEL
Czech Republic	1993	200	TWA
		1000	STEL
Denmark	1993	217	TWA
Finland	1998	220	TWA
France	1993	435	TWA
Germany	1999	440 (sk)	TWA
Hungary	1993	100 (sk)	TWA
		200	STEL
Ireland	1997	435	TWA
		545	STEL
Japan	1993	435	TWA
Netherlands	1997	215 (sk)	TWA
Philippines	1993	435	TWA
Poland	1998	100 (sk)	TWA
		350	STEL
Russian Federation	1993	435	TWA
		50	STEL
Slovakia	1993	200	TWA
		1000	STEL
Sweden	1993	200	TWA
		450	STEL
Switzerland	1993	435	TWA
		1275	STEL
Turkey	1993	435	TWA
United Kingdom	1993	435	TWA

Table 7 (contd)

Country	Year	Concentration (mg/m^3)	Interpretation[a]
United States			
ACGIH (TLV)[b]	1999	435	TWA
NIOSH (REL)	1999	545	STEL
OSHA (PEL)	1999	435	TWA
		545	STEL
		435	TWA

From Finnish Ministry of Social Affairs and Health (1998); American Conference of Governmental Industrial Hygienists (ACGIH) (1999); Deutsche Forschungsgemeinschaft (1999)

[a] Abbreviations: TWA, time-weighted average; STEL, short-term exposure limit; sk, skin designation; PEL, permissible exposure limit; REL, recommended exposure limit; TLV, threshold limit value

[b] The following countries follow the exposure limits suggested by the ACGIH: Bulgaria, Colombia, Jordan, Republic of Korea, New Zealand, Singapore and Viet Nam

the last four hours of the last shift of the working week. The BEI Committee recommends the determination of ethylbenzene in end-exhaled air collected before the shift as a confirmatory test for ethylbenzene exposure. The concentration in end-exhaled air collected 16 hours after the fourth exposure of the working week should be about 2 ppm [8.7 mg/m^3] if the exposure at the TLV of 100 ppm [434 mg/m^3] is maintained (American Conference of Governmental Industrial Hygienists, 1999).

2. Studies of Cancer in Humans

Some 200 ethylbenzene-production workers in Czechoslovakia [exact number not stated] between 1964 and 1985 were monitored twice a year for mandelic acid excretion (Bardodej & Círek, 1988). The mean age of workers exposed to ethylbenzene was 36.6 years and their mean length of employment was 12.2 years. The authors stated that cancer incidence among chemical workers in the industrial complex (of comparable age and length of employment) not engaged in ethylbenzene production was about three times the national average, whereas in the group of ethylbenzene production workers, no tumours had been reported over the 10 previous years. [The Working Group noted that no precise figures were given to substantiate these assertions; in addition, co-exposure to benzene was present, and the age of the workers and length of follow-up were not sufficient for a proper evaluation of cancer risk in relation to exposure to ethylbenzene.]

A mortality study was conducted among 560 styrene production and polymerization workers employed for at least five years on 1 May 1960 at a plant in the United States (Nicholson *et al*., 1978). Exposures other than ethylbenzene included benzene, toluene and styrene. Follow-up covered the period from 1 May 1960 (or the 10th anniversary of employment in the plant) through 31 December 1975. Eighty-three deaths were observed versus 106.4 expected, including 17 cancer deaths (versus 21.0 expected). Among these, one death from leukaemia (0.79 expected) and one death from lymphoma (1.25 expected) occurred. A further review of additional death certificates from recent years revealed additional cases of leukaemia and lymphoma. [The Working Group noted that these figures were not formally included in the follow-up period for which the analysis was performed and should be interpreted as a case report. Ethylbenzene was a raw material used in the production of styrene, and it is reasonable to assume its presence through the remainder of the process, albeit at low levels, because it has been detected in polystyrene food packaging (Section 1.4.3(*d*)). This study does not permit the evaluation of cancer risk among ethylbenzene-exposed workers, since mortality data are not presented separately for this group].

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

Rat: Groups of 50 male and 50 female Sprague-Dawley rats, seven weeks of age, were administered 0 or 800 mg/kg bw ethylbenzene (purity, 99.57%) by stomach tube in 1 mL extra-virgin olive oil solution daily on four days per week for 104 weeks. The experiment was terminated at 123 weeks. In a second experiment, groups of 40 male and 40 female Sprague-Dawley rats received 500 mg/kg bw ethylbenzene per day according to the same regimen, while 50 male and 50 female Sprague-Dawley rats comprised control groups receiving olive oil only. In this second experiment, the rats were permitted to live out their life span, up to 145 weeks. Survival was affected by treatment in both experiments, being recorded as an ‘intermediate reduction’ in animal numbers in both males and females. At 800 mg/kg, there was an increase in the incidence of tumours of the nasal cavity [type unspecified] (2% incidence in females versus 0% in controls) and neuroesthesioepitheliomas (6% in males versus 0% in controls) and a borderline increase in oral cavity cancer (6% in females versus 2% in controls) (Maltoni *et al.*, 1985, 1997). [The Working Group noted the lack of details on numbers of animals with specific tumours, adjustments for survival, historical control data, and statistical analysis.]

3.2 Inhalation exposure

3.2.1 *Mouse*

Groups of 50 male and 50 female B6C3F$_1$ mice, six weeks of age, were exposed to ethylbenzene (purity, > 99%; impurities included 62 ± 3 ppm cumene) by inhalation in whole-body exposure chambers at concentrations of 0, 75, 250 or 750 ppm [0, 325, 1083 or 3250 mg/m^3] for 6 h per day on five days per week for 103 weeks. The dose levels were selected on the basis of results from 13-week studies. Survival and body weights of the exposed and control groups were similar. There were statistically significant increases in the incidences of alveolar/bronchiolar adenomas in high-dose (750 ppm) males and of hepatocellular adenomas + carcinomas in high-dose (750 ppm) females (see Table 8). These neoplastic lesions were accompanied by statistically significant increases in the incidence of alveolar epithelial metaplasia in the lungs of high-dose males and of eosinophilic foci of cellular alteration in the livers of high-dose females (Chan *et al*., 1998; National Toxicology Program, 1999). [The Working Group noted that the statistically significant increases related only to adenomas in both liver and lung and that these increased incidences were within the historical control range.]

3.2.2 *Rat*

Groups of 50 male and 50 female Fischer 344/N rats, six weeks of age, were exposed to ethylbenzene (purity, > 99%) by inhalation in whole-body exposure chambers at concentrations of 0, 75, 250 or 750 ppm for 6 h per day on five days per week for 104 weeks. Survival was similar among the female groups (31/50, 31/50, 34/50 and 35/49 at 0, 75, 250 and 750 ppm, respectively) but was significantly decreased in the high-dose males compared with that of control males (15/50, 14/50, 13/50 and 2/50 at 0, 75, 250 and 750 ppm, respectively). The mean body weights of exposed males and females were 5–10% lower than those of the control animals. As shown in Table 9, there was a statistically significant increase in incidence at the high dose (750 ppm) in males of renal tubule adenomas and carcinomas combined after standard (single section) evaluation. After step-sectioning of the kidney, additional adenomas elevated the increase in the incidence of renal tumours in females also to statistical significance. Accompanying the neoplastic lesions in the kidneys was a significant increase in the incidence of focal renal tubule hyperplasia, judged to be a precursor stage of adenoma, in high-dose males after standard (single section) evaluation and at the high dose in both males and females after step-sectioning of the kidney (Chan *et al*., 1998; National Toxicology Program, 1999).

Table 8. Incidence of primary tumours in B6C3F$_1$ mice exposed to ethylbenzene by inhalation

Tumour site	Animals with tumours							
	Males				Females			
	0 ppm	75 ppm	250 ppm	750 ppm	0 ppm	75 ppm	250 ppm	750 ppm
Liver adenomas	0/50	0/50	0/50	0/50	6/50	9/50	12/50	16/50*
Liver carcinomas	0/50	0/50	0/50	0/50	7/50	4/50	3/50	12/50
Lung adenomas	5/50	9/50	10/50	16/50**	4/50	4/50	5/49	8/50
Lung adenocarcinomas	2/50	1/50	5/50	3/50	0/50	2/50	0/49	0/50

From National Toxicology Program (1999)
* $p < 0.05$, logistic regression test
** $p < 0.01$, logistic regression test

Table 9. Incidence of primary tumours in Fischer 344 rats exposed to ethylbenzene by inhalation

Tumour site	Animals with tumours							
	Males				Females			
	0 ppm	75 ppm	250 ppm	750 ppm	0 ppm	75 ppm	250 ppm	750 ppm
Single sections								
Kidney adenomas	0/50	3/50	2/50	4/50*	0/50	0/50	0/50	1/50
Kidney carcinomas	0/50	0/50	1/50	3/50				
Kidney adenomas and carcinomas combined	0/50	3/50	3/50	7/50**				
Step sections								
Kidney adenomas and carcinomas	3/50	2/50	8/50	18/50**	0/50	0/50	1/50	7/50*
Combined								
Kidney adenomas and carcinomas	3/50	5/50	8/50	21/50**	0/50	0/50	1/50	8/50**

From National Toxicology Program (1999)
* $p < 0.05$, logistic regression test
** $p < 0.01$, logistic regression test

3.3 Carcinogenicity of metabolites

1-Phenylethanol [α-methylbenzyl alcohol]

3.3.1 *Oral administration*

Mouse: Groups of 50 male and 50 female B6C3F_1 mice, nine to 10 weeks of age, were administered 1-phenylethanol (food grade) by gavage at doses of 0, 375 and 750 mg/kg bw in corn oil five times per week for 103 weeks. Body weight was decreased in both males (6–18%) and females (8–16%) at the high dose. No significant difference in survival was observed in either sex. There was no increase in the incidence of tumours in either sex (National Toxicology Program, 1990).

Rat: Groups of 50 male and 50 female Fischer 344 rats, seven to eight weeks of age, were administered 1-phenylethanol (food grade) by gavage at doses of 0, 375 and 750 mg/kg bw in corn oil on five days per week for 103 weeks. Survival in males in both the low-dose (after week 86) and high-dose groups was significantly lower than that of the controls. The survival of the high-dose female rats was significantly lower than that of the vehicle controls after week 40. Renal tubule adenomas were observed in males: 0/50 control, 1/50 mid-dose and 5/50 high-dose groups ($p < 0.05$, Fisher's exact test; $p = 0.011$, Cochran-Armitage trend test). One carcinoma was observed in low-dose males. Additional step-sections on kidneys resulted in a further increase in the number of adenomas: 1/50 control, 7/50 low-dose and 10/50 high-dose animals. No significant tumour response was observed at any other site in either males or females (National Toxicology Program, 1990) [The Working Group noted the poor survival both in males and females due to accidental deaths related to gavage.]

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

Ethylbenzene is a significant component of technical xylenes (see Section 1.4.2). The toxicology of these products has been reviewed (WHO, 1997). Xylenes themselves have been evaluted by IARC as not classifiable as to their carcinogenicity to humans (Group 3) (IARC, 1999).

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Ethylbenzene is rapidly absorbed after inhalation exposure of humans, as shown by the excretion of ethylbenzene metabolites and tissue retention of ethylbenzene in exposed workers and volunteers (Engström & Bjurström, 1978; Engström, 1984a; Drummond *et al.*, 1989). There occurs rapid absorption upon dermal application of

ethylbenzene as the neat liquid (absorption rate, 22–33 mg/cm^2/h) or as an aqueous solution (rate, 118–215 μg/cm^2/h) (Dutkiewicz & Tyras, 1967).

The metabolism of ethylbenzene in humans occurs along one major pathway which is oxidation at the α-carbon, yielding 1-phenylethanol (also called α-methylbenzyl alcohol) as the primary product. A metabolic scheme is presented in Figure 1. The α-carbon of ethylbenzene is a prochiral centre and hydroxylation thus yields a chiral product. The issue of stereoselectivity has been addressed in animal studies (see Section 4.1.2).

While some 1-phenylethanol is excreted in the urine as its glucuronic acid conjugate, its major fate is oxidation to acetophenone, which involves loss of the chiral centre. The majority of the acetophenone undergoes oxidation at the ω-carbon, giving the α-keto alcohol, ω-hydroxyacetophenone. In addition, small amounts of the ring-hydroxylation products *para*- and *meta*-hydroxyacetophenone are excreted in the urine. The further metabolism of ω-hydroxyacetophenone is both complex and obscure. This compound undergoes carbonyl reduction giving the chiral diol, 1-phenyl-1,2-ethanediol (also referred to as phenylethyleneglycol), while the primary alcohol may be oxidized to an aldehyde, giving phenylglyoxal. Taken together, these three compounds, ω-hydroxyacetophenone, phenylglyoxal and 1-phenyl-1,2-ethanediol are the precursors of three metabolites, namely mandelic acid, phenylglyoxylic acid and benzoic acid (excreted as hippuric acid, its glycine conjugate), but the exact interrelationships are uncertain (Engström, 1984a; Korn *et al.*, 1992).

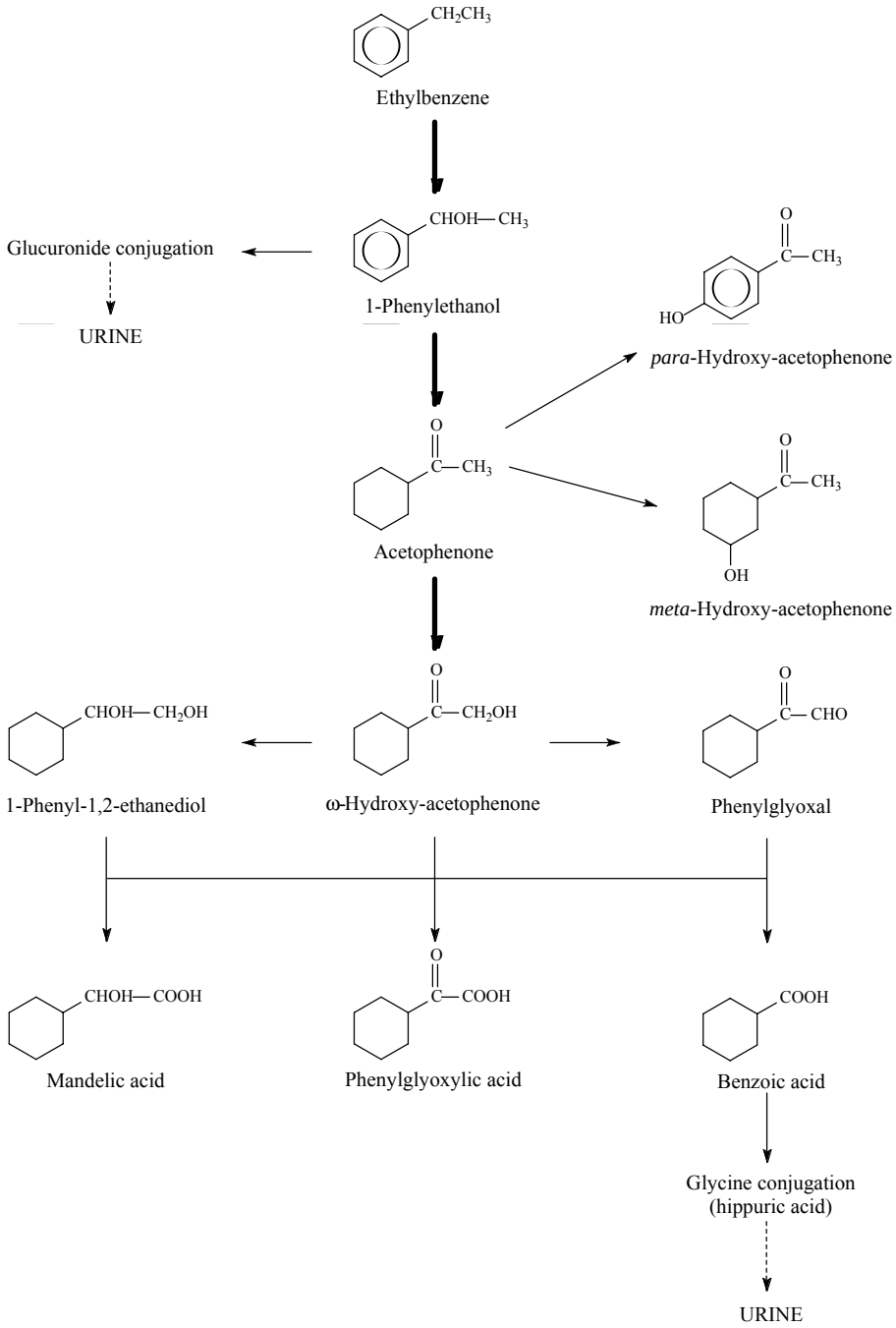
It is interesting to note that the mandelic acid excreted in human urine after exposure to ethylbenzene is predominantly the *R*-enantiomer, in contrast to the 1.2:1 mixture of *R*- and *S*-mandelic acid excreted after styrene exposure. Styrene and ethylbenzene share many common metabolic pathways, but there are evident differences in their stereoselectivity between the two compounds and this provides a means for selective monitoring of exposure to these solvents (Drummond *et al*., 1989; Korn *et al.,* 1992).

4.1.2 *Experimental systems*

When rats were kept for 6 h in an atmosphere containing [^{14}C]ethylbenzene, radioactivity was found in organs and tissues, including intestine, kidney, liver and adipose tissue for up to 42 h afterwards (Chin *et al*., 1980). Blood concentrations of ethylbenzene in rats after a 2-h inhalation were proportional to its concentration in the atmosphere (Freundt *et al*., 1989). After exposure to an atmosphere containing 600 ppm [2.60 g/m^3] ethylbenzene for 6 h, peak blood levels of ethylbenzene occurred at the end of the exposure, falling rapidly thereafter. Ethylbenzene was detected in brain, liver, kidney and adipose tissue; the time courses of brain, liver and kidney concentrations were broadly similar to those in the blood, but there was considerable retention in adipose tissue (Elovaara *et al.,* 1990).

Ethylbenzene was well absorbed through the skin of HRS/J hairless mice, the absorption rate being 37 ± 31 μg/cm^2/min (Susten *et al*., 1990).

Figure 1. Metabolism of ethylbenzene



From Engström (1984b)

The pattern of urinary metabolites in animals is qualitatively similar to that described for humans (see Section 4.1.1). In the rat, the principal pathway is α-hydroxylation to 1-phenylethanol, ultimately leading to excretion of *R*-mandelic acid and phenylglyoxylic acid as major metabolites. Another pathway is oxidation at the ω-carbon of the side-chain giving rise to 2-phenylethanol, which is further oxidized to phenylacetic acid. Minor metabolites found in rat urine include 1-phenylethanol, ω-hydroxyacetophenone and benzoic and phenylacetic acids, together with their glycine conjugates, hippuric and phenaceturic acids (Engström, 1984a,b; Drummond *et al.*, 1989).

After exposure to atmospheres containing 300 and 600 ppm [1.30 and 2.60 g/m^3] ethylbenzene for 6 h, Wistar rats excreted 83% and 59% of the estimated dose as ethylbenzene metabolites in the urine in 48 h, respectively. The principal metabolites were 1-phenylethanol, ω-hydroxyacetophenone and phenylacetic, mandelic, phenylglyoxylic and benzoic acids, accompanied by smaller amounts of 1-phenyl-1,2-ethanediol, phenylglyoxal, acetophenone and *para*-hydroxyacetophenone (Engström, 1984b).

When Wistar rats were exposed to 50, 300 or 600 ppm [0.22, 1.30 and 2.60 g/m^3] ethylbenzene intermittently for up to 16 weeks, the urinary recovery of metabolites increased with dose but not linearly. The metabolic pattern of ethylbenzene was affected by exposure level but not by the duration of administration. The amounts of 1-phenylethanol and ω-hydroxyacetophenone increased with increasing exposure, but those of phenylglyoxylic acid and hippuric acid decreased (Engström *et al.*, 1985).

The stereochemical aspects of the fates of 1-phenyl-1,2-ethanediol and mandelic acid in rats have been examined by Drummond *et al*. (1990). The proportions of a dose of 1-phenyl-1,2-ethanediol converted to phenylglyoxylic and mandelic acids depend upon its stereochemistry. The *R*-diol is preferentially converted to *R*-mandelic acid (30% of the dose in 48 h) with 15% of the dose as phenylglyoxylic acid. In contrast, after administration of the *S*-diol, the major product is phenylglyoxylic acid (46% of the dose) with 16% as mandelic acid (*R*/*S* 80:20).

S-Mandelic acid undergoes a chiral inversion, possibly by reversible oxidation to phenylglyoxylic acid. When *S*-mandelic acid was administered, some 80% of the dose was recovered as phenylglyoxylic acid in 48 h with 16% as mandelic acid (*R*/*S* 80:20). However, when racemic mandelic acid was given, 46% was excreted as phenylglyoxylic acid and 47% as *R*-mandelic acid.

4.2 Toxic effects

4.2.1 *Humans*

In a long-term study (~20 years) of about 200 ethylbenzene production workers exposed to an undefined concentration of this compound, none of the workers showed changes in haematological parameters or serum enzyme levels as a measure of liver function (Bardodej & Círek, 1988).

4.2.2 *Experimental systems*

The acute oral LD_{50} (lethal dose for 50% of the animals) of ethylbenzene has been estimated to be 3.5–5.5 g/kg body weight (bw) in rats (Wolf *et al*., 1956; Smyth *et al*., 1962). Smyth *et al*. (1962) reported the LC_{50} (lethal concentration in air for 50% of the animals) in female rats to be 4000 ppm [17.3 g/m^3] for a 4-h exposure.

(*a*) *In-vivo studies*

Male New Zealand White rabbits (2200 g) were exposed to 750 ppm [3.25 g/m^3] ethylbenzene for 12 h per day for seven days. Twelve or 24 h following the final day of exposure, the rabbits were killed and their brains dissected. Ethylbenzene depleted both striatal and tubero-infundibular dopamine levels (Mutti *et al*., 1988). In male Sprague-Dawley rats exposed to 2000 ppm [8.70 g/m^3] ethylbenzene for 6 h per day for three consecutive days and killed 16–18 h following the last exposure, ethylbenzene increased dopamine and noradrenaline levels and turnover in the hypothalamus and the median eminence. Ethylbenzene exposure also reduced the secretion of prolactin and increased dopamine turnover within the dopamine–cholecystokinin-8 immunoreactive nerve terminals of the nucleus accumbens (Andersson *et al*., 1981).

In a series of studies described by Wolf *et al.* (1956), female rats and male and female rats (strain not indicated) were administered ethylbenzene by gavage (13.6, 136, 408 or 680 mg/kg bw per day) or inhalation (400, 600 or 1250 ppm [1.74, 2.60 or 5.42 g/m^3] for 7–8 h per day respectively, for 6–7 months. Rabbits (1 or 2 per concentration) and guinea-pigs (5–10 per concentration) were exposed to 400, 600 or 1250 ppm ethylbenzene and rhesus monkeys (1 or 2 per concentration) to 400 and 600 ppm ethylbenzene for 6–7 months. Toxicity was evaluated by the following criteria: appearance and behaviour, haematological findings, blood urea nitrogen, organ and body weights, histopathological findings and bone marrow counts [no details were provided on these specific measurements of toxicity and the results were reported as indicators of an effect: slight (+) or moderate (++)]. No haematological changes were induced by ethylbenzene in any species. In rats, ethylbenzene caused an increase in kidney and liver weight with slight cloudy swelling in the liver and of the renal tubular epithelium, following both gavage and inhalation exposure. Liver weights were slightly increased in guinea pigs and monkeys exposed to 600 ppm ethylbenzene, with slight histopathological effects noted in the testes of rabbits and monkeys.

Male and female Fischer 344 rats (six weeks of age) and $B6C3F_1$ mice (seven to nine weeks of age) and New Zealand White rabbits were exposed by inhalation to 0, 99, 382 or 782 ppm [0, 0.43, 1.66 or 3.40 g/m^3] ethylbenzene and 0, 382, 782 or 1610 ppm [0, 1.66, 3.40 or 6.99 g/m^3] ethylbenzene, respectively, for 6 h per day on five days per week for four weeks. At 782 ppm, both rats and mice exhibited an increase in mean liver weight and liver-to-body weight ratio. There were no alterations in clinical chemistry, urinalysis or gross or microscopic changes in any of the species tested that were attributable to exposure to ethylbenzene. The authors noted that the

absence of abnormalities in liver histopathology and clinical chemistry indicates that these increases were due to adaptive induction of hepatic mixed-function oxidase rather than toxicity (Crag *et al*., 1989). This has been supported by the investigations described below, demonstrating that ethylbenzene induces rat cytochrome P450 (Toftgård & Nilsen, 1982; Elovaara *et al*., 1985).

Liver and kidney microsomes were prepared from male Sprague-Dawley rats (200–300 g) exposed by inhalation to 2000 ppm [8.70 g/m^3] ethylbenzene for 6 h per day for three days. Exposure to ethylbenzene caused an increase in hepatic cytochrome P450 concentration and in the hydroxylation of *n*-hexane and benzo[*a*]pyrene. NADPH-cytochrome c reductase and 7-ethoxyresorufin *O*-deethylation activity was increased in both liver and kidney microsomes (Toftgård & Nilsen, 1982). Elovaara *et al*. (1985) demonstrated that exposure of male Wistar rats (~ 342 g) by inhalation to 0, 50, 300 or 600 ppm [0, 0.22, 1.30 or 2.60 g/m^3] ethylbenzene for two, five, nine or 16 weeks caused a dose-related increase in hepatic microsomal protein content along with increased NADPH-cytochrome c reductase, 7-ethoxycoumarin-*O*-deethylase and UDP-glucuronosyl-transferase activities. The latter two activities increased in a concentration-dependent manner in kidney microsomes. Ethylbenzene did not deplete liver glutathione, but slightly increased kidney glutathione levels. At 600 ppm ethylbenzene, there was no increase in serum alanine aminotransferase activity and liver cells showed slight proliferation of smooth endoplasmic reticulum but no necrosis.

In one study, Yuan *et al*. (1997a) demonstrated that daily intraperitoneal injections for three days of 10 mmol/kg bw ethylbenzene to male Holtzman rats (seven weeks of age) modulated the levels of various cytochrome P450 isozymes, each of which exhibited different temporal characteristics. Yuan *et al*. (1997b) also evaluated the induction pattern of various cytochrome P450s with time following a single intraperitoneal injection of 10 mmol/kg bw ethylbenzene to male Holtzman rats. The forms found to be induced were CYP1A1, CYP2B1/2 and CYP2E1.

A 13-week inhalation study of ethylbenzene was conducted by exposing male and female Fischer 344/N rats and B6C3F$_1$ mice to 0, 100, 250, 500, 750 or 1000 ppm [0, 0.43, 1.1, 2.2, 3.3 or 4.3 g/m^3] ethylbenzene for 6 h per day for five days per week. No mortality was observed and the mean body weight gains of the exposed rats and mice did not differ from those of the respective controls. Signs of toxicity included increased absolute and relative liver, lung and kidney weights in exposed rats and an increase in liver weights in exposed mice. There was no evidence of histological changes in these studies (National Toxicology Program, 1992).

Chronic inhalation exposure to 0, 75, 250 or 750 ppm [0, 0.32, 1.1 or 3.3 g/m^3] ethylbenzene for 6 h per day on five days per week for 104 weeks caused an increased incidence of renal tubule hyperplasia in male Fischer 344/N rats and increased severity of spontaneous, age-related chronic progressive nephropathy in males and females. Male B6C3F$_1$ mice developed an increased incidence of alveolar epithelial metaplasia, syncytial alterations of hepatocytes, hepatocellular hypertrophy, hepatocyte necrosis and thyroid gland follicular-cell hyperplasia. Exposure to ethylbenzene also caused an

increase incidence of eosinophilic foci of the liver, pituitary gland hyperplasia and thyroid gland follicular-cell hyperplasia in female mice (National Toxicology Program, 1999).

(*b*) *In-vitro studies*

Neural membranes isolated from primary astrocyte cultures established from new-born Sprague-Dawley rat cerebella were exposed to 3, 6 or 9 mmol/L ethylbenzene for 1 h. ATPase activity decreased linearly with log concentration of ethylbenzene (Naskali *et al*., 1994). In the same astrocyte cultures, ethylbenzene (3, 6 or 9 mmol/L; 1-h exposure) decreased in a dose-dependent manner the activity of important membrane integral proteins such as Na^+/K^+-ATPase and Mg^{2+}-ATPase (Vaalavirta & Tahti, 1995).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

(*a*) *Developmental toxicity studies*

In an inhalation study, rabbits were exposed to 100 or 1000 ppm [0.43 or 4.3 g/m^3] ethylbenzene for 6–7 h per day on gestational days 1–24 and sacrificed on the day before term. A significantly reduced number of live fetuses per litter was found at both exposure levels, although the number of implantations per litter and the number of dead or resorbed fetuses per litter did not differ significantly from those of the controls. In rats (strain not specified) similarly exposed during gestational days 1–19, there was a significant increase in the incidence of extra ribs at both doses (Hardin *et al*., 1981).

An increased rate of anomalies (of uropoietic apparatus + extra ribs) and weight retardation were seen in CFY rats exposed to 2400 mg/m^3 ethylbenzene for 24 h per day during days 6–15 of gestation. Similar exposure to 600 or 1200 mg/m^3 ethylbenzene induced skeletal retardation in the fetuses. Maternal effects were moderate and dose-dependent (not specified further). In CFLP mice exposed to 500 mg/m^3 ethylbenzene intermittently 4 h three times per day on gestational days 6–15, increased rates of anomalies (the same as in rats) were found. In New Zealand White rabbits, inhalation exposure to 1000 mg/m^3 ethylbenzene for 24 h per day on gestational days 7–20 induced abortions (with decreased maternal weight gain). Exposure to 500 mg/m^3 led to lower fetal weight in female offspring (Ungváry & Tátrai, 1985).

(*b*) *Reproductive toxicity studies*

No studies of the effects of ethylbenzene on fertility were available.

No testicular abnormalities were reported in Fischer 344 rats and B6C3F_1 mice exposed to ethylbenzene concentrations of up to 782 ppm [3.40 g/m^3] and in New Zealand White rabbits exposed to ethylbenzene at concentrations of up to 1610 ppm [6.99 g/m^3] for 6 h per day on five days per week for four weeks (Crag *et al.*, 1989). [The Working Group noted the small number of animals (five per sex per group) used].

Sperm or vaginal cytological evaluations of Fischer 344/N rats and B6C3F_1 mice exposed to ethylbenzene concentrations of up to 1000 ppm [4.34 g/m^3] for 6 h per day on five days per week for 13 weeks revealed no changes from normal (National Toxicology Program, 1992).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 10 for references)

Ethylbenzene has been found consistently to be non-mutagenic in bacteria, yeast and insects. It did not cause chromosomal aberrations in mammalian cells. It was inactive in inducing sister chromatid exchanges in Chinese hamster embryo cells, but was very weakly positive in cultured human lymphocytes. It did not induce micronuclei in in-vivo test systems but, *in vitro*, it was positive in Syrian hamster embryo cells. It also caused cell transformation in these cells at the highest concentration tested. At the highest non-lethal concentration, an increase in mutant mouse lymphoma L5178Y cell colonies was induced by ethylbenzene in both the absence and presence of an exogenous metabolic system.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Ethylbenzene is a major industrial chemical produced by alkylation of benzene. The pure chemical is used almost exclusively for styrene production. It is also present at up to 25% in technical grades of mixed xylenes and up to 15% in gasoline.

Occupational exposure to ethylbenzene may occur by inhalation during its production and use. Most occupational exposures are related to technical grades of mixed xylenes used as solvents in various paints and coatings, inks, insecticides and in rubber and plastic production, as well as from the production and handling of gasoline and bitumen. Ethylbenzene from these sources as well as from vehicle emissions is ubiquitous at µg/m^3 levels in ambient air. It is a component of tobacco

Table 10. Genetic and related effects of ethylbenzene

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	318 µg/plate	Florin *et al.* (1980)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	400 µg/plate	Nestmann *et al.* (1980)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	2000 µg/plate	Dean *et al.* (1985)
Salmonella typhimurium TA100, TA1535, TA98, TA97, reverse mutation	–	–	1000 µg/plate	Zeiger *et al.* (1992); National Toxicology Program (1999)
Escherichia coli, WP2 and WP2 *uvrA*, reverse mutation	–	–	2000 µg/plate	Dean *et al.* (1985)
Pseudomonas putida, mutation	–		vapour; ≤ 40 d	Leddy *et al.* (1995)
Saccharomyces cerevisiae, mitotic gene conversion	–	–	NR	Dean *et al.* (1985)
Saccharomyces cerevisiae, mitotic gene conversion and reversion	–	NT	NR	Nestmann & Lee (1983)
Mutation, mouse lymphoma L5178Y cells *in vitro*	+	NT	80	McGregor *et al.* (1988); National Toxicology Program (1999)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	–	100	National Toxicology Program (1999)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	–	125	National Toxicology Program (1999)
Chromosomal aberrations, rat liver epithelial cells *in vitro*	–		NG	Dean *et al.* (1985)
Micronucleus formation, Syrian hamster embryo cells *in vitro*	+		25	Gibson *et al.* (1997)
Cell transformation, Syrian hamster embryo cells *in vitro*	+		200	Kerckaert *et al.* (1996)
Sister chromatid exchange, human lymphocytes *in vitro*	(+)		1060	Norppa & Vainio (1983)

Table 10 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, male mouse bone-marrow erythrocytes *in vivo*	–		650 ip × 2	Mohtashamipur *et al.* (1985)
Micronuclei, male and female B6C3F$_1$ mouse peripheral blood erythrocytes *in vivo*	–		1000 ppm inh, × 6 h/d, 5 d/w, 13 w	National Toxicology Program (1999)

[a] +, positive; (+), weak positive; –, negative
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; ip, intraperitoneal; inh, inhalation; d, day; w, week

smoke and of several household products. These various sources contribute to indoor air levels that are often higher than adjacent outdoor levels. Ethylbenzene is only rarely found in drinking-water but is found at μg/kg levels in a variety of foodstuffs.

5.2 Human carcinogenicity data

Two studies of workers potentially exposed to ethylbenzene in a production plant and a styrene polymerization plant were available. In the first study, no excess of cancer incidence was found but the description of methods was insufficient to allow proper evaluation of this finding. In the second study, no cancer mortality excess was observed during the follow-up of 15 years.

5.3 Animal carcinogenicity data

Ethylbenzene was tested by inhalation exposure in single experiments in mice and rats. In mice, it increased the incidence of lung adenomas in males and of liver adenomas in females. In male rats, it increased the incidence of renal tubule adenomas and carcinomas. An increase in the incidence of renal adenomas was seen in females only after step-sectioning. A study in rats by oral administration could not be evaluated. A metabolite of ethylbenzene, 1-phenylethanol, increased the incidence of renal tubule adenomas in male rats.

5.4 Other relevant data

Ethylbenzene is well absorbed from the skin, lungs and gastrointestinal tract. It is virtually completely metabolized, the primary pathways being hydroxylation of the two carbons of the side-chain, followed by further oxidation to a range of metabolites that are excreted principally in the urine. The fate of ethylbenzene is similar in animals and humans.

Limited data were available to evaluate the toxic effects of ethylbenzene in humans. Liver and kidney weights were increased in rats following exposure to ethylbenzene with no signs of hepatic necrosis. Cytochrome P450 enzymes were induced in both liver and kidney of ethylbenzene-exposed rats. Ethylbenzene caused changes in dopamine levels in brain and prolactin secretion in rats exposed for three to seven days. In rat brain cell cultures, ethylbenzene decreased the activity of several integral membrane enzymes.

No data on reproductive or developmental effects of ethylbenzene in humans were available. In rats and mice, developmental retardation and an increased incidence of variations were reported after inhalation exposure during pregnancy. Reduced numbers of live pups per litter or abortions were reported in rabbits exposed during pregnancy. No changes in sperm motility or estrous cyclicity were found in rats or mice exposed to ethylbenzene for 13 weeks.

Ethylbenzene was non-mutagenic in bacteria, yeast and insects. In mammalian cells, it was inactive in inducing sister chromatid exchanges in Chinese hamster embryo cells but very weakly positive in cultured human lymphocytes. It did not induce micronuclei *in vivo*, although it was positive in Syrian hamster embryo cells *in vitro*. It also caused cell transformation in these cells. Ethylbenzene induced mutations in the mouse lymphoma assay, but only at the highest non-lethal concentration tested.

5.5 Evaluation

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

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

Overall evaluation

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

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ortho-TOLUIDINE

This substance was considered by previous working groups, in June 1977 (IARC, 1978), February 1981 (IARC, 1982) and March 1987 (IARC, 1987a). Since that time, new data have become available, and these have been incorporated in the monograph and taken into consideration in the evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

ortho-Toluidine

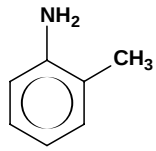
Chem. Abstr. Serv. Reg. No.: 95-53-4
Chem. Abstr. Name: 2-Methylbenzenamine
IUPAC Systematic Name: *ortho*-Toluidine
Synonyms: 1-Amino-2-methylbenzene; 2-amino-1-methylbenzene; 2-aminotoluene; *ortho*-aminotoluene; 2-methyl-1-aminobenzene; 2-methylaniline; *ortho*-methylaniline; *ortho*-methylbenzenamine; 2-methylphenylamine; *ortho*-tolylamine

ortho-Toluidine hydrochloride

Chem. Abstr. Serv. Reg. No.: 636-21-5
Chem. Abstr. Name: 2-Methylbenzenamine hydrochloride
IUPAC Systematic Name: *ortho*-Toluidine hydrochloride
Synonyms: 1-Amino-2-methylbenzene hydrochloride; 2-amino-1-methylbenzene hydrochloride; 2-aminotoluene hydrochloride; *ortho*-aminotoluene hydrochloride; 2-methyl-1-aminobenzene hydrochloride; 2-methylaniline hydrochloride; *ortho*-methylaniline hydrochloride; *ortho*-methylbenzenamine hydrochloride; 2-methylphenylamine hydrochloride; *ortho*-tolylamine hydrochloride

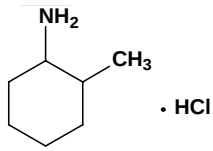
1.1.2 *Structural and molecular formulae and relative molecular mass*

***ortho*-Toluidine**



C_7H_9N Relative molecular mass: 107.16

***ortho*-Toluidine hydrochloride**



$C_7H_9N.HCl$ Relative molecular mass: 143.62

1.1.3 *Chemical and physical properties of the pure substances*

***ortho*-Toluidine**

(*a*) *Description*: Colourless to light yellow liquid becoming reddish brown on exposure to air and light (Verschueren, 1996; Budavari, 1998)
(*b*) *Boiling-point*: 200.3 °C (Lide & Milne, 1996)
(*c*) *Melting-point*: –16.3 °C (Lide & Milne, 1996)
(*d*) *Density*: 0.9984 g/cm^3 at 20 °C (Lide & Milne, 1996)
(*e*) *Spectroscopy data*: Infrared (prism [1543]; grating [8160]), ultraviolet [442], nuclear magnetic resonance (proton [107]; C-13 [225]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980; Lide & Milne, 1996)
(*f*) *Solubility*: Slightly soluble in water (15 mg/L at 25 °C) (Verschueren, 1996); miscible with carbon tetrachloride, diethyl ether and ethanol (Lide & Milne, 1996)
(*g*) *Volatility*: Vapour pressure, 0.013 kPa at 20 °C; relative vapour density (air = 1), 3.72 (Verschueren, 1996)
(*h*) *Stability*: Flash-point (closed cup), 85 °C; oxidizes with prolonged exposure to air and light (Budavari, 1998)
(*i*) *Octanol/water partition coefficient (P)*: log P, 1.32 (Hansch *et al*., 1995)
(*j*) *Conversion factor*[1]: mg/m^3 = 4.38 × ppm

[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

***ortho*-Toluidine hydrochloride**

From IARC (1982), National Toxicology Program (1991), New Jersey Department of Health and Senior Services (1994) and Chemfinder (2000), except where otherwise indicated.

(*a*) *Description*: Colourless to white crystalline solid
(*b*) *Boiling-point*: 242.2 °C
(*c*) *Melting-point*: 215 °C
(*d*) *Spectroscopy data*: Infrared (prism [6330]; grating [29769], ultraviolet [1740], nuclear magnetic resonance (proton [10946]) (Sadtler Research Laboratories, 1980) and mass spectral data (Mass Spectrometry Data Centre, 1974) have been reported.
(*e*) *Solubility*: Very soluble in water (< 1 g/L at 22 °C); soluble in ethanol and dimethyl sulfoxide; insoluble in benzene, and diethyl ether
(*f*) *Volatility:* Vapour pressure, 0.13 kPa at 43.9 °C
(*g*) *Stability*: Sensitive to exposure to light and moisture
(*h*) *Conversion factor*: $mg/m^3 = 5.87 \times ppm$

1.1.4 *Technical products and impurities*

ortho-Toluidine from one source is available with the following specifications: purity, 99.5% min.; *meta*-toluidine, 0.4% max.; *para*-toluidine, 0.1% max; sum of *meta*- and *para*-toluidine, 0.5% max.; and water, 0.1% max. (Bayer Organic Chemicals, 1995). *ortho*-Toluidine hydrochloride is available from another source as 98% pure (Chemfinder, 2000).

1.1.5 *Analysis*

Selected methods for the analysis of *ortho*-toluidine in various matrices are given in Table 1.

ortho-Toluidine has also been determined in biological samples (urine, blood) as the free amine or in derivatized form by gas chromatography (GC) or high-performance liquid chromatography with electrochemical or electron capture detection (El-Bayoumy *et al.*, 1986; Teass *et al.*, 1993; Brown *et al.*, 1995; Riffelmann *et al.*, 1995; Ward *et al.*, 1996). Food and beverage samples have been analysed by GC with flame ionization detection or mass spectrometry (Vitzthum *et al.*, 1975; Neurath *et al.*, 1977).

1.2 Production

Reacting *ortho*-chlorotoluene with sodium in liquid ammonia generates a mixture of 67% *ortho*-toluidine and 33% *meta*-toluidine (Lin & Krishnamurti, 1993). *ortho*-Toluidine can also be produced by reduction of *ortho*-nitrotoluene or obtained mixed with *para*-toluidine by reduction of crude nitrotoluene (Lewis, 1993).

Table 1. Selected methods for the analysis of *ortho*-toluidine

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Adsorb (silica gel); desorb (ethanol)	GC/FID	0.01 mg/sample	Eller (1994) [Method 2002]
Wastewater, municipal and industrial	Add isotope-labelled analogue; extract with dichloromethane; dry over sodium sulfate; concentrate	GC/MS	10 µg/L	Environmental Protection Agency (1999a) [Method 1625]
Solid waste matrices	Concentrate by azeotropic distillation	GC/MS	13 µg/L	Environmental Protection Agency (1996a) [Method 8260B]
Air sampling media, water samples, solid waste matrices, soil samples	Liquid–liquid extraction or water dilution	GC/MS	10 µg/L (aqueous) (EQL)	Environmental Protection Agency (1996b) [Method 8270C]
Ground and surface water	Solvent extraction or direct injection	GC/FID	NR	Environmental Protection Agency (1996c) [Method 8015B]

GC, gas chromatography; FID, flame ionization detector; MS, mass spectrometry; EQL, estimated quantitation limit (the EQL of Method 8270 for determining an individual compound is approximately 660 µg/kg (wet weight) for soil/sediment samples, 1–200 mg/kg for wastes (dependent on matrix and method of preparation), and 10 µg/L for groundwater samples); NR, not reported

ortho-Toluidine was first produced commercially in the United Kingdom in 1880. It has been produced commercially in the United States for over 70 years and commercial production of the hydrochloride salt was first reported in 1956. Production of *ortho*-toluidine in the United States in the late 1970s was reported to range from 500 to 5000 tonnes per year (IARC, 1982); production ranged from 6600 to 12 800 tonnes in the early 1990s (Environmental Protection Agency, 1999b).

Information available in 1999 indicated that *ortho*-toluidine was manufactured by 16 companies in China, 11 companies in India, six companies in the United States, three companies each in Germany and Russia, two companies each in Japan and Poland, and one company each in Brazil, France, Italy, Mexico, Romania and Spain, and that *ortho*-toluidine hydrochloride was manufactured by one company each in Germany and India (Chemical Information Services, 1999).

1.3 Use

The major uses of *ortho*-toluidine and its hydrochloride salt are as intermediates in the manufacture of over 90 dyes and pigments, including acid-fast dyestuffs, optical brighteners, synthetic rubber and rubber chemicals, pharmaceuticals and pesticides (IARC, 1982; Bayer Organic Chemicals, 1995; American Conference of Governmental Industrial Hygienists, 1999).

1.4 Occurrence

1.4.1 *Natural occurrence*

ortho-Toluidine has been detected in tea (Vitzthum *et al.*, 1975) and possibly in some fresh vegetables (Neurath *et al.*, 1977).

1.4.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1999), as many as 30 000 workers in the United States were potentially exposed to *ortho*-toluidine and its hydrochloride salt (see General Remarks). Occupational groups included workers in the chemical industry, laboratory workers, machine operators and cleaners and janitors. Ninety laboratory workers, health-care workers and university teachers were identified as exposed to *ortho*-toluidine or its salts in the Finnish Register of Employees Exposed to Carcinogens in 1997 (Savela *et al.*, 1999). National estimates of workers exposed were not available from other countries.

In the former USSR (Khlebnikova *et al.*, 1970), workers at a plant manufacturing *ortho*-toluidine from *ortho*-nitrotoluene were exposed to concentrations of *ortho*-toluidine in the air (n = 215) generally exceeding the maximum permissible concentrations [of 3 mg/m^3, IARC, 1982] by 2–7 times . The highest airborne exposure levels were observed during distillation and extraction processes (25–28.6 mg/m^3). Concurrently, *ortho*-nitrotoluene levels in the air in 80–90% of the samples exceeded the maximum permissible concentration of 1 mg/m^3; levels up to approximately 5 mg/m^3 were also reported (the highest levels were observed during extraction and heating of the reduction reaction). Dermal deposition of 0.01–0.03 mg *ortho*-toluidine per square decimetre of skin was measured by collecting 1% acetic acid washes from wrists, chest and back of individuals at the end of work-shifts (n = 168). After post-shift showers, dermal levels of *ortho*-toluidine decreased by a factor of 10. Patches of cloth placed on the workers' overalls collected about 0.10 mg *ortho*-toluidine per square decimetre (n = 46).

Measurements in the 1940s in a United States dye production plant indicated that the concentration of *ortho*-toluidine was < 0.5 ppm [2.19 mg/m^3] in the workroom air in the breathing zone and area samples and from < 0.3 ppm to 1.7 (mg/L) ppm in the urine of workers engaged in the production of thioindigo. In addition to inhalatory

exposure, there was concern about exposure from ingestion and skin contact (Ott & Langner, 1983).

Exposure to *ortho*-toluidine was reported to occur in an Italian plant producing fuchsin (magenta) and safranine T-based dyes (Rubino *et al.*, 1982), in a German plant producing 4-chloro-*ortho*-toluidine (Stasik, 1988) and in a plant producing rubber chemicals in the United Kingdom (Sorahan *et al.*, 2000), but no data on exposure levels were provided.

ortho-Toluidine, aniline, hydroquinone and toluene were used to synthesize a rubber antioxidant in a United States chemical plant. Despite low air concentrations (< 1 ppm) [4.38 mg/m^3], elevated levels of *ortho*-toluidine were detected in the urine of exposed workers (mean, 104 μg/L post-shift), suggesting dermal exposure, in measurements carried out in 1990 (Ward *et al*., 1991; Teass *et al*., 1993). A more recent exposure study showed similar levels of *ortho*-toluidine (mean, 99 μg/L post-shift) in the urine of workers in the rubber chemicals department of the plant. Exposed workers also had significantly increased levels of *ortho*-toluidine–haemoglobin adducts in blood (Ward *et al*., 1996) (see Section 2.2 for further details).

1.4.3 *Environmental occurrence*

The production of *ortho*-toluidine and its use as an intermediate in the production of dyes and pigments, rubber chemicals and other products may result in its release to the environment through various waste streams. The primary routes of potential human exposure to *ortho*-toluidine and its hydrochloride salt are inhalation and dermal contact. Consumer exposure may occur from residues present in commercial dyes and on textiles and via smoking (IARC, 1982; Department of Health and Human Services, 1982; Environmental Protection Agency, 1984, 1997; Department of Health and Human Services, 1999).

(*a*) *Air*

According to the Toxics Release Inventory (Environmental Protection Agency, 1997), air emissions of *ortho*-toluidine from 23 industrial facilities were approximately 5260 kg in 1995 in the United States.

(*b*) *Water*

Surface water discharges of *ortho*-toluidine from 23 industrial facilities in the United States in 1995 amounted to 116 kg, as reported in the Toxics Release Inventory (Environmental Protection Agency, 1997), reduced from a value of 242 kg in 1994 (Environmental Protection Agency, 1996d).

ortho-Toluidine has been reported in surface water samples taken from three rivers in Germany at levels of 0.3–1 μg/L (Neurath *et al*., 1977).

ortho-Toluidine has been identified in one secondary effluent from seven industrial and publicly owned treatment works (Ellis *et al*., 1982) and in wastewaters from

synthetic fuel production (Leenheer *et al*., 1982; Stuermer *et al*., 1982). It has also been detected in effluents from refineries and chemical production facilities, in process water, river water, groundwater and seawater in the United States (Shackelford & Keith, 1976; Environmental Protection Agency, 1984; Department of Health and Human Services, 1999).

(*c*) *Soil*

Soil discharges of *ortho*-toluidine from 23 industrial facilities in 1995 in the United States amounted to only 5.5 kilogrammes, as reported in the Toxics Release Inventory (Environmental Protection Agency, 1997). Additionally, an estimated 10 000 kg of *ortho*-toluidine were released via underground injection.

(*d*) *Food and beverages*

Unspecified isomers of toluidine were found in samples of kale and celery (1.1 mg/kg) and carrots (7.2 mg/kg) (Neurath *et al*., 1977). *ortho*-Toluidine has been identified in the volatile aroma components of black tea (Vitzthum *et al*., 1975).

(*e*) *Tobacco smoke*

ortho-Toluidine has been reported to be present in mainstream cigarette smoke at 32–162 ng per cigarette and at 3 μg per cigarette in sidestream smoke (IARC, 1986).

ortho-Toluidine was identified in the urine of both smokers and nonsmokers at levels of 6.3 ± 3.7 μg/24 h in all 10 subjects tested and 4.1 ± 3.2 μg/24 h in all nine subjects tested, respectively, suggesting that sources other than cigarette smoke contribute significantly to *ortho*-toluidine exposures (El-Bayoumy *et al*., 1986). In a biological monitoring study of occupationally exposed workers, significantly higher concentrations of *ortho*-toluidine were also found in the urine of smokers (1.7 ± 1.6 μg/L) who were in the control (non-exposed) study group, in comparison with nonsmokers (0.0 ± 0.0 μg/L) in control groups (Riffelmann *et al*., 1995).

1.5 Regulations and guidelines

Occupational exposure limits and guidelines for *ortho*-toluidine are presented in Table 2.

Table 2. Occupational exposure limits and guidelines for *ortho*-toluidine[a]

Country	Year	Concentration (mg/ m^3)	Interpretation[b]
Australia	1993	9 (sk, ca)	TWA
Belgium	1993	9 (sk, ca)	TWA
Canada	1994	9 (sk, susp.h.ca)	TWA
Czech Republic	1993	5	TWA
		20	STEL
Denmark	1993	9 (sk, ca)	TWA
Finland	1998	22 (sk)	TWA
		44	Ceiling
France	1993	9 (ca)	TWA
Germany	1999	(2, sk), 0.5	TRK
Ireland	1997	9 (sk)	TWA
Japan	1998	4.4 (sk, ca)	TWA
Philippines	1993	22 (sk)	TWA
Poland	1998	3 (sk)	TWA
		9	STEL
Russian Federation	1993	0.5 (sk, ca)	TWA
		1	STEL
Sweden	1993	none (ca)	
Switzerland	1993	9 (sk, ca)	TWA
Turkey	1993	22 (sk)	TWA
United Kingdom	1993	0.9 (sk)	TWA
United States			
ACGIH[c]	1999	9 (sk, A3)	TWA
NIOSH	1999	lfc (sk, ca)	TWA
OSHA	1999	22 (sk)	TWA

[a] From Finnish Ministry of Social Affairs and Health (1998); American Conference of Governmental Industrial Hygienists (ACGIH) (1999); Deutsche Forschungsgemeinschaft (1999); United Nations Environment Programme (1999); Occupational Safety and Health Administration (OSHA) (1999)

[b] TWA, time-weighted average; STEL, short-term exposure limit; 2 (Germany), Substances that are considered to be carcinogenic for man because sufficient data from long-term animal studies or limited evidence from animal studies substantiated by evidence from epidemiological studies indicate that they can make a significant contribution to cancer risk. Limited data from animal studies can be supported by evidence that the substance causes cancer by a mode of action that is relevant to man and by results of *in vitro* tests and short-term animal studies; TRK (Germany), technical exposure limit; A3 (ACGIH), confirmed animal carcinogen with unknown relevance to humans; ca, carcinogen; lfc, lowest feasible concentration; sk, skin notation; susp.h.ca, suspected human carcinogen

[c] These countries follow the recommendations of the ACGIH threshold limit values: Bulgaria, Colombia, Jordan, Republic of Korea, New Zealand, Singapore and Viet Nam.

2. Studies of Cancer in Humans

2.1 Case reports

One case report pertaining to *ortho*-toluidine has been published since the previous review (IARC, 1982). Conso and Pontal (1982) reported three cases of bladder cancer occurring among 50 workers employed in a factory where *para*-toluenediamine (IARC, 1987b) was synthesized from *ortho*-toluidine and *ortho*-aminoazotoluene (see IARC, 1987c). [The Working Group noted that although a formal estimate of expected numbers of bladder cancer cases was not provided, three bladder cancers among 50 persons is likely to represent a substantial excess. The relationship of these bladder cancers to *ortho*-toluidine exposure cannot be determined because of co-exposure to *ortho*-aminoazotoluene, which has been classified as possibly carcinogenic to humans (IARC, 1975, 1987c).]

2.2 Cohort studies (see Table 3)

Among the epidemiological studies of cancer in humans identified in the previous IARC review (1982), only the study by Rubino *et al*. (1982) provides adequate information on the study population and methods. In that study, 906 men first employed in a dyestuff factory in northern Italy between 1922 and 1970 were followed from 1946 to 1976. Mortality was compared with that of the Italian male population as a whole. Follow-up was 95.8% complete and, among those traced (868), 260 (30%) had died. Thirty-six deaths from urinary bladder cancer were observed, with 1.23 expected. Thirty-one of these deaths occurred among 610 men with exposure to benzidine, 1-naphthylamine, 2-naphthylamine or several of these, but five (with 0.08 expected) occurred among 53 men engaged solely in the manufacture of fuchsin (magenta; see IARC, 1993) and safranine T, involving exposure either to a combination of toluene, *ortho*-nitrotoluene, *ortho*-toluidine and 4,4′-methylene bis(2-methylaniline) (three deaths from bladder cancer) or to a combination of *ortho*-toluidine, 4,4′-methylene bis(2-methylaniline), *ortho*-nitrotoluene, 2,5-diaminotoluene, *ortho*-aminoazotoluene, aniline, fuchsin and safranine T (two deaths from bladder cancer). The standardized mortality ratio (SMR) for bladder cancer in this group was 62.5 [95% confidence interval (CI), 20.3–145.6]. No quantitative exposure measurements or data on smoking were available. [The Working Group noted that the excess of bladder cancer could not be attributed with certainty specifically to exposure to *ortho*-toluidine or to any one of the other compounds involved.]

Three pertinent epidemiological studies have been published since the last IARC review. Ott and Langner (1983) studied the mortality of 342 employees assigned to three aromatic amine-based dye production areas from 1914 to 1958 in the United States. One of these areas, the bromoindigo and thioindigo production area, involved potential exposure to *ortho*-toluidine, 4-chloro-*ortho*-toluidine and 4-chloroacetyl-*ortho*-toluidine.

Table 3. Summary of cohort studies of workers exposed to *ortho*-toluidine

Reference	Rubino *et al.* (1982)	Ott & Langner (1983)	Stasik (1988)	Ward *et al.* (1991); Prince *et al.* (2000)	Sorahan *et al.* (2000)
Country	Italy	USA	Germany	USA	UK
Industry	Dyestuff production	Dye production	4-Chloro-*ortho*-toluidine production and processing	Rubber chemicals	Rubber chemicals
Size of the total study	906 men	275 men	116 men	1749 (1643 men)	2160 men
Cohort definition	First employed 1922–70	Employed any time during 1940–58	Employed before 1970	Employed any time during 1946–88	Employed six months between 1955–84
Period of follow-up	1946–76	1940–75	< 1967–86	1973–88	1955–96 (mortality) 1971–92 (incidence)
Deaths					
All causes					
N	260	98	19	[190]	1131
SMR (95% CI)	1.5 [1.4–1.7]	1.0 [0.8–1.2]	1.12 (0.68–1.7)	[0.9] [0.8–1.0]	1.01 (0.96–1.1)
All cancers					
N	96	23	5	[49]	305
SMR (95% CI)	2.6 $p < 0.001$	1.3 [0.8–2.0]	1.4 (0.5–3.4)	[1.0] [0.7–1.3]	1.02 (0.91–1.1)
Bladder cancer mortality					
N	36	0		[2]	
SMR (95% CI)	29.3 $p < 0.001$	1.2 expected		[2.1] [0.3–7.6]	17 SMR, 1.4 (0.8–2.2)
Bladder cancer incidence					
N			8	13	19
SIR (95% CI)			72.7 (31.4–143.3)	3.6 [1.92–6.2]	SIR, 1.1 (0.6–1.7)

Table 3 (contd)

Reference	Rubino *et al.* (1982)	Ott & Langner (1983)	Stasik (1988)	Ward *et al.* (1991); Prince *et al.* (2000)				Sorahan *et al.* (2000)
Subgroup exposed to *ortho*-toluidine				Duration of exposure (y)				
Size	53	117	Same as full cohort	Total 708	< 5 584	5–9.99 51	≥ 10 73	53
Bladder cancer mortality								
N	5	0						3
SMR (95% CI)	62.5 [20.3–145.6]							SMR, [15.8] [3.3–46.4]
								RR (internal analysis)
								1–4 years: *n* = 2;
								6.7 (1.6–28.4)
Bladder cancer incidence								≥ 5 years; *n* = 1;
N				7	0	1	6	7.6 (1.0–56.9)
SIR (95% CI)				6.5 [2.6–13.3]	–	8.8 [0.2–49.0]	27.2 [10.0–59.3]	[SIR 7.0; 95% CI, 1.4–20.4]
Co-exposures[a]	4,4′-Methylene bis (2-methylaniline) (2B) Magenta (2B) Safranine T *ortho*-Nitrotoluene (3) 2,5-Diaminotoluene (3) Aniline (3) *ortho*-Amino-azotoluene (2B)	Multiple exposures including 4-chloro-*ortho*-toluidine and 4-chloroacetyl-*ortho*-toluidine	*N*-Acetyl-*ortho*-toluidine 6-Chloro-*ortho*-toluidine 4-Chloro-*ortho*-toluidine (2A)	Aniline (3) Hydroquinone (3) Toluene (3) Carbon disulfide Sulfur Benzothiazole 4-Aminobiphenyl (contaminant) (1) 2-Mercaptobenzothiazole (Ward *et al.*, 1996) (Proprietary chemical)				Aniline (3) 2-Mercaptobenzothiazole Phenyl-β-naphthylamine (3)

[a] Previous IARC overall evaluations of carcinogenicity are given in parentheses.
N, number; SMR, standardized mortality ratio; SIR, standardized incidence ratio

ortho-Toluidine concentrations measured in breathing zone and area samples were consistently below 0.5 ppm [2.19 mg/m^3] and urinary levels measured in 1948 ranged from undetected (< 0.3 mg/L) to 1.7 mg/L in workers engaged in the production of thioindigo. In order to be included in the study, employees had to be currently working as of 1 January 1940 or hired after that date. Mortality was followed from 1 January 1940 through 31 December 1975; US white male referent rates were used to calculate expected number of deaths. Individuals not known to be deceased based on company records and social security follow-up were assumed to be alive. Mortality was analysed separately for 275 individuals not exposed to arsenicals, vinyl chloride or asbestos. Among 98 deaths identified, 94 death certificates (95.9%) were obtained. No deaths due to bladder cancer were observed, with 1.2 deaths expected from malignant neoplasms of the urinary organs. There were 23 deaths due to malignant neoplasms (17.5 expected; SMR, 1.3; [95% CI, 0.8–2.0]), 10 of which were coded to digestive organs (5.7 expected; SMR, 1.8; [95% CI, 0.8–3.2]). The expected number of bladder cancers in the subcohort of 117 workers exposed to *ortho*-toluidine in the production of bromoindigo and thioindigo was not provided. There were no data on the smoking habits of the cohort. [The Working Group noted that the conclusions of this study were limited by the small size of the population exposed to *ortho*-toluidine.]

In a historical mortality study of 335 male employees involved in the production and processing of 4-chloro-*ortho*-toluidine between 1929 and 1982 in Essen, Germany, no deaths from bladder cancer were identified. Four monocyclic amines had been used at the plant: *N*-acetyl-*ortho*-toluidine, 6-chloro-*ortho*-toluidine, *ortho*-toluidine and 4-chloro-*ortho*-toluidine; exposure to 4-chloro-*ortho*-toluidine was reported to be predominant. Quantitative exposure data were not available. Urothelial carcinomas were noted in eight of the employees, between 1967 and 1985, two of whom had died as of December 1986. All eight had been employed in the 4-chloro-*ortho*-toluidine production plant before improvements in industrial hygiene were made in 1970. As a result of this discovery, an incidence study was initiated and the vital status ascertainment for a subcohort of 116 subjects who had been employed in the 4-chloro-*ortho*-toluidine production plant before 1970 was extended through 1986. The expected number of incident bladder cancers in the cohort of 116 men, calculated based on the 1983 rates from the Saarland Cancer Registry, was 0.11. The standardized incidence ratio (SIR) based on eight observed cases was 72.7 (95% CI, 31.4–143) (Stasik, 1988). [The Working Group noted that the definition of the subcohort was made *a posteriori*, but this was justified by the comment that improvements in industrial hygiene were introduced in 1970. It was also unclear in what year the follow-up started. The excess of bladder cancer could not be attributed with certainty specifically to *ortho*-toluidine or to any one of the other compounds present.]

A bladder cancer incidence study was conducted among workers exposed to *ortho*-toluidine at a plant manufacturing rubber chemicals in New York State, United States. The study was initiated at the request of the union representing workers at the plant, who had noted a number of bladder cancers among workers in a department where an

antioxidant and an accelerator were produced. Among the major reactants used in these processes were two primary aromatic amines, *ortho*-toluidine and aniline; other reactants and intermediates included 2-mercaptobenzothiazole (Ward *et al.*, 1996), hydroquinone, toluene, carbon disulfide, sulfur, benzothiazole and a proprietary chemical which was introduced into the process in 1970 (Ward *et al.*, 1991). 4-Aminobiphenyl was identified as a potential low-level contaminant (< 1 ppm) [4.38 mg/m^3] in some bulk samples of process chemicals in 1990 (Ward & Dankovic, 1991). A study cohort was identified from personnel records of the chemical plant, which opened in 1946 for production of poly(vinyl chloride). The antioxidant was produced in a separate building that opened in 1957 and in 1970 an expansion of this building was completed and production of a new accelerator was begun. There were 1749 (1643 male) individuals employed in the plant between 1946 and 1988, 708 of whom had been assigned to the department where the accelerator and antioxidant were produced (and were considered to be definitely exposed to *ortho*-toluidine and aniline); 288 had been assigned to departments such as maintenance in which possible exposure was considered to have occurred, and 753 of whom had never worked in either definitely or possibly exposed jobs. Within the exposed department, it was not possible to separate individuals with exposure to *ortho*-toluidine from individuals with exposure to other chemicals. Vital status was identified from company records and records of the United States social security administration. Bladder cancer cases were identified from company and union records and confirmed through medical records, or through matching with the cancer registry in the state where the plant was located. Cancer incidence rates were compared with bladder cancer incidence rates in the same state. Person–years at risk began on 1 January 1973 (the first year in which matching with the registry through social security number was possible) or on the date of starting employment, whichever occurred later. Follow-up was from 1973 to 1988. Race was recorded in the personnel records for only 670 subjects, among whom 91% were white. There were 13 cases of bladder cancer in 1973–88 in the cohort overall (SIR, 3.6; [95% CI, 1.9–6.2]), seven of which occurred in the definitely exposed group (SIR, 6.5; [95% CI, 2.6–13.3]) and four in the possibly exposed group (SIR, 3.7; [95% CI, 1.0–9.4]). The SIR was not elevated among workers in the probably unexposed group (two observed; SIR, 1.4; [95% CI, 0.17–5.0]). Six of the seven bladder cancer cases occurred among workers employed in the exposed department for over 10 years; the SIR for this group was 27.2 [95% CI, 10.0–59.2]. Data on smoking were available for 143 members of the cohort and showed that cohort members were slightly more likely to be current or former smokers than the general United States population. It was estimated, using a method of indirect adjustment (Axelson & Steenland, 1988), that differences in smoking habits between the cohort and the United States general population would account for an SIR of 1.05 for bladder cancer. A subsequent mortality analysis of the same cohort followed through 1994 (Prince *et al.*, 2000) showed no elevation in all-cause, all-cancer or bladder cancer mortality in the total cohort (Table 3).

The carcinogenicity of some of the reactants, intermediates and end-products present in the previous cohort has been evaluated by IARC. Aniline is not known to induce bladder cancer in humans or animals (IARC, 1987d).

4-Aminobiphenyl, which was present as a potential contaminant, is classified by IARC in Group 1 and is known to be a highly potent human bladder carcinogen (IARC, 1972, 1987e). Potential for 4-aminobiphenyl contamination arose from use of diphenylamine as a reactant intermittently from 1972 to 1985 (Ward *et al.*, 1994) (commercial diphenylamine was contaminated with low levels of 4-aminobiphenyl in the 1970s and earlier (Safe *et al*., 1977)) and from its possible formation in one of the reactions in the antioxidant process. 2-Mercaptobenzothiazole has not been reviewed by IARC, but has shown some evidence of carcinogenicity in rats and equivocal evidence of carcinogenicity in mice (National Toxicology Program, 1988).

An exposure assessment study was conducted at the plant in February–March 1990 (Ward *et al*., 1996). This study demonstrated substantially higher urinary concentrations and levels of haemoglobin adducts of aniline and *ortho*-toluidine among exposed workers compared with in-plant controls. Levels of 4-aminobiphenyl adducts were much lower than those of *ortho*-toluidine and aniline and did not differ between the exposed and unexposed groups (Table 4). Haemoglobin adducts reflect only recent exposures (Hemminki, 1992), and it is therefore possible that higher levels of 4-aminobiphenyl contamination existed in the past. [The Working Group noted that, while *ortho*-toluidine was a plausible cause of the bladder cancer excess, the contribution of other chemicals cannot be ruled out. The presence of a proprietary chemical was noted, for which the Working Group had no data on carcinogenicity.]

Sorahan *et al*. (2000) updated a study of workers exposed to several aromatic amines in a factory manufacturing chemicals for the rubber industry in the United Kingdom (Sorahan & Pope, 1993). All subjects had at least six months' employment in the factory and some employment in the period 1955–84. Mortality was examined for the period 1955–96 and cancer incidence was examined for the period 1971–92. The updated study included 2160 male production workers, 605 of whom had been exposed to aniline, 2-mercaptobenzothiazole, phenyl-β-naphthylamine or *ortho*-toluidine. There were nine bladder cancer deaths observed and 3.25 expected among the 605 individuals (SMR, 2.8; 95% CI, 1.3–5.3). A total of 30 incident bladder cancers were identified, of which nine occurred among [357] individuals who had been exposed to mercaptobenzothiazole [SIR, 2.6; 95% CI, 1.2–4.9], five occurred among 94 individuals who had been exposed to phenyl-β-naphthylamine [SIR, 3.8; 95% CI, 1.2–8.8], seven occurred among individuals exposed to aniline [SIR, 1.5; 95% CI, 0.6–3.1] and three occurred among 53 individuals who had been exposed to *ortho*-toluidine [SIR, 7.0; 95% CI, 1.4–20.4]. All of the bladder cancer cases among workers exposed to *ortho*-toluidine had also had exposure to one or more of the other chemicals. Poisson regression analyses revealed no association between estimated duration of mercaptobenzothiazole exposure and risk of bladder cancer, some evidence for an exposure–response relationship with duration of exposure to *ortho*-toluidine (RR internal analysis: 1–4 years, n = 2, RR, 6.7; 95% CI,

Table 4. Air and urine levels and haemoglobin adduct levels measured in 1990 among chemical workers employed at a plant where excess bladder cancer incidence was observed

Exposure matrix	Exposure group	No.	Mean of total group (SD)	*p* value
Aniline levels in air ($\mu g/m^3$)	Exposed	28	187 (181)	
ortho-Toluidine levels in air ($\mu g/m^3$)	Exposed	28	412 (366)	
Aniline levels in post-shift urine (μg/L)	Unexposed Exposed	25 42	3.9 (2.8) 29.8 (25.7)	0.0001
ortho-Toluidine in post-shift urine (μg/L)	Unexposed Exposed	25 42	2.8 (1.4) 98.7 (119.4)	0.0001
Aniline adducts (pg/g Hb)	Unexposed Exposed	27 46	3163 (1302) 17 441 (8867)	0.0001
ortho-Toluidine adducts (pg/g Hb)	Unexposed Exposed	27 46	3515 (6036) 40 830 (32 518)	0.0001
4-Aminobiphenyl adducts (pg/g Hb)	Unexposed Exposed	27 42	74.5 (63.8) 81.7 (106.1)	0.48

From Ward *et al.* (1996)
Hb: haemoglobin

1.6–28.4; ≥ 5 years, n = 1, RR, 7.6; 95% CI, 1.0–56.9), and the strongest evidence for an exposure–response relationship with duration of exposure to phenyl-β-naphthyl-amine (RR internal analysis: 1–4 years, n = 1, RR, 1.3; 95% CI, 0.17–9.3; ≥ 5 years, n = 4, RR,7.5; 95% CI, 2.6–21.5).

[The Working Group made two observations relevant to the interpretation of all five cohort studies. Individual smoking habits had not been taken into account in the analysis of most of the reported studies; however, the excesses of bladder cancer reported in the four positive studies were much too large to have been due to smoking alone.

Negative results on mortality from bladder cancer might be caused by limited power due to high survival from this disease. Therefore, differences between results of analyses based on mortality and morbidity data might reflect the lower sensitivity of the former.]

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 25 male and 25 female Swiss CD-1 mice, four to six weeks of age, were treated with *ortho*-toluidine hydrochloride (purity, 97–99%) in the diet at dose levels of 16 000 or 32 000 mg/kg diet (ppm) for three months and then at levels of 8000 or 16 000 ppm for a further 15 months. Animals were kept without treatment for an additional three months and then killed. The doses were chosen on the basis of preliminary tests, the higher being the maximum tolerated dose. A simultaneous control group of 25 untreated mice of each sex was used, plus additional controls used for the other compounds tested in the study, and tumour incidences of matched and pooled controls were compared statistically (both separately and together) with those of treated groups. Animals that died during the first six months of the study were discarded without necropsy. In male mice, the incidence of vascular tumours (haemangiomas and haemangiosarcomas combined, observed in abdominal viscera) was 0/14, 5/99, 5/14 ($p < 0.025$, Fisher's exact test) and 9/11 ($p < 0.025$, Fisher's exact test) in concurrent controls, pooled controls, low-dose and high-dose groups, respectively. In female mice, the incidence of vascular tumours (haemangiomas and haemangiosarcomas combined, observed in abdominal viscera) was 0/15, 9/102, 5/18 ($p < 0.05$, Fisher's exact test) and 9/21 ($p < 0.025$, Fisher's exact test) in concurrent controls, pooled controls, low-dose and high-dose groups, respectively [the separate incidences for haemangiomas and haemangiosarcomas were not reported] (Weisburger *et al*., 1978).

Groups of 50 male and 50 female B6C3F_1 mice, six weeks of age, were administered *ortho*-toluidine hydrochloride (purity, > 99%) in the diet at 1000 or 3000 ppm for 102–103 weeks. Concurrent control groups consisted of 20 male and 20 female untreated mice. Mean body weights of both treated males and females were lower than those of the corresponding controls. Mortality was not significantly related to treatment in either sex. In male mice, the incidence of haemangiomas or haemangiosarcomas (combined, all sites, mainly observed in the abdominal viscera) was increased: 1/19, 2/50 and 12/50 ($p < 0.002$, Cochran–Armitage trend test) in control, low-dose and high-dose groups, respectively. In female mice, the incidence of hepatocellular adenomas or carcinomas (combined) was increased: 0/20, 4/49 and 13/50 ($p < 0.007$, Fisher's exact test; $p = 0.001$ trend test) in control, low-dose and high-dose groups, respectively (National Cancer Institute, 1979).

3.1.2 *Rat*

Groups of 25 male Sprague-Dawley CD rats, four to six weeks of age, were treated with *ortho*-toluidine hydrochloride (purity, 97–99%) in the diet at dose levels of 8000 or 16 000 ppm for three months and then at levels of 4000 or 8000 ppm for a further

15 months. Animals were kept without treatment for an additional six months and then killed. The doses were chosen on the basis of preliminary tests, the higher being the maximum tolerated dose. A concurrent control group of 25 untreated male rats was used, plus additional controls used for the other compounds tested in the study, and tumour incidences of matched and pooled controls were compared with those of treated groups. Animals that died during the first six months of the study were discarded without necropsy. The incidence of subcutaneous fibromas and fibrosarcomas (combined) was 0/16, 18/111, 18/23 ($p < 0.025$, Fisher's exact test) and 21/24 ($p < 0.025$, Fisher's exact test, compared with all controls) in simultaneous controls, pooled controls, low-dose and high-dose groups, respectively. A non-statistically significant increase in the incidence of transitional-cell carcinomas of the urinary bladder was also observed: 0/16, 5/111, 3/23 and 4/24 in simultaneous controls, pooled controls, low-dose and high-dose groups, respectively (Weisburger *et al*., 1978).

Groups of 30 male Fischer 344 rats, eight weeks of age, were treated with *ortho*-toluidine hydrochloride [purity not specified, recrystallized product] in the diet at a concentration of 4000 ppm (0.028 mol/kg of diet) for 72 weeks. A control group of 30 untreated male rats was used. The experiment was terminated at 93 weeks. Mean body weights were lower in the treated than in the control group. The incidence of fibromas of the skin was 1/27 and 25/30 [$p < 0.001$, Fisher's exact test]; that of fibromas of the spleen was 0/27 and 10/30 [$p < 0.001$, Fisher's exact test]; that of mammary fibroadenomas was 0/27 and 11/30 [$p < 0.001$, Fisher's exact test]; and that of peritoneal sarcomas was 0/27 and 9/30 [$p < 0.01$, Fisher's exact test] in control and treated groups, respectively (Hecht *et al*., 1982).

Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were administered o*rtho*-toluidine hydrochloride (purity, > 99%) in the diet at concentrations of 3000 or 6000 ppm for 101–104 weeks. Matched control groups consisted of 20 male and 20 female untreated rats. Mean body weights of treated male and female rats were lower than those of the corresponding controls. Mortality was significantly affected by treatment of male and female rats ($p < 0.001$, Tarone test for positive trend). In males, the incidence of sarcomas, fibrosarcomas, angiosarcomas or osteosarcomas (combined) of multiple organs (mainly subcutis and spleen or bone) was 0/20, 15/50 ($p = 0.003$, Fisher's exact test) and 37/49 ($p < 0.001$, Fisher's exact test); that of subcutaneous integumentary fibromas was 0/20, 28/50 ($p < 0.001$, Fisher's exact test) and 27/49 ($p < 0.001$, Fisher's exact test); and that of mesotheliomas of multiple organs or tunica vaginalis was 0/20, 17/50 ($p < 0.001$, Fisher's exact test) and 9/49 ($p = 0.036$, Fisher's exact test) in control, low- and high-dose groups, respectively. In females, the incidence of transitional cell carcinomas of the urinary bladder was 0/20, 9/45 ($p = 0.028$, Fisher's exact test) and 22/47 ($p < 0.001$, Fisher's exact test); that of sarcomas, fibrosarcomas, osteosarcomas or angiosarcomas (combined) of multiple organs (mainly subcutis and spleen or bone) was 0/20, 3/50 and 21/49 ($p = 0.001$, Fisher's exact test); and that of mammary adenomas and fibroadenomas (combined) was 7/20, 20/50 and 35/49

($p = 0.006$) in control, low- and high-dose groups, respectively (National Cancer Institute, 1979).

Male Fischer 344/N rats, 45 days of age, were administered *ortho*-toluidine hydrochloride at a concentration of 5000 ppm in the diet for 13 weeks and then kept for observation for a further 13 weeks, when animals were killed. Mesotheliomas in the epididymis were observed in 2/20 male rats exposed to *ortho*-toluidine hydrocloride. No mesotheliomas were seen in concurrent controls (0/10) (National Toxicology Program, 1996).

3.2 Subcutaneous injection

Hamster: Groups of 15 male and 15 female Syrian golden hamsters, eight weeks of age, were given subcutaneous injections of 1.9 mmol/kg bw (2 mg/kg bw) *ortho*-toluidine (free base) [purity not specified, recrystallized product] in peanut oil once per week for 52 weeks. Control groups of 15 male and 15 female hamsters were given 52 subcutaneous injections of peanut oil vehicle. The experiment was terminated at 87 weeks. Mean body weights in the treated groups were similar to those of the control groups. Mean survival times were shorter in the treated groups, being 61.3 and 57.8 weeks in male and female treated hamsters, respectively, compared with 75.5 and 68.7 weeks in male and female controls, respectively. The incidence of tumours in the treated groups was not significantly different from that in the control groups [details on the incidence of specific tumours not reported] (Hecht *et al*., 1983). [The Working Group noted the small number of animals, low dose and short duration of treatment.]

3.3 Carcinogenicity of metabolites

Rat: Groups of 30 male Fischer 344 rats, eight weeks of age, were treated with *ortho*-nitrosotoluene [purity not specified, recrystallized product] in the diet at a concentration of 3380 ppm (0.028 mol/kg of diet) for 72 weeks. A control group of 30 untreated male rats was used. The experiment was terminated at 93 weeks. Mean body weights were lower in the treated than in the control group. The incidence of fibromas of the skin was 1/27 and 19/29 [$p < 0.001$, Fisher's exact test]; that of fibromas of the spleen was 0/27 and 14/29 [$p < 0.001$, Fisher's exact test]; that of hepatocellular carcinomas was 0/27 and 18/29 [$p < 0.001$, Fisher's exact test]; and that of urinary bladder tumours was 0/27 and 15/29 [$p < 0.01$, Fisher's exact test] in control and treated groups, respectively (Hecht *et al*., 1982).

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

ortho-Toluidine is absorbed via the respiratory tract and skin. It can be inhaled as dust, fumes or vapour (ILO, 1971). Ward *et al*. (1996), while not able to determine the relative contributions of airborne versus dermal exposure, found strong evidence that *ortho*-toluidine was absorbed in an exposed group of workers at a chemical manufacturing facility (see Section 2.2). While *ortho*-toluidine is a component of cigarette smoke (IARC, 1986), El-Bayoumy *et al*. (1986) found that the levels in urine of smokers and nonsmokers were not significantly different (see Section 1.4.3(*e*)). The authors suggested that nitrobenzene, nitrotoluenes and dietary factors may be major contributors to *ortho*-toluidine levels in human urine, which might in part explain the marked variability (~ 100-fold) in *ortho*-toluidine–haemoglobin adducts reported in unexposed groups between nonsmokers and smokers in three studies (Stillwell *et al*., 1987; Bryant *et al*., 1988; Ward *et al*., 1996). In two of these studies (Stillwell *et al.*, 1987; Bryant *et al.*, 1988), smoking significantly increased the level of *ortho*-toluidine–haemoglobin adducts, whereas, in the study by Ward *et al*. (1996), adduct levels were similar in smokers (40 494 pg/g Hb) and nonsmokers (41 028 pg/g Hb) in the exposed group, as well as in the unexposed group in smokers (3510 pg/g Hb) and nonsmokers (3518 pg/g Hb). They concluded that the possibility of some exposure to *ortho*-toluidine in the 'unexposed' workers could not be ruled out.

4.1.2 *Experimental systems*

The tissue distribution of radioactivity in male Fischer 344 rats 48 h after subcutaneous injection of 50 or 400 mg/kg bw *ortho*-[*methyl*-^{14}C]toluidine was mainly in the liver, kidney, lung, spleen, colon and bladder. The major excretory route was via the urine, with ~ 84% of the 400-mg/kg bw dose being eliminated via this pathway within the first 48 h. Approximately 1% of the 400-mg/kg bw dose was eliminated via the lungs and ~ 3% in the faeces (Son *et al*., 1980). Following oral administration of a single 50-mg/kg bw dose of *ortho*-[*methyl*-^{14}C]toluidine to male Sprague-Dawley rats, > 92% of the administered radiolabel was eliminated in the urine within 72 h. The amount of unchanged compound appearing in the urine within 24 h varied according to the isomer of toluidine administered at 500 mg/kg bw: 21% for *ortho*-toluidine, 2.5% for *meta*-toluidine and 2.5% for *para*-toluidine. Such differences may help to explain why only the *ortho*-isomer causes tumours in the urinary bladder of rats (Cheever *et al.*, 1980). However, in contrast to this finding, after subcutaneous doses of 400 mg/kg bw (Son *et al.*, 1980) or 0.82 mmol/kg [88 mg/kg bw] (Kulkarni *et al.*, 1983), Fischer 344 rats

excreted only approximately 5% of the dose in the urine as *ortho*-toluidine over 48 and 6 h, respectively.

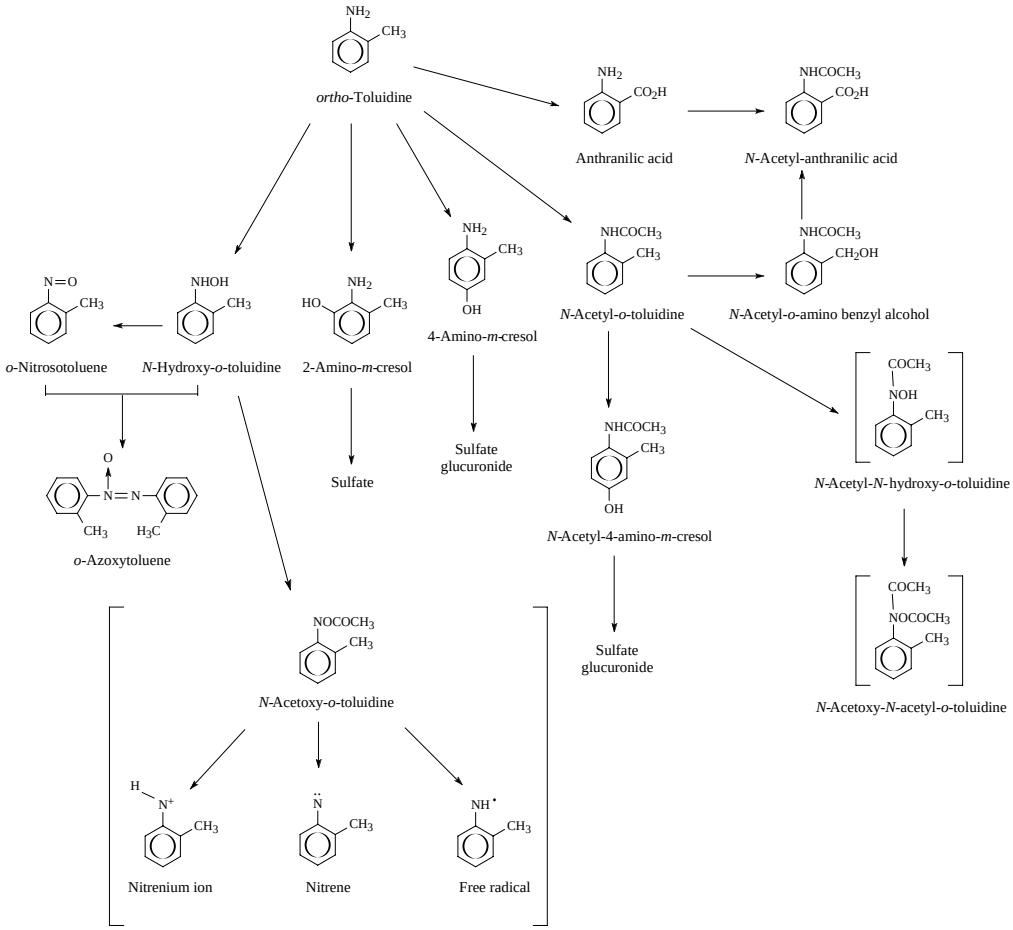
The metabolism of *ortho*-toluidine in rats proceeds primarily through ring hydroxylation with subsequent conjugation (Cheever *et al*., 1980; Son *et al*., 1980). *N*-Acetylation of *ortho*-toluidine is also a major metabolic pathway in male Fischer 344 rats. The major ether-extractable urinary metabolites of a 400-mg/kg dose of *ortho*-toluidine are azoxytoluene, *ortho*-nitrosotoluene, *N*-acetyl-*ortho*-toluidine, *N*-acetyl-*ortho*-aminobenzyl alcohol, *N*-acetyl-4-amino-*meta*-cresol, 4-amino-*meta*-cresol, anthranilic acid and *N*-acetylanthranilic acid (Son *et al*., 1980). *N*-Hydroxy-*ortho*-toluidine is also a urinary metabolite of male Fischer 344 rats (Kulkarni *et al*., 1983). After a 400-mg/kg bw dose of *ortho*-[*methyl*-^{14}C]toluidine administered subcutaneously to male Fischer 344 rats, 51% of urinary metabolites were conjugates, with sulfate conjugates of 4-amino-*meta*-cresol (27.8% of the dose), *N*-acetyl-4-amino-*meta*-cresol (8.5% dose) and 2-amino-*meta*-cresol (2.1%), dominating over glucuronides of 4-amino-*meta*-cresol (2.6%), *N*-acetyl-4-amino-*meta*-cresol (2.8%) and *N*-acetyl-*ortho*-aminobenzyl alcohol by a ratio of 6:1. A double acid conjugate of 4-amino-*meta*-cresol was also identified. *ortho*-Toluidine can be oxidized to yield *N*-hydroxy-*ortho*-toluidine and *ortho*-nitrosotoluene (Son *et al*., 1980) (see Figure 1).

On the basis of data from the *Salmonella*/mammalian microsomal mutagenicity assay, Gupta *et al*. (1987) proposed that *N*-hydroxy-*ortho*-toluidine undergoes further metabolic activation via an acetylation reaction catalysed by *N*-acetyltransferase to form *N*-acetoxy-*ortho*-toluidine. The latter metabolite could undergo non-enzymatic breakdown to yield a highly reactive nitrenium ion, nitrene or free radical that can covalently bind to tissue macromolecules (Figure 1). The same authors also postulated that *N*-acetyl-*ortho*-toluidine can be metabolized to produce *N*-acetyl-*N*-hydroxy-*ortho*-toluidine, which could undergo further acetylation to form *N*-acetoxy-*N*-acetyl-*ortho*-toluidine. However, there are no in-vivo data to substantiate the existence of *N*-acetyl-*N*-hydroxy-*ortho*-toluidine or *N*-acetoxy-*N*-acetyl-*ortho*-toluidine in rats fed *ortho*-toluidine; the suggestion that they are formed is based on results with other aromatic amines and amides (Thorgeirsson *et al*., 1983; Gupta *et al*., 1987). The secondary metabolite *ortho*-azoxytoluene is postulated to be formed by an interaction between *ortho*-nitrosotoluene and *N*-hydroxy-*ortho*-toluidine (Son *et al*., 1980). Another putative secondary metabolite, *ortho*-azotoluene, is proposed to be formed through a reaction of *ortho*-toluidine with *ortho*-nitrosotoluene, but no data on its in-vivo formation exist (Gupta *et al*., 1987).

Cheever *et al*. (1980) also reported finding 4-amino-3-methylphenol in the urine of rats fed *ortho*-toluidine. Leslie *et al*. (1988) have shown that administration of *ortho*-toluidine to male Sprague-Dawley rats induces various metabolic activities associated with the cytochrome P450 system.

In male Crl:CD rats, after a single oral dose of 500 mg/kg bw *ortho*-toluidine, binding to hepatic DNA and RNA appeared to be maximal 24–48 h after dosing (Brock *et al*., 1990). The highest levels of binding were found in the blood, spleen, kidney and liver.

Figure 1. Metabolic disposition of *ortho*-toluidine in rats



Adapted from Cheever *et al.* (1980); Son *et al.* (1980); Kulkarni *et al.* (1983) and Gupta *et al.* (1987)
Brackets indicate postulated proximate or reactive metabolites of *ortho*-toluidine, based on data from the metabolism of other aromatic amines.

ortho-Toluidine forms haemoglobin adducts in female Wistar rats and female B6C3F$_1$ mice after administration of a single oral dose of 0.47–0.6 mmol/kg bw [50.4–64.3 mg/kg bw] (Birner & Neumann, 1988). In male Sprague-Dawley rats given intraperitoneal doses of [^{14}C]*ortho*-toluidine ranging from 10 to 100 mg/kg bw, peak albumin binding occurred after 4 h at 50 mg/kg bw and peak haemoglobin binding at 24 h following a dose of 100 mg/kg bw (DeBord *et al.*, 1992). A linear dose–response relationship was seen for [^{14}C]*ortho*-toluidine binding to haemoglobin. The biological half-lives of [^{14}C]*ortho*-toluidine bound to albumin and haemoglobin were calculated to be 2.6 and 12.3 days, respectively. The route of [^{14}C]*ortho*-toluidine administration

significantly affected binding to haemoglobin, with rats injected intraperitoneally having approximately twofold higher [^{14}C]*ortho*-toluidine–haemoglobin levels than animals treated orally.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

The following intraperitoneal LD_{50} values have been reported for *ortho*-toluidine hydrochloride: 179 mg/kg bw in male mice, 113 mg/kg bw in female mice, 164 mg/kg bw in male rats and 246 mg/kg bw in female rats (Weisburger *et al*., 1978). Administration of 225 mg/kg bw *ortho*-toluidine per day by gavage (estimated oral LD_{50}, 900 mg/kg bw) to male Fischer 344 rats for either five, 10 or 20 days led to splenic congestion, increased haematopoiesis, haemosiderosis and bone marrow hyperplasia (after 10 days) consistent with enhanced erythrocytic destruction. Histopathological examination of the livers did not reveal any overt hepatotoxic effect (Short *et al*., 1983). In both male and female Fischer 344 rats, 3000 and 6000 ppm (mg/kg) *ortho*-toluidine hydrochloride given in the diet over 104 weeks induced proliferative mesenchymal lesions in the spleen, including capsular and parenchymal fibrosis (Goodman *et al*., 1984).

ortho-Toluidine given to male Wistar rats by intraperitoneal injection (75 mg/kg bw for three consecutive days) increased the microsomal arylhydrocarbon hydroxylase activity, presumably reflecting CYP1A isoenzymes, in the liver, kidney and lung. The activity of NADPH-cytochrome c reductase and the content of cytochrome b_5 were enhanced only in the liver. No significant effect was observed on epoxide hydrolase or glutathione *S*-transferase activity using benzo[*a*]pyrene 4,5-oxide and 1-chloro-2,4-dinitrobenzene, respectively, as substrates (Gnojkowski *et al*., 1984).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

[The Working Group noted that many aromatic amines induce methaemoglobinaemia (Watanabe *et al*., 1976; Coleman & Coleman, 1996). The effect of methaemoglobinaemia on fetal development has not been well studied, but may be associated with suboptimal fetal outcome (Fan & Steinberg, 1996; Kilpatrick & Laros, 1999).]

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Tables 5 and 6 for references)

The genetic toxicology of *ortho*-toluidine was extensively studied in two international collaborative trials for evaluation of short-term tests for carcinogens (de Serres & Ashby, 1981; Ashby *et al.*, 1985). A review (Danford, 1991) summarizes the conclusions of these trials. The genetic toxicology of *ortho*-toluidine has also been reviewed more briefly, in the context of carcinogenesis, by Sellers and Markowitz (1992). There are some difficulties in evaluating the significance of much of the available data, since there seems to be substantial variation in results between different laboratories and minor variations in protocols.

By far the majority of the data from bacterial or bacteriophage assay systems show negative or, at most, weakly positive results. *ortho*-Toluidine gave positive results for induction of bacteriophage lambda, but only when tested in the presence of exogenous metabolic activation. However, it failed to induce SOS activity in *Salmonella typhimurium* TA1535/PSK1002. At very high concentrations (20 mg per plate), it was differentially toxic towards *Escherichia coli* strains differing in capacity for recombinational repair, in the absence of S9 mix. However, this result was not reproduced in two further studies carried out in other laboratories, using lower concentrations. *ortho*-Toluidine gave positive results for forward mutation in recombination-deficient strains of *Bacillus subtilis*. A large series of studies have been reported using *S. typhimurium* strains TA100, TA102, TA1535, TA1537, TA1538, TA98 and TA97. Almost all of the results were negative, although there are sporadic reports of positive responses, only in the presence of S9 mix, with strains TA100, TA98, TA1535 and TA1538. Uniformly negative results were found for reverse mutation in *E. coli* strains WP2 or WP2 *uvrA*. Where positive response have been seen in microbial assays, they have generally required variations to the standard test procedures, including the use of the fluctuation protocol, or incorporating the addition of norharman or lithocholic acid. High concentrations of S9 mix, or special types of S9 mix may also be important.

In *Saccharomyces cerevisiae*, treatment with *ortho*-toluidine resulted in differential toxicity between repair-proficient and -deficient strains. However, inconsistent data were seen in all other assays with this species of yeast. Of eight assays for gene conversion carried out in different laboratories, one positive result was reported only when exogenous metabolic activation was present and another only when it was absent. Although *ortho*-toluidine caused a recombinogenic event leading to deletion (in either the presence or absence of exogenous metabolic activation), it failed to cause intrachromosomal recombination in the same yeast strain. It was a mitochondrial ‘petite’ mutagen, but failed to give a positive response for forward mutation in a nuclear gene.

Table 5. Genetic and related effects of *ortho*-toluidine

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Prophage induction, SOS repair, DNA strand breaks or cross-links	NT	+	2500	Thomson (1981)
Prophage induction, SOS repair, DNA strand breaks or cross-links (*Salmonella typhimurium* TA1535/pSK1002)	–	–	1670	Nakamura *et al.* (1987)
Escherichia coli pol A/W3110-P3478, differential toxicity (liquid suspension tests)	–	–	250 µg/plate	Rosenkranz *et al.* (1981)
Escherichia coli rec strains, differential toxicity	+	NT	20 µL/disc	Rosenkranz & Poirier (1979)
Escherichia coli rec strains, differential toxicity	?	–	2500	Green (1981)
Escherichia coli rec strains, differential toxicity	–	–	1000	Tweats (1981)
Bacillus subtilis rec strains, forward mutation	+	+	20 µL/disc	Kada (1981)
Salmonella typhimurium TM677, forward mutation	NT	–	500	Skopek *et al.* (1981)
Salmonella typhimurium TM677, forward mutation	–	–	500	Liber (1985)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	10 000 µg/plate	McCann *et al.* (1975)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	1000 µg/plate	Simmon (1979)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	1000 µg/plate	Tanaka *et al.* (1980)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	10 µL/plate	Baker & Bonin (1981)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA92, TA98, reverse mutation	–	–	2000 µg/plate	Brooks & Dean (1981)
Salmonella typhimurium TA100, TA98, TA1537, reverse mutation	–	–	5000 µg/plate	MacDonald (1981)
Salmonella typhimurium TA100, TA1537, TA98, reverse mutation	–	–	1000 µg/plate	Nagao & Takahashi (1981)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	10 000 µg/plate	Richold & Jones (1981)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	2000 µg/plate	Rowland & Severn (1981)
Salmonella typhimurium TA100, reverse mutation	NT	–	200 µg/plate	Sugimura *et al.* (1982)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, G46, C3076, reverse mutation	–	–	1000 µg/mL agar	Thompson *et al*. (1983)
Salmonella typhimurium TA100, TA98, TA97, TA102, reverse mutation	–	–	10 000 µg/plate	Baker & Bonin (1985)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	1000 µg/mL	Falck *et al*. (1985)
Salmonella typhimurium TA100, TA102, TA98, TA97, reverse mutation	–	–	2000 µg/plate	Matsushima *et al*. (1985)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	5000 µg/plate	Rexroat & Probst (1985)
Salmonella typhimurium TA100, reverse mutation	–	+[c]	2000 µg/plate	Zeiger & Haworth (1985)
Salmonella typhimurium BA13 (L-arabinose resistance), forward mutation	NT	+	480 µg/plate	Dorado & Pueyo (1988)
Salmonella typhimurium TA1535, TA1538, reverse mutation	–	–	250 µg/plate	Rosenkranz & Poirier (1979)
Salmonella typhimurium TA1535, TA1537, TA98, reverse mutation	–	–	300 µg/mL	Gatehouse (1981)
Salmonella typhimurium TA1535, reverse mutation	–	–	1000 µg/plate	Zeiger & Haworth (1985)
Salmonella typhimurium TA1538, reverse mutation	–	–	100 µg/plate	Ferreti *et al*. (1977)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA1538, reverse mutation	–	–	100 µg/plate	Garner & Nutman (1977)
Salmonella typhimurium TA1538, reverse mutation	–	+[d]	10 µg/mL	Gatehouse (1981)
Salmonella typhimurium TA98, reverse mutation	NT	–	200 µg/plate	Nagao *et al*. (1977)
Salmonella typhimurium TA98, reverse mutation	NT	–	50 µg/plate	Nagao *et al*. (1978)
Salmonella typhimurium TA98, reverse mutation	NT	+	2.5 µg/plate	Kawalek *et al*. (1983)
Salmonella typhimurium TA98, TA97, reverse mutation	–	–	1000 µg/plate	Zeiger & Haworth (1985)
Escherichia coli WP2 *uvrA*, reverse mutation	–	–	1000	Gatehouse (1981)
Escherichia coli WP2 and WP2 *uvrA*, reverse mutation	–	–	1000	Thompson *et al*. (1983)
Escherichia coli WP2 *uvrA*, reverse mutation	–	–	1000	Falck *et al*. (1985)
Aspergillus nidulans, forward mutation	–	NT	504	Carere *et al*. (1985)
Aspergillus nidulans, genetic crossing-over	–	NT	2520	Carere *et al*. (1985)
Saccharomyces cerevisae, DNA repair-deficient strains, differential toxicity	+	+	300	Sharp & Parry (1981a)
Saccharomyces cerevisiae, gene conversion	–	–	333 µg/plate	Jagannath *et al*. (1981)
Saccharomyces cerevisiae, gene conversion	+	NT	50	Sharp & Parry (1981b)
Saccharomyces cerevisiae, gene conversion	NT	–	2 µL/mL	Zimmermann & Scheel (1981)
Saccharomyces cerevisiae, gene conversion, reverse mutation	–	–	500	Arni (1985)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Saccharomyces cerevisiae, gene conversion	–	–	2000	Brooks *et al.* (1985)
Saccharomyces cerevisiae, gene conversion, forward/reverse mutation	–	–	1000	Inge-Vechtomov *et al.* (1985)
Saccharomyces cerevisiae, gene conversion, reverse mutation	–	–	500	Parry & Eckardt (1985a)
Saccharomyces cerevisiae, deletion assay	+	+	1000	Carls & Schiestl (1994)
Saccharomyces cerevisiae, interchromosomal recombination	–	–	5000	Carls & Schiestl (1994)
Saccharomyces cerevisiae, forward 'petite' mutation	+	NT	2500	Ferguson (1985)
Saccharomyces cerevisiae, strain XV185-14C, reverse mutation	–	–	2222	Mehta & von Borstel (1981)
Saccharomyces cerevisiae, reverse mutation	–	–	21.2	Harrington & Nestmann (1985)
Saccharomyces cerevisiae strain D7-144, gene conversion	–	+[e]	378	Mehta & von Borstel (1985)
Saccharomyces cerevisiae strain XV185-14C & RM52, reverse mutation	+	+[e]	378	Mehta & von Borstel (1985)
Saccharomyces cerevisiae, aneuploidy	+	+	50	Parry & Sharp (1981)
Saccharomyces cerevisiae, aneuploidy	+	+	NR	Parry & Eckardt (1985b)
Saccharomyces cerevisiae, aneuploidy	–	NT	1.5 µL/mL	Zimmermann *et al.* (1985)
Drosophila melanogaster, genetic recombination	+		2 mM in feed	Vogel (1985)
Drosophila melanogaster, genetic crossing-over, somatic mutation or recombination	+		0.94 mM in feed[f]	Würgler *et al.* (1985)
Drosophila melanogaster, somatic mutation (and recombination)	(+)		10 700	Fujikawa *et al.* (1985)
Drosophila melanogaster, somatic mutation (and recombination)	+		1 mM in feed	Vogel (1985)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Drosophila melanogaster, somatic mutation (and recombination)	+		1 mM in feed	Batiste-Alentorn *et al.* (1991)
Drosophila melanogaster, somatic mutation	–		2 mM in feed	Batiste-Alentorn *et al.* (1994)
Drosophila melanogaster, somatic mutation (and recombination), wing-spot test	+		5 mM in feed	Batiste-Alentorn *et al.* (1995)
DNA strand breaks, cross-links or related damage, rat hepatocytes *in vitro*	+	NT	319	Bradley (1985)
DNA strand breaks, cross-links or related damage, Chinese hamster ovary cells *in vitro*	+	+	4280	Douglas *et al.* (1985)
DNA strand breaks, cross-links or related damage, Chinese hamster ovary cells *in vitro*	–	(+)	2140	Lakhanisky & Hendrickx (1985)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	54	Thompson *et al.* (1983)
Unscheduled DNA synthesis, Sprague-Dawley rat primary hepatocytes *in vitro*	–	NT	10.7	Kornbrust & Barfknecht (1984)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	53.5	Probst & Hill (1985)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	10	Williams *et al.* (1985)
Unscheduled DNA synthesis, Sprague-Dawley rat primary hepatocytes *in vitro*	–	NT	10.7	Barfknecht *et al.* (1987)
Unscheduled DNA synthesis, golden Syrian hamster primary hepatocytes *in vitro*	–	NT	10.7	Kornbrust & Barfknecht (1984)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Unscheduled DNA synthesis, golden Syrian hamster primary hepatocytes *in vitro*	–	NT	10.7	Barfknecht *et al.* (1987)
Gene mutation, *Hprt* locus, ouabain resistance, Chinese hamster ovary cells *in vitro*	–	–	500	Zdzienicka & Simons (1985)
Gene mutation, Chinese hamster lung V79 cells *Hprt* locus *in vitro*	–	NT	2000	Fox & Delow (1985)
Gene mutation, Chinese hamster lung V79 cells, *Hprt* locus *in vitro*	(+)	–	500	Kuroda *et al*. (1985)
Gene mutation, Chinese hamster lung V79 cells *Hprt* locus *in vitro*	–	–	NR	Lee & Webber (1985)
Gene mutation, Chinese hamster lung V79 cells, oubain resistance *in vitro*	–	–	535	Kuroki & Munakata (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	800	Amacher & Turner (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus and *Hprt* locus *in vitro*	–	–	1.3 µL/mL	Knaap & Langebroek (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	NR	Lee & Webber (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	+	NT	300	Myhr *et al*. (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	500[g]	Oberly *et al*. (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	(+)	–	1004	Styles *et al*. (1985)
Gene mutation, mouse lymphoma L5178Y cells, ouabain or thioguanine resistance *in vitro*	–	+	200	Garner & Campbell (1985)
Gene mutation, mouse lymphoma L5178Y cells, ouabain or trifluorothymidine resistance, *in vitro*	–	–	1004	Styles *et al.* (1985)
Gene mutation, Balb/c 3T3 cells, ouabain resistance *in vitro*	NT	(+)	250	Matthews *et al*. (1985)
Sister chromatid exchange, Chinese hamster cells *in vitro*	+	–	300	Perry & Thomson (1981)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	–	1070	Douglas *et al*. (1985)
Sister chromatid exchange, Chinese hamster cells *in vitro*	+	+	50	Gulati *et al*. (1985)
Sister chromatid exchange, Chinese hamster cells *in vitro*	+	–	500	Lane *et al*. (1985)
Sister chromatid exchange, Chinese hamster cells *in vitro*	–	–	2140	Natarajan *et al*. (1985)
Sister chromatid exchange, Chinese hamster cells *in vitro*	+	+	268	van Went (1985)
Sister chromatid exchange, RL_4 rat liver cells *in vitro*	+	NT	21.8	Priston & Dean (1985)
Micronucleus formation, Chinese hamster ovary cells *in vitro*	–	–	1070	Douglas *et al*. (1985)
Micronucleus formation, Syrian hamster embryo cells *in vitro*	+	NT	NR	Fritzenschaf *et al*. (1993)
Chromosomal aberrations, Chinese hamster CH1-L liver fibroblasts *in vitro*	+	NT	12	Danford (1985)
Chromosomal aberrations, Chinese hamster cells *in vitro*	+	+	250	Gulati *et al*. (1985)
Chromosomal aberrations, Chinese hamster cells *in vitro*	–	+	1000	Ishidate & Sofuni (1985)
Chromosomal aberrations, Chinese hamster cells *in vitro*	–	–	2140	Natarajan *et al*. (1985)
Chromosomal aberrations, Chinese hamster cells *in vitro*	–	(+)	300	Palitti *et al*. (1985)
Chromosomal aberrations, RL_4 rat liver cells *in vitro*	+	NT	700	Priston & Dean (1985)
Aneuploidy, Chinese hamster CH1-L liver fibroblasts *in vitro*	+	NT	60	Danford (1985)
Cell transformation, C3H/10T½ mouse cells	–	(+)	600	Lawrence & McGregor (1985)
Cell transformation, BALB/c3T3 mouse cells	–	+[h]	150	Matthews *et al*. (1985)
Cell transformation, C3H/10T½ mouse cells	+	NT	500	Nesnow *et al*. (1985)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	1	Barrett & Lamb (1985)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	100	Sanner & Rivedal (1985)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	750	Kerckaert *et al.* (1998)
Cell transformation, baby hamster kidney BHK-21 cells	+	+	NR	Daniel & Dehnel (1981)
Cell transformation, baby hamster kidney BHK-21 cells	NT	+	25	Styles (1981)
Cell transformation, Chinese hamster ovary cells	–	–	500	Zdzienicka *et al*. (1985)
Cell transformation, RLV/Fischer rat embryo cells	(+)	NT	10	Suk & Humphreys (1985)
Cell transformation, SA7/Syrian hamster embryo cells	(+)	NT	965	Hatch & Anderson (1985)
DNA strand breaks (Comet assay), MCL-5 cells	+	NT	454	Martin *et al.* (1999)
Gene mutation, human TK6 cells *in vitro*	–	+	450	Crespi *et al.* (1985)
Gene mutation, human AHH-1 cells *in vitro*	+	NT	300	Crespi *et al.* (1985)
Sister chromatid exchange, human lymphocytes *in vitro*	?	–	600	Obe *et al*. (1985)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	21.4	Lindahl-Kiessling *et al*. (1989)
Micronucleus formation, human lymphocytes *in vitro*	+	–	214	Vian *et al*. (1993)
Body fluids from Sprague-Dawley rats (urine), microbial mutagenicity (*S. typhimurium* TA98)	–	+[c]	300 po × 1	Tanaka *et al*. (1980)
Body fluids from WAG/Rij rats (plasma), sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+		400 ip × 1	Darroudi & Natarajan (1985)
DNA strand breaks, cross-links or related damage, animal cells *in vivo*	+		100	Cesarone *et al*. (1982)
Sister chromatid exchange, B6C3F_1 mouse bone marrow cells *in vivo*	(+)		200	Neal & Probst (1983)
Sister chromatid exchange, animal cells *in vivo*	+		600	McFee *et al*. (1989)
Micronucleus formation, B6C3F_1 mice *in vivo*	–		338 ip × 2	Salamone *et al*. (1981)
Micronucleus formation, CD-1 mice *in vivo*	–		160 ip × 2	Tsuchimoto & Matter (1981)
Chromosomal aberrations, B6C3F_1 mice bone marrow cells *in vivo*	–		300 ip × 1	McFee *et al*. (1989)
Micronucleus formation, B6C3F_1 mice bone marrow cells *in vivo*	–		300 ip × 1	McFee *et al*. (1989)
Micronucleus test, *Pleurodeles waltl in vivo*	+		20 µg/mL	Fernandez *et al*. (1989)
Binding (covalent) to DNA, RNA or protein, Crl:CD rat liver *in vivo*	+		500 po × 1	Brock *et al*. (1990)

Table 5 (contd)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Inhibition of intercellular communication, Chinese hamster V79 cells rodent *in vitro*	+	NT	5	Elmore *et al.* (1985)
Inhibition of intercellular communication, Chinese hamster V79 cells rodent *in vitro*	+	NT	5	Scott *et al.* (1985)
Inhibition of intercellular communication, Chinese hamster V79 cells rodent *in vitro*[i]	–	NT	535	Umeda *et al.* (1985)
Sperm morphology, (CBA × BALB/c)F_1 mice *in vivo*	?		250 ip × 5	Topham (1981)
Sperm morphology, (CBA × BALB/c)F_1 mice *in vivo*	–		400 ip × 5	Topham (1980)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested; ?, inconclusive; NR, not reported
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro test, µg/mL; in-vivo test, mg/kg bw/day; ip, intraperitoneal; po, oral
[c] Active only with 30% hamster liver S9; not with rat liver S9
[d] S9 from phenobarbital-treated rats
[e] Activity detected in YEPD medium
[f] Acute feeding
[g] Toxicity higher in the presence of S9
[h] Activation by co-cultivation with X-irradiated primary rat hepatocytes
[i] Growth of V79 (T2-14) 6-thioguanine-resistant cells

Table 6. Genetic and related effects of metabolites of *ortho*-toluidine

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
***N*-Hydroxy-*ortho*-toluidine**				
Salmonella typhimurium TA100, reverse mutation	–	+	0.16 µg/plate	Gupta *et al*. (1987)
Salmonella typhimurium TA98, reverse mutation	–	–	0.62 µg/plate	Gupta *et al*. (1987)
***N*-Acetyl-*ortho*-toluidine**				
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	3.75 µg/plate	Gupta *et al*. (1987)
***N*-Acetyl-*N*-hydroxy-*ortho*-toluidine**				
Salmonella typhimurium TA100, reverse mutation	–	–	4.1 µg/plate	Gupta *et al*. (1987)
Salmonella typhimurium TA98, reverse mutation	–	+	2.1 µg/plate	Gupta *et al*. (1987)
***N*-Acetoxy-*N*-acetyl-*ortho*-toluidine**				
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	5.2 µg/plate	Gupta *et al*. (1987)

[a] +, positive; –, negative

[b] LED, lowest effective dose; HID, highest ineffective dose

It gave a positive result in one out of seven assays for reverse mutation. However, it caused aneuploidy in two out of three assays. Assays for forward mutation or genetic crossing-over in *Aspergillus nidulans* gave completely negative results, as did a forward mutation assay in *Schizosaccharomyces pombe*.

ortho-Toluidine caused DNA strand breakage in various animal cell lines *in vitro*, apparently in the absence of exogenous metabolic activation. The alkaline single-cell gel electrophoresis ('comet') assay revealed DNA breakage after *ortho*-toluidine treatment in a metabolically competent human mammary cell line, MCL-5, as well as in primary cultures of cells isolated from human breast milk. The response was substantially increased when the cells were incubated in the presence of the DNA repair inhibitors, hydroxyurea and cytosine arabinoside. Only one out of eight studies showed that treatment with *ortho*-toluidine could lead to unscheduled DNA synthesis. A single study suggested a weak positive effect in gene mutation at the *Hprt* locus in V79 Chinese hamster cells, although two other similar studies gave negative results. *ortho*-Toluidine failed to cause mutation to ouabain resistance in V79 Chinese hamster cells. Two out of five studies suggested a positive response at the *Tk* locus but not usually at other loci in mouse lymphoma L5178Y cells. However, there are isolated reports of *ortho*-toluidine increasing gene mutations at loci other than *Tk* in L5178Y cells or in other animal cells *in vitro*, but only in the presence of exogenous metabolic activation.

There have been occasional reports of *ortho*-toluidine causing chromosomal aberrations or micronuclei in various cultured cell lines. Manifestation of these effects seems to require incubation times longer than 3 h. In some of these studies, S9 mix was required, while in others it appeared to reduce the effect. Most studies of effects on sister chromatid exchanges, in either animal or human cells, have revealed positive results, even in the absence of exogenous metabolic activation. *ortho*-Toluidine caused aneuploidy in mammalian cells *in vitro*, and increased cell transformation in all but one of 11 studies. The latter effects did not generally appear to require exogenous metabolic activation, although it should be noted that the cell types have some endogenous metabolic capability. In two out of three studies, *ortho*-toluidine inhibited intracellular communication.

A number of in-vivo studies have been conducted. *ortho*-Toluidine gave a positive result in a host-mediated assay for bacterial mutagenesis. It increased somatic mutation but not genetic crossing-over in *Drosophila melanogaster*, and enhanced sister chromatid exchanges in rodent models. Only one out of four studies in mice, but one study in a newt model, suggested that it enhanced micronucleus frequency. Studies on sperm morphology have given equivocal data.

Metabolites of ortho-*toluidine*

Gupta *et al*. (1987) synthesized the various *N*-oxidized putative metabolites and esters of *ortho*-toluidine and tested them for mutagenic activity in the *Salmonella* microsome mutagenicity assay. On the basis of these data, they proposed a metabolic

pathway involving nitrenium ion/nitrene/free radicals which could bind covalently to DNA (see Table 6).

4.5 Mechanistic considerations

ortho-Toluidine undergoes extensive metabolism *in vivo* and, like other aromatic amines, *N*-hydroxylation is thought to be the first step in its metabolic activation.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

ortho-Toluidine and its hydrochloride salt have been widely produced commercially throughout the twentieth century for use in manufacture of dyestuffs, pigments, optical brighteners, rubber chemicals, pharmaceuticals and pesticides. Human exposure has been reported during its use in production of dyestuffs and rubber chemicals. Non-occupational exposure to *ortho*-toluidine may result from its occurrence in certain foods and from exposure to tobacco smoke.

5.2 Human carcinogenicity data

Five studies were available for evaluation. Two mortality studies were conducted in the 1980s among dye production workers in Italy and in the United States. In each case, the subgroups of workers exposed to *ortho*-toluidine were small. Two recent cohort studies in Germany, the United Kingdom and a larger study in the United States among workers in 4-chloro-*ortho*-toluidine production and in rubber chemical manufacturing looked at bladder cancer incidence. Of these five studies, four observed a very high excess of bladder cancer among *ortho*-toluidine-exposed workers. The fifth study had limited power to detect a risk. In the two studies with data on duration of exposure to *ortho*-toluidine, the highest risk was observed in the subgroup with the longest duration of exposure. In none of these studies, however, could confounding by concomitant exposure to various other potential bladder carcinogens be ruled out with confidence, although co-exposures differed between studies.

5.3 Animal carcinogenicity data

ortho-Toluidine was tested for carcinogenicity as its hydrochloride salt in two experiments in mice and in three experiments in rats and as the free base in one limited experiment in hamsters. After oral administration to mice, it induced an increased incidence of haemangiomas and haemangiosarcomas and hepatocellular carcinomas

or adenomas. In rats, oral administration of *ortho*-toluidine increased the incidence of tumours in multiple organs, including fibromas, sarcomas, mesotheliomas, mammary fibroadenomas and transitional-cell carcinomas of the urinary bladder.

5.4 Other relevant data

ortho-Toluidine undergoes extensive metabolism *in vivo*, with the bulk of the dose being excreted in the urine within 24 h. Like other aromatic amines, it is thought to undergo metabolic activation initially via *N*-hydroxylation, leading to covalent binding to tissue macromolecules. Evidence that *ortho*-toluidine undergoes metabolic activation *in vivo* is supported by the fact that it forms both haemoglobin and albumin adducts in laboratory animals and haemoglobin adducts in humans.

In rats, repeated administration of *ortho*-toluidine led to haemosiderosis, splenic congestion, bone marrow and splenic proliferation and splenic fibrosis consistent with a response to erythrocyte destruction.

Bacterial or bacteriophage assay systems showed negative or inconsistent data or, at most, weakly positive results. In the yeast *Saccharomyces cerevisiae*, *ortho*-toluidine caused reverse mutation at some loci and occasionally recombinational events. It caused gain or loss of whole chromosomes and mutation of mitochondrial DNA. In cultured mammalian cells, it generally caused sister chromatid exchanges and sometimes also increased gene mutations, chromosomal aberrations and micronuclei. It induced aneuploidy and increased cell transformation in such cells. *ortho*-Toluidine may inhibit intercellular communication. It has been demonstrated to be a mutagen but not a recombinogen in *Drosophila melanogaster*. In rodent models *in vivo*, it enhanced sister chromatid exchanges but gave equivocal results for micronuclei and sperm morphology.

5.5 Evaluation

There is *limited evidence* in humans for the carcinogenicity of *ortho*-toluidine.

There is *sufficient evidence* in experimental animals for the carcinogenicity of *ortho*-toluidine.

Overall evaluation

ortho-Toluidine is *probably carcinogenic to humans (Group 2A)*.

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4-CHLORO-*ortho*-TOLUIDINE

This substance was considered by previous working groups in June 1977 (IARC, 1978), June 1982 (IARC, 1983), March 1987 (IARC, 1987a) and February 1989 (IARC, 1990). Since that time, new data have become available, and these have been incorporated in the monograph and taken into consideration in the evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

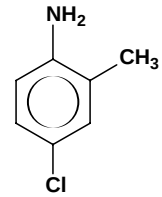
4-Chloro-*ortho*-toluidine

Chem. Abstr. Serv. Reg. No.: 95-69-2
Chem. Abstr. Name: 4-Chloro-2-methylbenzenamine
IUPAC Systematic Name: 4-Chloro-ortho-toluidine
Synonyms: 2-Amino-5-chlorotoluene; 3-chloro-6-aminotoluene; 5-chloro-2-amino-toluene; 4-chloro-2-methylaniline; 4-chloro-6-methylaniline; 4-chloro-2-toluidine; *para*-chloro-*ortho*-toluidine; 2-methyl-4-chloroaniline; 2-methyl-4-chlorobenzene-amine

4-Chloro-*ortho*-toluidine hydrochloride

Chem. Abstr. Serv. Reg. No.: 3165-93-3
Chem. Abstr. Name: 4-Chloro-2-methylbenzenamine hydrochloride
IUPAC Systematic Name: 4-Chloro-*ortho*-toluidine hydrochloride
Synonyms: 4-Chloro-2-methylaniline hydrochloride; C.I. Azoic Diazo Component 11; 2-methyl-4-chloroaniline hydrochloride; *para*-chloro-*ortho*-toluidine hydro-chloride

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_7H_8ClN Relative molecular mass: 141.6

Hydrochloride

$C_7H_8ClN.HCl$ Relative molecular mass: 178.07

1.1.3 *Chemical and physical properties of the pure substance*

4-Chloro-*ortho*-toluidine

(*a*) *Description*: Crystalline solid (Lewis, 1993)
(*b*) *Boiling-point*: 244 °C (Lide, 1999)
(*c*) *Melting-point*: 30.3 °C (Lide, 1999)
(*d*) *Spectroscopy data*: Infrared (grating [669], ultraviolet [5411], nuclear magnetic resonance (proton [558]) and mass [NIST 69505] spectral data have been reported (Lide & Milne, 1996)
(*e*) *Solubility*: Soluble in ethanol; slightly soluble in carbon tetrachloride (Lide, 1999)
(*f*) *Conversion factor*[1]: $mg/m^3 = 5.79 \times ppm$

4-Chloro-*ortho*-toluidine hydrochloride

(*a*) *Description*: Buff-coloured or light-pink powder (Department of Health and Human Services, 1999)
(*b*) *Conversion factor*[1]: $mg/m^3 = 7.28 \times ppm$

1.1.4 *Technical products and impurities*

Trade names for 4-chloro-*ortho*-toluidine include: Daito Red Base TR; Fast Red Base TR; Fast Red 5CT Base; Fast Red TR Base; Fast Red TR-T Base; Fast Red TRO Base; Kako Red TR Base; Mitsui Red TR Base; Red Base NTR; Red TR Base; Sanyo Fast Red TR Base.

[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

1.1.5 *Analysis*

Gas chromatography (GC) with alkali flame ionization, thin-layer chromatography and GC with electron capture detection (ECD)/flame ionization detection have been used to determine 4-chloro-*ortho*-toluidine in food and plant materials (Kossman *et al.*, 1971; Fan & Ge, 1982). GC/ECD and reversed-phase high-performance liquid chromatography (HPLC) with ultraviolet detection have been used to determine 4-chloro-*ortho*-toluidine in human urine samples (Geyer & Fattal, 1987). Liquid chromatography with electrochemical detection has been used to determine haemoglobin adducts of 4-chloro-*ortho*-toluidine (Riffelmann *et al*., 1995).

1.2 Production

Commercial production of 4-chloro-*ortho*-toluidine began in Germany in 1924 (Uebelin & Pletscher, 1954) and was first reported in the United States in 1939 (Tariff Commission, 1940). It has been sold as both the free amine and the hydrochloride salt (Tariff Commission, 1940, 1945). Production of both forms has been discontinued in most countries (Stasik, 1988; Environmental Protection Agency, 1988).

Information available in 1999 indicated that 4-chloro-*ortho*-toluidine was manufactured by two companies in China (Chemical Information Services, 1999).

1.3 Use

4-Chloro-*ortho*-toluidine and its hydrochloride salt have been used to produce azo dyes *in situ* on cotton, silk, acetate and nylon and as intermediates in the production of Pigment Red 7 and Pigment Yellow 49 (Society of Dyers and Colourists, 1971; Environmental Protection Agency, 1988). 4-Chloro-*ortho*-toluidine was also used from the 1960s until the 1980s in the manufacture of chlordimeform [*N*′-(4-chloro-2-methylphenyl)-*N*,*N*-dimethylformamide; see IARC, 1983], an acaricide and insecticide that is believed to be no longer produced or used worldwide (WHO, 1998).

1.4 Occurrence

1.4.1 *Natural occurrence*

4-Chloro-*ortho*-toluidine and its hydrochloride salt are not known to occur as natural products.

1.4.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1999), 250 chemists employed in health services in the United States were potentially exposed to 4-chloro-*ortho*-toluidine (see General Remarks). Only three laboratory workers were

identified as exposed to 4-chloro-*ortho*-toluidine or its salts by the Finnish Register of Employees Exposed to Carcinogens in 1997 (Savela *et al*., 1999). No data on the extent of exposure in the manufacture of 4-chloro-*ortho*-toluidine, textile dyes, pigments or insecticide chlordimeform or in the use of 4-chloro-*ortho*-toluidine-based dyes were available to the Working Group.

4-Chloro-*ortho*-toluidine is a major metabolite of chlordimeform and it has been detected in the urine of workers exposed to chlordimeform in packaging and agriculture (Folland *et al*., 1978; Geyer & Fattal, 1987). Exposure to 4-chloro-*ortho*-toluidine and chlordimeform were measured in the urine of chlordimeform production workers and was reported to be minimal after substantial improvement of working conditions in a chlordimeform-manufacturing plant in 1980 (Popp *et al*., 1992). Stasik (1991) reported that 4-chloro-*ortho*-toluidine was detected in the urine of two workers at a chemical plant 12 and 24 h after exposure at concentrations of 1.7 and 2.1 mg/L, respectively.

1.4.3 *Environmental occurrence*

The principal source of 4-chloro-*ortho*-toluidine in the environment was as an impurity and as a decomposition (metabolic) product of chlorodimeform. This pesticide is no longer produced (WHO, 1998).

(*a*) *Water*

4-Chloro-*ortho*-toluidine occurred in water as a result of the hydrolysis of chlordimeform via hydrolysis of the intermediate *N*-formyl-4-chloro-*ortho*-toluidine (WHO, 1998).

(*b*) *Soil*

The microbial degradation of chlordimeform in soils by a number of bacterial and fungal species has led to formation of 4-chloro-*ortho*-toluidine (Johnson & Knowles, 1970). When ^{14}C-labelled 4-chloro-*ortho*-toluidine was incubated at a concentration of 5 ppm [mg/kg] in non-autoclaved soil, almost 20% of 4-chloro-*ortho*-toluidine was metabolized to carbon dioxide within 40 days (Bollag *et al*., 1978).

(*c*) *Plants and foods*

4-Chloro-*ortho*-toluidine has been identified in field samples of plant materials treated with chlordimeform, e.g., in young bean leaves at concentrations of less than 0.1–0.2 ppm [mg/kg], in grape stems at 0.02–0.3 ppm [mg/kg], in a mixture of grape stems and berries at 0.02–0.5 ppm [mg/kg] and in prunes and apples at less than 0.04 ppm [mg/kg] (Kossmann *et al*., 1971). In an experimental field application (one to three treatments with chlordimeform, harvesting 42 days after last treatment), 4-chloro-*ortho*-toluidine was found in rice grains at 3–61 ppb [µg/kg] and in straw parts at 80–7200 ppb [µg/kg] (Iizuka & Masuda, 1979). 4-Chloro-*ortho*-toluidine was detected as a metabolic product in cotton plants following treatment with chlordimeform (Bull, 1973).

1.5 Regulations and guidelines

4-Chloro-*ortho*-toluidine is listed as a carcinogen in Finland, Germany (Class 1 substances that cause cancer in man) and Switzerland. Switzerland has set an occupational exposure limit (time-weighted average) [8 h time-weighted average] of 12 mg/m^3 with a skin irritation notation for 4-chloro-*ortho*-toluidine (American Conference of Governmental Industrial Hygienists, 1999; Deutsche Forschungsgemeinschaft, 1999).

2. Studies of Cancer in Humans

2.1 Cohort studies

Two epidemiological studies (Ott & Langner, 1983; Stasik, 1988) concerning workers exposed to both 4-chloro-*ortho*-toluidine and *ortho*-toluidine are more fully described in the monograph on *ortho*-toluidine in this volume.

Ott and Langner (1983) studied the mortality of 342 employees assigned to three aromatic amine-based dye production areas from 1914 to 1958 in the United States. One of these areas, the bromoindigo and thioindigo production area, involved potential exposure to *ortho*-toluidine, 4-chloro-*ortho*-toluidine and 4-chloro-acetyl-*ortho*-toluidine. In a separate analysis of 275 individuals not exposed to arsenicals, vinyl chloride or asbestos, no deaths due to bladder cancer were observed, with 1.2 deaths expected from malignant neoplasms of the urinary organs. There were 23 deaths due to malignant neoplasms (17.5 expected; standardized mortality ratio (SMR), 1.3; [95% confidence interval (CI), 0.8–2.0]), 10 of which were coded to digestive organs (5.7 expected; SMR, 1.8; [95% CI, 0.8–3.2]). [The Working Group noted that the conclusions of this study were limited by the small size of the population exposed to 4-chloro-*ortho*-toluidine and the ascertainment of only deceased and not incident bladder cancer cases.]

In a historical mortality study of 335 male employees involved in the production and processing of 4-chloro-*ortho*-toluidine between 1929 and 1982 in Essen, Germany, no deaths from bladder cancer were identified. Four monocyclic amines had been used at the plant: *N*-acetyl-*ortho*-toluidine, 6-chloro-*ortho*-toluidine, *ortho*-toluidine and 4-chloro-*ortho*-toluidine; exposure to 4-chloro-*ortho*-toluidine was reported to be predominant. Urothelial carcinomas were subsequently recorded in eight of the employees between 1967 and 1985, two of whom had died as of December 1986. All eight had been employed in the 4-chloro-*ortho*-toluidine production plant before improvements in industrial hygiene were made in 1970. As a result of this discovery, an incidence study was initiated and the vital status ascertainment for 116 subjects with exposure before 1970 was extended through 1986. The expected number of incident bladder cancers in the cohort of 116 men was 0.11. The standardized incidence ratio (SIR) based on eight

observed cases from 1967 to 1985 was 72.7 (95% CI, 31.4–143) (Stasik, 1988). [The Working Group noted that the definition of the subcohort was made *a posteriori*, but this was justified by the authors'comment that improvements in industrial hygiene were introduced in 1970. It was also unclear in what year the follow-up started. The excess of bladder cancer could not be attributed with certainty specifically to 4-chloro-*ortho*-toluidine or to any one of the other compounds present.]

Popp *et al*. (1992) reported the results of a bladder cancer incidence study conducted among a group of 49 male workers exposed to 4-chloro-*ortho*-toluidine in the synthesis of chlordimeform (evaluated as not classifiable as to its carcinogenicity to humans, Group 3 (IARC, 1987b)) from 1950 to 1986 in a German chemical plant. The period of follow-up was 1950–90. Seven cases of bladder cancer were identified between 1982 and 1990 in workers exposed to 4-chloro-*ortho*-toluidine while synthesizing chlordimeform before 1976, when working conditions were improved. Expected numbers of bladder cancers were calculated based on cancer registry data from the former German Democratic Republic, Denmark and Saarland. SIRs based on the three estimates of expected numbers of deaths were 89.7 (95% CI, 35.6–168.6) (German Democratic Republic), 35.0 (95% CI, 13.9–65.7) (Denmark) and 53.8 (95% CI, 21.3–101.1) (Saarland). No bladder tumours were noted among individuals exposed only to the final product, chlordimeform; however, the size of this group is unknown. The only other aromatic amine to which there was potential exposure was 4-chloroaniline (classified as possibly carcinogenic to humans, Group 2B (IARC, 1993)), which was used for appreciably shorter periods and in smaller quantities than 4-chloro-*ortho*-toluidine. [The Working Group noted that the nature of the facility and the methods of this study were not fully described. Concomitant exposure to chlordimeform and 4-chloroaniline could not be excluded as confounders. The Working Group considered that the excesses of bladder cancer reported were too large to have been due to smoking alone.]

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 25 male and 25 female Swiss CD-1 mice, four to six weeks of age, were fed 4-chloro-*ortho*-toluidine hydrochloride (97–99% pure) in the diet at dose levels of 0, 750 or 1500 mg/kg diet (ppm) (males) or 0, 2000 or 4000 ppm (females) for 18 months. Animals were kept without treatment for three further months and then killed. The doses were chosen on the basis of preliminary tests, the higher being the maximum tolerated dose. Additional control groups were prepared for the other compounds tested in the study, and tumour incidences of concurrent and pooled controls were compared statistically (both separately and together) with those of treated groups. Animals that

died during the first six months of the study were discarded without necropsy. In male mice, the incidence of vascular tumours (haemangiomas and haemangiosarcomas combined, observed mainly in the spleen and in the subcutaneous or subperitoneal adipose tissue) was 0/14, 5/99, 12/20 ($p < 0.025$, Fisher's exact test) and 13/20 ($p < 0.025$, Fisher's exact test) in concurrent controls, pooled controls, low-dose and high-dose groups, respectively. In female mice, the incidence of vascular tumours (haemangiomas and haemangiosarcomas combined) was 0/15, 9/102, 18/19 ($p < 0.025$, Fisher's exact test) and 12/16 ($p < 0.025$, Fisher's exact test) in concurrent controls, pooled controls, low-dose and high-dose groups, respectively [separate incidences for haemangiomas and haemangiosarcomas were not reported] (Weisburger *et al*., 1978).

Groups of 50 male and 50 female B6C3F$_1$ mice, six weeks of age, were administered 4-chloro-*ortho*-toluidine (purity, > 99%) in the diet at concentrations of 3750 or 15 000 ppm (males) and 1250 or 5000 ppm (females) for 99 weeks, except high-dose females which had all died by 92 weeks. Concurrent controls consisted of 20 male and 20 female untreated mice. Mean body weights of all treated groups were lower than those of the corresponding controls and were dose-related. Mortality was dose-related for both males and females ($p < 0.001$, Tarone test for dose-related trend). In male mice, the incidence of haemangiosarcomas (originating in the adipose tissue adjacent to the genital organs) was 0/20, 3/50 and 37/50 ($p < 0.025$, Fisher's exact test) in control, low- and high-dose groups, respectively. In female mice, the incidence of haemangiosarcomas was 0/18, 40/49 ($p < 0.025$, Fisher's exact test) and 39/50 ($p < 0.025$, Fisher's exact test) in control, low- and high-dose groups, respectively (National Cancer Institute, 1979).

3.1.2 *Rat*

Groups of 25 male Sprague-Dawley CD rats, four to six weeks of age, were treated with 4-chloro-*ortho*-toluidine hydrochloride (97–99% pure) in the diet at dose levels of 2000 or 4000 ppm for three months and then at levels of 500 or 1000 ppm for a further 15 months. Animals were kept without treatment for an additional six months and then killed. The doses were chosen on the basis of preliminary tests, the higher being the maximum tolerated dose. A concurrent control group of 25 untreated male rats was used, plus additional controls used for the other compounds tested in the study, and tumour incidences of matched and pooled controls were compared with those of treated groups. Animals that died during the first six months of the study were discarded without necropsy. There was no treatment-related increase in the incidence of tumours at any site (Weisburger *et al*., 1978). [The Working Group noted the non-standard protocol and that no information on survival was given.]

Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were administered 4-chloro-*ortho*-toluidine (purity, > 99%) in the diet at concentrations of 1250 or 5000 ppm for 107 weeks. Concurrent controls consisted of 20 male and 20 female untreated rats. Mean body weights of the high-dose males and females were

lower than those of the corresponding controls. Mortality was not significantly affected by treatment in rats of either sex. At the end of the study, survival in all groups was more than 50% in males and more than 70% in females. No tumours occurred at incidences that could be related to the treatment (National Cancer Institute, 1979).

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

In Sprague-Dawley rats treated orally with 4-chloro-*ortho*-[^{14}C]toluidine, 71% of a 3 μCi (specific activity, 1.43 mCi/mmol) dose was excreted in the urine and 24.5% in the faeces after 72 h. In addition to 4-chloro-*ortho*-toluidine, the metabolites 5-chloro-anthranilic acid and 4-chloro-2-methylacetanilide were isolated from the urine. A number of other metabolites were isolated but their chemical structures were not determined. The highest levels of 4-chloro-*ortho*-[^{14}C]toluidine equivalents were found in the fat, liver and kidney (Knowles & Gupta, 1970). 4-Chloro-*ortho*-toluidine was given orally to female Wistar rats and female B6C3F$_1$ mice (0.5 mmol [71 mg]/kg bw) and found to bind to haemoglobin. It was concluded that binding of such monocyclic aromatic amines occurs via a reaction of the respective nitrosoarene metabolites with haemoglobin (Birner & Neumann (1988). In Osborne-Mendel rats administered 4-chloro-*ortho*-[*methyl*-^{14}C]toluidine intraperitoneally at a dose of 14 mg/kg bw, the level of adducts bound to protein, DNA and RNA was significantly higher in the liver than in 10 other tissues examined. In in-vitro studies, the major and minor microsomal metabolites were identified as 5-chloro-2-hydroxyaminotoluene and 4,4′-dichloro-2,2′-dimethylazobenzene, respectively. Binding to microsomal protein was increased in Osborne-Mendel rats pretreated with phenobarbital intraperitoneally at a dose of 100 mg/kg bw for two days, implying that cytochrome P450 is involved in the reaction (Hill *et al.*, 1979). Following oral administration of 25 mg/kg bw 4-chloro-*ortho*-toluidine to male Sprague-Dawley-derived [Tif:RAIf (SPF] rats and male mice [Tif:MAGf (SPF)] both from CIBA-GEIGY, covalent binding to both DNA and protein occurred (Bentley *et al.*, 1986a). Administration of 4-chloro-*ortho*-toluidine to male Sprague-Dawley rats induces a number of enzymes involved in xenobiotic metabolism: cytochrome P450, ethoxyresorufin-*O*-deethylase, ethoxycoumarin-*O*-deethylase, glutathione *S*-transferase and epoxide hydrolase (Leslie *et al.*, 1988).

4.2 Toxic effects

4.2.1 *Humans*

Toxic effects due to 4-chloro-*ortho*-toluidine result from inhalation or skin contact (Stasik, 1991). The first symptom of 4-chloro-*ortho*-toluidine toxicity is macroscopic or microscopic haematuria. Further symptoms include dysuria, reduced bladder capacity and generalized pain in the lower abdomen. Haemorrhagic cystitis is the leading symptom of acute toxicity.

Acute intoxications with 4-chloro-*ortho*-toluidine have occurred in workers in the chemical industry involved in the production and use of the compound. A single dermal exposure was sufficient to produce signs of toxicity. Endoscopic inspection of the urinary bladder revealed necrotic epithelial damage, bleeding and oedema. Methaemoglobinaemia was observed in about 50% of the intoxicated individuals (reviewed by Stasik, 1991). [The Working Group noted the incomplete reporting of these data.]

4.2.2 *Experimental systems*

The following LD_{50} values have been reported for intraperitoneally administered 4-chloro-*ortho*-toluidine: 720 mg/kg bw in male mice, 680 mg/kg bw in female mice, 560 mg/kg bw in male rats and 700 mg/kg bw in female rats (Weisburger *et al*., 1978). Administration of 4-chloro-*ortho*-toluidine in the diet for 99 weeks to B6C3F_1 mice (3750 and 15 000 ppm [mg/kg diet] for male mice; 1250 and 5000 ppm for female mice) led to a high incidence of haemosiderin deposit in the renal tubular epithelium, particularly in mice with haemangiosarcoma (National Cancer Institute, 1979).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

[The Working Group noted that many aromatic amines, including 4-chloro-*ortho*-toluidine, induce methaemoglobinaemia (Watanabe *et al*., 1976; Sachsse *et al*., 1980, cited in WHO 1998; Stasik, 1991; Coleman & Coleman, 1996). The effect of methaemoglobinaemia on fetal development has not been well studied, but may be associated with suboptimal fetal outcome (Fan & Steinberg, 1996; Kilpatrick & Laros, 1999).]

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

The data available up to 1990 and 1993 have been reviewed by IARC (1990) and Jackson *et al.* (1993), respectively.

4-Chloro-*ortho*-toluidine has been tested in a number of laboratories with a range of *Salmonella* strains, and in general the results have been negative. There were single positive results with TA100 and TA98 (with metabolic acivation) and with TA1535 (without metabolic activation). Mutation tests (with and without activation) and DNA repair assays (without activation only) in *Escherichia coli* were negative, while a positive response was observed for differential toxicity in *S. typhimurium*, indicating DNA damage.

However, positive results have been obtained in several tests in mammalian systems, including induction of DNA strand breaks *in vitro*, unscheduled DNA synthesis in rat primary hepatocytes, sister chromatid exchanges and chromosomal aberrations (only in the presence of an external metabolizing system) in Chinese hamster cells *in vitro*, and transformation of BALB/c 3T3 mouse cells. The mouse spot test was also positive. 4-Chloro-*ortho*-toluidine bound to hepatic DNA and RNA in mice and rats *in vivo*, with a greater extent of binding in mice than in rats. In contrast, it gave negative results in both the sister chromatid exchange and chromosomal aberration assays in human lymphocytes *in vitro* and in the mouse heritable translocation assay *in vivo*.

4.5 Mechanistic considerations

Like other aromatic amines 4-chloro-*ortho*-toluidine has been shown to undergo metabolic activation resulting in covalent binding to tissue proteins, DNA and RNA both *in vivo* and *in vitro* (Hill *et al*., 1979; Bentley *et al*., 1986a,b; Birner & Neumann, 1988).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

4-Chloro-*ortho*-toluidine and its hydrochloride salt were produced commercially in substantial amounts as intermediates in the manufacture of azo dyes and chlordimeform, an insecticide. Since the 1980s, production and use of 4-chloro-*ortho*-toluidine have been discontinued in most countries.

5.2 Human carcinogenicity data

Three small cohort studies of workers exposed to 4-chloro-*ortho*-toluidine, one each among dye, 4-chloro-*ortho*-toluidine and chlordimeform production workers, were

Table 1. Genetic and related effects of 4-chloro-*ortho*-toluidine

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium, repair-deficient strains, TA1538, TA1978, differential toxicity	+	NT	250 mg/disc	Rashid *et al*. (1984)
Escherichia coli rec strains, differential toxicity	+	NT	1000 mg/disc	Rashid *et al*. (1984)
Salmonella typhimurium TA100, reverse mutation[c]	–	+	17.8 µg/plate	Zimmer *et al*. (1980)
Salmonella typhimurium TA100, reverse mutation[c]	–	–	333 µg/plate	Haworth *et al*. (1983)
Salmonella typhimurium TA1535, TA1537, TA98, reverse mutation[c]	–	–	1000 µg/plate	Haworth *et al*. (1983)
Salmonella typhimurium TA100, TA1537, TA1538, TA98, reverse mutation[c]	–	NT	325 µg/plate	Rashid *et al*. (1984)
Salmonella typhimurium TA100, reverse mutation	–	+	100 µg/plate	Göggelmann *et al*. (1996)
Salmonella typhimurium TA1535, reverse mutation[c]	+	NT	200 µg/plate	Rashid *et al*. (1984)
Salmonella typhimurium TA1535, TA1537, reverse mutation	–	–	1500 µg/plate	Göggelmann *et al*. (1996)
Salmonella typhimurium TA1537, TA98, reverse mutation[c]	–	–	NR	Zimmer *et al*. (1980)
Salmonella typhimurium TA98, reverse mutation	–	+	375 µg/plate	Göggelmann *et al*. (1996)
Escherichia coli WP2 *uvrA*, WP2, other strains, reverse mutation[c]	–	–	2000 µg/plate	Rashid *et al*. (1984)
DNA strand breaks, cross-links or related damage, Chinese hamster V79 lung cells *in vitro*[c]	(+)	NT	534	Zimmer *et al*. (1980)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	+	NT	14.2	Williams *et al*. (1989)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*[c]	+	+	50	Galloway *et al*. (1987)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*[c]	–	+	400	Galloway *et al*. (1987)
Cell transformation, BALB/c 3T3 mouse cells[c]	+	NT	75	Matthews *et al*. (1993)
Sister chromatid exchange, human lymphocytes *in vitro*	–	–	283	Göggelmann *et al*. (1996)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, human lymphocytes *in vitro*	(+)	(+)	283	Göggelmann *et al.* (1996)
Mouse spot test, C57BL6J×T mice[c]	+		100 po × 3[d]	Lang (1984)
Mouse heritable translocation test, NMRI/SPF mice[c]	–		200 po; 7 d/w, 7 w	Lang & Adler (1982)
Binding (covalent) to DNA, rat and mouse liver *in vivo*[c]	+		25 po × 1	Bentley *et al.* (1986a)
Binding (covalent) to RNA or protein, rat and mouse liver *in vivo*[c]	+		25 po × 1	Bentley *et al.* (1986b)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; NR, not reported
[c] Test performed with the hydrochloride salt of 4-chloro-*ortho*-toluidine
[d] Treatments on days 8, 9 and 10 of embryonic development

available. Two of them showed high relative risks of bladder cancer. Despite problems in the cohort definitions in these two studies, the high relative risks observed for bladder cancer most likely represent a true excess. However, confounding cannot be excluded due to the presence of other exposures including potential bladder carcinogens. The third study had limited power to detect a risk due to use of mortality data only in a small cohort.

5.3 Animal carcinogenicity data

4-Chloro-*ortho*-toluidine or its hydrochloride was tested for carcinogenicity by oral administration in two experiments in mice and in two experiments in rats. The compounds increased the incidence of haemangiosarcomas in the spleen and adipose tissue in both male and female mice, but no increase in the incidence of tumours was observed in rats.

5.4 Other relevant data

4-Chloro-*ortho*-toluidine undergoes extensive metabolism in rodents *in vivo*. Like other aromatic amines, it undergoes metabolic activation via initial formation of the *N*-hydroxy derivative. The further metabolic processing of this metabolite has not been investigated.

In humans, 4-chloro-*ortho*-toluidine induces acute toxicity in the urinary bladder and causes methaemoglobinaemia. In rodents, 4-chloro-*ortho*-toluidine and/or its metabolites bind to macromolecules in liver cells.

4-Chloro-*ortho*-toluidine gave variable results in the majority of bacterial tests for mutagenicity. While most of the mammalian tests were positive, chromosomal aberration assays gave conflicting results. These data overall indicate that 4-chloro-*ortho*-toluidine causes DNA damage in mammalian cells.

5.5 Evaluation

There is *limited evidence* in humans for the carcinogenicity of 4-chloro-*ortho*-toluidine.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 4-chloro-*ortho*-toluidine.

Overall evaluation

4-Chloro-*ortho*-toluidine is *probably carcinogenic to humans (Group 2A)*.

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5-CHLORO-*ortho*-TOLUIDINE

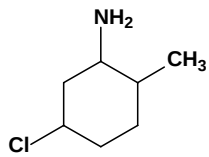
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 95-79-4
Chem. Abstr. Name: 5-Chloro-2-methylbenzenamine
IUPAC Systematic Name: 5-Chloro-*ortho*-toluidine
Synonyms: 2-Amino-4-chlorotoluene; 4-chloro-2-aminotoluene; 3-chloro-6-methylaniline; 5-chloro-2-methylaniline; 5-chloro-2-toluidine; 2-methyl-5-chloroaniline

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_7H_8ClN Relative molecular mass: 141.6

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Off-white solid or light brown oil which tends to darken on storage (Lewis, 1993)
(*b*) *Boiling-point*: 239 °C (Lide & Milne, 1996)
(*c*) *Melting-point*: 26 °C (Lide & Milne, 1996)
(*d*) *Spectroscopy data*: Infrared [COB, 2315], ultraviolet [1302] and mass [NIST, 71121] spectral data have been reported (Lide & Milne, 1996)
(*e*) *Solubility*: Very soluble in ethanol (Lide & Milne, 1996)

(*f*) *Conversion factor*[1]: $mg/m^3 = 5.79 \times ppm$

1.1.4 *Technical products and impurities*

Trade names for 5-chloro-*ortho*-toluidine include: Acco Fast Red KB base; Ansibase Red KB; Azoene Fast Red KB base; Fast Red KB amine; Fast Red KB salt; Fast Red KB salt Supra; Fast Red KB-T Base; Fast Red KBS salt; Genazo Red KB soln; Hiltonil Fast Red KB base; Metrogen Red Former KB soln; Naphthosol Fast Red KB base; Pharmazoid Red KB; Red KB base; Spectrolene Red KB; Stable Red KB base.

1.1.5 *Analysis*

No methods have been reported for the analysis of 5-chloro-*ortho*-toluidine in environmental matrices.

1.2 Production

Information available in 1999 indicated that 5-chloro-*ortho*-toluidine was manufactured by three companies in China and by one company each in Germany, India, Japan and the United Kingdom (Chemical Information Services, 1999).

1.3 Use

5-Chloro-*ortho*-toluidine is used as a dye intermediate in the synthesis of Pigment Red 11, Pigment Yellow 77 and for Azoic Coupling Component 21. It is also used as a dye for cotton, silk and nylon (National Library of Medicine, 1999)

1.4 Occurrence

1.4.1 *Natural occurrence*

5-Chloro-*ortho*-toluidine is not known to occur as a natural product.

1.4.2 *Occupational exposure*

No data were available to the Working Group.

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[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

1.4.3 *Environmental occurrence*

5-Chloro-*ortho*-toluidine is produced in relatively small volume as a dye intermediate and thus may be released into the environment in various waste streams as a result of its production, distribution and use. However, no data on its occurrence in the environment were available that would permit assessment of exposures by the Working Group.

1.5 Regulations and guidelines

5-Chloro-*ortho*-toluidine is listed as a Class 3 carcinogenic substance in Germany; these are substances which cause concern that they could be carcinogenic for man but which cannot be assessed conclusively because of lack of data (Deutsche Forschungsgemeinschaft, 1999).

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 B6C3F_1 mice, six weeks of age, were administered 5-chloro-*ortho*-toluidine (technical grade, chromatography showing a single homogeneous peak) in the diet at concentrations 2000 or 4000 mg/kg diet (ppm) for 78 weeks, followed by an observation period of 13 weeks. Concurrent controls consisted of 20 male and 20 female untreated mice. Mean body weights of treated groups of each sex were lower than those of the corresponding control groups. Mortality was dose-related for each sex ($p < 0.001$ and $p = 0.039$ for male and female groups, respectively, Tarone test for dose-related trend). In male mice, the incidence of haemangiosarcomas (mostly originating in the adipose tissue adjacent to the genital organs) was 1/20, 11/50 and 37/47 ($p < 0.001$, Fisher's exact test; $p < 0.001$, trend test) in control, low- and high-dose groups, respectively. In female mice, the incidence of haemangiosarcomas (mostly originating in the adipose tissue adjacent to the genital organs) was 0/20, 6/50 and 22/43 ($p < 0.001$, Fisher's exact test; $p < 0.001$, Cochran-Armitage trend test) in control, low- and high-dose groups, respectively. In male mice, the incidence of hepatocellular carcinomas was 4/20, 19/50 and 25/47 ($p = 0.011$, Fisher's exact test; $p = 0.007$, trend

test) in control, low- and high-dose groups, respectively. In female mice, the incidence of hepatocellular carcinomas was 0/20, 19/50 ($p < 0.001$, Fisher's exact test) and 26/43 ($p < 0.001$, Fisher's exact test; $p < 0.001$, trend test) in control, low- and high-dose groups, respectively (National Cancer Institute, 1979).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, six weeks of age, were administered 5-chloro-*ortho*-toluidine (technical grade, chromatography showing a single homogeneous peak) in the diet at concentrations of 2500 or 5000 ppm for 78 weeks, followed by an observation period of 25–26 weeks. Concurrent controls consisted of 20 male and 20 female untreated rats. Mean body weights of low- and high-dose females were lower than those of the corresponding control group. Mortality was not affected by treatment in either sex. Although a positive association between dose and the incidence of phaeochromocytomas of the adrenal gland was observed in male rats (control, 0/20; low-dose, 2/49; high-dose, 7/48; $p = 0.019$, trend test), neither of the Fisher's exact tests for comparison of the treated groups with the control group showed statistical significance (National Cancer Institute, 1979). [The Working Group noted the small numbers of animals in the control group.]

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

5-Chloro-*ortho*-toluidine was given orally to female Wistar rats and female B6C3F_1 mice (0.5 mmol [71 mg]/kg bw) and found to bind covalently to haemoglobin. It was concluded that binding of such monocyclic aromatic amines occurs via a reaction of the respective nitrosoarene metabolites with haemoglobin. The haemoglobin binding index of 5-chloro-*ortho*-toluidine was 28-fold higher in rats than in mice. This difference may reflect the proportions of the dose of 5-chloro-*ortho*-toluidine that undergo *N*-oxidation in rats compared with mice (Birner & Neumann, 1988).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

5-Chloro-*ortho*-toluidine given orally to male mice at a dose of 200 mg/kg bw inhibited testicular DNA synthesis, as measured by tritiated thymidine incorporation (Seiler, 1977).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

[The Working Group noted that many aromatic amines induce methaemglobinaemia (Watanabe *et al*., 1976; Coleman & Coleman, 1996). The effect of methaemoglobinaemia on fetal development has not been well studied, but may be associated with suboptimal fetal outcome (Fan & Steinberg, 1996; Kilpatrick & Laros, 1999).]

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 1 for references)

There are few data available on the genetic toxicology of 5-chloro-*ortho*-toluidine. In single assays, it did not induce mutagenicity in *Salmonella typhimurium*, prophage lambda in *Escherichia coli* or unscheduled DNA synthesis in cultured rat hepatocytes.

4.5 Mechanistic considerations

Like other aromatic amines, 5-chloro-*ortho*-toluidine undergoes an initial metabolic activation step, probably *N*-oxidation, to form a nitrosoarene that can bind covalently to haemoglobin.

Ashby and Tennant (1988) have identified this chemical as showing structural features predictive of genotoxicity. No genotoxic effects were seen in three different assays, but the data-set is inadequate to allow firm conclusions.

Table 1. Genetic and related effects of 5-chloro-*ortho*-toluidine

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Prophage induction, SOS repair, DNA strand breaks or cross-links	–	–	4.4	DeMarini & Brooks (1992)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	666 µg/plate	Haworth *et al*. (1983)
Cell transformation, BALB/c 3T3 mouse cells	+	NT	160	Matthews *et al.* (1993)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	14.2	Yoshimi *et al*. (1988)

[a] –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro test, µg/mL

5. Summary of Data Reported and Evaluation

5.1 Exposure data

5-Chloro-*ortho*-toluidine is an aromatic amine which is produced in relatively small quantities as an intermediate in the manufacture of some pigments and azo dyes. No data were available on human exposure.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

5-Chloro-*ortho*-toluidine was tested for carcinogenicity by oral administration in one experiment in mice and in one experiment in rats. In mice, it increased the incidence of haemangiosarcomas (mostly of adipose tissue) and of hepatocellular carcinomas in both males and females. In rats, no carcinogenic effect was observed.

5.4 Other relevant data

5-Chloro-*ortho*-toluidine undergoes an initial metabolic activation step, probably via *N*-oxidation, to form a nitrosoarene that can bind covalently to haemoglobin.

The few available genotoxicity test results on 5-chloro-*ortho*-toluidine were negative.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of 5-chloro-*ortho*-toluidine were available.

There is *limited evidence* in experimental animals for the carcinogenicity of 5-chloro-*ortho*-toluidine.

Overall evaluation

5-Chloro-*ortho*-toluidine is *not classifiable as to its carcinogenicity to humans (Group 3)*.

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Yoshimi, N., Sugie, S., Iwata, H., Niwa, K., Mori, H., Hashida, C. & Shimizu, H. (1988) The genotoxicity of a variety of aniline derivatives in a DNA repair test with primary cultured rat hepatocytes. *Mutat. Res.*, **206**, 183–191

DIETHANOLAMINE

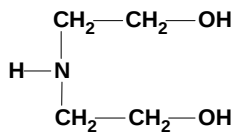
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 111-42-2
Deleted CAS Reg. No.: 8033-73-6
Chem. Abstr. Name: 2,2′-Iminobis[ethanol]
IUPAC Systematic Name: 2,2′-Iminodiethanol
Synonyms: Bis(hydroxyethyl)amine; bis(2-hydroxyethyl)amine; *N*,*N*-bis(2-hydroxyethyl)amine; DEA; *N*,*N*-diethanolamine; 2,2′-dihydroxydiethylamine; di-(β-hydroxyethyl)amine; di(2-hydroxyethyl)amine; diolamine; 2-(2-hydroxyethyl-amino)ethanol; iminodiethanol; *N*,*N*′-iminodiethanol; 2,2′-iminodi-1-ethanol

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_4H_{11}NO_2$ Relative molecular mass: 105.14

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Deliquescent prisms; colourless, viscous liquid with a mild ammonia odour (Budavari, 1998; Dow Chemical Company, 1999)
(*b*) *Boiling-point*: 268.8 °C (Lide & Milne, 1996)
(*c*) *Melting-point*: 28 °C (Lide & Milne, 1996)
(*d*) *Density*: 1.0966 g/cm^3 at 20 °C (Lide & Milne, 1996)
(*e*) *Spectroscopy data*: Infrared (proton [5830]; grating [33038]), nuclear magnetic resonance (proton [6575]; C-13 [2936]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980; Lide & Milne, 1996)
(*f*) *Solubility*: Very soluble in water (954 g/L) and ethanol; slightly soluble in benzene and diethyl ether (Lide & Milne, 1996; Verschueren, 1996)

(*g*) *Volatility*: Vapour pressure, < 0.01 mm Hg [1.33 Pa] at 20 °C; relative vapour density (air = 1), 3.6; flash-point, 149 °C (Verschueren, 1996)
(*h*) *Stability*: Incompatible with some metals, halogenated organics, nitrites, strong acids and strong oxidizers (Dow Chemical Company, 1999)
(*i*) *Octanol/water partition coefficient (P)*: log P, –2.18 (Verschueren, 1996)
(*j*) *Conversion factor*[1]: $mg/m^3 = 4.30 \times ppm$

1.1.4 *Technical products and impurities*

Diethanolamine is commercially available with the following specifications: purity, 99.3% min.; monoethanolamine, 0.45% max.; triethanolamine (see monograph in this volume), 0.25% max.; and water content, 0.15% max. (Dow Chemical Company, 1998a). Diethanolamine is also available as a blend of 85% diethanolamine and 15% deionized water which is a low freeze-grade product for use in colder temperatures (Dow Chemical Company, 1998b).

1.1.5 *Analysis*

Diethanolamine can be determined in workplace air by drawing the air sample through aqueous hexanesulfonic acid and analysing by ion chromatography. The limit of detection for this method is 13 µg per sample (Eller, 1994).

Diethanolamine can be determined in water samples by gas chromatography (GC) and by high-performance liquid chromatography (HPLC) with fluorescence detection (Melnick *et al.*, 1994a,b; Pietsch *et al.*, 1997); in metalworking and cutting fluids by GC–mass selective detection of silylated derivatives, by isotachophoresis, by capillary zone electrophoresis with indirect ultraviolet detection, and by spectrophotometry (Kenyon *et al.*, 1993; Fernando, 1995; Schubert *et al.*, 1996; Sollenberg, 1997); and in cosmetics and pharmaceuticals by GC with flame ionization detection, by ion-exclusive chromatography, and by reversed-phase HPLC (Fukui *et al.*, 1992; Maurer *et al.*, 1996; Chou, 1998).

1.2 Production

Ethanolamines became available commercially in the early 1930s; they assumed steadily growing commercial importance as intermediates after 1945, because of the large-scale production of ethylene oxide. Since the mid-1970s, economical production of very pure, colourless ethanolamines has been possible. Ethanolamines are produced on an industrial scale exclusively by reaction of ethylene oxide (see IARC, 1994) with excess ammonia. This reaction takes place slowly but is accelerated by water. An

[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

anhydrous procedure uses a fixed-bed ion-exchange resin catalyst (Hammer *et al*., 1987).

Worldwide production of ethanolamines in 1985 was approximately (thousand tonnes per year): United States, 220; western Europe, 145; south-east Asia, 40; South America, 18; eastern Europe, 4. About 50% of world production of ethanolamines in 1985 was monoethanolamine, 30–35% diethanolamine and 15–20% triethanolamine (Hammer *et al*., 1987). Estimated annual production of diethanolamine in the United States is presented in Table 1.

Table 1. Estimated annual production of diethanolamine in the USA (thousand tonnes)

Year	1960	1965	1970	1975	1980	1985	1989[a]
Production	24	35	42	39	56	76	92

From Bollmeier (1992)
[a] National Toxicology Program (1992)

Information available in 1999 indicated that diethanolamine was manufactured by seven companies in the United States, three companies each in China and Germany, two companies each in France and India, and one company each in Belgium, Brazil, Canada, Iran, Japan, Mexico, Netherlands, the Russian Federation, Spain, Sweden and the United Kingdom (Chemical Information Services, 1999).

1.3 Use

Diethanolamine is used as surface-active agent in metal-cutting fluids and oils (see General Remarks), as a corrosion inhibitor, as a dispersant in agricultural chemical formulations, and as an intermediate in the production of other compounds such as fatty acid condensates of diethanolamine which are extensively used in soaps and cosmetics as emulsifiers, thickeners, wetting agents and detergents (Beyer *et al.*, 1983). In the cosmetic formulations, the concentration of diethanolamine may range from 1 to 25% (National Toxicology Program, 1999a).

Other applications of diethanolamine are in adhesives, antistatic agents, cement and concrete work, coatings, electroplating, an epoxy hardener, a fuel-gelling agent, printing inks, metal cleaning and lubricating, mining, natural gas treatment, paint and pigments, paper, petroleum and coal production, a pharmaceutical intermediate and an ointment-emulsifier, polymers and polymer production, rubber processing, soldering flux, textile finishing and polyurethane production and use (Hammer *et al*., 1987; Bollmeier, 1992; Knaak *et al.*, 1997; Dow Chemical Company, 1998b). Table 2 presents estimates of percentages used in major applications in the United States (Knaak *et al.*, 1997).

Table 2. Major uses of diethanolamine in the United States

Applications	Percentage of production
Surfactants	39
Gas purification	30
Textile processing	15
Metalworking fluids	10
Miscellaneous	8
Laundry detergents	2
Agricultural chemicals	2

From Knaak *et al*. (1997)

Free diethanolamine is reported to be a contaminant in fatty acid-diethanolamine condensates (amides of coconut oil acid, oleic acid and lauric acid) at levels ranging from < 1% to nearly 10% (National Toxicology Program, 1999b,c,d). Diethanolamine also occurs as a contaminant in triethanolamine products (National Toxicology Program, 1999e).

1.4 Occurrence

1.4.1 *Natural occurrence*

Diethanolamine is not known to occur as a natural product.

1.4.2 *Occupational exposure*

Diethanolamine is present in machining and grinding fluids and has been detected in workplace air in the metal manufacturing industry. It was present in bulk cutting fluids at levels ranging from 4 to 5% (Kenyon *et al*., 1993). Diethanolamine has also been reported to be present in wetting fluids used in road paving. A level of 0.05 mg/m^3 was detected in a stationary sample at a slurry machine discharging a bitumen emulsion containing 0.2% of the amine. All personal exposures were below the detection limit (0.02 mg/m^3) (Levin *et al*., 1994). In a German study (1992–94), diethanolamine was measured in samples of metalworking fluids in a range of 0–44% (n = 69). The number of samples with diethanolamine present steadily declined from 90% to 60% over the study period (Pfeiffer *et al*., 1996).

According to the 1981–83 National Occupational Exposure Survey (NOES, 1999), as many as 800 000 workers (many of whom were metalworkers) in the United States were potentially exposed to diethanolamine (see General Remarks).

1.4.3 *Environmental occurrence*

Production of diethanolamine and its wide use in industrial and consumer products may result in its release to the environment (Yordy & Alexander, 1981; Beyer *et al*., 1983; Environment Canada, 1995; Mathews *et al*., 1995; Knaak *et al*., 1997).

(*a*) *Air*

According to the Environmental Protection Agency Toxics Release Inventory, air emissions of diethanolamine from 358 industrial facilities in 1994 were approximately 149 200 kg in the United States (Environmental Protection Agency, 1996). According to the National Pollutant Release Inventory (NPRI) of Canada, on-site releases of diethanolamine to air from 74 facilities amounted to about 40 000 kg (Environment Canada, 1995).

(*b*) *Water*

Surface water discharges of diethanolamine from 358 industrial facilities in 1994 in the United States amounted to 100 350 kg, as reported in the Toxics Release Inventory (Environmental Protection Agency, 1996). On-site releases of diethanolamine (and its salts) to water from 74 facilities in Canada amounted to about 26 000 kg, as reported to the NPRI (Environment Canada, 1995).

Because of the spectrum of industrial and consumer uses of diethanolamine and its miscibility with water, large amounts of the chemical can be discharged into wastewater and sewage in an unaltered form (Yordy & Alexander, 1981; Mathews *et al*., 1995).

(*c*) *Soil*

Releases of diethanolamine to land and underground from 358 industrial facilities in the United States in 1994 (as reported to the Toxics Release Inventory) amounted to 77 050 kg and 36 850 kg respectively (Environmental Protection Agency, 1996). Canadian on-site releases of diethanolamine (and its salts) to land and underground amounted to about 118 000 kg and 497 000 kg, respectively, as reported to the NPRI (Environment Canada, 1995).

1.5 Regulations and guidelines

Occupational exposure limits and guidelines for diethanolamine are presented in Table 3.

The Food and Drug Administration permits the use of diethanolamine as a component of adhesives in food packaging, as an indirect food additive, as a component of the uncoated or coated food contact surface of paper and paperboard for use with dry solid foods with no free fat or oil on the surface, and for use only as an adjuvant to control pulp absorbance and pitch content in the manufacture of paper and paperboard or for use only in paper mill boilers in the United States (Food and Drug Administration, 1999).

Table 3. Occupational exposure limits and guidelines for diethanolamine[a]

Country	Year	Concentration (mg/ m^3)	Interpretation[b]
Australia	1993	15	TWA
Belgium	1993	15	TWA
Denmark	1993	15	TWA
France	1993	15	TWA
Ireland	1997	15	TWA
Netherlands	1997	2	TWA
Russian Federation	1993	5 (sk)	STEL
Switzerland	1993	15	TWA
United Kingdom	1997	15	TWA
United States			
ACGIH[c]	1999	2	TWA
NIOSH	1999	15	TWA

[a] From American Conference of Governmental Industrial Hygienists (1999); National Library of Medicine (1999)
[b] TWA, time-weighted average; STEL, short-term exposure limit; sk, skin notation
[c] These countries follow the recommendations of the ACGIH threshold limit values: Bulgaria, Colombia, Jordan, Republic of Korea, New Zealand, Singapore and Viet Nam

2. Studies of Cancer in Humans

The Working Group was not aware of any study that specifically examined the risk of cancer among persons exposed to diethanolamine. However, ethanolamines have been used as additives for metalworking fluids since the 1950s and are present in wetting fluids used in asphalt paving. Results from cohort and case–control studies of asphalt and road-maintenance workers suggest elevations in the risk of several cancers, including lung, stomach, non-melanoma skin cancer and leukaemia (reviewed by Partanen & Boffetta, 1994). These groups of workers are also exposed to known or suspected carcinogens present in road paving and roofing materials (see Table 4). These compounds include benzene (Group 1) (IARC, 1987a), 1,3-butadiene (Group 2A) (IARC, 1999) and coal-tar pitches (Group 1) (IARC, 1987b). In the light of these concomitant exposures, any observed risk elevations cannot be specifically attributed to diethanolamine or to any other constituent of the complex mixtures. The Working Group, therefore, did not make a detailed evaluation of these studies.

There are three major types of metalworking fluid; straight (generally mineral oils), soluble (straight oils diluted with water and additives) and synthetic (water and addi-

Table 4. Degrees of evidence for carcinogenicity in humans and experimental animals and overall evaluation of carcinogenicity to humans for agents to which asphalt workers and roofers may be or may have been exposed, as evaluated by IARC as of 1993[a]

Agent [CAS No.]	Human	Animal	Overall evaluation
Asbestos [1332-21-4]	S	S	1
Benzene [71-43-2]	S	S	1
Bitumens [8052-42-4], undiluted, steam-refined (straight-run)	I	L	3
Bitumens [92062-05-0], undiluted, cracking-residue	I	L	3
Bitumens [64742-93-4], undiluted, air refined (air-blown)	I	L	3
Extracts of steam-refined bitumens	I	S	2B
Extracts of air-refined bitumens	I	S	2B
1,3-Butadiene [106-99-0]	L	S	2A
Coal-tars [8007-45-2]	S	S	1
Coal-tar pitches [65996-93-2]	S	S	1
Diesel engine exhaust	L	S	2A
Gasoline	I	L	2B
Gasoline engine exhaust	I	I	2B
Kerosene [8008-20-6]	I	I	3
Petroleum solvents	I	I	3
Polyaromatic hydrocarbons			
Anthracene [120-12-7]	I	I	3
Phenanthrene [85-01-8]	I	I	3
Fluoranthene [206-44-0]	I	I	3
Pyrene [129-00-0]	I	I	3
Chrysene [218-01-0]	I	L	3
Benzo[*a*]pyrene [50-32-8]	I	S	2A
Benz[*a*]anthracene [56-55-3]	I	S	2A
Perylene [198-55-0]	I	I	3
Benzo[*b*]fluoranthene	I	S	2B
Benzo[*j*]fluoranthene	I	S	2B
Benzo[*k*]fluoranthene [207-08-9]	I	S	2B
Anthanthrene [191-26-4]	I	L	3
Silica, crystalline [7631-86-9]	L	S	2A
Solar radiation	S	S	1
Styrene [100-42-5]	I	L	2B

From Partanen and Boffetta (1994)

[a] I, inadequate evidence; L, limited evidence; S, sufficient evidence. Overall evaluation: 1, carcinogenic to humans; 2A, probably carcinogenic to humans; 2B, possibly carcinogenic to humans; 3, not classifiable as to its carcinogenicity to humans.

tives) (see General Remarks). Ethanolamines, either diethanolamine or triethanolamine, are very common additives to both soluble and synthetic metalworking fluids (see Sections 1.3 and 1.4.2). Metalworking fluids are complex mixtures that may vary considerably depending on the type of fluid and the additives used. These mixtures may contain many potential carcinogens and, in particular, there is potential for exposure to *N*-nitrosodiethanolamine (see monograph in this volume) in all of the studies considered. A number of studies have examined the risk of cancer among workers exposed to metalworking fluids. Only studies which stated that ethanolamines (no studies indicated diethanolamine alone) were used as additives or that presented results for workers primarily exposed to soluble or synthetic fluids were considered by the Working Group. The characteristics of these studies are presented in Table 5 and a summary of the results for specific cancer sites is presented in Table 6. The use of ethanolamines and nitrites together as additives to metalworking fluids can lead to the formation of *N*-nitrosodiethanolamine. Studies stating that ethanolamines and nitrites were used as additives or which presented results for exposure to nitrosamines are described in detail in the monograph in this volume on *N*-nitrosodiethanolamine. The other studies are described in detail below.

Järvholm and Lavenius (1987) examined the risk for cancer among Swedish men employed for at least five years and any time between 1950 and 1966 in the grinding or turning departments of a company producing bearing rings. This was an extension of an earlier study reported by Järvholm *et al*. (1981) in which a two-fold excess of stomach cancer morbidity was reported among workers in the grinding department during 1958–76. A total of 792 employees met the entrance criteria (4.4% were lost to follow-up). Of these, 559 men had been employed in the grinding department where soluble and some synthetic oils (acid-refined from 1940–75 and solvent-refined mineral oils from 1975) were used. Ethanolamines were introduced as additives in the metalworking fluids used in the department in the mid-1950s. Mortality and cancer incidence follow-up was conducted from 1958 until 1983 and expected numbers were calculated using reference rates from the same city. There were 209 deaths (standardized mortality ratio (SMR), [0.83]; 95% confidence interval (CI), 0.71–0.94) and 67 incident cancers (standardized incidence ratio (SIR), [0.69]; 95% CI, 0.54–0.87) in the full cohort. Among the subcohort of 559 workers in grinding departments, there were 41 incident cancers (SIR, [0.63]; 95% CI, 0.45–0.86), with the only notable excess being for stomach cancer (SIR, [1.5]; 95% CI, 0.7–3.0). [The Working Group noted that part of this cohort was also studied in relation to exposure to *N*-nitrosodiethanolamine. The results of this investigation (Järvholm *et al*., 1986) are reported in the monograph on *N*-nitrosodiethanolamine in this volume.]

Eisen *et al*. (1992) performed a cohort mortality study of 46 384 workers employed for three or more years before 1985 in three United States auto parts manufacturing facilities. Exposure to all three types of metalworking fluid (straight oils (insoluble or cutting oils), soluble oils (water-miscible or emulsifier oils) and synthetic oils (chemical fluids, containing ethanolamines)), the last two introduced in the 1940s, existed and no

Table 5. Characteristics of studies on diethanolamine exposure

Study/country	Study design	Study population	Follow-up period	Potential exposures
Järvholm & Lavenius (1987) Sweden	Cohort	792 men employed > 5 years, any time 1950–66 in the grinding and turning departments of a bearing rings company (may overlap with Järvholm *et al.*, 1986)	1958–83	Analysis of the subgroup of 559 grinders exposed to soluble or synthetic oils
Eisen *et al.* (1992)[a] USA	Cohort	46 384 employed for > 3 years before 1985 at three auto parts manufacturing facilities	1941–84	All three types of metalworking fluid; no analysis by sub-group
Tolbert *et al.* (1992) USA	Cohort	33 619 (two of the three facilities in Eisen *et al*., 1992)	1941–84	Analysis of three sub-groups exposed to each type of metalworking fluid by years of exposure
Eisen *et al.* (1994) USA	Nested case–control of laryngeal cancer	108 fatal and incident cases; 538 controls (study base: Eisen *et al.*, 1992 cohort)	1941–84	Cumulative exposure to straight and soluble types of metalworking fluid and metalworking fluid particulate exposure during grinding; duration of exposure to metalworking fluid and other components.
Sullivan *et al*. (1998) USA	Nested case–control of oesophageal cancer	53 fatal cases; 971 controls (study base: Eisen *et al*., 1992 cohort)	1941–84	Cumulative exposure to the three types of metalworking fluid; duration of exposure to metalworking fluid and other components, incl. nitrosamines.

[a] The results of this study were not considered by the Working Group, but it is included because it forms the study base of the nested case–control studies considered.

Table 6. Results of epidemiological studies of cohorts exposed to soluble and synthetic metalworking fluids

Reference	Stomach		Oesophagus		Larynx		Leukaemia		Pancreas		All cancer		All mortality	
	Obs.	SMR/ PMR	Obs.	SMR/ PMR/OR	Obs.	SMR/ PMR/OR	Obs.	SMR/ PMR	Obs.	SMR/ PMR	Obs.	SMR/ PMR	Obs.	SMR/ PMR
Järvholm & Lavenius (1987) (incidence)														
All grinders (SIR)	8	[1.5] (0.7–3.0)	2	[2.0] (0.2–7.2)	NR		NR		NR		41	[0.63] (0.45–0.86)		
> 20 years latency (SIR)	7	[1.7] (0.7–3.5)	2	[2.4] (0.3–8.8)	NR		NR		NR		33	[0.66] (0.46–0.93)		
Tolbert *et al*. (1992) (mortality)														
Synthetic oils														
White males	21	1.3 (0.8–2.0)	8	0.99 (0.4–1.9)	8	1.6 (0.7–3.1)	16	1.2 (0.7–2.0)	19	1.03 (0.6–1.6)	333	0.97 (0.87–1.1)	1632	1.01 (0.96–1.1)
Soluble oils														
White males	99	1.2 (1.0–1.4)	35	1.03 (0.7–1.4)	30	1.4 (1.0–2.0)	75	1.3 (1.0–1.7)	61	0.8 (0.6–1.0)	1479	1.02 (0.97–1.1)	7287	1.00 (0.98–1.03)
Black males	17	1.0 (0.6–1.6)	10	0.7 (0.3–1.3)	6	1.5 (0.5–3.2)	4	0.7 (0.2–1.9)	19	1.6 (1.0–2.5)	200	0.90 (0.78–1.0)	922	0.81 (0.76–0.87)
Eisen *et al*. (1994) (mortality)														
Soluble fluids (mg/m^3–years)														
0	NA		NA		9	1.00	NA		NA		NA		NA	
0.1–2.0					41	1.34 (0.6–3.0)								
> 2.0–6.0					29	1.22 (0.5–2.9)								
> 6.0					29	1.16 (0.5–2.7)								
Sullivan *et al*. (1998) (20-year lag) (mortality)														
5 mg/m^3–years synthetic oil	NA		–	2.8 (1.1–7.5)	NA		NA		NA		NA		NA	
5 mg/m^3–years soluble oil			–	1.0 (1.0–1.1)										

NA, not applicable; NR, not reported

separate analyses for subgroups were presented (Tolbert *et al.,* 1992). This cohort formed the study base for the three subsequent studies in this monograph.

Tolbert *et al*. (1992) reported the results of a cohort study of 33 619 persons who had worked for at least three years before 1985 in two of the three facilities studied by Eisen *et al*. (1992) where metalworking fluids were used extensively. Mortality was followed from 1941 to 1984 and vital status could be determined for 94% of the cohort at the end of follow-up. In total, 9349 deaths were identified and death certificates were obtained for 92%. Plant records and industrial hygiene data were used in combination with detailed work history records to identify which persons were exposed to different types of machining fluid and their duration of exposure. Among white men exposed to soluble oils (n = 23 488), there were 7287 deaths (SMR, 1.00) and small excesses were observed for cancers of the stomach (SMR, 1.2; 95% CI, 1.0–1.4), larynx (SMR, 1.4; 95% CI, 1.0–2.0) and brain (SMR, 1.2; 95% CI, 0.9–1.7) and leukaemia (SMR, 1.3; 95% CI, 1.0–1.7). Among white men exposed to synthetic fluids (n = 8446), there were 1632 deaths (SMR, 1.01) and small excesses were observed for cancers of the stomach (SMR, 1.3; 95% CI, 0.8–2.0) and larynx (SMR, 1.6; 95% CI, 0.7–3.1) and leukaemia (SMR, 1.2; 95% CI, 0.7–2.0). Among black men exposed to soluble oils (n = 4964), there were 922 deaths (SMR, 0.81) and small excesses were observed for pancreatic cancer (SMR, 1.6; 95% CI, 1.0–2.5) and laryngeal cancer (SMR, 1.5; 95% CI, 0.5–3.2). Results for black men exposed to synthetic fluids or women exposed to any fluids were not presented because of small numbers. Poisson regression analyses were performed to examine the relationships between duration of exposure to each of the three types of metalworking fluid and specific cancer sites after adjustment for plant, sex, race, length of follow-up, year of birth and age at risk. With the exception of statistically significantly negative associations between lung cancer and synthetic fluids (p = 0.006) (for soluble oils, p = 0.09), no strong dose–response relationship was observed. Mild excesses were observed among persons exposed to soluble fluids for 20 or more years for stomach cancer (rate ratio, 1.2; 95% CI, 0.7–2.1) and pancreatic cancer (rate ratio, 1.4; 95% CI, 0.5–3.7). Slightly larger excesses were observed among persons exposed to synthetic fluids for eight or more years for colon cancer (rate ratio, 1.6; 95% CI, 0.8–3.4) and pancreatic cancer (rate ratio, 2.0; 95% CI, 0.9–4.7).

Eisen *et al*. (1994) reported the results of a nested case–control study of laryngeal cancer among the members of the cohort studied by Eisen *et al*. (1992). Potential cases were individuals who had, or died from, laryngeal cancer between 1941 and 1984 and people with laryngeal cancer identified using regional tumour registries or based on other information included on death certificates. Cases were verified using tumour registry or hospital records and a total of 108 cases were eligible for inclusion (all but one being squamous-cell carcinomas). Incidence density sampling was used to select five controls for each case matched on the basis of year of birth, plant, race and sex. Exposure was assessed based on air sampling data, plant records and interviews with plant personnel. Indices of exposure were developed for duration and cumulative

exposure to the straight and soluble metalworking fluids and duration of exposure to biocides, sulfur and various metals. Matched analyses were performed using conditional logistic regression models with additional adjustment for time since hire. The risk for laryngeal cancer was not found to be associated with either cumulative level (mg/m^3–years) or duration of exposure to soluble metalworking fluids. The relationship with exposure to synthetic fluids or ethanolamines was not presented.

Sullivan *et al*. (1998) conducted a nested case–control study of oesophageal cancer among the members of the cohort studied by Eisen *et al*. (1992). Potential cases were 60 individuals who died of oesophageal cancer between 1941 and 1984. Incidence density sampling was used to select 20 controls for each case matched on the basis of year of birth, plant, race and sex, but because of missing data, 53 cases and 971 controls remained. Work history data and an exposure matrix developed for the study were used to assign exposure. The same indices of exposure were used as those described for Eisen *et al*. (1994), with the addition of duration and cumulative exposure to synthetic fluids and duration of exposure to nitrosamines. Matched analyses were performed using conditional logistic regression with additional adjustment for time since hire. Lagging was used to account for latency. After allowing for a 20-year latency, oesophageal cancer was associated with cumulative exposure to synthetic fluids (odds ratio, 2.8; 95% CI, 1.1–7.5 for 5 mg/m^3–years) and duration of exposure to synthetic fluids (odds ratio, 3.3; 95% CI, 1.1–9.6 for five years). Analyses for exposure specifically to ethanolamines and the risk for oesophageal cancer were not presented. [The Working Group noted that in the last studies, data on tobacco smoking and alcohol drinking were not directly presented.]

The Working Group was aware of several other cohort and proportionate mortality studies which included workers exposed to metalworking fluids but did not include analyses of sub-groups of workers exposed to soluble or synthetic fluids. The Working Group was also aware of a number of population-based case–control studies that reported risks associated with exposure to unspecified metalworking fluids or employment in occupations with potential exposure to metalworking fluids. However, these studies were not considered informative for the evaluation because of the unknown probability of exposure to ethanolamines and the potential for confounding from exposure to other known or suspected carcinogens.

[The Working Group noted that the mixed and varying exposures may explain the variability of the results of the different studies and also make it very difficult to ascribe the excesses of cancer observed to any single agent.]

3. Studies of Cancer in Experimental Animals

3.1 Skin application

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F$_1$ mice, six weeks of age, were administered 0, 40, 80 or 160 mg/kg bw diethanolamine (purity, > 99%) in 95% ethanol by dermal application on five days per week for two years. Survival of dosed male mice was similar to that of the vehicle control group, but survival of dosed female mice was reduced (44/50, 33/50, 33/50 and 23/50 for the control, low-, mid- and high-dose groups, respectively). The mean body weights of the mid- and high-dose males were lower than those of the vehicle controls after weeks 88 and 77, respectively. The mean body weights of the low- and mid-dose females were lower than those of the vehicle controls from week 73, but those of the high-dose females were reduced compared with the vehicle controls from week 53. In male mice, the incidences of hepatocellular adenoma and of hepatocellular adenoma and carcinoma (combined) in all dosed groups were significantly greater than those in the vehicle control group (hepatocellular adenoma: 31/50, 42/50, 49/50 and 45/50 ($p < 0.001$, Poly-3 trend test); hepatocellular carcinoma: 12/50, 17/50, 33/50 and 34/50 ($p < 0.001$, Poly-3 trend test), for the control, low-, mid- and high-dose groups, respectively). In addition, the incidences of hepatoblastoma in the mid- and high-dose groups were significantly increased compared with the vehicle control (0/50, 2/50, 8/50 ($p = 0.004$) and 5/50 ($p = 0.028$, pairwise comparisons) in the control, low-, mid- and high-dose groups, respectively). In the female mice, the incidences of hepatocellular neoplasms were significantly higher than those in the vehicle control group (hepatocellular adenoma: 32/50, 50/50, 48/50 and 48/50 ($p < 0.001$, Poly-3 trend test); hepatocellular carcinoma: 5/50, 19/50, 38/50 and 42/50 ($p < 0.001$, Poly-3 trend test) in the control, low-, mid- and high-dose groups, respectively). Renal tubule adenomas in males showed a marginal increase after standard single-section examination (1/50, 4/50, 6/50 and 6/50 ($p = 0.05$, Poly-3 trend test) in the control, low-, mid- and high-dose groups, respectively). When combining single with extended step-sectioning, the incidences were: 1/50, 6/50, 8/50 and 7/50 ($p = 0.055$, Poly-3 trend test) for the control, low-, mid- and high-dose groups, respectively (National Toxicology Program, 1999a).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344/N rats, six weeks of age, were administered diethanolamine (purity, > 99%) in 95% ethanol by dermal application on five days per week for two years. Males received 0, 16, 32 or 64 mg/kg bw and females 0, 8, 16 or 32 mg/kg bw. Survival rates for dosed male and female groups were similar to those of corresponding vehicle control groups. The mean body weight

of the high-dose male group was lower than that of the vehicle controls from week 8 and the mean body weight of the high-dose female group was lower than that of the vehicle controls from week 97. There were no increases in tumours in treated groups compared with the vehicle controls (National Toxicology Program, 1999a).

3.2 Genetically modified mouse

Groups of 15–20 female Tg.AC mice, which carry a zeta-globin promoted v-Ha-*ras* gene on an FVB background, 14 weeks of age, were administered diethanolamine topically in 95% ethanol (the diethanolamine used was from the same chemical batch as that used in the mouse National Toxicology Program study (National Toxicology Program, 1999a). The diethanolamine was administered in 200-µL volumes, five times per week for 20 weeks. The concurrent negative control groups were treated with 200 µL 95% ethanol. The positive control group was treated with 1.25 µg 12-*O*-tetradecanoylphorbol 13-acetate (TPA; approximately 99% pure) twice per week for 20 weeks. The doses of diethanolamine selected were based on the maximum tolerated dose used earlier (National Toxicology Program, 1999a) and were 5, 10 or 20 mg diethanolamine per mouse per application (higher than the MTD). Survival was high in both the control (90%) and treated groups (80–95%). Lesions were diagnosed as papillomas when they reached at least 1 mm in diameter and persisted for three weeks. Animals that did not survive until the end of week 10 were not included in the data summaries or calculations. Six weeks after the last application, all surviving mice were killed. There was no evidence of chronic irritation or ulceration at the site of application. In contrast to the positive controls, which developed multiple papillomas in 18/20 animals, there was no increase in the incidence of skin tumours in diethanolamine-treated animals in this model (Spalding *et al.*, 2000).

[The Working Group was aware of three carcinogenicity bioassays (dermal application studies) in $B6C3F_1$ mice and Fischer 344/N rats of fatty acid-diethanolamine condensates conducted by the National Toxicology Program. These were coconut oil acid, lauric acid and oleic acid diethanolamine condensates (National Toxicology Program, 1999b,c,d). The same three condensates were also tested in the transgenic Tg.AC and $p53^{+/-}$ mouse models (Spalding *et al.*, 2000). The Working Group concluded that these studies could not be used in the evaluation of the carcinogenicity of diethanolamine *per se*. This judgement was based on the fact that the substances tested were complex mixtures of imprecise composition, that the actual diethanolamine content had not been measured in any of the three studies and therefore the precise levels of exposure were indeterminable, and the fact that these studies were not designed as, and did not represent, conventional or adequate carcinogenesis bioassays of diethanolamine.]

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

(*a*) *Absorption and distribution*

Data on the toxicokinetics of diethanolamine have been reviewed (Beyer *et al*., 1983; Melnick & Tomaszewski, 1990; Gillner & Loeper, 1995; Knaak *et al*., 1997).

Evidence of dermal absorption and the effect of grooming were reported by Stott *et al.* (2000). Diethanolamine was administered (160 mg/kg bw per day) to $B6C3F_1$ mice by dermal application (with or without access to the application site) or by oral gavage for two weeks. After the final dose (1–2 h), the blood levels of diethanolamine were 5, 6.6 and 7.7 μg/g for dermal (collared mice), oral + dermal (grooming allowed) and oral gavage treatment, respectively. The dermal dosing method for diethanolamine (90 mg/mL in ethanol; 1.78 mL/kg bw per 4-cm^2 area) was the same as in the carcinogenicity bioassay (Section 3.1.1; National Toxicology Program, 1999a).

Skin penetration rates and permeability constants (k_p) for ^{14}C-labelled diethanolamine (Table 7) were determined *in vitro* using full-thickness skin preparations from rats, mice, rabbits and humans (female mammoplasty patients). Human skin proved to be the best barrier against aqueous diethanolamine (37%, w/w) followed by rat, rabbit and mouse skin when the chemical was applied as an 'infinite dose' (20 mg/cm^2 to cm^2 of skin for 6 h). The total absorbed dose from aqueous diethanolamine was greater (0.23–6.68%) than that from undiluted material (0.02–1.3%) (Sun *et al*., 1996).

Dermal doses of [^{14}C]diethanolamine applied in 95% ethanol (for 48 h) to a 1-cm^2 area of $B6C3F_1$ mouse skin (8–81 mg/kg bw, 15 μL volume, and protected non-occlusively by a dome of wire mesh) were more efficiently absorbed (27–58%) than the test doses (2–28 mg/kg bw, 25 μl volume) applied to a 2-cm^2 area of Fischer 344 rat skin (3–16%) (Mathews *et al*., 1997).

Dermal absorption was also studied in rats. [^{14}C]Diethanolamine was applied to 19.5 cm^2 of the dorsal skin (20 mg/cm^2, 1500 mg/kg bw) and covered for 48 h (no washing) or for 6 h before it was removed by washing. Absorbed [^{14}C]diethanolamine was determined in 48-h urine and faeces and from sampled tissues. Unwashed rats absorbed 1.4% and washed animals 0.64% of the dose, while the majority of [^{14}C]diethanolamine was recovered in the occlusive wrappings (80%) and in skin of the dose site (3.6%). The radioactivity was found in carcass, liver or kidneys but very little in urine (0.11%), faeces or blood (Waechter *et al.*, 1995, cited by Knaak *et al.*, 1997).

Table 7. Skin penetration characteristics of undiluted and aqueous solutions of [^{14}C]diethanolamine

Species	Cumulative dose absorbed (%)	Lag time (h)[a]	Steady-state penetration rate[b] (μg/cm^2/h)	Permeability constant, k_p[c] (cm/h × 10^{-4})
Undiluted diethanolamine				
Rat	0.04 ± 0.01[d]	0.6	1.8	0.02
Mouse	1.30 ± 1.15[d]	0.9	46.3	0.42
Rabbit	0.02 ± 0.01[d]	1.3	0.9	0.01
Human	0.08 ± 0.03[e]	3.2	5.7	0.05
Aqueous diethanolamine (37% w/w)				
Rat	0.56 ± 0.43[d]	0.8	23.0	0.60
Mouse	6.68 ± 5.28[d]	0.8	294.4	7.62
Rabbit	2.81 ± 2.39[d]	1.5	132.2	3.42
Human	0.23 ± 0.09[e]	2.4	12.7	0.34

From Sun *et al*. (1996)

[a] Extrapolated from the intercept of the linear segment (regression) line with the abscissa

[b] Penetration rate at steady state, derived from the slope of the linear segment of a plot of the cumulative mg/cm^2 absorbed versus time

[c] k_p = Steady-state penetration rate (mg/cm^2/h) / Initial concentration (mg/cm^3)

[d] Mean ± SE (n = 3)

[e] Mean ± SE (n = 6)

Combined data from several studies (cited above) showed that in rats the absorption rate increased linearly with the [^{14}C]diethanolamine dose (single), and that a 100-fold increase in the dose of diethanolamine (188–19 720 μg/cm^2) resulted in a 450-fold increase in absorption rate (0.113–45.0 μg/cm^2 per h) (Knaak *et al*., 1997).

Non-radiolabelled diethanolamine was applied to the dorsal skin of rats (1500 mg/kg bw, *ca.* 20 mg/cm^2 to 25 cm^2 of skin and covered) once per day for 6 h per day for three or six days. [^{14}C]Diethanolamine (1500 mg/kg bw) was then applied to the skin for a 48-h penetration test. Animals in the three-day and six-day pretreatment groups absorbed 21% and 41% of the applied dose, respectively. Liver, kidney or carcass contained the majority of absorbed radioactivity, urine from three-day and six-day groups contained 4.3% and 13%, respectively, and less than 0.3% was found in brain, fat or heart (Waechter *et al.*, 1995, cited by Knaak *et al.*, 1997).

[^{14}C]Diethanolamine (7 mg/kg bw) was given orally to male Fischer 344 rats once or by daily repeat dosing for up to eight weeks. Single oral doses (0.7–200 mg/kg bw) were well absorbed but excreted very slowly. About 20–30% of oral and intravenous doses (7 mg/kg bw) was found in urine (mainly as unchanged diethanolamine), with less than 3% in faeces and only 0.2% or less was exhaled (CO_2) within 48 h. Most of the diethanolamine was retained in tissues at high concentrations. The tissue-to-blood

ratios were 150–200 for the liver and kidney, 30–40 for the lung and spleen and 10–20 for the heart, brain and muscle. Tissue radioactivity was found mainly in aqueous extracts (up to 90%) and 5–10% was organic-extractable (Mathews *et al*., 1995, 1997).

(*b*) *Metabolism and excretion*

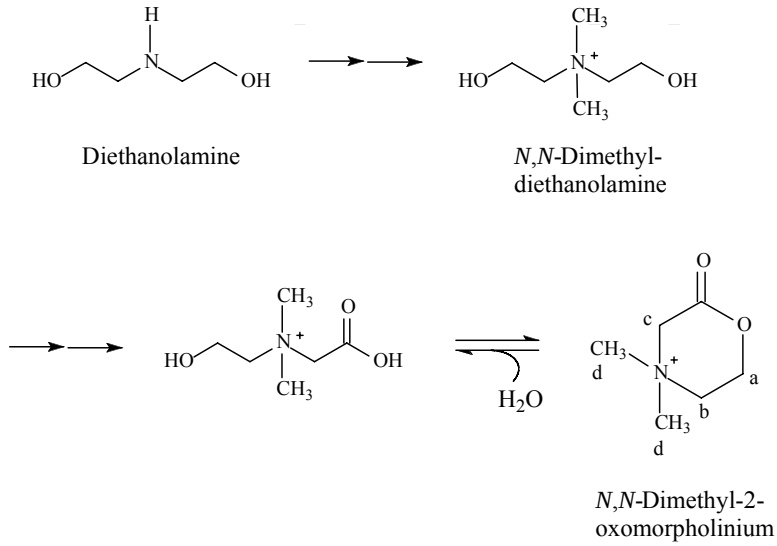
Diethanolamine is incorporated into membrane phospholipids (Artom *et al*., 1949, 1958) and interacts with lipid metabolism *in vivo*, for example by inhibiting incorporation of ethanolamine and choline into phospholipids in rat liver and kidney. The synthesis of liver phospholipids *in vitro* was competitively inhibited by diethanolamine (K_i ~ 3 mM). Diethanolamine was a less effective precursor (K_m = 12 mM) in phospholipid synthesis than the natural substrates choline (K_m = 0.076 mM) and ethanolamine (K_m = 0.054 mM) (Barbee & Hartung, 1979a). The catabolism of diethanolamine-containing lipids was slower than that of the corresponding choline- and ethanolamine-containing derivatives (Artom *et al*., 1958; Barbee & Hartung, 1979a). Diethanolamine is conserved and metabolized by biosynthetic routes common to ethanolamine, resulting in *O*-phosphorylated, *N*-methylated and *N*,*N*-dimethylated derivatives that are incorporated as polar head groups into aberrant phospholipids which are, in turn, incorporated into critical membranes (Mathews *et al*., 1997). Functional and structural alterations induced by diethanolamine in liver mitochondria may ensue from its adverse effects on lipid metabolism in subcellular membranes (Barbee & Hartung, 1979b). About 30% of the diethanolamine-derived phospholipids in rat liver were ceramides (sphingomyelins) and about 70% were phosphoglycerides following a single oral dose of diethanolamine (7 mg/kg bw). After repeated administration (7 mg/kg bw on five days per week for eight weeks), the bioaccumulation of diethanolamine to plateau levels at between four and eight weeks was accompanied by an increasing degree of methylation and accumulation of aberrant sphingomyelinoid lipids in tissues. The highest concentrations of diethanolamine-associated radioactivity measured 72 h after the final dose given in the eight-week period were found in the liver (0.3 mg equivalent/g). The blood was a notable exception in that it continued to bioaccumulate diethanolamine throughout the eight-week dosing period. Uptake, retention and metabolism of diethanolamine in human and rat liver slices are reported to be similar (Mathews *et al*., 1995, 1997).

Hepatic levels of choline, phosphocholine and glycerophosphocholine were reduced as much as 64, 84 and 70%, respectively in male B6C3F_1 mice after two weeks' administration of diethanolamine (160 mg/kg bw per day) via oral gavage or skin painting. These levels were inversely related to the blood diethanolamine levels (uptake) after the final dose. In contrast, the hepatic levels of sphingomyelin were increased relative to those in control mice, and were directly correlated with blood diethanolamine levels (Stott *et al*., 2000).

The metabolism of diethanolamine leading to urinary elimination is illustrated in Figure 1. After single oral and intravenous administrations of diethanolamine to Fischer 344 rats, the compound is excreted predominantly unchanged in urine, only a small

portion being found as its mono- and dimethylated derivatives. As a result of progressive methylation after repeated oral administration (eight weeks), the relative amounts of *N*-methyldiethanolamine and *N*,*N*-dimethyl-2-oxomorpholinium appearing together with unchanged diethanolamine in urine increased markedly. The quaternized lactone was formed from *N*,*N*-dimethyldiethanolamine *in vivo* and in incubations with rat liver microsomes *in vitro* (Mathews *et al.*, 1995, 1997).

Figure 1. Proposed pathway of diethanolamine metabolism in the rat, based on urinary excretion data from an eight-week oral dosing study (7 mg/kg bw per day, five days per week for eight weeks)



From Mathews *et al.* (1997)

After four weeks' repeated administration of [^{14}C]diethanolamine (7 mg/kg bw per day on five days per week) to rats, the urinary excretion of radiolabel (during the 'washout phase') was followed for another four weeks. The log-linear response with time was a first-order process with a whole-body elimination half-life of about six days (Mathews *et al.*, 1997).

Excretion of *N*-nitrosodiethanolamine in urine was evident in Sprague-Dawley rats receiving diethanolamine via the skin (100–400 mg/animal) and sodium nitrite in the drinking water (2000 ppm [mg/L]) for six days but not in the absence of sodium nitrite (Preussmann *et al.*, 1981). *N*-Nitrosodiethanolamine was detected in a gastric rinse of rats treated with 100 µmol diethanolamine [59 mg/kg bw] and 400 µmol sodium nitrite [153 mg/kg bw] by gavage (Konishi *et al.*, 1987). No evidence of formation of *N*-nitrosodiethanolamine *in vivo* was found, however, in B6C3F$_1$ mice treated with diethanolamine (160 mg/kg bw per day) and sodium nitrite (140 ppm in drinking-water; 40 mg/kg bw) (Stott *et al.*, 2000).

4.2 Toxic effects

4.2.1 *Humans*

The only experimental data available on human exposure to airborne diethanolamine come from clinical provocation tests. Diethanolamine-induced occupational asthma was diagnosed following specific bronchial provocation tests in an exposure chamber. The positive reaction was observed in a 39-year-old male metal worker after a 30-min or 45-min inhalation exposure to aerosols from a warmed cutting fluid (40 °C) containing 0.15% diethanolamine and 0.32% triethanolamine, as well as after a 15-min exposure to pure diethanolamine at aerosol concentrations of 0.75 and 1.0 mg/m^3 (Piipari *et al.*, 1998).

4.2.2 *Experimental systems*

The toxicity of diethanolamine (as well as of mono- and triethanolamine) has been reviewed (Knaak *et al*., 1997).

In Swiss Webster mice, the LD_{50} for diethanolamine (by intraperitoneal injection) was 2.3 g/kg bw. At this dose, marked liver changes, including extensive vacuolization and fat droplets, were observed 4 h after dosing. By 24 h, no vacuoles were visible in hepatocytes and fatty droplets were reduced in number (Blum *et al.*, 1972).

Extensive information is available on toxic effects following oral and dermal application of diethanolamine in a 13-week subchronic study (National Toxicology Program, 1992; Melnick *et al*., 1994a,b). Groups of 10 male Fischer 344/N rats were given 0, 320, 630, 1250, 2500 or 5000 ppm [mg/L] diethanolamine in the drinking-water (equivalent to 25–440 mg/kg bw per day), while groups of 10 females were given 0, 160, 320, 630, 1250 or 2500 ppm (equivalent to 15–240 mg/kg bw per day). Two male rats died in the highest-dose group; both male and female rats lost weight in a dose-dependent fashion. Poorly regenerative microcytic anaemia developed within two weeks, without observed changes in bone marrow. Moreover, increased kidney weight, tubular necrosis and loss of kidney function occurred after two weeks. Epithelial cell necrosis in kidney tubules was seen only at the highest dose in both sexes. Some mild changes in the liver were observed, such as weight increase. Demyelination in the medulla oblongata (brain) and spinal cord was found after 13 weeks in both males and females (Melnick *et al.*, 1994a).

In a concurrent study, B6C3F$_1$ mice were given to 0, 630, 1250, 2500, 5000 and 10 000 ppm [mg/L] in the drinking-water; exposures were equivalent to 100–1700 mg/kg bw per day for males and 140–1100 mg/kg bw per day for females. At the three higher dose levels, the mice lost weight and all males and females in the two highest-dose groups died before the end of the study. In both males and females, a dose-dependent increase in liver weight was observed after two weeks; the effect was present even at the lowest dose after 13 weeks in both males and females. Hepatocellular necrosis was found with doses ≥ 2500 ppm. Cytological changes in hepatocytes were found at all doses after 13 weeks. Kidney toxicity, including tubular necrosis, was seen

only in male mice after 13 weeks. In both males and females, degeneration of cardiac myocytes was seen at doses of 2500 ppm and above (Melnick *et al.*, 1994b).

In the same study (Melnick *et al.*, 1994a,b), the effects of dermal exposure were observed during a 13-week study. Groups of 10 male and 10 female rats received applications of 32–500 mg/kg bw on five days per week. At the highest dose, some rats died during the study period. Ulcerative skin lesions at the site of application developed, accompanied by inflammation, hyperkeratinosis and acanthosis of the epidermis. Microcytic anaemia also developed, similarly to that observed after oral exposure. Kidney toxicity, including tubular necrosis and mineralization, was observed, especially in females. Liver weights were increased in both males and females, but no histopathological changes were observed in the liver. Demyelination in the medulla oblongata (brain) and spinal cord also occurred. In mice, after skin application of doses of 80–1250 mg/kg bw on five days per week, the highest dose induced a decrease in body weight compared with controls. Skin toxicity was observed at the site of application and liver weight increased, but hepatocellular necrosis occurred only in male mice. Kidney toxicity, including tubular necrosis, and cardiac myocyte degeneration were found in both males and females.

Irritation of the eye and skin after application of pure (98%) diethanolamine was investigated in New Zealand White rabbits. After 72 h, irritation of the skin was moderate, whereas irritation of the eye was severe (Dutertre-Catella *et al*., 1982).

Diethanolamine has been shown to inhibit choline uptake into cultured Syrian hamster embryo (SHE) and Chinese hamster ovary cells and to inhibit the synthesis of phosphatidylcholine in in-vitro systems in a concentration-dependent, competitive and reversible manner (Lehman-McKeeman & Gamsky, 1999, 2000). Diethanolamine treatment caused a marked reduction in hepatic choline metabolite concentrations in mice following two weeks of dermal dosing. The most pronounced reduction was in the hepatic concentration of phosphocholine, the intracellular storage form of choline (Stott *et al.*, 2000). Moreover, the pattern by which choline metabolites were altered was similar to the pattern of change that has been observed following dietary choline deprivation in rodents (Pomfret *et al.*, 1990). Excess choline also prevented diethanolamine-induced inhibition of phosphatidylcholine synthesis and incorporation of diethanolamine into SHE cell phospholipids (Lehman-McKeeman & Gamsky, 2000).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

The reproductive and developmental toxicity of diethanolamine tested has been reviewed (Knaak *et al*., 1997).

Diethanolamine was administered by gavage to Sprague-Dawley rats on days 6–15 of gestation at dose levels of 0, 50, 200, 500, 800 or 1200 mg/kg bw per day. The rats were killed on day 20 and the uteri examined for number of implantation sites and for live and dead implantations. Rats receiving 500 mg/kg bw or higher doses either died or were in a moribund condition and were killed. Maternal body weight gain was reduced in the 200-mg/kg bw group, but none of the gestational parameters in the treated groups was significantly different from those of the controls (Environmental Health Research & Testing, 1990; cited by Knaak *et al.*, 1997).

Diethanolamine was painted as an aqueous solution on the skin of CD rats on days 6–15 of gestation at dose levels of 0, 150, 500 and 1500 mg/kg bw per day. The two higher dose levels produced severe skin irritation. There was no effect of any treatments on fetal weight or on the incidence of external, visceral or skeletal abnormalities, but delayed ossification of the axial skeleton and distal appendages was observed in fetuses of the 1500-mg/kg bw group (Marty *et al.*, 1999).

Diethanolamine was applied as an aqueous solution to the skin of New Zealand White rabbits on days 6–18 of gestation at dose levels of 0, 35, 100 or 350 mg/kg bw per day. The highest dose level produced marked skin irritation. There was no effect of any treatments on development or on the incidence of external, visceral or skeletal abnormalities (Marty *et al.*, 1999).

In a 13-week subchronic study in male Fischer 344/N rats, testis and epididymis weights were decreased at diethanolamine doses of 1200 ppm or more in the drinking water (Melnick *et al.*, 1994a). Reduced sperm count and motility as well as degeneration of the seminiferous tubules were found at a dose of 2500 ppm.

Inhalation exposure of pregnant Wistar rats to 0.2 mg/m^3 diethanolamine aerosols for 6 h per day on days 6–15 of gestation caused an increased incidence of cervical ribs in the fetuses. No treatment-related malformations were observed (Gamer *et al.*, 1993, cited in Marty *et al.*, 1999).

4.4 Genetic and related effects

The genetic toxicity of diethanolamine has been reviewed by an expert panel for the cosmetic ingredient review (Beyer *et al.*, 1983) and by Knaak *et al.* (1997).

4.4.1 *Humans*

No data were available to the Working Group.

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

Diethanolamine was not mutagenic to *Salmonella typhimurium* strains TA100, TA1535, TA1537, TA1538 or TA98 in three studies, or to *Escherichia coli* WP2 *uvrA* in a single study, in the presence or absence of exogenous metabolic activation. It did

Table 8. Genetic and related effects of diethanolamine

Test system	Result[a]		Dose (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, reverse mutation	–	–	3333 µg/plate	Haworth *et al.* (1983)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	3333 µg/plate	National Toxicology Program (1999a)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	4000 µg/plate	Dean *et al.* (1985)
Escherichia coli WP2/WP2*uvr*A, reverse mutation	–	–	4000 µg/plate	Dean *et al.* (1985)
Saccharomyces cerevisiae JD1, mitotic gene conversion in stationary and log-phase cultures	–	–	5000	Dean *et al.* (1985)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	330	National Toxicology Program (1999a)
Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	2176	Sorsa *et al.* (1988)
Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	1500	National Toxicology Program (1999a)
Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	3010	National Toxicology Program (1999a)
Chromosomal aberrations, rat liver RL cells *in vitro*	–	–	$0.5 \times GI_{50}$	Dean *et al.* (1985)
Cell transformation, Syrian hamster embryo cells (8-day treatment)	–	NT	500	Inoue *et al.* (1982)
Cell transformation, Syrian hamster embryo cells (24-h treatment)	+	NT	4500	Kerckaert *et al.* (1996)
Cell transformation, Syrian hamster embryo cells (7-day treatment)	+	NT	250	Kerckaert *et al.* (1996)
Cell transformation, Syrian hamster embryo cells (7-day treatment)	+[c]	NT	500	Lehman-McKeeman & Gamsky (2000)

Table 8 (contd)

Test system	Result[a]		Dose (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, newt larvae (*Pleurodeles waltl*) blood cells *in vivo*	–		75 ppm; 12 d	Fernandez *et al.* (1993)
Micronucleus formation, newt larvae (*Pleurodeles waltl*) blood cells *in vivo*[d]	–		75 ppm; 12 d	L'Haridon *et al.* (1993)

[a] +, positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day
[c] Negative in the presence of 30 mM choline
[d] In the presence of sodium nitrite or nitrate at pH 8.6 and 5
GI_{50}, concentration causing 50% growth inhibition

not induce gene conversion in *Saccharomyces cerevisiae* strain JD1 in the presence or absence of exogenous metabolic activation. Exposure of the larvae of the newt *Pleurodeles waltl* to diethanolamine did not induce micronuclei in their blood cells and this result remained unaffected by changing the pH or by the addition of sodium nitrite or nitrate.

Diethanolamine did not induce mutations in mouse lymphoma L5178Y cells at the *Tk* locus in the presence or absence of exogenous metabolic activation in one study. It did not induce sister chromatid exchanges in Chinese hamster ovary cells in two studies with or without exogenous metabolic activation. A single study using cultured rat liver cells found no induction of chromosomal aberrations and one study in Chinese hamster ovary cells also found no induction of chromosomal aberrations in either the presence or absence of exogenous metabolic activation.

In one study of cell transformation in the Syrian hamster embryo clonal assay, diethanolamine had no effect after an eight-day treatment. A much larger study revealed induction of cell transformation at a similar dose after a seven-day treatment and at a much higher dose after a 24-h treatment with diethanolamine.

A further seven-day treatment cell transformation study demonstrated a positive dose-related response to diethanolamine up to 500 μg/mL that was abolished by co-administration with 30 mM choline.

4.5 Mechanistic considerations

In mice, diethanolamine alters choline homeostasis in a manner resembling choline deficiency. Stott *et al.* (2000) showed that diethanolamine induced choline deficiency and depleted several choline-containing compounds in B6C3F_1 mice, while Lehman-McKeeman & Gamsky (1999, 2000) found that diethanolamine inhibited the uptake of choline into mammalian cells.

It is known that deprivation of choline in the diet of rodents predisposes to the appearance of hepatocellular carcinomas (Zeisel, 1996). Diethanolamine-induced choline deficiency thus provides a mechanism for the tumorigenesis noted in mice but not in rats.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Diethanolamine is a viscous liquid widely used as a chemical intermediate and as a corrosion inhibitor and surface-active agent in various products including metal-working fluids, oils, fuels, paints, inks, cosmetic formulations and agricultural products. Occupational exposure may occur by inhalation and dermal contact, particularly in metal-machining occupations. No data were available on environmental exposure to

this substance. The general population may be exposed through contact with a variety of personal care products.

5.2 Human carcinogenicity data

Two cohort studies and two nested case–control studies looked at cancer mortality or incidence among workers using metalworking fluids with ethanolamines as additives, with or without sodium nitrite. Small excesses were observed for cancers at various sites, in particular the stomach, oesophagus and larynx. In most of these studies, only associations with use of soluble oils or synthetic fluids were presented and no results were given specifically in relation to diethanolamine exposure. It is difficult to draw conclusions regarding diethanolamine using data from studies of exposures to these complex mixtures.

5.3 Animal carcinogenicity data

Diethanolamine was tested for carcinogenicity by dermal application in one study in mice and in one study in rats. In the mouse study, there was a treatment-related increase in the incidences of both hepatocellular adenomas and carcinomas in both males and females, as well as an increase in the incidence of hepatoblastomas in males. There was also a marginal increase of renal tubule adenomas in males. In rats, no treatment-related increase in the incidence of tumours was seen in either males or females.

In a Tg.AC transgenic mouse model using similar doses to the first mouse study, there was no treatment-related increase in the incidence of skin tumours after skin application.

5.4 Other relevant data

Diethanolamine is metabolized by biosynthetic routes common to endogenous alkanolamines (ethanolamine and choline) and incorporated into phospholipids. It is excreted predominantly unchanged with a half-life of approximately one week in urine. In the absence of sodium nitrite, no conversion to *N*-nitrosodiethanolamine is observed. Diethanolamine competitively inhibits the cellular uptake of choline *in vitro* and hepatic changes in choline homeostasis, consistent with choline deficiency, are observed *in vivo*.

No data on reproductive and developmental effects in humans were available.

Oral or dermal exposure of rats to diethanolamine during organogenesis was not associated with any sign of developmental toxicity, while inhalation exposure to diethanolamine aerosols caused signs of developmental toxicity. Dermal exposure of rabbits during organogenesis caused no sign of developmental toxicity.

Testicular effects have been found after exposure of rats to diethanolamine in the drinking water.

No data on genetic and related effects of diethanolamine in humans were available to the Working Group.

Diethanolamine induced cell transformation in Syrian hamster embryo cells *in vitro* in two studies but not in another. It did not induce gene mutations, sister chromatid exchanges or chromosomal aberrations. Diethanolamine did not induce micronucleus formation in larval newt blood cells in either the absence or presence of sodium nitrite or nitrate. It was without effect on gene conversion in yeast and was not mutagenic in bacteria.

The limited data available to the Working Group do not indicate that diethanolamine is genotoxic.

5.5 Evaluation

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

There is *limited evidence* in experimental animals for the carcinogenicity of diethanolamine.

Overall evaluation

Diethanolamine is *not classifiable as to its carcinogenicity to humans (Group 3)*[1].

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[1] Dr Mirer dissociated himself from the conclusions of the Working Group.

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TRIETHANOLAMINE

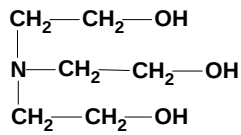
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 102-71-6
Deleted CAS Reg. Nos: 36549-53-8; 36549-54-9; 36549-55-0; 36659-79-7; 105655-27-4; 126068-67-5
Chem. Abstr. Name: 2,2′,2″-Nitrilotris[ethanol]
IUPAC Systematic Name: 2,2′,2″-Nitrilotriethanol
Synonyms: Alkanolamine 244; nitrilotriethanol; TEA; TEA (amino alcohol); TEOA; triethanolamin; tris(β-hydroxyethyl)amine; tris(2-hydroxyethyl)amine

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_6H_{15}NO_3$ Relative molecular mass: 149.19

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Hygroscopic crystals, or colourless, viscous liquid with a mild ammoniacal odour (Lide & Milne, 1996; Budavari, 1998)
(*b*) *Boiling-point*: 335.4 °C (Lide & Milne, 1996)
(*c*) *Melting-point*: 20.5 °C (Lide & Milne, 1996)
(*d*) *Density*: 1.1242 g/cm^3 at 20 °C (Lide & Milne, 1996)
(*e*) *Spectroscopy data*: Infrared (proton [10636]; grating [18547]), nuclear magnetic resonance (proton [7209]; C-13 [1871]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980; Lide & Milne, 1996)

(*f*) *Solubility*: Miscible with water, acetone, ethanol and methanol; soluble in chloroform; slightly soluble in benzene, diethyl ether and lignans (Lide & Milne, 1996; Budavari, 1998)
(*g*) *Volatility*: Vapour pressure, < 1.3 Pa at 20 °C; relative vapour density (air = 1), 5.14 (Verschueren, 1996); flash-point, 185 °C (Budavari, 1998)
(*h*) *Stability*: Incompatible with metals such as aluminium and copper, halogenated organics, strong acids, oxidizing materials and absorbent materials (cellulose, sawdust) (Dow Chemical Company, 1999a)
(*i*) *Octanol/water partition coefficient (P)*: log P, –2.3 (Verschueren, 1996)
(*j*) *Conversion factor*[1]: $mg/m^3 = 6.10 \times ppm$

1.1.4 *Technical products and impurities*

Triethanolamine is commercially available with the following specifications: purity, 99.0% min.; monoethanolamine, 0.05% max.; diethanolamine, 0.40% max. (see monograph in this volume); and water content, 0.20% max. (Dow Chemical Company, 1999b). Triethanolamine is also available in several other grades, including a blend of 85% triethanolamine and 15% diethanolamine [TEA 85]; a low freeze-grade blend (85% TEA 85 and 15% deionized water) for use in colder temperatures; and a blend of 85% triethanolamine and 15% deionized water [TEA 99 Low Freeze Grade] (Dow Chemical Company, 1998).

Trade names for triethanolamine include Daltogen, Sterolamide, Sting-Kill, Thiofaco T-35, and Trolamine.

1.1.5 *Analysis*

Triethanolamine can be determined in workplace air by drawing the air sample through aqueous hexanesulfonic acid and analysing by ion chromatography. The limit of detection for this method is 20 µg per sample (Eller, 1994).

Triethanolamine can be determined in metalworking and cutting fluids by gas chromatography–mass selective detection of silylated derivatives, by isotachophoresis, by capillary zone electrophoresis with indirect ultraviolet detection, and by spectrophotometry (Kenyon *et al.*, 1993; Fernando, 1995; Schubert *et al.*, 1996; Sollenberg, 1997); and in cosmetics and pharmaceuticals by ion-exclusion chromatography and by reversed-phase high performance liquid chromatography (Fukui *et al.*, 1992; Maurer *et al.*, 1996).

[1] Calculated from: $mg/m^3 = (\text{relative molecular mass}/24.45) \times ppm$, assuming a temperature of 25 °C and a pressure of 101 kPa

1.2 Production

Ethanolamines became available commercially in the early 1930s; they assumed steadily growing commercial importance as intermediates after 1945, because of the large-scale production of ethylene oxide. Since the mid-1970s, economical production of very pure, colourless ethanolamines has been possible. Ethanolamines are produced on an industrial scale exclusively by reaction of ethylene oxide (see IARC, 1994) and excess ammonia. This reaction takes place slowly, but is accelerated by water. An anhydrous procedure uses a fixed-bed ion-exchange resin catalyst (Hammer *et al*., 1987).

Estimated annual production of triethanolamine in the United States is presented in Table 1. Worldwide production has been estimated at 100 000–500 000 tonnes per year and European production at 50 000–100 000 tonnes per year (United Nations Environment Program Chemicals, 2000).

Table 1. Estimated annual production of triethanolamine in the United States (thousand tonnes)

Year	1960	1965	1970	1975	1980	1985	1990
Production	13	25	38	41	56	71	98

From Bollmeier (1992)

Information available in 1999 indicated that triethanolamine was manufactured by six companies in India, five companies in the United States, three companies each in China, France, Germany and Mexico, two companies each in Italy and the Russian Federation and one company each in Australia, Belgium, Brazil, Czech Republic, Iran, Japan, Spain and the United Kingdom (Chemical Information Services, 1999).

1.3 Use

Triethanolamine is used as a corrosion inhibitor in metal-cutting fluids (see General Remarks), a curing agent for epoxy and rubber polymers, as a copper–triethanolamine complex to control freshwater algae on lakes and ponds and as a neutralizer–dispersing agent in agricultural herbicide formulations. It is also extensively used in emulsifiers, thickeners and wetting agents in the formulation of consumer products such as cosmetics, detergents, shampoos and other personal products (Beyer *et al*., 1983; Santa María *et al*., 1996; West & Gonsior, 1996).

Other applications of triethanolamine include: adhesives, antistatic agents, cement and concrete work, coatings, in electroless and electroplating, in fuels, printing inks, lithography, metal-cleaning and lubricating, mining, paint and pigments, petroleum and coal production, as a pharmaceutical intermediate and an ointment-emulsifier, in

polymers and polymer production, rubber processing, soldering flux, textile finishing, polyurethane production and use and wood pulping (Dow Chemical Company, 1998).

Table 2 presents estimates of the percentages used in major applications (Knaak *et al.*, 1997) in the United States.

Table 2. Major uses of triethanolamine in the United States

Applications	Percentage of production
Metalworking fluids	33
Concrete/cement	25
Surfactants	20
Textile processing	8
Miscellaneous	6
Agricultural chemicals	3
Cosmetics	2

From Knaak *et al*. (1997)

1.4 Occurrence

1.4.1 *Natural occurrence*

Triethanolamine is not known to occur as a natural product.

1.4.2 *Occupational exposure*

No data on the number of workers exposed to triethanolamine were available from the 1981–83 National Occupational Exposure Survey (NOES, 1999) conducted by the National Institute for Occupational Safety and Health (NIOSH).

Triethanolamine is present in machining and grinding fluids and has been measured in the metal manufacturing industry. It was present in bulk cutting fluids at levels ranging from 0.3 to 40%. Personal air exposures ranged from 0.02 to 244 μg/m^3 (n = 110) (Kenyon *et al.*, 1993). Concentrations were generally higher for workers engaged in transfer operations and lowest for assembly workers (who did not use machining fluids themselves).

In a German study (1992–94), triethanolamine was measured in metalworking fluid samples (n = 69). The proportion of samples containing triethanolamine varied over time between 50 and 85% (Pfeiffer *et al.*, 1996).

1.4.3 *Environmental occurrence*

The broad utility of triethanolamine in a large number of industrial applications and consumer products may result in its release to the environment through various

waste streams (Beyer *et al*., 1983; Santa María *et al*., 1996; West & Gonsior, 1996).

Dermal exposure to triethanolamine-containing products (principally personal care products) is the primary route of general population exposure to triethanolamine (Jones & Kennedy, 1988; Batten *et al*., 1994).

In 1981, triethanolamine was reported to be an ingredient (generally at a concentration of less than or equal to 5%) in 2720 out of 22 572 cosmetic products which may be applied to or come into contact with skin, eyes, hair, nails, mucous membrane and respiratory epithelium. Small amounts may be ingested from lipsticks. Product formulations containing also monoethanolamine (triethanolamine–ethanolamine) may be in contact with the skin for variable periods of time following each application. Daily or occasional use may extend over many years (Beyer *et al*., 1983).

1.5 Regulations and guidelines

Occupational exposure limits and guidelines for triethanolamine are presented in Table 3.

Table 3. Occupational exposure limits and guidelines for triethanolamine[a]

Country	Year	Concentration (mg/ m^3)	Interpretation[b]
Ireland	1997	5	TWA
Netherlands	1997	5	TWA
Russian Federation	1988	3 (aerosol)	Ceiling
		5 (vapour aerosol)	Ceiling
Sweden	1993	5	TWA
		10	STEL
United States ACGIH	1999	5	TWA

[a] From American Conference of Governmental Industrial Hygienists (ACGIH) (1999); United Nations Environment Programme (1999)
[b] TWA, time-weighted average; STEL, short-term exposure limit

The Food and Drug Administration (1999) permits the use of triethanolamine as a component of adhesives in food packaging as an indirect food additive, as a component of the uncoated or coated food contact surface of paper and paper board for use with dry solid foods with no free fat or oil on the surface, and to adjust pH during the manufacture of amino resins permitted for use as components of paper and paper board in the United States.

2. Studies of Cancer in Humans

The Working Group was not aware of any studies that specifically examined the risk of cancer among persons exposed to triethanolamine. However, ethanolamines have been used as additives for metalworking fluids since the 1950s (see General Remarks). There are three major types of metalworking fluid; straight (generally mineral oils), soluble (straight oils diluted with water and additives) and synthetic (water and additives). Ethanolamines, either triethanolamine or diethanolamine, are very common additives to both soluble and synthetic metalworking fluids (see Sections 1.3 and 1.4.2). A number of studies have examined the risk of cancer among workers exposed to metalworking fluids. Only studies which stated that ethanolamines (no study indicated triethanolamine alone) were used as additives or that presented results for workers primarily exposed to soluble or synthetic fluids were considered by the Working Group. The characteristics of these studies are presented in Table 4 of the monograph on diethanolamine in this volume and a summary of the results of these studies for specific cancer sites is presented in Table 5 of the same monograph. The use of ethanolamines and nitrites together as additives to metalworking fluids can lead to the formation of *N*-nitrosodiethanolamine. Studies stating that ethanolamines and nitrites were used together as additives or which presented results for exposure to nitrosamines are described in detail in the monograph in this volume on *N*-nitroso-diethanolamine. The other studies are described in detail in the monograph on diethanolamine.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 40 male and 40 female ICR-JCL mice, six weeks of age, were fed diets prepared by adding 0.03 (low dose) or 0.3% w/w (high dose) triethanolamine (analytical grade) to a powdered diet (heated for 40 min at 100 °C and formed into pellets) throughout their lifespan. Control animals received untreated diet [heating not specified]. The survival rate was 50% at 85 weeks in females and at 65 weeks in males for both treated and control animals. There was a statistically significant ($p < 0.05$, test unspecified) increase in the incidence of lymphomas in female mice (controls, 1/36; low dose, 7/37; high dose, 9/36), but no increase in the incidence of tumours at any site in male mice (Hoshino & Tanooka, 1978). [The Working Group noted the lack of historical control data on the incidence of lymphomas in female mice, as well as the possibility that heating of the triethanolamine in the diet may have produced degradation products.]

Groups of 50 male and 50 female B6C3F$_1$ mice, six weeks of age, were given drinking-water containing triethanolamine (reagent grade, containing 1.9% diethanolamine as a contaminant) at concentrations of 0% (control), 1% (low dose) or 2% (high dose) for 82 weeks, at which time the study was terminated. The high dose was estimated to be the maximum tolerated dose. The percentage of mice surviving at week 82 was: females—all groups, 100%; males—control, 86%; low-dose, 92%; high-dose, 96%. There was no significant difference between the body weights of treated and untreated mice. There was no treatment-related increase in tumour incidence in either sex (Konishi *et al.*, 1992).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer (F344/DuCrj) rats, six weeks of age, were given drinking-water containing triethanolamine (reagent grade, containing 1.9% diethanolamine as a contaminant) at concentrations of 0% (control), 1% (low dose) or 2% (high dose) for two years. At approximately experimental week 60, loss of body weight gain and mortality increased in the female 2% group. Administration of triethanolamine was therefore ceased in both female treatment groups at week 68 for one week and thereafter, from week 69, dietary concentrations in both female treatment groups were reduced by half. After week 104, untreated drinking-water was given to all rats and observation was continued until week 113, when all surviving animals were killed. Treatment with triethanolamine produced a reduction in weight in both males and females and, in females, a dose-dependent increase in mortality due to nephrotoxicity was seen. The percentage mortality in the control, low- and high-dose groups was 32, 32 and 34 in males, and 16, 32 and 42 in females, respectively. No treatment-related increase in the incidence of tumours was observed (Maekawa *et al.*, 1986).

3.2 Skin application

3.2.1 *Mouse*

Groups of 60 male and 60 female B6C3F$_1$ mice, six weeks of age, were administered triethanolamine (purity, 99%) topically in acetone on five days per week for 103 weeks. Male mice received 0, 200, 630 or 2000 mg/kg bw and female mice received 0, 100, 300 or 1000 mg/kg bw triethanolamine. Although there was an apparent association with hepatocellular tumours in both sexes and hepatoblastomas in males, the authors noted that the mice were chronically infected with *Helicobacter hepaticus*, an organism that is known to induce hepatitis, so that interpretation of any relationship between triethanolamine and liver neoplasms was inconclusive (Fox *et al*., 1998; National Toxicology Program, 1999). [The Working Group did not consider this study in its evaluation of carcinogenicity since it was considered inadequate by the National Toxicology Program.]

3.2.2 *Genetically modified mouse*

Groups of 15–20 female Tg.Ac mice, which carry a zeta-globin v-Ha-*ras* gene on an FVB background, 14 weeks of age, were administered triethanolamine in acetone topically (the triethanolamine used was from the same chemical batch as that used in the National Toxicology Program mouse study (National Toxicology Program, 1999)). Triethanolamine was administered in 200-µL volumes, five times per week for 20 weeks. The concurrent negative control groups were treated with 200 µL acetone. The positive control group was treated with 1.25 µg 12-*O*-tetradecanoylphorbol 13-acetate (TPA; approximately 99% pure) three times per week or with 1.5 µg twice per week for 20 weeks. The doses of triethanolamine selected were based on the maximum tolerated dose (MTD) used earlier (National Toxicology Program, 1999) and were 3, 10 or 30 mg triethanolamine per mouse per application. Lesions were diagnosed as papillomas when they reached at least 1 mm in size and persisted for at least three weeks. Animals that did not survive until the end of week 10 were not included in the data summaries or calculations. Six weeks after the last application, all surviving mice were killed. There was no evidence of chronic irritation or ulceration at the site of application during the exposure period. In contrast to the positive controls, which developed multiple papillomas in 19/20 animals, there was no increase in the incidence of skin tumours in triethanolamine-treated animals (Spalding *et al*., 2000).

3.2.3 *Rat*

Groups of 60 male and 60 female Fischer 344/N rats, six weeks of age, were administered triethanolamine (purity, 99%) topically in acetone on five days per week for 103 weeks. Male rats received 0, 32, 63 or 125 mg/kg bw and females received 0, 63, 125 or 250 mg/kg bw triethanolamine. The survival rates of males were 21/50, 11/50, 18/49 and 19/50 and of females were 25/50, 29/50 and 18/50 in the control, low-, mid- and high-dose rats respectively. The mean body weight of females receiving 250 mg/kg bw ranged from 9% to 12% lower than that of the vehicle controls from weeks 73 to 93, and by the end of the study, was 7% lower than that of the vehicle control group. There was no significant increase in the incidence of tumours at any site (National Toxicology Program, 1999).

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No toxicokinetic data related directly to triethanolamine were available.

4.1.2 *Experimental systems*

(*a*) *Absorption, distribution, metabolism and excretion*

Toxicokinetic data for triethanolamine have been reviewed (Beyer *et al*., 1983; Melnick & Tomaszewski, 1990; Gillner & Loeper, 1993; Knaak *et al*., 1997; National Toxicology Program, 1999).

Absorption in the gastrointestinal tract of triethanolamine administered orally to Wistar rats is rapid; 63% of the dose disappeared from intestines within 65 min (Kohri *et al*., 1982). In dermal toxicity studies, the peak blood levels of [^{14}C]triethanolamine were observed 2 h after its application in C3H/HeJ mice (2000 mg/kg bw), whereas in Fischer 344 rats (1000 mg/kg bw), the blood levels (expressed as radioactivity) indicated that triethanolamine was absorbed less rapidly than by mice. Data from various studies in mice and rats (1000–2000 mg/kg bw) suggest that absorption of dermally administered triethanolamine is almost complete in 24 h (Waechter & Rick, 1988, cited in Knaak *et al.,* 1997).

The elimination of [^{14}C]triethanolamine from the blood of mice administered 1.0 mg/kg bw intravenously showed first-order biphasic kinetics with a rapid (0.58-h half-life) and a slow phase (10.2-h half-life). The slow phase half-lives for elimination of triethanolamine in mice after dermal exposure to 1000 and 2000 mg/kg bw in acetone were 9.7 h and 18.6 h. Skin absorption rates (as blood concentration–time curves) after dermal application of aqueous and neat [^{14}C]triethanolamine to mouse skin (2000 mg/kg bw, enclosed by a glass ring) showed no significant change with the use of water as the vehicle (Waechter & Rick, 1988, cited in Knaak *et al.,* 1997).

After topical application of 2000 mg/kg bw [^{14}C]triethanolamine in acetone to 2 cm^2 of mouse back skin, blood levels, expressed as radioactivity/mL, were about fivefold higher than those after application of 1000 mg/kg [^{14}C]triethanolamine to 1 cm^2 of skin, and about 5000-fold higher than that after a 1.0 mg/kg bw intravenous dose (Waechter & Rick, 1988, cited in Knaak *et al*. 1997).

After a single application of [^{14}C]triethanolamine for 48 h to the skin of rats (1000 mg/kg bw; 1.75 cm^2 area) and mice (2000 mg/kg bw; 1.75 cm^2 area), the absorption rate through rat skin (2.4 mg/cm^2/h) was estimated to be greater than that through mouse skin (0.4 mg/cm^2/h). This was evaluated by analysis of [^{14}C]triethanolamine-equivalents in the blood. The sixfold difference in the absorption rates was explained by the sixfold higher concentration of triethanolamine applied to rat skin rather than by a greater permeability (k_p) of rat skin, since the k_p values for rats (1.85×10^{-2} cm/h) and mice ($1.8–2.0 \times 10^{-2}$ cm/h) were similar (Waechter & Rick, 1988, cited in Knaak *et al.,* 1997). Triethanolamine thus enhances its own penetration.

About 60% of the radioactivity in [^{14}C]triethanolamine applied to mouse skin (1000 mg/kg bw) was excreted in 48 h in urine and 20% in faeces, with less than 10% found in the skin at the site of application. The biotransformation of [^{14}C]triethanolamine to monoethanolamine and diethanolamine was specifically investigated in mice after both intravenous and dermal treatments. Neither of the hypothetical metabolites

was detected in urine (by mass spectral analysis), whereas more than 95% of the radioactivity detected in urine was identified as unchanged triethanolamine (Waechter & Rick, 1988, cited in Melnick and Thomaszewski, 1990). *In vitro*, triethanolamine had an inhibitory effect on the incorporation of [^{32}P]phosphate into phospholipids from rabbit and human tissues (Morin & Lim, 1970). Cytochrome P450 monooxygenase-dependent oxidative *N*-dealkylation of triethanolamine does occur in microorganisms, with formation of diethanolamine, ethanolamine and glyoxylate as reaction products (Fattakhova *et al*., 1991).

The disposition of triethanolamine in male Wistar (180–250 g) rats after administration of a single oral dose (350 mg/rat) was measured in a four-day follow-up study. The cumulative percentage recoveries reported for unchanged triethanolamine were 53 and 57% in urine and 20 and 23% in faeces at day 1 and day 3, respectively. Also, after multiple oral administration to male and female rats, triethanolamine was mainly excreted unchanged. The urinary and faecal excretion ratio of unchanged triethanolamine remained constant throughout the treatment period (for five to six days) in both males and females. A small amount of triethanolamine (1.4–2.7%) was excreted as glucuronide conjugates (Kohri *et al*., 1982).

4.2 Toxic effects

4.2.1 *Humans*

Triethanolamine appeared to be without irritating effects below a concentration of 5% in most people; above this concentration (mild) skin irritation is observed. In some volunteers, however, skin irritation was observed at lower concentrations of triethanolamine, particularly in persons who had a history of contact dermatitis and scarified skin (Beyer *et al.*, 1983). Among patients with contact dermatitis, triethanolamine was found to be the most frequent sensitizer (Tosti *et al*., 1990). When a total of 1357 patients suspected of having allergic eczematous contact dermatitis were patch-tested with triethanolamine, 41 had positive reactions. Of these, 29 had applied lotion-based medicaments locally for some time (Scheuer, 1983). Many case reports on people exposed to cosmetics and investigations in groups of workers exposed to cutting fluids (containing triethanolamine among many other components) indicate that contact dermatitis and allergic reactions to triethanolamine occur (e.g., Calas *et al.*, 1978; Herman, 1983; Shrank, 1985; Jones & Kennedy, 1988; Batten *et al*., 1994; Hüner *et al*., 1994; Hamilton & Zug, 1996; Blum & Lischka, 1997).

Triethanolamine has been clinically tested with other model irritant compounds for potency to stimulate signal release of proinflammatory mediators in human skin in order to find biomarkers of irritancy. Neat or aqueous triethanolamine was applied to the lower arm of 12 male volunteers; after 24 h, suction blister fluid specimens were taken from the site of treated skin. Triethanolamine caused no significant increase in arachidonic acid and prostaglandin concentrations in suction blister fluid samples, in

contrast to the irritants sodium lauryl sulfate, benzalkonium chloride and Tween 80 that gave positive test results (Müller-Decker *et al*., 1998).

4.2.2 *Experimental studies*

The toxicology of triethanolamine has been reviewed (Knaak *et al*., 1997). In general, rather high doses of triethanolamine are well tolerated by rats and mice. Major sites of toxicity in rats and mice are liver and kidney, while skin toxicity occurs after dermal application, especially when undiluted triethanolamine is applied.

Irritation to the eye and skin was minimal to slight 72 h after application of pure (98%) triethanolamine to New Zealand White rabbits (Dutertre-Catella *et al.*, 1982).

Subchronic inhalation exposure to triethanolamine aerosols has been studied in both male and female Fischer 344 rats and B6C3F_1 mice at concentrations of 125–2000 mg/m^3 for 6 h per day on five days a week over a 16-day period (an unpublished study reported by Mosberg *et al*., 1985, Battelle Columbus Division Laboratories, and reviewed in Knaak *et al*., 1997). In rats, several minor changes were observed such as a decrease in body weight and an increase in kidney weight, but no histopathological changes were apparent, indicating little toxicity under these conditions. In mice, various haematological changes were considered not to be dose-related.

Signs of kidney and liver toxicity were observed by Kindsvatter (1940) in guinea-pigs and albino rats during oral administration of triethanolamine (200–1600 mg/kg bw per day). Maekawa *et al*. (1986) reported that exposure to triethanolamine in the drinking-water led to kidney toxicity at a concentration of 1–2% (approximately 1000 mg/kg bw per day intake) in both male and female Fischer 344 rats. Body weight gain was depressed by 10–14% after two years on this regimen, compared with controls. Kidney weights were greatly increased, and major histopathological changes were observed in the kidneys, suggesting a dose-related acceleration of chronic nephropathy, which is common in ageing Fischer 344 rats. Mineralization and necrosis of the renal papilla were found. In a 14-day study, no histopathological changes were observed in Fischer 344 rats exposed to 2% triethanolamine in the drinking-water, equivalent to an intake of approximately 2500–2800 mg/kg bw per day (an unpublished study reported by Hejtmancik *et al*., 1985, cited by Knaak *et al*., 1997).

In a chronic study in B6C3F_1 mice given 1 or 2% triethanolamine (containing 2% diethanolamine) in the drinking-water (see Section 3.1.1), little toxicity was observed in either sex (Konishi *et al*., 1992). [The mice consumed up to 3000 mg/kg bw per day at the high dose.]

Dermal application of high concentrations and, in particular, of undiluted triethanolamine to rats and mice in (sub-)chronic studies led to a necrotizing, chronic–active inflammatory process on the skin as observed in several studies and reported extensively in a National Toxicology Program (1999) study. In a 13-week study in Fischer 344 rats given 125–2000 mg/kg bw dermally per day on five days per week, animals receiving 2000 mg/kg bw per day showed body weight gain reductions and

kidney weight increases. Hypertrophy of the pars intermedia of the pituitary gland also occurred. The effects were dose-related and were similar in both males in females. In a subsequent 103-week study, doses of 32–125 mg/kg bw per day were applied to the skin of males and of 63–250 mg/kg per day to the skin of females. At the 15-month interim evaluation, inflammation and ulceration were present at the site of application in both males and females. In females, kidney weights increased after application of 250 mg/kg bw per day. At the end of the study, hyperplasia in the kidney was observed, which was more severe in males than in controls, although the incidence of hyperplasia was the same.

The National Toxicology Program (1999) study also included B6C3F_1 mice. In the 13-week study (250–4000 mg/kg bw per day on five days per week), acanthosis and inflammation at the site of application were observed in both males and females at 4000 mg/kg bw per day, and liver and kidney weights were increased at that dose. In the subsequent 103-week study, females received doses of 100–1000 mg/kg bw per day and males 200–2000 mg/kg bw per day. Skin inflammation was found in both males and females at the site of application. Infection with *Helicobacter hepaticus* in these mice (Fox *et al*., 1998) complicates the interpretation of the results. Another subchronic study of triethanolamine administered dermally to C3H/HeJ mice (140–2000 mg/kg bw in males and 160–2300 mg/kg bw in female mice, three times per week for 95 days) found no signs of toxicity except for slight epidermal hyperplasia at the application site; the internal organs showed no change in weight and no histopathological signs were observed (DePass *et al*., 1995).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Triethanolamine was administered to groups of 10 male and 10 female Fischer 344/N rats and B6C3F_1 mice for 13 weeks by topical application at dose levels of 0, 500, 1000 or 2000 mg/kg bw per day and 0, 1000, 2000 or 4000 mg/kg bw per day, respectively. Body weight gains were significantly lower in the highest-dose group of male and female rats, but there was no change in mice at any dose level. In neither rats nor mice was there any significant change in sperm motility, morphology or number and there was no change in the mean duration of the estrous cycle (National Toxicology Program, 1999).

No embryotoxic or teratogenic effects were produced when pregnant rats were exposed by topical administration to their shaved skin of semipermanent hair-dye preparations containing 0.1–1.5% triethanolamine on gestational days 1, 4, 7, 10, 13, 16 and 19 (Burnett *et al*., 1976). [This study was not reviewed in detail by the Working

Group because of the low proportion of triethanolamine in the complex mixtures tested.]

The reproductive and developmental toxicity of triethanolamine tested without the complication of many other accompanying substances has been reviewed (Knaak *et al*., 1997). This review is used as the reference source to the following studies because they have not been reported in the open literature (Battelle reports).

Triethanolamine was administered as a solution in acetone to the skin of male and female Fischer 344 rats at dose levels of 0 or 500 mg/kg bw per day for 10 weeks before mating and then to the females during gestation and lactation. No effect on mating, fertility or offspring growth and survival was observed. In a similar study with CD-1 mice administered triethanolamine doses of 0 or 2000 mg/kg bw per day, no effect of treatment was observed.

4.4 Genetic and related effects

Triethanolamine has been reviewed by an expert panel for cosmetic ingredient review (Beyer *et al.*, 1983), by Knaak *et al.* (1997) and by the National Toxicology Program (1999).

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 4 for references)

Triethanolamine was not mutagenic to *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 or TA1538 in the presence or absence of exogenous metabolic activation in a number of studies. Triethanolamine did not induce mutations in *Escherichia coli* WP2 *uvrA* and WP2 *try*$^-$ in the presence or absence of exogenous metabolic activation in two studies. In a single study, triethanolamine was not mutagenic to *Bacillus subtilis* strains carrying *uvr*A or *uvr*A and *pol*A mutations in the presence or absence of exogenous metabolic activation. However, when triethanolamine was mixed with sodium nitrite, mutations were induced in this system without exogenous metabolic activation; this activity was lost in the presence of exogenous metabolic activity.

Triethanolamine did not induce gene conversion in *Saccharomyces cerevisiae* in the presence or absence of exogenous metabolic activation in one study. In a single study, sex-linked recessive lethal mutations were not induced in *Drosophila melanogaster* by treatment with triethanolamine either by diet or injection.

Unscheduled DNA synthesis was not induced in rat primary hepatocytes exposed to triethanolamine in two studies.

Triethanolamine did not induce sister chromatid exchanges in Chinese hamster ovary cells in either the presence or absence of exogenous metabolic activation.

Table 4. Genetic and related effects of triethanolamine

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	100 mg/plate	Beyer *et al.* (1983)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	20 000 µg/plate	Inoue *et al.* (1982)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	4000 µg/plate	Dean *et al.* (1985)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	3333 µg/plate	Mortelmans *et al.* (1986)
Escherichia coli WP2 *try*$^{-}$ reverse mutation	–	–	10 000 µg/plate	Inoue *et al.* (1982)
Escherichia coli WP2 and WP2 *uvr*A, reverse mutation	–	–	4000 µg/plate	Dean *et al.* (1985)
Bacillus subtilis TKJ5211 *uvr*A^{-}, mutation	–	–	10 000	Hoshino & Tanooka (1978)
Bacillus subtilis WT, mutation (plus sodium nitrite)	+	–	8580	Hoshino & Tanooka (1978)
Bacillus subtilis TKJ5211 *uvr*A^{-}, mutation (plus sodium nitrite)	+	–	8580	Hoshino & Tanooka (1978)
Bacillus subtilis TKJ6321 *uvr*A^{-} *pol*A^{-}, mutation (plus sodium nitrite)	+	–	8580	Hoshino & Tanooka (1978)
Bacillus subtilis H17 *rec*$^{+}$/M45 *rec*$^{-}$, DNA damage assay	–	NT	4000 µg/disk	Inoue *et al.* (1982)
Saccharomyces cerevisiae JD1, gene conversion	–	–	5000	Dean *et al.* (1985)
Drosophila melanogaster, sex-linked recessive lethal mutation	–		30 000 ppm[c]	Yoon *et al.* (1985)
Unscheduled DNA synthesis, primary rat hepatocytes *in vitro*	–	NT	14 900	Beyer *et al.* (1983)
Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	–	–	10 100	Galloway *et al.* (1987)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, Chinese hamster lung CHL cells *in vitro*	–	NT	100; 24/48 h	Inoue *et al.* (1982)
Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	10 100	Galloway *et al.* (1987)
Chromosomal aberrations, rat liver RL cells *in vitro*	–	NT	$0.5 \times GI_{50}$	Dean *et al.* (1985)
Cell transformation, Syrian hamster embryo cells *in vitro*	–	NT	500; 8 d	Inoue *et al.* (1982)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; NR, not reported; w, week
[c] Feeding or injection
GI_{50}, concentration causing 50% growth inhibition

Chromosomal aberrations were not induced in rat liver cells, Chinese hamster lung cells or Chinese hamster ovary cells by in-vitro exposure to triethanolamine. It did not induce cell transformation in Syrian hamster embryo cells.

Treatment of male and female mice with triethanolamine for 13 weeks by dermal application did not result in any change in the frequency of micronuclei in their blood cells.

4.5 Mechanistic considerations

Triethanolamine is rapidly absorbed and excreted in urine (about 60%) and faeces (about 20%) mainly in the unchanged form. Biodegradation of triethanolamine to monoethanolamine or diethanolamine or to any other putative metabolite has not been shown in rodents, nor its incorporation into natural products. In spite of the search for a possible mode of bioactivation of triethanolamine, no mechanism has been reported in mammals to date.

It has been hypothesized that endogenous nitrosation of triethanolamine may produce a potent liver carcinogen, *N*-nitrosodiethanolamine (Lijinsky *et al*., 1980; Lijinsky & Kovatch, 1985), or that some other endogenous reactions convert triethanolamine to a putative carcinogen (Hoshino & Tanooka, 1978). The formation of *N*-nitrosodiethanolamine in amounts that would cause liver cancer *in vivo* appears, however, unlikely since no treatment-related liver cancers have been observed in oral or dermal triethanolamine carcinogenicity studies in mice or rats (Hoshino & Tanooka, 1978; Maekawa *et al*., 1986; Konishi *et al*., 1992). The potential reported for triethanolamine to undergo nitrosative dealkylation and form *N*-nitrosodiethanolamine under physiological conditions (including gastric pH) is, in general, considered negligible in comparison with the nitrosation of secondary amines (Knaak *et al*., 1997).

In human keratinocyte cultures, triethanolamine was categorized as a weak inducer of a delayed (≥ 4 h) stimulation of the release of key mediators (arachidonic acid, eicosanoids, interleukin-1α) that are known to be indicative of hyperproliferative and inflammatory events in human skin (Müller-Decker *et al*., 1994). In line with the in-vitro irritancy tests, triethanolamine was found to be a non-irritant in a clinical patch testing study of human skin in 20 male volunteers (Müller-Decker *et al*., 1998).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Triethanolamine is a viscous liquid widely used as a corrosion inhibitor, a surface-active agent and an intermediate in various products including metalworking fluids, oils, fuels, paints, inks, cement, cosmetic and personal products, as well as herbicide and algicide formulations. Occupational exposure may occur by inhalation and dermal

contact, particularly in metal-machining occupations. No data were available on environmental exposure to this substance. The general population may be exposed through contact with a variety of personal care products.

5.2 Human carcinogenicity data

Two cohort studies, two proportionate mortality studies and two nested case–control studies looked at cancer mortality or incidence among workers using metalworking fluids with ethanolamines as additives, with or without sodium nitrite. Small excesses were observed for cancers at various sites, in particular, stomach, oesophagus and larynx. In most of these studies, only associations with use of soluble oils or synthetic fluids were presented and no results were given specifically in relation to triethanolamine exposure. It is difficult to draw conclusions regarding triethanolamine using data from studies of exposures to these complex mixtures.

5.3 Animal carcinogenicity data

Triethanolamine was adequately tested for carcinogenicity in one study in mice and in one study in rats by oral administration in the drinking-water. No increase in the incidence of tumours was observed. It was also tested by dermal application in one study in rats and no increase in the incidence of tumours was found.

In a Tg.AC transgenic mouse model, dermal application of triethanolamine produced no increase in tumours.

5.4 Other relevant data

Triethanolamine is rapidly absorbed and excreted in rodent urine (about 60%) and faeces (about 20%), mainly in the unchanged form. Biodegradation of triethanolamine to monoethanolamine or diethanolamine or to any other putative metabolite has not been shown in rodents, nor has its incorporation into endogenous macromolecules. There is no evidence for formation of *N*-nitrosodiethanolamine from triethanolamine under physiological conditions.

In humans, triethanolamine is reported to be a skin sensitizer. Repeated dermal application of high concentrations of triethanolamine to rats led to a necrotizing inflammatory process in the skin.

Data on reproductive and developmental effects in humans were not available. No reproductive or developmental effects were produced when rats and mice were exposed by topical administration. Other routes of exposure have not been studied.

No data on the genetic and related effects of triethanolamine in humans were available to the Working Group.

Triethanolamine did not induce mutations in bacteria, unless nitrite was also present. It did not influence the frequency of micronuclei in mouse peripheral blood *in vivo* after

dermal application. Triethanolamine did not induce unscheduled DNA synthesis, sister chromatid exchange, chromosomal aberrations or cell transformation in rodent cells *in vitro*. Triethanolamine had no effect on sex-linked recessive lethal mutations in *Drosophila melanogaster* or on gene conversion in *Saccharomyces cerevisiae.*

5.5 Evaluation

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

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

Overall evaluation

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

6. References

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N-NITROSODIETHANOLAMINE

This substance was considered by previous working groups, in October 1977 (IARC, 1978) and March 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated in the monograph and taken into consideration in the evaluation.

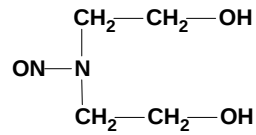
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 1116-54-7
Chem. Abstr. Name: 2,2′-(Nitrosoimino)bis[ethanol]
IUPAC Systematic Name: 2,2′-Nitrosoiminodiethanol
Synonyms: Diethanolnitrosamine; *N*,*N*-diethanolnitrosamine; *N*-nitrosobis(2-hydroxyethyl)amine; nitrosodiethanolamine; NDELA

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_4H_{10}N_2O_3$ Relative molecular mass: 134.14

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Light yellow oil (Budavari, 1998)
(*b*) *Boiling-point*: 125 °C at 0.01 mm Hg [1.33 Pa] (Budavari, 1998)
(*c*) *Density*: 1.26 g/cm^3 (TCI America, 1999)

(*d*) *Solubility*: Miscible with water; soluble in polar solvents; insoluble in non-polar organic solvents (National Toxicology Program, 1998)
(*e*) *Volatility*: Vapour pressure, 5×10^{-5} mm Hg [0.007 Pa] at 20 °C; flash point, 11.6 °C (TCI America, 1999)
(*f*) *Stability*: Sensitive to light, especially ultraviolet light, and undergoes relatively rapid photolytic degradation (US National Toxicology Program, 1998)
(*g*) *Octanol/water partition coefficient (P)*: log P, –1.583 (TCI America, 1999)
(*h*) *Conversion factor*[1]: $mg/m^3 = 5.48 \times ppm$

1.1.4 *Technical products and impurities*

N-Nitrosodiethanolamine is not known to be a commercial product. It is available in research quantities (TCI America, 1999).

1.1.5 *Analysis*

N-Nitrosodiethanolamine can be determined in air and bulk process samples. Air samples are collected on glass fibre filters, extracted with 2-propanol and analysed by gas chromatography with thermal energy analyser detection. The limit of detection is 200 ng per sample (0.42 $\mu g/m^3$). Bulk samples can be screened for *N*-nitrosodiethanolamine by high-performance liquid chromatography (HPLC) with ultraviolet detection (Occupational Safety and Health Administration, 1990).

N-Nitrosodiethanolamine has been found in many complex matrices such as cutting and grinding fluids and cosmetics. Analysis for *N*-nitrosodiethanolamine is complicated by the matrix and a clean-up technique with derivatization is typically required before quantitation of the analyte to achieve adequate sensitivity and selectivity. Ammonium sulfamate may be added to the sample to prevent the artifactual formation of *N*-nitrosamines. Derivatives of *N*-nitrosodiethanolamine have been prepared by acylation, trifluoroacylation, trimethylsilylation and methylation. The derivatives have been analysed by gas chromatography using flame ionization and mass spectrometric detectors (Occupational Safety and Health Administration, 1990).

1.2 Production

N-Nitrosodiethanolamine is formed by the action of nitrosating agents (nitrites; 2-bromo-2-nitropropane-1,3-diol; nitrogen oxides) on diethanolamine and triethanolamine (Schmeltz & Wenger, 1979; Hoffmann *et al.*, 1982; Budavari, 1998). The rate of formation of *N*-nitrosodiethanolamine in aqueous solutions of ethanolamines is pH-, temperature- and time-dependent. The reaction was originally thought to occur only

[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

under acidic conditions, but it can also occur at near-neutral pH by the action of bacteria and at basic pH with appropriate catalysts. For instance, technical-grade triethanolamine (85% triethanolamine, 15% diethanolamine) yielded concentrations of *N*-nitrosodiethanolamine approximately 8- to 12-fold greater than reagent-grade triethanolamine (99% pure) under the same experimental conditions. The increase was attributed to the faster nitrosation rate of diethanolamine (10 to 20 times, depending on pH). Tertiary amines nitrosate at a slower rate than secondary amines because the reaction involves a nitrosative dealkylation rate-limiting step to yield a secondary amine which is then available for further nitrosation. Extensive analysis of representative nitrite-containing metalworking fluids for *N*-nitrosodiethanolamine suggested that the levels of *N*-nitrosodiethanolamine formed were related primarily to the amount of diethanolamine originally present (Lijinsky *et al.*, 1972; Zingmark & Rappe, 1977a,b; Rappe & Zingmark, 1978; Schmeltz & Wenger, 1979; Ong & Rutherford, 1980; Lucke & Ernst, 1993).

1.3 Use

There is no known commercial use for *N*-nitrosodiethanolamine.

1.4 Occurrence

1.4.1 *Natural occurrence*

N-Nitrosodiethanolamine is not known to occur as a natural product.

1.4.2 *Occupational exposure*

The formation of *N*-nitrosamines (mostly *N*-nitrosodiethanolamine) in cutting fluids has been documented since the mid-1970s (Zingmark & Rappe, 1977a,b). Reported concentrations in undiluted, unused fluids have varied considerably (0–30 000 ppm [mg/L]) depending, among other factors, on the presence of sodium nitrite in the fluids (see General Remarks). Concentrations in diluted fluids have been reported to be much lower (Zingmark & Rappe, 1977a,b; Fan & Fajen, 1978; Williams *et al*., 1978; Berg *et al*., 1979; Rounbehler & Fajen, 1983; Spiegelhalder *et al*., 1984, 1987; Fadlallah *et al.*, 1990; Järvholm *et al*., 1991a,b). Acidification, heat, catalysts such as formaldehyde preservatives and transition metal complexes can markedly increase the inherent tendency of diethanolamine and triethanolamine-containing alkaline cutting fluids to react with nitrite to form *N*-nitrosodiethanolamine. Alkanolamines in pH 9 cutting fluids can also react rapidly with nitric oxide in air to form *N*-nitrosodiethanolamine (Loeppky *et al*., 1983). A survey in a plant manufacturing cutting fluids and lubricant revealed concentrations of 20–500 mg/L in bulk samples from separate areas in the manufacturing process (Wolf & Young, 1977).

Fan *et al*. (1977a) reported concentrations of *N*-nitrosodiethanolamine varying between 200 and 29 900 ppm in metalworking fluids used in the United States. Analysis of some German grinding fluids containing di- and triethanolamines in combination with up to 30% nitrite showed concentrations of up to 593 mg/kg [mg/L] *N*-nitrosodiethanolamine in the original, concentrated fluid and up to 90 mg/kg in ready-to-use emulsions (Spiegelhalder *et al*., 1984). *N*-Nitrosodiethanolamine levels between 100 and 3600 mg/L were reported in some French metalworking fluids (Ducos *et al*., 1979) and Järvholm *et al*. (1991a) found a concentration of 650 ppm *N*-nitrosodiethanolamine in a diluted commercial Swedish machine-working fluid.

A recent Canadian study reported *N*-nitrosodiethanolamine concentrations in metalworking fluids to vary between 0.02 and 7.53 ppm in soluble oils, whereas it was absent in insoluble oils (Fadlallah *et al*., 1997). The same authors reported levels of *N*-nitrosodiethanolamine ranging from trace amounts to 0.193 μg/m^3 in the air of metal factories. No correlation was found between *N*-nitrosodiethanolamine concentrations in metalworking fluids and air concentrations (Fadlallah *et al*., 1996). A German study in the metal industry (1991–93) reported an arithmetic mean concentration of *N*-nitrosodiethanolamine of 0.2 μg/m^3 (N = 132, range < 0.01–3.66 μg/m^3). Analyses of metalworking fluids showed no *N*-nitrosodiethanolamine present in 50% of the fluids, while it varied between 0.1 and 16 mg/kg in the other 50% (Pfeiffer *et al*., 1996). Another recent study from Italy reported *N*-nitrosodiethanolamine levels from traces to high concentrations (0.3–1900 mg/kg in used cutting fluids, and 0.4–31 mg/kg in new cutting fluids) in 21% of the 63 cutting fluids tested (Monarca *et al*., 1993). Keefer *et al*. (1990) reported mean concentrations of 1.5 ppm (0.5–4.5 ppm) for all six semi-synthetic fluids, 0.07 ppm (0.11–0.16 ppm) for three of six petroleum-based lubricants and 11.4 ppm (0.5–59 ppm) for five of six synthetic metalworking fluids in the United States.

Comparison of data from three studies (Williams *et al*., 1978; 410–4150 mg/kg; Fadlallah *et al*., 1990; synthetic, 36–95 ppm; soluble, 5–35 ppm; Fadlallah *et al*., 1997; soluble, 0.02–7.5 ppm; none in insoluble oils) indicates that concentrations of *N*-nitrosodiethanolamine in metalworking fluids used in Canada have declined over a period of two decades.

No information was available on the number of exposed workers in the United States from the 1981–83 National Occupational Exposures Survey (NOES, 1999).

N-Nitrosodiethanolamine is easily absorbed through the skin and uptake can therefore occur both via the dermal route as well as the respiratory route. In preliminary investigations, *N*-nitrosodiethanolamine was found in the urine of metal grinders: of 264 analysed, 166 showed positive results (> 0.5 μg/kg (detection limit) with levels up to 103 μg/kg) (Spiegelhalder *et al*., 1984). During biological monitoring of workers using cutting fluids with detectable levels of *N*-nitrosodiethanolamine, only workers working with cutting fluids containing ≥ 5 mg *N*-nitrosodiethanolamine/L excreted trace amounts of *N*-nitrosodiethanolamine in their urine (68% had values < 0.5 μg/kg (detection limit) and 32% had values between 0.6 and 2.7 μg/kg urine). Workers with low exposure and a control group were all *N*-nitrosodiethanolamine-negative (Monarca *et al*., 1996).

Järvholm *et al*. (1991a) reported levels of 19 mg/kg and < 0.02 mg/kg (detection limit) urine for two workers at the end of a shift. The first worker had considerably higher dermal exposure.

1.4.3 *Environmental occurrence*

The presence of *N*-nitrosodiethanolamine is widespread in the environment as a contaminant of cutting fluids and oils, certain pesticides, antifreeze, a broad range of consumer products (including cosmetics, lotions and shampoos) and tobacco and foods at concentrations ranging from 1 to 130 000 ppb (Fan *et al*., 1977a,b; IARC, 1978; Williams *et al*., 1978; Elder, 1980; Brunnemann *et al*., 1982–83; Loeppky *et al*., 1983; Spiegelhalder *et al*., 1984; Eisenbrand *et al*., 1991; Department of Health and Human Services, 1999).

(*a*) *Cosmetics*

Ninety-nine samples representative of 17 cosmetic products were evaluated and *N*-nitrosodiethanolamine was detected in 21 of the samples at concentrations of 50 to greater than 1000 ppb, and in traces in nine samples (Beyer *et al*., 1983).

In a study of off-the-shelf cosmetic formulations carried out by the United States Food and Drug Administration from 1978 to 1980, *N*-nitrosodiethanolamine was detected in 110 of 252 products containing triethanolamine and in 25 of 64 products not containing triethanolamine (however, the products without triethanolamine may have contained diethanolamine or monoethanolamine) (Beyer *et al*., 1983). Levels of *N*-nitrosodiethanolamine as high as 130 ppm have been reported (Edwards *et al.*, 1979).

N-Nitrosodiethanolamine at levels of 600–7386 ppb was detected in 11 samples of cosmetics in the United States which included hand creams, face creams, shampoos, cocoa butter cream, moisturizing lotion and a make-up remover (all products listed diethanolamine and/or triethanolamine as ingredients) (Tunick *et al*., 1982). *N*-Nitrosodiethanolamine was found in all seven cosmetic formulations; one with traces (< 10 ng/g) and six at levels of 41–47 000 ng/g and in 12 of 13 lotions (seven with traces < 10 ng/g and five with 14–140 ng/g) and in eight of nine hair shampoos (three with traces < 10 ng/g and five with 17–280 ng/g) in the United States (Fan *et al*., 1977b). Of 191 cosmetics analysed, 77 contained from 10 to more than 2000 ppb *N*-nitrosodiethanolamine (Elder, 1980). Westin *et al*. (1990) analysed 20 different suntan lotions in Israel and found that three were contaminated with 17–27 ppb *N*-nitrosodiethanolamine (with traces: ~ 5–10 ppb).

Levels of *N*-nitrosodiethanolamine ranging from 100 to 380 μg/kg have been found in 15 of 20 cosmetic and dermo-pharmaceutical preparations commercially available in Belgium (Rollmann *et al*., 1981). Among commercial samples of cosmetics from the German market examined during the summer of 1986, 40% were contaminated with *N*-nitrosodiethanolamine (7–2000 μg/kg). In 1987, the German Federal Health Office recommended that secondary amines no longer be used in cosmetics. Six to 18 months

later, a survey showed that 13% of commercially available cosmetic products from the German market were still contaminated with *N*-nitrosodiethanolamine (10–235 μg/kg) (Eisenbrand *et al*., 1991).

Fan *et al*. (1977b) and Brunnemann *et al.* (1982–83) calculated that women who use 2 g of cosmetics daily would absorb 0.41 μg *N*-nitrosodiethanolamine through the skin. They estimated that women using 1–2 cosmetics daily are exposed to 50–100 μg *N*-nitrosodiethanolamine from these cosmetic products daily.

After application of a cosmetic contaminated with 980 μg of *N*-nitrosodiethanolamine (77 μg/g), *N*-nitrosodiethanolamine (< 50 ng/mL [μg/kg]) was detected in human urine with a half-life greater than 13 hours (Edwards *et al.*, 1979).

(*b*) *Tobacco products*

N-Nitrosodiethanolamine has been detected in cigarette smoke at concentrations of 24–36 ng per cigarette, in smoking tobacco at concentrations up to 420 μg/kg, and in smokeless tobacco products (chewing tobacco, snuff) at concentrations up to 6800 μg/kg (Brunnemann & Hoffmann, 1981; Brunnemann *et al*., 1982–1983; IARC, 1985). The presence of *N*-nitrosodiethanolamine in tobacco and tobacco smoke was attributed, at least in part, to a maleic hydrazide–diethanolamine herbicidal combination commonly applied to tobacco to prevent sucker growth and levels of *N*-nitrosodiethanolamine have declined (< 100 ppb) in some tobaccos since the herbicide was phased out (Brunnemann & Hoffmann, 1991)

N-Nitrosodiethanolamine levels in five United Kingdom samples of oral tobacco (mean, 105 μg/kg; range, not detected–220 μg/kg), in five samples of Swedish moist snuff (mean, 19 μg/kg; range, 8–31 μg/kg), in 11 samples of Indian zarda (mean, 9.5 μg/kg; range, not detected–54 μg/kg) and in 10 samples of European nasal snuff (mean, 12 μg/kg; range, not detected–42 μg/kg) in 1987–88, and in one sample each of moist snuff (94 μg/kg) and chewing tobacco (74 μg/kg) in the United States in 1990 have been reported (Brunnemann & Hoffmann, 1991; Tricker & Preussmann, 1991). *N*-Nitrosodiethanolamine was found in five Nigerian tobacco products (16 brands analysed), at levels of 3.88–34.1 μg/kg (Okieimen & Akintola, 1985).

(*c*) *Food products*

N-Nitrosodiethanolamine was found in a number of Nigerian dairy products (1.5–7 μg/kg) and meat (0.87–1.89 mg/kg) (Okieimen & Akintola, 1985; Anucha *et al.*, 1986).

(*d*) *Miscellaneous products*

N-Nitrosodiethanolamine was found in five of 23 samples of antifreeze (used in the protection of internal combustion engines of cars and trucks) at concentrations varying from 15 to 5700 mg/L in several brands which contained both sodium nitrite and triethanolamine borate (Ducos *et al*., 1983).

1.5 Regulations and guidelines

N-Nitrosodiethanolamine is listed as a class 2 carcinogenic substance in Germany (Deutsche Forschungsgemeinschaft, 1999; substances that are considered to be carcinogenic for man because sufficient data from long-term animal studies or limited evidence from animal studies substantiated by evidence from epidemiological studies indicate that they can make a significant contribution to cancer risk). The European Commission has listed *N*-nitrosodiethanolamine as category 2 carcinogen. Germany has set an 8-h TRK (technical exposure limit) value for combined exposure to 10 *N*-nitroso compounds, including *N*-nitrosodiethanolamine, of 0.0025 mg/m^3 and a 15-min STEL of 0.01 mg/m^3 for some rubber, polyacrylonitrile and amine handling operations, and an 8-h TRK value of 0.001 mg/m^3 for all other operations (Pflaumbaum *et al.*, 1999; United Nations Environment Programme, 1999).

2. Studies of Cancer in Humans

The Working Group was not aware of any studies that specifically examined the risk of cancer among persons exposed to *N*-nitrosodiethanolamine. However, ethanolamines and sodium nitrite have been used as additives for metalworking fluids since the 1950s and together these can lead to the formation of *N*-nitrosodiethanolamine. There are three major types of metalworking fluid; straight (generally mineral oils), soluble (straight oils diluted with water and additives), and synthetic (water and additives) (see General Remarks). Ethanolamines and nitrites are additives used in both soluble and synthetic metalworking fluids (see Sections 1.3 and 1.4.2). A number of studies have examined the risk of cancer among workers exposed to metalworking fluids. Studies stating that ethanolamines and nitrites were used together as additives or which presented results for exposure to nitrosamines are described in detail below. A summary of their characteristics is presented in Table 1 and a summary of their results for specific cancer sites is presented in Table 2. Tables 4 and 5 in the monograph on diethanolamine in this volume also include information on other studies of workers exposed to soluble or synthetic metalworking fluids where ethanolamines are a likely additive. These additional studies are described in detail in the monograph on diethanolamine but are of relevance for this monograph because it is likely that some of these workers were also exposed to *N*-nitrosodiethanolamine through the use of sodium nitrite as an additive.

Järvholm *et al.* (1986) examined the risk for cancer among 219 Swedish men who had worked for at least one year on a machine in which cutting fluids containing amines (mostly triethanolamine, diethanolamine and monoethanolamine) and sodium nitrite were used. This population overlaps with a larger cohort of men employed for at least five years and any time between 1950 and 1966 in the grinding and turning departments of a company producing bearing rings, described in detail in the monograph on

Table 1. Characteristics of studies of cancer in potentially *N*-nitrosodiethanolamine-exposed workers

Study/ country	Study design	Study population	Follow-up period	Potential exposures
Järvholm *et al.* (1986) Sweden	Cohort	219 men employed for at least one year on a machine using fluids containing ethanolamines and sodium nitrite	1966–83	No sub-group or exposure analysis. Population presumed to be exposed to *N*-nitroso-diethanolamine
Park *et al.* (1988) United States	Industry-based proportionate mortality study	755 decedents; employed for 10 or more years in bearing manufacturing plant	1969–82	Both straight and soluble metalworking fluids. Different types of metalworking fluids examined in a nested case–control analysis.
Park & Mirer (1996) United States	Industry-based proportionate mortality study	1870 decedents; employed for > 2 years at two auto engine manufacturing plants 1966–87	1970–89	Population primarily exposed to soluble fluids. Different types of metalworking fluids examined in nested case-control analysis.
Sullivan *et al.* (1998) United States	Nested case–control of oesophageal cancer	53 fatal cases; 971 controls (study base: Eisen *et al.*, 1992 cohort)	1941–84	Cumulative exposure to the three types of metalworking fluids; duration of exposure to metalworking fluids and other components, incl. nitrosamines.

Table 2. Results of epidemiological studies of cohorts exposed to soluble and synthetic metalworking fluids potentially containing *N*-nitrosodiethanolamine (mortality studies)

Reference	Stomach		Oesophagus		Larynx		Leukaemia		Pancreas		All cancer		All mortality	
	Obs.	SMR/ PMR	Obs.	SMR/ PMR/OR	Obs.	SMR/ PMR/OR	Obs.	SMR/ PMR	Obs.	SMR/ PMR	Obs.	SMR/ PMR	Obs.	SMR/ PMR
Järvholm *et al*. (1986)														
≥ 1 year's exposure	NR		NR		NR		NR		NR		4	[0.5] (0.1–1.3)	29	[1.0] (0.6–1.4)
≥ 5 years' exposure	NR		NR		NR		NR		NR		3	[0.5] (0.1–1.5)	20	[0.8] (0.5–1.3)
Park *et al*. (1988)														
White males	11	2.0 (1.1–3.5)	6	1.8 (0.7–4.0)	NR		1	0.2 (0.0–1.3)	8	1.1 (0.6–2.2)	157	1.15 (1.0–1.3)	NA	(PMR study)
Park & Mirer (1996)														
Plant 1							All lymphopoietic							
White males	8	2.1 (0.9–4.1)	NR		1	0.7 (0.0–3.8)	10	1.0 (0.5–1.8)	10	1.8 (0.9–3.3)	107	1.0 (0.8–1.2)	NA	(PMR study)
Black males	2	1.2 (0.2–4.4)	NR		1	1.5 (0.0–8.4)	4	1.5 (0.4–3.9)	0	0.0 (0.0–1.9)	35	1.1 (0.8–1.4)	NA	
Plant 2														
White males	8	1.3 (0.6–2.6)	NR		4	1.7 (0.5–4.3)	15	0.9 (0.6–1.5)	11	1.2 (0.6–2.2)	199	1.1 (1.0–1.3)	NA	
Black males	5	0.8 (0.3–2.0)	NR		1	0.5 (0.0–2.8)	7	1.0 (0.4–2.1)	6	1.1 (0.4–2.4)	111	1.0 (0.9–1.2)	NA	
Sullivan *et al*. (1998) (20-year lag)														
5 years of exposure to nitrosamines			–	3.7 (1.2–11.1)										

NA, not applicable; NR, not reported; PMR, proportionate mortality ratio

diethanolamine in this volume (Järvholm & Lavenius, 1987). Mortality and cancer incidence follow-up was conducted from 1966 (10 years after the first use of fluids containing both types of additives) to 1983 and expected numbers were calculated using reference rates from the same city. There were 29 deaths (30.5 expected), four cancer deaths (7.6 expected) and seven incident cancers (13.0 expected). No notable excesses for specific cancer sites were observed, although the numbers were very small.

Park *et al*. (1988) conducted a proportionate mortality study of workers employed at a bearing-manufacturing plant in the United States. The study population included many workers employed in grinding operations using soluble metalworking fluids, at least one of which was known to contain nitrites and organic amines. The study population consisted of any person who was employed at the facility for at least 10 years and who had died between 1 January 1969 and 31 July 1982. Employment in various work areas and exposure to straight and water-based (soluble) metalworking fluids were classified based on the last job held 15 years before death using available work history records. Deaths were identified using death benefit records, pension records and local union files. Of the 768 decedents meeting the entrance criteria, 13 (2%) were excluded due to missing death certificates (755 left). Proportionate mortality ratios (PMR) were calculated using national rates. Among all deceased white males (n = 610), a somewhat greater number of cancers was observed than expected (PMR, 1.15) and excesses of oesophageal (PMR, 1.8; 95% confidence interval [CI], 0.7–4.0), stomach (PMR, 2.0; 95% CI, 1.1–3.5), rectal (PMR, 3.1; 95% CI, 1.5–5.5) and skin (PMR, 1.9; 95% CI, 0.5–4.8) cancers. An excess of stomach cancer among white women was also observed (PMR, 3.1; based on three observed cases; p = 0.15). No detailed results were presented for white women (n = 124) or the small number of non-white men (n = 20) and women (n = 2) in the population. The risk for cancer in relation to employment in various job and exposure groups was examined in a mortality odds ratio analysis (case–control study). An additional 53 decedents (7%) were excluded (702 left) from the analyses because of a lack of work history information. Mortality odds ratios were calculated using Mantel–Haenszel techniques to adjust for age. Decedents with causes of death thought to be unrelated to potential exposures at the facilities were used as controls. An excess of stomach cancer (mortality odds ratio, 6.6; p = 0.02) was associated with exposure to water-based cutting fluids. [The Working Group expressed some concern about the validity of exposure classification based on one job title and about the representativeness of the control group used.]

Park and Mirer (1996) conducted a proportionate mortality study of workers employed at two large automotive engine manufacturing plants in the United States. The study population included many workers employed in operations using soluble or synthetic metal working fluids. *N*-Nitrosodiethanolamine was detected in synthetic fluids containing nitrites and ethanolamines in 1978. Levels ranged from 1 to 4 μg/mL in used fluids and from 6 to 140 μg/mL in new, undiluted fluids. The study population consisted of any person who was employed at the facilities for at least two years some time between 1966 and 1987 and who died between 1 January 1970 and 31 December

1989, inclusive. Exposure was assessed on the basis of industrial hygiene reports and work history data. Detailed work history data were only available after 1966 and employment before 1966 was assigned to the same department as in 1966. Ranks (0, 1, 2, 4, 8) were assigned for exposure to metalworking fluids, oil smoke, solvents, coal tar and cobalt. Deaths were identified using state vital records, insurance claims and pension records and the authors estimated that over 90% of deaths were identified. Of the 1914 decedents eligible for the PMR analysis, 44 (2.3%) were excluded due to missing cause of death (1870 left). PMRs were presented only for deceased white (1170) and black (613) men employed at each facility and the total number of cancers was similar to that expected (white men—plant 1: PMR, 1.0; white men—plant 2: PMR, 1.1; black men—plant 1: PMR, 1.1; black men—plant 2: PMR, 1.0). The risk for cancer in relation to potential exposures and duration of employment in various operations was examined in a mortality odds ratio analysis. Mortality odds ratios were calculated using logistic regression and adjusting for age, sex, race, year and overall duration of employment at the facilities. Decedents with causes of death thought to be unrelated to potential exposures at the facilities were used as controls. Employment in camshaft and crankshaft grinding at plant 1, where exposure to nitrosamines had been documented, was associated with an increased risk for stomach cancer (mortality odds ratio, 5.1; 95% CI, 1.6–17, at the mean duration of the exposed cases). Exposure to soluble or synthetic metalworking fluids in grinding was found to be significantly associated with non-Hodgkin lymphoma and multiple myeloma combined (mortality odds ratio, 4.1; 95% CI, 1.1–15.4 at the mean cumulative exposure of the exposed cases). No other association with soluble or synthetic fluids was reported.

Sullivan *et al*. (1998) conducted a nested case–control study of oesophageal cancer among the members of the cohort studied by Eisen *et al*. (1992). Potential cases were 60 individuals who died of oesophageal cancer between 1941 and 1984, seven of whom were excluded due to missing work history data. Incidence density sampling was used to select 20 controls for each case matched on the basis of year of birth, plant, race and sex and 58 of these were excluded because of missing data. Extensive efforts were made to assess exposure to biocides (odds ratio, 16.0; 95% CI, 1.8–143 for a five-year exposure and a 20-year latency). It was stated that many of the same workers were exposed to both nitrosamines and biocides. Co-exposure to nitrites and ethanolamines was considered to indicate exposure to nitrosamines. Matched analyses were performed using conditional logistic regression with additional adjustment for time since hire and lagging was used to account for latency. After allowing for a 20-year latency, the incidence of oesophageal cancer was associated with duration of exposure to nitrosamines (odds ratio, 3.7; 95% CI, 1.2–11.1 for five years), to straight, synthetic and soluble fluids, as well as to nitrosamines, biocides, sulfur, asbestos, solvents and various metals. [The Working Group noted that data on tobacco smoking and alcohol drinking were not directly presented.]

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

Mouse: In a mouse lung screening bioassay, a group of 40 female A/J mice, aged 8–10 weeks, received 0.2 μmol/mL of *N*-nitrosodiethanolamine (reported to be pure by chromatography) in the drinking water for 10 weeks followed by tap water alone for the next 20 weeks. A group of 40 mice receiving tap water alone served as concurrent controls. At 30 weeks, all mice were killed and lung adenomas were counted [survival not reported]. In the control group, 40% of animals had lung tumours compared with 70% in treated animals. The number of lung tumours per mouse (mean ± SD) was 0.5 ± 0.6 in controls versus 1.4 ± 1.2 ($p < 0.01$) in treated animals (Hecht *et al*., 1989).

Rat: Groups of 10 male and 10 female Fischer 344 rats, six to seven weeks of age, were given solutions of *N*-nitrosodiethanolamine [purity not specified] in neutral deionized tap-water as the drinking water. Doses of 20 mL *N*-nitrosodiethanolamine solution (3900, 7800, 15 600, 31 250 or 62 500 ppm [mg/L]) were given to each animal on five days per week for 34 weeks. On the remaining two days, the animals were given tap-water without *N*-nitrosodiethanolamine (animals were dosed on seven days per week *ad libitum* for the first 12 weeks of the experimental period). Due to toxicity, treatment of the group given the highest concentration of *N*-nitrosodiethanolamine was discontinued. A few animals that received the 15 600 and 31 250 ppm concentrations died before week 34 [number not stated]. At week 34, all surviving animals were killed and necropsied. The incidence of hepatocellular carcinomas in the remaining four treatment groups was 100% compared with 0% in the controls. Cholangiocellular carcinomas also occurred at the higher doses (males: 0/10, 1/10, 6/10, 8/10, 10/10 and females: 0/10, 3/10, 5/10, 8/10 and 7/10 in the 0, 3900, 7800, 15 600 and 32 500 ppm groups respectively). At the 15 600- and 31 250-ppm doses, many of the induced liver carcinomas metastasized to the lungs and peritoneal cavity (Lijinsky *et al*., 1980).

Groups of 36 to 88 male Sprague-Dawley rats, 100 days old, were administered 5 mL of an aqueous solution of *N*-nitrosodiethanolamine (purity > 99%) in the drinking-water (tap-water) on five days per week for life. There were five groups dosed at concentrations of *N*-nitrosodiethanolamine calculated to provide 1.5, 6, 25, 100 or 400 mg/kg bw daily. Controls received tap-water only. All animals were allowed to die naturally or were killed when moribund. In all groups, *N*-nitrosodiethanolamine was well tolerated without any sign of acute or subchronic toxicity. During the first six months of the study, body weights of all treated animal groups did not significantly differ from those of the control group. This continued for the lifespans for the two lowest-dose groups, but in the three higher-dose groups, body weights were reduced. Survival times did not differ significantly between the two low-dose groups and the controls. In the other three groups, survival times decreased sharply with increasing daily dose. There was a dose-related increase in the incidences of hepatocellular neoplasms and neoplasms of the nasal cavity. The liver tumours were principally adenocarcinomas, with a total

incidence of 0/88, 7/72, 43/72, 33/36, 32/36 and 31/36 in the groups receiving 0, 1.5, 6, 25, 100 and 400 mg/kg bw *N*-nitrosodiethanolamine daily. The nasal cavity neoplasms were mainly squamous-cell carcinomas as well as neuro-epitheliomas of the olfactory epithelium, with total incidences of 0/88, 2/72, 0/72, 6/36, 6/36 and 1/36 in the groups receiving 0, 1.5, 6, 25, 100 and 400 mg/kg bw *N*-nitrosodiethanolamine daily (Preussmann *et al.*, 1982).

Groups of 16–20 male and 16–20 female Fischer 344 rats [age unspecified] were administered 20 mL of an aqueous solution of *N*-nitrosodiethanolamine (purity, > 99%) in the drinking-water (tap-water) (a cage of four animals shared 80 mL of solution) on five days per week for life. The doses administered and the duration of the exposure periods for the various groups were as follows: 2500 mg/L drinking-water for 45 weeks, 1000 mg/L drinking-water for 50 weeks or 400 mg/L drinking-water for 75 weeks. A control group consisted of 40 males and 40 females received tap-water *ad libitum*. All treated rats had died by week 110 and all surviving control rats were killed at 130 weeks; 12 males were killed at 95 weeks and the remainder at 130 weeks. Almost all of the treated animals were reported to have died due to tumour development, principally in the liver and nasal cavity. There was an increased incidence of hepatocellular carcinomas (females: controls, 0/40; 400 mg/L for 50 weeks, 15/16; 400 mg/L for 75 weeks, 16/16; 1000 mg/L for 50 weeks, 19/20; 2500 mg/L for 45 weeks, 20/20; males: controls, 1/28; 400 mg/L for 50 weeks, 14/16; 400 mg/L for 75 weeks, 14/16; 1000 mg/L for 50 weeks, 18/20; 2500 mg/L for 45 weeks, 20/20) together with a few cholangiocarcinomas. In almost all of the treated groups with hepatocellular carcinomas, 30–40% of the rats had lung metastases. In the nasal cavity, the principal tumours induced were adenocarcinomas, with some squamous-cell carcinomas (Lijinsky & Reuber, 1984).

Groups of male and female Fischer 344 rats, seven to eight weeks of age, received 20 mL per animal (a cage of four animals shared 80 mL of solution) of *N*-nitrosodiethanolamine [purity not specified] in deionized water at three concentrations: 28 mg/L, 64 mg/L or 160 mg/L on five days per week. The low dose was given to 39 male and 39 female rats for 100 weeks (total dose, 280 mg per rat). The medium dose was given to 20 male and 20 female rats for 50 weeks (total dose, 320 mg per rat) and 20 males and 20 females for 100 weeks (total dose, 640 mg per rat). The highest dose was given to 27 male and 27 female rats for 50 weeks (total dose, 800 mg per rat). Controls consisting of 20 males and 20 females received deionized water *ad libitum*. Animals were allowed to die naturally or were killed when moribund. The survival of treated male rats beyond 100 weeks was reduced compared with controls, but not in females. A significant increase in the incidence of liver tumours (neoplastic nodules, hepatocellular carcinomas, cholangiocarcinomas or adenomas) was observed in the groups treated with 64 mg/L *N*-nitrosodiethanolamine solution for 100 weeks and 160 mg/L for 50 weeks compared with the control group (males: controls, 4/20; 28 mg/L for 100 weeks, 6/39; 64 mg/L for 100 weeks, 11/20 [$p = 0.024$]; 64 mg/L for 50 weeks, 2/20; 160 mg/L for 50 weeks, 19/27 [$p < 0.001$]; females: controls, 1/20; 28 mg/L for

100 weeks, 10/39 [$p = 0.05$]; 64 mg/L for 100 weeks, 14/20 [$p < 0.001$]; 64 mg/L for 50 weeks, 5/20; 160 mg/L for 50 weeks, 27/27 [$p < 0.001$]) (Lijinsky & Kovatch, 1985).

Groups of 80 male Sprague-Dawley rats, 100 ± 10 days old, received *N*-nitrosodiethanolamine (purity, > 99%) in the drinking-water at doses of 0.2 (low), 0.63 (mid) or 2.0 mg/kg bw (high) on five days per week for life. On the other two days per week, all rats received normal tap-water *ad libitum*. Control animals (500 rats) received tap-water only. There were no signs of acute or subchronic toxicity at any of the dose levels. The incidence of hepatocellular neoplasms was increased (controls, 3/500; low, 2/80 ($p < 0.05$); mid, 1/80; and high, 6/80 ($p < 0.001$)). The gastrointestinal tract had a significantly increased incidence of tumours only in the low-dose group compared with the controls, with the numbers of animals affected as follows: controls, 26/500; low, 7/80 ($p < 0.05$); mid, 3/80; and high, 6/80. The authors noted that this latter increase was due to a slightly higher rate of tumours in the oral cavity and in the forestomach. In addition, there were significant increases in the incidence of tumours in the neurogenic tissue in the medium-dosed group (controls, 54/500; low 8/80; mid, 16/80 ($p < 0.05$); high, 11/80) and of the haematopoietic and lymphatic system in the low- and high-dosed groups (controls, 23/500; low, 8/80 ($p < 0.05$); mid, 5/80; high, 7/80 ($p < 0.1$) (Berger *et al.*, 1987).

A group of 20 female Fischer 344 rats, eight weeks of age, received approximately 20 mL per day on five days per week, of tap-water containing 150 mg/L *N*-nitrosodiethanolamine. On the other two days per week, tap-water alone was provided. A group of 20 control rats received tap-water alone. Treatment was for 50 weeks and surviving animals were maintained on tap-water alone. All surviving animals were killed at weeks 120–124. Survival was 18/20 in both controls and treated groups at 80 weeks and 17/20 versus 11/20 at 100 weeks. No tumours were reported in the control group but in the treated animals, 14/20 had hepatocellular carcinomas (Hecht *et al.*, 1989).

3.2 Subcutaneous administration

Hamster: Groups of 15 male and 15 female Syrian golden hamsters, 8–10 weeks of age, received either seven subcutaneous injections (twice weekly) of 2260 mg/kg bw of *N*-nitrosodiethanolamine (1/5th of the LD_{50}) in 0.5 mL saline over three weeks or 27 subcutaneous injections of 565 mg/kg bw (1/20th of the LD_{50}) over 45 weeks. Injection-site necrosis required interruption of the dosing schedule with cessation of dosing after seven applications in the high-dose group and the need for injection-free intervals of one to two weeks for the low-dose group. This latter group received a total of 27 injections over 45 weeks. Negative controls received 78 injections of 0.5 mL saline solution alone. After 78 weeks, all surviving animals were killed and necropsied, as well as animals found to be moribund during the study. Survival rates to the appearance of the first tumour (33 weeks) of the treated groups (92%) did not differ significantly from the controls (90%). The following incidences of tumours were reported: nasal tumours (mainly adenocarcinomas): males: controls, 0/15; lose-dose, 7/13; high-dose 6/15; and

females: controls, 0/12; low-dose, 5/14; and high-dose, 4/13; tracheal tumours: males: controls, 0/15; low-dose, 2/13; high-dose, 5/15; and females: controls, 0/12; low-dose, 5/14; and high-dose, 3/13 (Hilfrich *et al.*, 1977). [No statistical analysis was reported].

Groups of 15 male and 15 female Syrian golden hamsters, 8–10 weeks of age, were given weekly subcutaneous injections [vehicle not specified but presumed to be saline] of 250, 500 or 1000 mg/kg bw *N*-nitrosodiethanolamine [chromatographically homogeneous] for life. The control group received saline only. *N*-Nitrosodiethanolamine was not found to be toxic, even at the highest dose tested. A few animals died of spontaneous infectious diseases early in the observation period and were excluded from the study. Average body weight in treated animals did not vary significantly from that of control animals. An increase in the incidence of tumours of the nasal cavity (adenocarcinomas) was seen in all treatment groups (males—0/15, 8/13, 8/14 and 11/15; females—0/15, 5/14, 6/15 and 11/15 in the control, low-, mid- and high-dose groups, respectively) (Pour & Wallcave, 1981).

Groups of 15 male and 15 female Syrian golden hamsters, 8–10 weeks of age, received weekly subcutaneous injections of 58, 170 or 500 mg/kg bw *N*-nitrosodiethanolamine (purified by HPLC) in 0.9% NaCl solution for 27 weeks. Groups of 10 male and 10 female hamsters received similar treatment but with only the 0.9% NaCl solution and served as controls. The experiment was terminated after 20 months when approximately 20% of animals were still alive [further details not reported]. Nasal cavity tumours occurred in males: 0/10, 0/15, 2/15, 11/15 and females: 0/8, 0/14, 2/14 and 5/15 in the control, low-, mid- and high-dose groups respectively. These tumours were described as ranging from squamous papillomas to olfactory esthesioneuroepitheliomas. Three of the latter in the high-dose female group invaded the brain. Also reported in these groups were tracheal tumours; males: 0/0, 2/15, 1/15, 3/15 and in females: 0/8, 0/14, 3/14, 4/15 in the control, low-, mid- and high-dose groups, respectively, and single laryngeal tumours in one male and one female in the high-dose group (Hoffman *et al.*, 1983). [No statistical analysis was reported.]

3.3 Topical application

Hamster: Groups of 15 male and 15 female Syrian golden hamsters, 8–10 weeks of age, received topical application to the shaved back of 2.5, 8 or 25 mg *N*-nitrosodiethanolamine (purified by HPLC) three times per week in 0.5 mL acetone for 36 weeks. Groups of 10 male and 10 female hamsters received similar treatment with 0.5 mL acetone and served as controls. The experiment was terminated at 20 months when approximately 20% of animals were still alive [further details not reported]. The treatment-related tumours reported were 2/15 and 2/15 nasal cavity tumours in the male and female high-dose (25 mg) groups respectively and 4/15 tracheal tumours in the high-dose male group (Hoffman *et al.*, 1983). [The Working Group noted the small group sizes and the inadequate reporting.]

3.4 Other routes of administration

Hamster: Groups of 20 male and 20 female Syrian golden hamsters, 8–10 weeks of age, received applications by swab to the oral cavity (including lips and cheek pouches) of 20 mg *N*-nitrosodiethanolamine in 0.9% saline solution (0.4 mL) three times per week for 45 weeks. Groups of 10 male and 10 female hamsters received the saline solution alone and served as controls. The experiment was terminated at 20 months when approximately 20% of animals were still alive [further details not reported]. Nasal cavity tumours were reported in 12/20 treated males and 4/18 treated females compared with none in the control group. Tracheal tumours were seen in the treated groups (4/20 males, 2/18 females) but not in controls (Hoffman *et al*., 1983).

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Dermal absorption of *N*-nitrosodiethanolamine was studied in a 40-year-old man following application of 12.7 g of a facial cream containing 77 μg/g *N*-nitrosodiethanolamine [980 μg]. The cream was applied for 7.75 h (after which it was removed by washing) to a 2090-cm^2 area of skin on the chest and back and covered by a plastic undershirt. About 2% (17.3 μg) of the applied dose was found in the urine at 21.5 h. *N*-Nitrosodiethanolamine was excreted in urine at a high rate (0.98–1.24 μg/h) both before (at 4–7.75 h) and after the cream was removed (at 7.75–11 h), and somewhat more slowly (0.80 μg/h) 13 h after removal of the cream. The half-life of *N*-nitrosodiethanolamine appeared to be long enough [no specific figure given] to allow accumulation of the compound in subjects exposed on a daily basis (Edwards *et al*., 1979).

In 264 metal grinders analysed for urinary excretion of *N*-nitrosodiethanolamine, 166 showed positive results (> 0.5 μg/kg (L)) with levels up to 103 μg/kg (L) urine. The amount of *N*-nitrosodiethanolamine in grinding fluids (0.04–90 mg/kg) was predictive of the amounts detected in workers' urine. Data from quantitative excretion studies (24-h urines) indicated accumulation of *N*-nitrosodiethanolamine during a working week (Spiegelhalder *et al*., 1984, 1987).

The excretion of *N*-nitrosodiethanolamine and thioethers in urine (for the first four hours of the shift on Thursday) was studied in 62 male subjects of whom 25 had high exposure to *N*-nitrosodiethanolamine from metal factories using cutting fluids with an *N*-nitrosodiethanolamine content of up to 1900 mg/kg (L), and in 37 control male subjects. Only the workers using cutting fluids with *N*-nitrosodiethanolamine concentrations of ≥ 5 mg/L (high exposure) excreted detectable amounts in their urine

(0.6–2.7 ng/g). These workers had also a higher mean value of urinary thioethers than the low-exposure and control subjects. Smoking did not affect the excretion of *N*-nitrosodiethanolamine but increased the mean value of the biomarkers (D-glucaric acid, thioethers and number of sister chromatid exchanges per metaphase) (Monarca *et al*., 1993, 1996).

4.1.2 *Experimental systems*

(*a*) *Absorption*

Percutaneous penetration of *N*-nitrosodiethanolamine was measured using cryopreserved human trunk skin and three vehicle formulations (isopropyl myristate, sunscreen cream or a 10% shampoo) containing *N*-nitroso[^{14}C]diethanolamine. The absorption rate of a low dermal dose (10 μg/cm^2) of *N*-nitrosodiethanolamine was a linear function of the concentration (0.06, 0.2 or 0.6 μg/μL) applied to the skin. The peak rates for the isopropyl myristate and shampoo vehicles were seen within five hours and for the sunscreen somewhat later. Total 48-h absorption ranged from 35 to 65% of the dose and was formulation-dependent (isopropyl myristate > shampoo ≥ sunscreen). A total absorption of 4–6 μg/cm^2 was estimated to equate to an applied *N*-nitrosodiethanolamine dose of 10 μg/cm^2. When applied as a large 'infinite' dose (0.5 mg/cm^2), total *N*-nitrosodiethanolamine absorption (4–35% of the applied dose) followed a different rank order (shampoo ≥ isopropyl myristate > sunscreen), probably due to the barrier-damaging properties of the vehicles. The permeability coefficient for isopropyl myristate was 3.5×10^{-3} cm/h (Franz *et al*., 1993).

Percutaneous absorption of *N*-nitrosodiethanolamine through human skin was studied in diffusion cells to determine the permeability constants for water (5.5×10^{-6} cm/h), propylene glycol (3.2×10^{-6} cm/h), a popular lotion (an oil in water emulsion) (6.2×10^{-5} cm/h) and isopropyl myristate (1.1×10^{-3} cm/h). The *N*-nitrosodiethanolamine membrane (stratum corneum):vehicle partition coefficients obtained were 1.8 for water, 1.0 for propylene glycol and 230 for isopropyl myristate. The skin penetration of *N*-nitrosodiethanolamine was greatly increased when lipoidal formulations (isopropyl myristate) were used. The use of cosmetic products over large areas of the skin and for long periods of time (e.g., sun-tanning lotion) results in the greatest absorption of *N*-nitrosodiethanolamine (Bronaugh *et al*., 1981).

Dermal penetration of *N*-nitroso[^{14}C]diethanolamine (4 μg/cm^2) *in vivo* was investigated by applying skin lotion and acetone to 3–15 cm^2 of the skin of monkeys (the abdomen) and pigs (the back) for a 24-h contact time. The skin penetration capacity was greater in monkeys (23% in skin lotion, 34% in acetone) than in pigs (4% in skin lotion, 11.5% in acetone) and the permeability was greater from acetone than skin lotion (Marzulli *et al*., 1981).

Rats were treated with *N*-nitrosodiethanolamine to compare the absorption through the skin with that from the stomach. Skin painting with *N*-nitrosodiethanolamine in water (20 mg/100 μL) and in cutting oil (25 mg/25 μL) yielded ≤ 25 μg/mL

blood levels of *N*-nitrosodiethanolamine. In contrast, a 50 mg dose of *N*-nitrosodiethanolamine applied undiluted to the skin or given in 500 μL water by gavage yielded blood levels of 130–220 μg/mL and 150–190 μg/mL after 1 h, respectively. From 20 to 30% of the *N*-nitrosodiethanolamine applied undiluted or by gavage was recovered in the 24-h urine (Sansone *et al*., 1980; Lijinsky *et al*., 1981).

(*b*) *Body distribution*

Whole-body autoradiography of male Sprague-Dawley rats given intravenous injections (2.7 mg/kg bw) of *N*-nitroso[^{14}C]diethanolamine showed an even distribution in most tissues except for tissue-bound radioactivity that was localized in the liver and the nasal olfactory mucosa. A lower level of labelling in the central nervous system probably indicated that *N*-nitrosodiethanolamine penetrated the blood–brain barrier poorly, while higher labelling in the kidney and urinary bladder may reflect elimination of *N*-nitroso[^{14}C]diethanolamine in urine (Löfberg & Tjälve, 1985).

Oral administration of *N*-nitroso[^{14}C]diethanolamine to Osborne-Mendel rats resulted in rapid distribution throughout the entire body, with little difference between the test doses of 0.5 and 50 mg/kg bw. Radioactivity was highest in tissues at 8 h and detectable up to one week. After a single topical dose, *N*-nitroso[^{14}C]diethanolamine (0.5 or 50 mg/kg bw in acetone) was slowly absorbed over one week and distributed as in the orally dosed rats. In most tissues, the radioactivity was highest after four to seven days (Lethco *et al*., 1982).

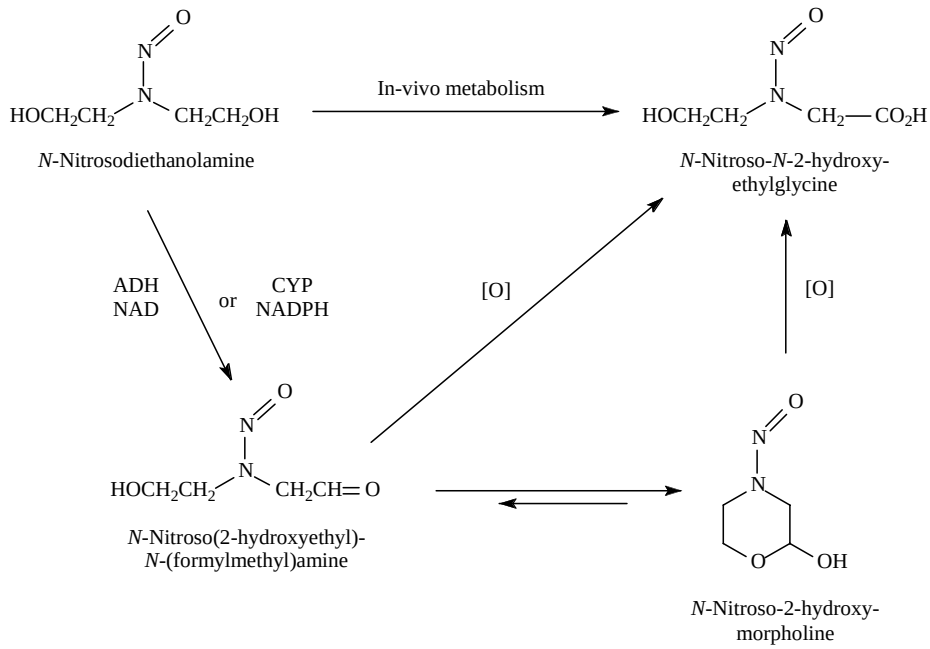
A three-compartment model has been described for the toxicokinetics of *N*-nitrosodiethanolamine studied in CD-COBS rats after a low intravenous dose (5 mg/kg bw). Blood levels of *N*-nitrosodiethanolamine reflected the levels in the liver, suggesting that the liver may not accumulate *N*-nitrosodiethanolamine. The overall elimination rate corresponded to a half-life of 5.77 h (Airoldi *et al*., 1984a).

(*c*) *Metabolism*

The metabolism of *N*-nitrosodiethanolamine has been reviewed (Eisenbrand & Janzowski, 1994; Loeppky, 1999).

The β-oxidation pathway (Figure 1) is the major route for the metabolic disposition of *N*-nitrosodiethanolamine in rodents (Bonfanti *et al*., 1986, 1987). The conversion of *N*-nitrosodiethanolamine to *N*-nitroso-*N*-2-hydroxyethylglycine [*N*-nitroso(2-hydroxyethyl)-*N*-carboxymethyl)amine] proceeds through formation of *N*-nitroso(2-hydroxyethyl)-*N*-(formylmethyl)amine, which exists in equilibrium with its cyclic hemiacetal form, *N*-nitroso-2-hydroxymorpholine. The latter is produced in rat liver preparations as well as by alcohol dehydrogenase (ADH)-mediated oxidation (Airoldi *et al*., 1984b; Eisenbrand *et al*., 1984; Hecht, 1984; Loeppky *et al*., 1987). The β-oxidation pathway shown with S9 fraction from rodent livers was preferentially catalysed by ADH/aldehyde dehydrogenase (Airoldi *et al*., 1983, 1984b; Eisenbrand *et al*., 1984; Bonfanti *et al*., 1986; Denkel *et al*., 1987) rather than by microsomal monooxygenases (Knasmüller *et al*., 1986; Loeppky, 1999). Some of the metabolic discrepancies in

Figure 1. Proposed metabolism of *N*-nitrosodiethanolamine by the β-oxidation pathway



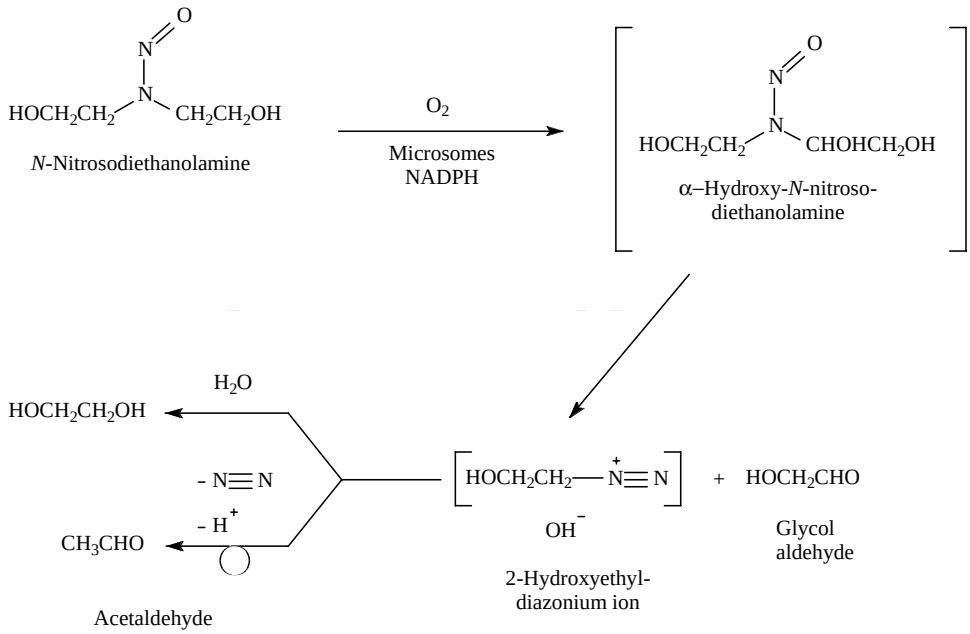
From Loeppky (1999)
ADH, alcohol dehydrogenase; CYP, cytochrome P450; NAD, nicotinamide-adenine dinucleotide; NADPH, nicotinamide-adenine dinucleotide phosphate (reduced form)

bioactivation of *N*-nitrosodiethanolamine may be explained by impurities in commercial *N*-nitrosodiethanolamine products or by vehicles (such as dimethyl sulfoxide) that are inhibitory (Mori *et al*., 1987; Henn *et al*., 1989).

The α-oxidation pathway of *N*-nitrosodiethanolamine metabolism (Figure 2) leads to the formation of an α-hydroxynitrosamine that rapidly decomposes, producing glycol aldehyde, acetaldehyde, ethylene glycol and molecular nitrogen. The latter is assumed to arise from a reactive (2-hydroxyethyl)diazonium ion, which probably is responsible for the formation of 2-hydroxyethylated adducts in DNA (Scherer *et al*., 1991; Loeppky *et al*., 1998; Loeppky, 1999).

The metabolism of *N*-nitrosodiethanolamine by α-hydroxylation, which is a cytochrome P450 (CYP)-mediated pathway, was not detected in liver preparations from uninduced male Fischer 344 rats (Farrelly *et al*., 1984, 1987). The existence of α-hydroxylation was proved later, notably by the formation of glycol aldehyde in liver microsomes from rats pretreated with CYP2E1 inducers. The microsomal metabolism of *N*-nitrosodiethanolamine was slower by α-oxidation than by β-oxidation (Loeppky, 1999). Bioactivation tests of *N*-nitrosodiethanolamine in V79 Chinese hamster cells showed that cytotoxicity was observed only in cells transfected with human CYP2E1 but not in cells expressing CYP2B1 or in the controls (Janzowski *et al*., 1996; Loeppky, 1999).

Figure 2. Proposed metabolism of *N*-nitrosodiethanolamine by the α-oxidation pathway



From Loeppky (1999)
NADPH, nicotinamide-adenine dinucleotide phosphate (reduced form)

α-Deuteration reduces the α-hydroxylation of *N*-nitrosodiethanolamine by a primary isotope effect, causing a decrease in the formation of glycol aldehyde and 2-hydroxyethyldiazonium ion as well as a corresponding decrease in DNA alkylation. As a result of β-deuteration, α-hydroxylation is enhanced through metabolic switching, leading to an increased rate of DNA alkylation (Loeppky *et al.*, 1998). α-Hydroxylation of *N*-nitroso-2-hydroxymorpholine, which could lead to DNA alkylation by the formation of either glyoxal or 2-hydroxyethyldiazonium ion, is a pathway supported both by the lack of carcinogenicity (Hecht *et al.*, 1989) and by the formation of hydroxyethylguanine adducts in rats exposed to *N*-nitroso-2-hydroxymorpholine (Chung & Hecht, 1985; Loeppky, 1999).

DNA base deamination studies employing *N*-nitroso-2-hydroxymorpholine and calf thymus DNA indicate that *N*-nitroso-2-hydroxymorpholine may deaminate the primary amino groups in DNA via nitroso transfer reactions *in vivo* (Loeppky *et al*., 1994).

The role of CYP2E1 in α-oxidation of *N*-nitrosodiethanolamine was probed by using the deuterated isotopomers *N*-nitroso[α-D_4]diethanolamine and *N*-nitroso-[β-D_4]diethanolamine. *N*-Nitrosodiethanolamine and *N*-nitroso[β-D_4]diethanolamine were equally cytotoxic to human CYP2E1-transfected V79 cells, while *N*-nitroso-[α-D_4]diethanolamine was not. Significant DNA single-strand break levels were

observed in these cells for *N*-nitrosodiethanolamine and *N*-nitroso[β-D_4]diethanolamine but not for *N*-nitroso[α-D_4]diethanolamine. A kinetic deuterium isotope effect of 2.6 for V_{max}/K_m was observed for the horse liver ADH-mediated oxidation of *N*-nitroso[β-D_4]diethanolamine to *N*-nitroso-2-hydroxymorpholine, while k_H/k_D for *N*-nitroso[α-[D_4]diethanolamine was 1.05. These data suggest that the α- and β-hydroxylations of *N*-nitrosodiethanolamine are mediated by CYP2E1 and liver ADH, respectively (Loeppky *et al*., 1998).

Little evidence has been presented specifically for *N*-nitrosodiethanolamine to support the hypothesis that nitrosoamines could be activated by a process involving sulfation (Sterzel & Eisenbrand, 1986; Loeppky *et al*., 1987; Michejda *et al*., 1994; Loeppky *et al*., 1998).

Experiments *in vitro* showed that the liver and the nasal mucosa were capable of forming $^{14}CO_2$ from *N*-nitroso[^{14}C]diethanolamine (Löfberg & Tjälve, 1985).

(*d*) *Excretion*

About 60–90% of *N*-nitrosodiethanolamine administered orally, intravenously, cutaneously or intratracheally to rats was excreted unchanged in the urine (Preussmann *et al*., 1978, 1981; Lethco *et al*., 1982; Spiegelhalder *et al*., 1982, 1984). In some studies, the amount of *N*-nitrosodiethanolamine recovered in 24-h urine was only 3–30% of the dose (Lijinsky *et al*., 1981; Lethco *et al*., 1982; Airoldi *et al*., 1984a). Only 2–8% of the dose was found in the faeces and ≤ 2% was exhaled as $^{14}CO_2$ after oral or intravenous administration of *N*-nitroso[^{14}C]diethanolamine (Lethco *et al*., 1982; Löfberg & Tjälve, 1985; Farrelly *et al*., 1987). The amounts of *N*-nitrosodiethanolamine that were excreted in a conjugated form (glucuronide/sulfate) appeared to be marginal in CD-COBS and Fischer 344 rat urine (Bonfanti *et al*., 1985; Farrelly *et al*., 1987).

Excretion rates of *N*-nitrosodiethanolamine in urine (24 h) seemed not to be dose-dependent in rats following epicutaneous, intratracheal or oral administration of *N*-nitrosodiethanolamine at dose ranges of 0.03–300 mg per animal (Spiegelhalder *et al*., 1982), 100–1000 mg/kg bw (Preussmann *et al*., 1978) or 0.5–50 mg/kg bw (Lethco *et al*., 1982).

N-Nitrosomorpholine and *N*-nitrosodiethanolamine are both converted *in vivo* to *N*-nitroso-*N*-2-hydroxyethylglycine, which is excreted in rodent urine. The recovery of *N*-nitroso-*N*-2-hydroxyethylglycine in 24-h urine was lower in rats (8%) than in mice or hamsters (11–14%) dosed intraperitoneally with *N*-nitrosodiethanolamine (5 mg/kg bw), which was also found in urine of all the species (Bonfanti *et al*., 1986). Biliary excretion (a minor route of elimination) and enterohepatic recycling of *N*-nitrosodiethanolamine and its metabolite *N*-nitroso-*N*-2-hydroxyethylglycine has been shown in rats after intravenous administration of 5 mg/kg bw *N*-nitrosodiethanolamine (Bonfanti *et al*., 1985).

N-Nitrosodiethanolamine has been detected in urine (1–150 μg per animal) of male Sprague-Dawley rats given nitrite in their drinking water following skin treatment with diethanolamine (100–400 mg per rat) (Preussmann *et al*., 1981).

4.2 Toxic effects

No data were available to the Working Group.

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

One small study (Garry *et al*., 1986) examined peripheral blood samples from 11 tool and die workers and seven non-manufacturing workers (controls) for frequencies of sister chromatid exchanges and chromosomal aberrations. No significant differences were observed for either parameter.

In a larger study (Fuchs *et al*., 1995; Oesch *et al*., 1995), peripheral blood from 65 male metal workers from seven small–medium plants in central West Germany was examined for DNA single-strand breaks (primary, including reversible DNA damage). There was a significant correlation between the increased level of DNA damage in workers and the concentration of *N*-nitrosodiethanolamine in the air of the workplace, but a concomitant effect of other genotoxic agents in the environment could not be excluded. [The Working Group noted the absence of a control group.]

4.4.2 *Experimental systems* (see Table 3 for references)

N-Nitrosodiethanolamine was mutagenic to *Salmonella typhimurium* in most assays in the presence of exogenous metabolic activation systems and in some assays in the absence of such systems.

In two studies, *N*-nitrosodiethanolamine was not mutagenic to *Escherichia coli* K-12/343/113 in the absence of exogenous metabolic activation. When exogenous metabolic activation preparations from rats or mice were included, mutations were induced at one of three loci tested, i.e. *gal*$^+$. These two studies also reported that *N*-nitrosodiethanolamine induced mutations in *E. coli* K12/343/113 in animal-mediated assays in male and female mouse livers and spleens. In one of these studies, pyrazole, an ADH-blocking agent, did not completely abolish this mutagenicity, indicating that metabolic activation pathways not requiring ADH are involved in *N*-nitrosodiethanolamine mutagenicity.

In a single study in *Drosophila melanogaster* with a wide dose range (0.01–2.0 M applied topically on late embryos and newly hatched larvae), *N*-nitrosodiethanolamine was not mutagenic in germ cells by the classic sex-linked recessive lethal test, apart from a very weak effect after very high exposure. In contrast, *N*-nitrosodiethanolamine did induce genetic effects in somatic cells at much lower doses (0.01–0.26 M). In a single

Table 3. Genetic and related effects of *N*-nitrosodiethanolamine

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA98, TA100, TA1530, TA1538, reverse mutation	–	–	NR	Gilbert *et al.* (1979)
Salmonella typhimurium TA 100, reverse mutation	+	+[c]	6300 µg/plate	Hesbert *et al.* (1979)
Salmonella typhimurium TA1535, reverse mutation	+	NR	1260 µg/plate	Hesbert *et al.* (1979)
Salmonella typhimurium TA100, TA1530, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	10 000 µg/plate	Gilbert *et al.* (1981)
Salmonella typhimurium TA100, TA1535, reverse mutation	–	+[d]	5000 µg/plate	Prival *et al.* (1982)
Salmonella typhimurium TA100, reverse mutation	–	+[e]	3350 µg/plate	Eisenbrand *et al.* (1984)
Salmonella typhimurium TA98, TA100, reverse mutation	+	+[f]	13 400 µg/plate	Dahl (1985)
Salmonella typhimurium TA100, reverse mutation	(+)	(+)[g]	20 000 µg/plate	Mori *et al.* (1987)
Salmonella typhimurium TA1535, reverse mutation	–	–[h]	1000 µg/plate	Lijinsky & Andrews (1983)
Salmonella typhimurium TA1535, reverse mutation	–	–	6700 µg/plate	Eisenbrand *et al.* (1984)
Escherichia coli K-12/343/113, forward mutation (VAL^R)	NR	–[i]	20 000	Kerklaan *et al.* (1981)
Escherichia coli K-12/343/113, mutation (gal^+)	–	+[i,k]	2680	Knasmüller *et al.* (1986)
Drosophila melanogaster unstable *zeste/white* mutation assay (somatic)	+	NT	34 800[l]	Fahmy & Fahmy (1984)
Drosophila melanogaster unstable *zeste/white* mutation assay (germ)	–	NT	268 000[l]	Fahmy & Fahmy (1984)
Drosophila melanogaster unstable *white-ivory-16* mutation assay (somatic)	+	NT	34 800[l]	Fahmy & Fahmy (1984)
Drosophila melanogaster unstable *white-ivory-16* mutation assay (germ)	–	NT	268 000[l]	Fahmy & Fahmy (1984)
DNA single-strand breaks, primary Sprague-Dawley rat hepatocytes *in vitro*	+	NT	1675	Denkel *et al.* (1986)
DNA amplification, CO631 Chinese hamster embryo cells	–	NT	1340	Denkel *et al.* (1986)
DNA single-strand breaks, primary rat hepatocytes *in vitro*	+	NT	3350	Pool *et al.* (1990)
DNA single-strand breaks, primary hamster hepatocytes *in vitro*	+	NT	1675	Pool *et al.* (1990)
DNA single-strand breaks, primary pig hepatocytes *in vitro*	+	NT	1675	Pool *et al.* (1990)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	3570	Dittberner *et al.* (1988)
Sister chromatid exchange, human lymphocytes *in vitro*	NT	+[n]	1675 µg/tube[m]	Henn *et al.* (1989)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, human lymphocytes *in vitro*	NT	+[o]	840 µg/tube[m]	Henn *et al.* (1989)
Chromosomal aberrations, human lymphocytes *in vitro*	(+)	NT	13 800	Dittberner *et al.* (1988)
Chromosomal aberrations, human lymphocytes *in vitro*	NT	–[n]	8380 µg/tube[m]	Henn *et al.* (1989)
Chromosomal aberrations, human lymphocytes *in vitro*	NT	–[o]	4190 µg/tube[m]	Henn *et al.* (1989)
Micronucleus formation, human lymphocytes *in vitro*	+	NT	8770	Dittberner *et al.* (1988)
DNA single strand breaks in Namalva, human lymphoblastoid cell line	–	NT	40 240	Scherer *et al.* (1991)
DNA single strand breaks in rat (Sprague Dawley) or human kidney cells	–	NT	4290	Robbiano *et al.* (1996)
DNA single strand breaks in rat (Sprague Dawley) or human kidney cells	–	NT	6700	Brendler *et al.* (1992)
DNA strand breaks, single-cell gel electrophoresis assay, human mucosal cells of the upper aerodigestive tract *in vitro*	+	NT	6700	Harréus *et al.* (1999)
Host-mediated assay, *Escherichia coli* K-12/343/113 forward mutation (VAL^R) in female Swiss albino mouse livers *in vivo*	+		60.4 ip × 1; 3 h	Kerklaan *et al.* (1981)
Host-mediated assay, *Escherichia coli* forward mutation (*gal*$^+$) in male Swiss albino mouse livers *in vivo*	+		750 s.c. × 1; 3 h	Knasmüller *et al.* (1986)
Host-mediated assay, *E. coli* K-12/343/113 forward mutation (VAL^R) in male Swiss albino mouse liver and spleen *in vivo*	+		30 ip × 1; 3 h	Knasmüller *et al.* (1986)
Host-mediated assay, *E. coli* K-12 *uvr*B/*rec*A and *uvr*$^+$/*rec*$^+$ (differential DNA repair) in male Swiss albino mouse liver, lung and kidney *in vivo*	+		600 po × 1; 2 h	Knasmüller *et al.* (1993)
DNA single-strand breaks, Sprague-Dawley rat liver *in vivo*	+		50.3 po × 1	Denkel *et al.* (1986)
DNA single-strand breaks, male Wistar rat liver *in vivo*	+		100 po × 1	Sterzel & Eisenbrand (1986)
DNA strand breaks, male Sprague-Dawley rat liver *in vivo*	+		1.03 po × 1	Brambilla *et al.* (1987)
Unscheduled DNA synthesis, male Fischer 344 rat hepatocytes *in vivo*	–		1000 po × 1; 2 h/12 h	Mirsalis *et al.* (1989)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Unscheduled DNA synthesis, male B6C3F$_1$ mouse hepatocytes *in vivo*	–		600 po × 1; 2 h/12 h	Mirsalis *et al.* (1989)
Micronucleus assay in *Pleurodeles waltl* (newt) larvae red blood cells *in vivo*	+		12.5 µg/mL; 12 d	L'Haridon *et al.* (1993)
Micronucleus formation, BALB/c mice *in vivo*	–		10 000 ip × 1	Gilbert *et al.* (1981)
Chromosomal aberrations, BALB/c mice *in vivo*	–		10 000 ip × 1	Gilbert *et al.* (1981)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; po, oral; ip, intraperitoneal; d, day
[c] Mouse S9
[d] Hamster S9; inactive with rat S9
[e] Activation with NAD/ADH pH 7.4 and pH 9.5; higher doses were toxic.
[f] Nasal, lung and liver S9 from rat and rabbit
[g] Commercial grade; a purified sample was not mutagenic in the absence of S9.
[h] Rat or hamster liver S9
[i] Rat or mouse liver S9
[k] No mutation leading to arginine prototrophy or 5-methyl-DL-tryptophan resistance were detected.
[l] Topical application on embryos and larvae
[m] Volume not clearly stated
[n] Activation with alcohol dehydrogenase from yeast
[o] Activation with alcohol dehydrogenase from horse liver

study in larvae of the newt *Pleurodeles waltl*, exposure to *N*-nitrosodiethanolamine increased the frequency of micronuclei in a dose-dependent manner at concentrations from 12.5 to 50 ppm (93–373 μM (to larvae, feeding and renewal daily for 12 days).

DNA single-strand breaks were induced *in vitro* by *N*-nitrosodiethanolamine in rat, hamster and pig hepatocytes. However, no DNA single-strand breaks were found in Chinese hamster embryo cells, in a human lymphoblastoid cell line or in rat and human kidney cells *in vitro*.

In one study using cultured human lymphocytes from two female donors, sister chromatid exchanges, chromosomal aberrations and micronuclei were found at increased frequencies after exposure to *N*-nitrosodiethanolamine without exogenous metabolic activation. Dose-dependent increases were detected for all three end-points. In another study, the effect of ADH/NAD from horse liver and yeast was tested for induction of chromosomal aberrations and sister chromatid exchanges by *N*-nitrosodiethanolamine in human lymphocyte cultures. No significantly higher levels of numerical or structural chromosomal aberrations were seen, but the sister chromatid exchange frequency was significantly increased in a dose-dependent manner at dose levels which were significantly lower than those employed in the first study.

A study using the Comet assay in mucosal cells isolated from human biopsies of the upper aerodigestive tract indicated that *N*-nitrosodiethanolamine caused significant DNA damage in oral cavity epithelia and also in the mucosa of the pharynx and larynx.

Induction of DNA single-strand breakage in rat liver after in-vivo exposure to *N*-nitrosodiethanolamine was demonstrated in three studies and dose-dependent effects were shown. In one of these studies, the DNA strand-breaking potential of *N*-nitrosodiethanolamine was found to be abolished by inhibition of sulfotransferase by 2,6-dichloro-4-nitrophenol. Unscheduled DNA synthesis was not detected in rats or mice in an in-vivo/in-vitro hepatocyte DNA repair assay after treatment with *N*-nitrosodiethanolamine. A single study in mice exposed *in vivo* to *N*-nitrosodiethanolamine did not find any significant induction of structural or numerical chromosomal aberrations or micronuclei in bone-marrow cells.

4.5 Mechanistic considerations

Evidence from biological and synthetic model studies suggests that the metabolic activation of *N*-nitrosodiethanolamine may involve enzymatic oxidation at both α and β carbons (Eisenbrand & Janzowski, 1994; Loeppky *et al*., 1998; Park *et al*., 1998; Loeppky, 1999). *N*-Nitrosodiethanolamine has been found to be mutagenic in *Salmonella typhimurium*, mainly in the presence of metabolic activation systems. Eisenbrand *et al*. (1984) reported an activation mechanism for *N*-nitrosodiethanolamine by alcohol dehydrogenase and also investigated several of the metabolites generated during these processes. All were direct-acting mutagens in *S. typhimurium* TA100 (Denkel *et al*., 1986). Cytotoxicity in mammalian cells appears to be CYP2E1-dependent (Janzowski *et al.*, 1996; Loeppky, 1999).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

N-Nitrosodiethanolamine is a contaminant formed by the action of nitrites on ethanolamines in a wide range of products including metalworking fluids, pesticides, antifreeze and personal care products. Occupational exposure by inhalation and dermal contact may occur from water-diluted metalworking fluids contaminated with *N*-nitrosodiethanolamine. General population exposure is possible through contact with a variety of personal care products and the use of some tobacco products. Contamination levels in both metalworking fluids and personal care products have considerably decreased since the 1980s.

5.2 Human carcinogenicity data

Four studies showed inconsistent increases in cancer mortality or incidence at various sites among workers using metalworking fluids containing ethanolamines and sodium nitrite. Only one of them attempted indirectly to estimate exposure to nitrosamines, showing an increased risk for oesophageal cancer with increasing duration of exposure, but there was concomitant exposure to biocides, also associated with an increased risk for oesophageal cancer in this study.

5.3 Animal carcinogenicity data

N-Nitrosodiethanolamine was tested for carcinogenicity by addition to drinking-water in six studies in rats. It was also tested in hamsters by subcutaneous injection in three studies and in single studies by topical or buccal administration. In rats, it consistently produced liver tumours (principally hepatocellular carcinomas). It also produced adenocarcinomas and squamous-cell carcinomas of the nasal cavity. In hamsters, *N*-nitrosodiethanolamine consistently induced adenocarcinomas of the nasal cavity.

In a mouse lung screening assay, *N*-nitrosodiethanolamine increased the incidence and multiplicity of lung tumours.

5.4 Other relevant data

N-Nitrosodiethanolamine is metabolized *in vivo* slowly and only to a small extent, being principally eliminated unchanged in human and rodent urine. Bioactivation of *N*-nitrosodiethanolamine is associated with α- and β-hydroxylation pathways involving the enzymes CYP2E1 and alcohol dehydrogenase, resulting in DNA adduct formation.

Two studies have examined the potential genotoxic hazard of occupational exposure to *N*-nitrosodiethanolamine. The larger one, measuring DNA damage, indicated an association between single-strand breakage and the presence of *N*-nitrosodiethanolamine in

the air of the workplace, but the effects of other exposures could not be excluded. The small study measuring chromosome damage in tool-room workers did not find any significant effect.

N-Nitrosodiethanolamine is mutagenic to bacteria. Studies using cultured cells *in vitro* found induction of DNA single-strand breakage after exposure to *N*-nitrosodiethanolamine in human buccal cells and in rat, hamster and pig hepatocytes. Chromosomal damage was detected in human lymphocytes without exogenous metabolic activity in one study; sister chromatid exchange frequency alone was increased and was detected at lower doses in another study with an exogenous metabolic system including alcohol dehydrogenase.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of *N*-nitrosodiethanolamine.

There is *sufficient evidence* in experimental animals for the carcinogenicity of *N*-nitrosodiethanolamine.

Overall evaluation

N-Nitrosodiethanolamine is *possibly carcinogenic to humans (Group 2B).*

6. References

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2,3-DIBROMOPROPAN-1-OL

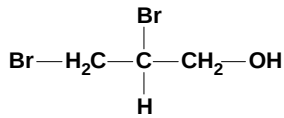
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 96-13-9
Deleted CAS Reg. Nos: 116499-75-3; 204570-16-1
Chem. Abstr. Name: 2,3-Dibromo-1-propanol
IUPAC Systematic Name: 2,3-Dibromopropan-1-ol
Synonyms: DBP; DBP (flame retardant); 1,2-dibromopropan-3-ol; 2,3-dibromopropyl alcohol

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_3H_6Br_2O$ Relative molecular mass: 217.89

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid (Lewis, 1993)
(*b*) *Boiling-point*: 219 °C (Lewis, 1993)
(*c*) *Density*: 2.120 g/cm^3 at 20 °C (Lewis, 1993)
(*d*) *Solubility*: Soluble in water (50–100 g/L), acetone, benzene, diethyl ether and ethanol (National Toxicology Program, 1991; Lewis, 1993)
(*e*) *Spectroscopy data*: infrared (prism [16246], grating [10042]), nuclear magnetic resonance (proton [4214], C-13[2148] and mass spectral data [NIST, 10146] have been reported (Sadtler Research Laboratories, 1980; National Institute of Standards and Technology, 1998)

(*f*) *Stability*: flash point, 112 °C (National Toxicology Program, 1991)
(*g*) *Conversion factor*[1]: $mg/m^3 = 8.91 \times ppm$

1.1.4 *Technical products and impurities*

Trade names for 2,3-dibromopropan-1-ol include: Brominex 257.

1.1.5 *Analysis*

2,3-Dibromopropan-1-ol can be analysed in air, water and food samples by gas chromatography with either electron capture or flame ionization detection (Yurawecz & Puma, 1986; Choudhary, 1987; Matthew & Anastasio, 2000).

1.2 Production

2,3-Dibromopropan-1-ol has been prepared by reaction of allyl alcohol, bromine, and aqueous lithium bromide (Jenkner & Rabe, 1967; Thomas & Levek, 1972).

Information available in 1999 indicated that 2,3-dibromopropan-1-ol was manufactured by three companies in Japan and one company each in Ukraine and the United States (Chemical Information Services, 1999).

1.3 Use

2,3-Dibromopropan-1-ol has been used as an intermediate in the preparation of flame retardants, insecticides and pharmaceuticals (Lewis, 1993). In particular, in the 1970s, the major use of 2,3-dibromopropan-1-ol was in the preparation of the flame retardant tris(2,3-dibromopropyl) phosphate, which was used in textiles; production of this flame retardant other than for research purposes has been discontinued (WHO, 1995; IARC, 1999).

1.4 Occurrence

1.4.1 *Natural occurrence*

2,3-Dibromopropan-1-ol is not known to occur as a natural product.

1.4.2 *Occupational exposure*

No data were available to the Working Group.

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[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

1.4.3 *Environmental occurrence*

The past use of 2,3-dibromopropan-1-ol as an intermediate in the preparation of the flame retardant tris(2,3-dibromopropyl) phosphate (and as an impurity in tris(2,3-dibromopropyl) phosphate) (Blum & Ames, 1977; Ulsamer *et al*., 1980; WHO, 1995), as a chemical intermediate for insecticidal and pharmaceutical preparations and as a flame retardant itself (Fishbein, 1979) may have resulted in its release into the environment through various waste streams (Ulsamer *et al*., 1980; WHO, 1995).

(*a*) *Industrial effluent discharges*

2,3-Dibromopropan-1-ol has been detected in industrial discharges at levels of 0.5 mg/L (CEC, 1976) and is a hydrolysis product of tris(2,3-dibromopropyl) phosphate (St John *et al.*, 1976). Tris(2,3-dibromopropyl) phosphate has been converted to 2,3-dibromopropan-1-ol by sewage sludge (Alvarez *et al.*, 1982).

(*b*) *Human tissues and secretions*

2,3-Dibromopropan-1-ol, a metabolite of tris(2,3-dibromopropyl) phosphate, has been found in urine samples (at levels of up to 29 ng/mL) from 10 children who were wearing or who had worn tris(2,3-dibromopropyl) phosphate-treated nightwear (Blum *et al*., 1978).

1.5 Regulations and guidelines

No occupational exposure limit or guideline has been established for 2,3-dibromopropan-1-ol.

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*

Groups of 50 male and 50 female B6C3F_1 mice, eight weeks of age, were administered doses of 0, 88 or 177 mg/kg bw 2,3-dibromopropan-1-ol (98% purity), applied in 95% ethanol onto the interscapular skin on five days per week for 36–39 weeks for male mice and 39–42 weeks for female mice (the study was terminated

earlier than the planned two years because lymphocytic choriomeningitis virus was detected in serum of sentinel mice). The chemical was administered by pipette at a constant volume of 100 μL. Survival at termination of the study was 100% in the male mice and almost 100% in the females. As shown in Table 1, there were statistically significant increases in the incidence of epithelial tumours of the skin at the site of application of the test substance (squamous papillomas and sebaceous adenomas in high-dose males and females), in squamous papillomas and/or carcinomas of the forestomach in both low- and high-dose females and in adenomas of the liver in high-dose males. In addition, there was a marginal increase in lung adenomas in high-dose males and females, accompanied by a significant increase in focal hyperplasia of the epithelial lining of bronchi, bronchioles or alveoli (National Toxicology Program, 1993; Eustis *et al*., 1995).

3.1.2 *Rat*

Groups of 50 male and 50 male Fischer 344/N rats, eight weeks of age, were administered total doses of 0, 188 or 375 mg/kg bw 2,3-dibromopropan-1-ol (98% purity, confirmed by gas chromatography) applied in 95% ethanol to the interscapular skin on five days per week for 48–51 weeks in male rats and 52–55 weeks in female rats. The study was terminated earlier than the planned two years because of reduced survival of the high-dose groups related to chemical-induced neoplasia, and because sentinel mice housed in the same room as the rats tested positively for lymphocytic choriomeningitis virus. The chemical was administered by pipette at a constant volume of 300 μL. Survival was reduced significantly in treated animals, particularly at the high dose, being 50/50, 41/50 and 17/50 for control, low-dose and high-dose males, respectively, and 48/50, 38/50 and 24/50 for control, low-dose and high-dose females, respectively. As shown in Table 2, there were significantly increased incidences of epithelial tumours of the skin at or near the site of application of the test substance (squamous carcinomas, basal-cell tumours, sebaceous adenomas, keratoacanthomas) in mid- and high-dose males and high-dose females, squamous-cell papillomas or carcinomas of the oral mucosa, oesophagus and/or forestomach in both males and females at both doses, adenocarcinomas of the small intestine of low- and high-dose males, adenomatous polyps of the large intestine in both males and females at both doses, adenomas of the nasal mucosa in both males and females at both doses, Zymbal gland adenomas or adenocarcinomas in either low- and/or high-dose males and females, hepatocellular neoplastic nodules [now generally classified as adenomas] or carcinomas in low- or high-dose females and mammary gland adenocarcinomas and clitoral gland adenocarcinomas in the high-dose females (National Toxicology Program, 1993; Eustis *et al*., 1995).

Table 1. Incidence of primary tumours in B6C3F$_1$ mice exposed to 2,3-dibromopropan-1-ol

Tumour site	Number of animals with tumours					
	Males			Females		
	0 mg/kg bw	88 mg/kg bw	177 mg/kg bw	0 mg/kg bw	88 mg/kg bw	177 mg/kg bw
Skin[a]	0/50	4/50	18/50**	0/50	4/50	9/50**
Forestomach[b]	0/50	14/50**	21/49**	0/50	18/49**	19/50**
Liver[c]	1/50	2/50	11/50*			
Lung[d]	1/50	1/50	6/50*	0/50	3/50	4/50

From National Toxicology Program (1993); Eustis *et al*. (1995)

[a] Squamous-cell papillomas or carcinomas and sebaceous gland adenomas

[b] Squamous-cell papillomas and carcinomas

[c] Hepatocellular adenomas

[d] Alveolar/bronchiolar adenomas

* $p \leq 0.05$ Fisher's exact test

** $p \leq 0.01$ Fisher's exact test

Table 2. Incidence of primary tumours in Fischer 344 rats exposed to 2,3-dibromopropan-1-ol

Tumour site	Number of animals with tumours					
	Males			Females		
	0 mg/kg bw	188 mg/kg bw	375 mg/kg bw	0 mg/kg bw	188 mg/kg bw	375 mg/kg bw
Skin[a]	1/50	22/50**	33/50**	0/50	3/50	18/50**
Mouth[b]	0/50	47/50**	48/50**	0/50	39/50**	49/50**
Oesophagus[c]	0/50	19/50**	33/50**	0/50	9/50**	38/50**
Forestomach[c]	0/50	1/50	17/50**	1/50	3/50	23/50**
Small intestine[d]	0/50	8/50**	11/50**	0/50	3/50	4/50
Large intestine[e]	1/50	13/50**	29/50**	0/50	12/50**	37/50**
Nose[f]	0/50	48/50**	48/50**	0/50	44/50**	49/50**
Liver[g]	0/50	4/50	5/50*	0/50	11/50**	14/50**
Zymbal gland[h]	0/50	9/50**	35/50**	1/50	9/50**	22/50**
Clitoral gland[h]				0/50	1/50	6/50*
Mammary gland[i]				0/50	0/50	5/50*

From National Toxicology Program (1993); Eustis *et al*. (1995)

[a] Squamous-cell papillomas or carcinomas, basal-cell tumours, sebaceous adenomas, keratoacanthomas

[b] Squamous-cell papillomas or carcinomas

[c] Squamous-cell papillomas

[d] Adenocarcinomas

[e] Adenomatous polyps

[f] Adenomas

[g] Neoplastic nodules or carcinomas

[h] Adenomas or adenocarcinomas

[i] Adenocarcinomas

* $p \leq 0.05$, Fisher's exact test

** $p \leq 0.01$, Fisher's exact test

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Following oral administration of 50 mg/kg bw 2,3-dibromopropan-1-ol to male Sprague-Dawley rats, two urinary mercapturic acid metabolites were identified as *N*-acetyl-*S*-(2,3-dihydroxypropyl)cysteine and *N,N′*-bis-acetyl-*S,S′*-(1,3-bis-cysteinyl)-propan-2-ol, respectively. It was inferred that 3-bromo-1,2-propane epoxide is an intermediate in the metabolism of 2,3-dibromopropan-1-ol. In addition, β-bromolactate was produced, presumably as a result of hydrolysis to the α-bromohydrin and subsequent oxidation (Jones & Fakhouri, 1979). Marsden and Casida (1982) identified small amounts of 2-bromoacrylic acid in the urine of rats injected intraperitoneally with 2,3-dibromopropan-1-ol and suggested that this arose by oxidation and dehydrobromination, with 2-bromoacrolein as an unstable intermediate.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

A single dose of 2,3-dibromopropan-1-ol (61 mg/kg bw) was administered intraperitoneally to male Sprague-Dawley rats (n = 8; 275–325 g) and the rats were killed 48 h later. Serum creatinine, a measure of glomerular filtration rate, was measured. Renal cortical slices were prepared from one kidney and incubated for 90 min in the presence of the organic acid, *para*-aminohippurate, and the organic base, *N*-[^{14}C]methylnicotinamide, to evaluate tubular function. The other kidney was processed by standard histological methods. At this dose, 2,3-dibromopropan-1-ol did not cause any functional or histological change in the kidney (Elliott *et al.*, 1982). In a separate study, male Sprague-Dawley rats (290–310 g) were given an intraperitoneal dose of 39 mg 2,3-dibromopropan-1-ol in 1 mL Emulphor and urine volume was measured for 10 days. There was a two- to threefold increase in urine volume in treated rats, which rapidly returned to normal (Lynn *et al.*, 1982).

In a study in which liver microsomes, prepared from male Wistar rats (200–250 g), were incubated with 5×10^{-4} mol/L 2,3-dibromo[^{3}H]propan-1-ol and an NADPH-generating system, covalent binding to protein was determined. Addition of the epoxide hydrolase inhibitor 1,1,1-trichloropropene-2,3-epoxide led to an increase in the protein-binding rate of 2,3-dibromopropan-1-ol (Söderlund *et al.*, 1981).

The National Toxicology Program (1993) conducted a series of toxicity studies on 2,3-dibromopropan-1-ol in Fischer 344 rats and B6C3F$_1$ mice. The first study involved 16-day dermal exposure to 2,3-dibromopropan-1-ol. Male and female rats and mice, 52 days and 59 days of age, respectively, were exposed by skin application to 0, 44, 88, 177, 375 or 750 mg/kg bw 2,3-dibromopropan-1-ol in 95% ethanol applied to the shaved subscapular skin. All rodents were treated on five days per week, for a total of 12 exposure days. Two rats and five mice died within two to three days following exposure to 750 mg/kg. However, no treatment-related clinical findings or gross observations were observed in male or female rats or mice exposed to 2,3-dibromopropan-1-ol.

In a 13-week study, male and female Fischer 344 rats and B6C3F$_1$ mice were exposed by skin application to 0, 44, 88, 177, 375 or 750 mg/kg bw 2,3-dibromopropan-1-ol in 95% ethanol applied to the clipped subscapular skin on five days per week for 13 weeks. Animals were approximately seven weeks (rats) or nine weeks (mice) of age when the study began. All rats survived until the end of the study. Eight male mice in the 750-mg/kg bw dose group died during the first four days of the study. Body weight gain decreased significantly in male and female rats exposed to 750 mg/kg bw, by 11% and 13% compared with male and female controls, respectively. Chemical-induced lesions were seen in the kidneys of male rats, the liver of female rats and the liver and lung of both male and female mice. In male rats, nephropathy, characterized by occasional clusters of cortical tubules with thickened basement membranes and basophilic epithelium, was observed in most animals; however, the severity was slightly greater in males rats receiving 375 or 750 mg/kg bw than in the controls. Individual hepatocyte necrosis was observed in all female rats in the 750-mg/kg bw dose group. A dose-related increase in the incidence of pleomorphism of the bronchial and bronchiolar epithelium was observed in male and female mice. This lesion was characterized by loss of nuclear and cellular polarity, cytomegaly with karyomegaly and syncytia formation. The low incidence of this lesion in male mice exposed to 750 mg/kg bw was attributed to the high and early mortality in this group. Centrilobular hepatocellular necrosis was seen in many of the male mice exposed to 750 mg/kg bw that died. There was an increased incidence of hepatocellular necrosis in female mice. Infrequent, scattered individual or small clusters of necrotic hepatocytes, surrounded by a few neutrophils or macrophages, characterized this lesion (National Toxicology Program, 1993; Eustis *et al.*, 1995).

In a long-term study, male and female Fischer 344 rats and B6C3F$_1$ mice received skin applications of 0, 188 or 375 mg/kg bw 2,3-dibromopropan-1-ol and 0, 88 or 177 mg/kg bw 2,3-dibromopropan-1-ol, respectively, in 95% ethanol to the subscapular

skin on five days per week. Due to the reduced survival of rats in the high-dose group and because of the detection of antibodies to lymphocytic choriomeningitis virus in sentinel mice, the study was terminated early. Rats received dermal applications for 48–55 weeks and mice received dermal applications for 36–42 weeks. 2,3-Dibromopropan-1-ol caused an increase in the incidence of hyperplasia in the skin, epithelial dysplasia of the forestomach and bronchiolar epithelial pleomorphism and hyperplasia in male and female mice. In rats, 2,3-dibromopropan-1-ol increased the incidences of hyperkeratosis in the skin, forestomach and oesophagus, and epithelial dysplasia in the nose. Cellular pleomorphism and basophilic and clear cell changes were observed in the liver. Nuclear enlargement in the kidney and dose-related increases in the incidences of forestomach ulcers and acanthosis, angiectasis in the liver and renal hyperplasia were also observed in male rats. In female rats, epithelial dysplasia of the forestomach and bile duct hyperplasia in the liver were also observed (National Toxicology Program, 1993).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 3 for references)

2,3-Dibromopropan-1-ol was mutagenic in several strains of *Salmonella typhimurium* both in the presence and in the absence of exogenous metabolic systems. It caused preferential cell killing in a polymerase-deficient strain of *Escherichia coli*. It also gave positive results in the mouse lymphoma assay in the absence of S9 activation and caused increases in sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells. It displayed mutagenic activity in male germ cells and caused chromosomal breakage in *Drosophila melanogaster*. However, the compound was inactive in an in-vivo bone-marrow micronucleus assay in male mice.

Table 3. Genetic and related effects of 2,3-dibromopropan-1-ol

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, reverse mutation	–	+	20 µg/plate	Blum and Ames (1977)
Salmonella typhimurium TA100, TA1535, reverse mutation	+	+	0.1 µL/plate	Carr & Rosenkranz (1978)
Salmonella typhimurium TA100, TA1535, reverse mutation	+	+	218 µg/plate	Nakamura *et al.* (1979)
Salmonella typhimurium TA100, TA1535, reverse mutation	(+)	+	54.5 µg/plate	Lynn *et al.* (1982)
Salmonella typhimurium TA100, TA1535, reverse mutation	+	+	3.3 µg/plate	Haworth *et al.* (1983)
Salmonella typhimurium TA100, reverse mutation	NT	+	10.9	Holme *et al.* (1983)
Salmonella typhimurium TA1535, reverse mutation	+	+	0.1 µL/plate	Prival *et al.* (1977)
Salmonella typhimurium TA1537, TA1538, reverse mutation	–	–	21 800 µg/plate	Nakamura *et al.* (1979)
Salmonella typhimurium TA1537, reverse mutation	–	–	1000 µg/plate	Haworth *et al.* (1983)
Salmonella typhimurium TA1538, reverse mutation	–	–	10 µL/plate	Prival *et al.* (1977)
Salmonella typhimurium TA1538, reverse mutation	–	NT	10 µL/plate	Carr & Rosenkranz (1978)
Salmonella typhimurium TA98, reverse mutation	–	(+)	NR	Nakamura *et al.* (1979)
Salmonella typhimurium TA98, reverse mutation	–	(+)	333 µg/plate	Haworth *et al.* (1983)
Salmonella typhimurium TA102, TA2638, reverse mutation	NT	+	625 µg/plate	Watanabe *et al.* (1998)
Escherichia coli WP2/pKM101, reverse mutation	NT	+	2000 µg/plate	Watanabe *et al.* (1998)
Escherichia coli WP2 *uvr*A/pKM101, reverse mutation	NT	+	313 µg/plate	Watanabe *et al.* (1998)
Drosophila melanogaster, sex-linked recessive lethal mutation	+		500 ppm in feed	Yoon *et al.* (1985); National Toxicology Program (1993)
Drosophila melanogaster, reciprocal translocations	+		440 ppm in feed	Yoon *et al.* (1985)
Drosophila melanogaster, aneuploidy	–[c]		50 mg/mL in feed	Zimmering (1983)

Table 3 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Drosophila melanogaster, aneuploidy	(+)		0.5 mg/mL in feed	Zimmering *et al.* (1986)
Drosophila melanogaster, loss of heterozygosity by mitotic recombination	+		21.8 µg/mL in feed	Vogel & Nivard (1993)
Mutation, V79 Chinese hamster lung cells *in vitro*	NT	+	4.36	Holme *et al.* (1983)
Mutation, mouse lymphoma, L5178Y cells *Tk* locus in *vitro*	+	NT	0.0625	National Toxicology Program (1993)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	+	50.9	National Toxicology Program (1993)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	+	+	626.4	National Toxicology Program (1993)
Micronucleus formation, male B6C3F_1 mouse bone-marrow cells *in vivo*	–		100 ip × 3	National Toxicology Program (1993)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; NR, not reported
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day
[c] Positive for DNA repair deficient strain Mei-9[a]

5. Summary of Data Reported and Evaluation

5.1 Exposure data

2,3-Dibromopropan-1-ol was used as an intermediate to produce the flame retardant tris(2,3-dibromopropyl) phosphate. In the past, it was detected in the urine of children wearing nightwear treated with tris(2,3-dibromopropyl) phosphate. It is still produced for use in the manufacture of other chemicals (possibly flame retardants, insecticides and pharmaceuticals).

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

2,3-Dibromopropan-1-ol was tested by skin application in one experiment in mice and in one experiment in rats. In mice, it produced tumours of the skin at the site of application and forestomach in both males and females and tumours of the liver in males. In rats, it produced tumours of the skin at the site of application and of the digestive tract including the mouth, oesophagus, forestomach and intestines, nasal mucosa and Zymbal gland in both males and females, and tumours of the liver, mammary gland and clitoral gland in females.

5.4 Other relevant data

Two mercapturic acid metabolites of 2,3-dibromopropan-1-ol have been identified in the urine of treated rats.

Application of 2,3-dibromopropan-1-ol to the skin of rats and mice for 13 weeks caused kidney lesions in male rats, liver lesions in female rats and liver and lung lesions in both male and female mice.

2,3-Dibromopropan-1-ol was mutagenic in bacterial assays both in the presence and absence of exogenous metabolic systems. It gave positive results in a mammalian cell mutagenesis assay and induced sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells. It was mutagenic in *Drosophila melanogaster*. It was inactive in an in-vivo bone-marrow micronucleus assay in male mice.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of 2,3-dibromopropan-1-ol were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2,3-dibromopropan-1-ol.

Overall evaluation

2,3-Dibromopropan-1-ol is *possibly carcinogenic to humans (Group 2B)*.

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2,2-BIS(BROMOMETHYL)PROPANE-1,3-DIOL

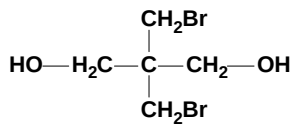
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 3296-90-0
Chem. Abstr. Name: 2,2-Bis(bromomethyl)propane-1,3-diol
IUPAC Systematic Name: 2,2-Bis(bromomethyl)propane-1,3-diol
Synonyms: 1,3-Dibromo-2,2-dihydroxymethylpropane; 1,3-dibromo-2,2-dimethylolpropane; 2,2-dibromomethyl-1,3-propanediol; dibromoneopentyl glycol; pentaerythritol dibromide; pentaerythritol dibromohydrin

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_5H_{10}Br_2O_2$ Relative molecular mass: 261.94

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Off-white crystalline powder (Ameribrom, 1996), with a slight, mild, musty odour (National Toxicology Program, 1996)
(*b*) *Boiling-point*: 134 °C at 0.13 kPa (Dead Sea Bromine Group, 1998)
(*c*) *Melting-point*: 109.5 °C (Ameribrom, 1996)
(*d*) *Density:* 2.23 g/cm^3 (Ameribrom, 1996)
(*e*) *Spectroscopy data*: Infrared (prism [48246], grating [35246]), ultraviolet/visible and nuclear magnetic resonance (proton [20723], C-13 [5652]) spectral data have been reported (Sadtler Research Laboratories, 1980)

(*f*) *Solubility*: Soluble in water (20 g/L at 25 °C) and benzene; very soluble in acetone, isopropanol and methanol; slightly soluble in carbon tetrachloride and xylene (Ameribrom, 1996)

(*g*) *Volatility*: Vapour pressure, 1.33 kPa at 178 °C; 3.33 kPa at 200 °C (Dead Sea Bromine Group, 1998)

(*h*) *Octanol/water partition coefficient (P)*: log P, 2.29 (Dead Sea Bromine Group, 1998)

(*i*) *Conversion factor*[1]: $mg/m^3 = 10.71 \times ppm$

1.1.4 *Technical products and impurities*

2,2-Bis(bromomethyl)propane-1,3-diol is currently available with a purity of 98.5% (Ameribrom, 1996; Dead Sea Bromine Group, 1998). 2,2- Bis(bromomethyl)-propane-1,3-diol was available earlier as a technical product with a purity of 79%. Several impurities were identified in the technical product: 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane, 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane and pentaerythritol (National Toxicology Program, 1996).

Trade names for 2,2-bis(bromomethyl)propane-1,3-diol include: FR-522 and FR-1138.

1.1.5 *Analysis*

No methods have been reported for analysis of 2,2-bis(bromomethyl)propane-1,3-diol in environmental matrices.

1.2 Production

2,2-Bis(bromomethyl)propane-1,3-diol can be produced by replacement of the hydroxyl groups of pentaerythritol with bromide (National Toxicology Program, 1996).

The total world market for flame retardants is estimated at just under 1 million tonnes per year (Roskill Information Services, 1997).

Information available in 1999 indicated that 2,2-bis(bromomethyl)propane-1,3-diol was manufactured by one company each in Israel and Ukraine (Chemical Information Services, 1999).

1.3 Use

2,2-Bis(bromomethyl)propane-1,3-diol is a reactive flame retardant that is used primarily in unsaturated polyester resins for moulded products and in rigid polyurethane

[1] Calculated from: $mg/m^3 = (\text{relative molecular mass}/24.45) \times ppm$, assuming a temperature of 25 °C and a pressure of 101 kPa

foams. It is increasingly used in CFC (chlorofluorocarbon)-free foam products designed to meet more stringent standards of flame retardancy (Ameribrom, 1996; National Toxicology Program, 1996).

1.4 Occurrence

1.4.1 *Natural occurrence*

2,2-Bis(bromomethyl)propane-1,3-diol is not known to occur as a natural product.

1.4.2 *Occupational exposure*

No data were available to the Working Group.

1.4.3 *Environmental occurrence*

2,2-Bis(bromomethyl)propane-1,3-diol may enter the environment as fugitive dust, through wastewater and through disposal of resins and foams which may contain the compound as an impurity. 2,2-Bis(bromomethyl)propane-1,3-diol may be persistent in water (Environmental Protection Agency, 1983; Elwell *et al*., 1989; Dunnick *et al*., 1997). No data were available to the Working Group on levels of 2,2-bis(bromomethyl)propane-1,3-diol in the environment.

1.5 Regulations and guidelines

No occupational exposure limit or guideline has been established for 2,2-bis(bromoethyl)propane-1,3-diol.

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 B6C3F_1 mice, six weeks of age, were fed diets containing 0, 312, 625 or 1250 mg/kg diet (ppm) 2,2-bis(bromomethyl)propane-1,3-diol (technical grade FR-1138®; with a composition of 78.6% 2,2-bis(bromomethyl)-

propane-1,3-diol, 6.6% 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane, 6.9% 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane, 0.2% pentaerythritol and 7.7% dimers and structural isomers) for 104–105 weeks. Average daily doses were 0, 35, 70 or 149 mg/kg bw for male mice and 0, 40, 80 or 170 mg/kg bw for female mice. Survival in both males and females treated with 1250 ppm was significantly lower than that of controls and this decrease in survival was associated with development of treatment-related tumours. Mean body weights of exposed male and female mice were similar to those of controls throughout the study. As shown in Table 1, male mice treated for two years had significantly increased incidences of Harderian gland, lung and forestomach tumours. In females, increased incidences of Harderian gland, lung, subcutaneous and forestomach tumours were observed. A marginal increase in the incidence of haemangiomas/haemangiosarcomas was observed in females (National Toxicology Program, 1996; Dunnick *et al*., 1997).

3.1.2 *Rat*

Groups of 49–50 male and 49–50 female Sprague-Dawley rats, eight to nine weeks of age, were fed diets containing FR-1138® (with a composition of 80% 2,2-bis(bromomethyl)propane-1,3-diol, 8% 2,2′-bis(bromomethyl)-1-bromo-3-hydroxypropane and 6% 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane) at doses of 0, 5 or 100 mg/kg bw per day for two years. Slight reductions in body weight were observed in treated males. Survival was not significantly different among the groups; most rats died or were killed at between 17 and 24 months. No treatment-related effects on tumour incidence were noted (Keyes *et al*., 1980).

Groups of 60 male and 60 female Fischer 344/N rats, six weeks of age, were fed diets containing FR-1138® (as used in the mouse study) at concentrations of 0, 2500, 5000 or 10 000 ppm for 104–105 weeks. A stop-exposure group of 60 male rats received 20 000 ppm in the diet for three months, after which animals received control diet for the remainder of the two-year study. Average daily doses were 0, 100, 200 or 430 mg/kg bw for males and 0, 115, 230 or 460 mg/kg bw for females. Stop-exposure male rats received an average daily dose of 800 mg/kg bw. Survival at two years of male and female rats exposed continuously to 5000 and 10 000 ppm and of the 20 000-ppm stop-exposure male rats was significantly lower than that of controls. Mean body weights of the 10 000-ppm male rats and 20 000-ppm stop-exposure male rats were lower than those of controls. Body weights of the other treated groups were in general similar to those of controls. Male rats in the stop-exposure group began to die of treatment-related tumours (particularly intestinal tumours) by one year of age. As shown in Table 2, significantly increased incidences of neoplasms in various organs of both male and female rats were observed, with males exhibiting a wider range of affected organs than females. In male rats that received 20 000 ppm for three months, then standard diet for the remainder of the study, essentially the same pattern of proliferative changes and tumours occurred as in male rats fed the agent continuously.

Table 1. Neoplastic lesions in mice treated with 2,2-bis(bromomethyl)-propane-1,3-diol in the diet

Tumour site/type	Number of animals with tumours			
Dose (mg/kg diet):	0	312	625	1250
Males				
Harderian gland				
Adenomas	3/50	6/51	12/50**	18/49**
Carcinomas	1/50	1/51	4/50	4/49
Adenomas and carcinomas combined	4/50	7/51	16/50**	22/49**
Lung (alveolar/bronchiolar)				
Adenomas	12/50	4/51	12/50	21/49**
Carcinomas	3/50	7/51	8/50	11/49**
Adenomas and carcinomas combined	15/50	11/51	16/50	25/49**
Forestomach				
Squamous-cell papillomas	0/50	3/51	2/50	2/49
Squamous-cell carcinomas	0/50	0/51	1/50	2/49
Papillomas and carcinomas combined	0/50	3/51	3/50	4/49*
Females				
Harderian gland				
Adenomas	2/52	6/50	8/51*	15/50**
Carcinomas	1/52	6/50	5/51	7/50*
Adenomas and carcinomas combined	3/52	12/50**	13/51**	19/50**
Lung (alveolar/bronchiolar)				
Adenomas	3/52	3/50	9/51*	17/50**
Carcinomas	2/52	2/50	6/51	5/50
Adenomas and carcinomas combined	5/52	5/50	15/51*	19/50**
Skin, subcutaneous				
Fibrosarcomas/sarcomas	0/52	1/50	4/51	12/50**
Forestomach				
Squamous-cell papillomas	0/52	1/50	5/51*	3/50
Circulatory system				
Haemangioma/haemangiosarcomas	1/52	2/50	0/51	5/50*

From National Toxicology Program (1996); Dunnick *et al*. (1997)
* $p < 0.05$, logistic regression test
** $p \leq 0.01$, logistic regression test

Significantly increased incidences of neoplasms of the skin, subcutaneous tissue, mammary gland, Zymbal gland, oral cavity, oesophagus, forestomach, small and large intestines, peritoneum, lung and thyroid were observed in males. Increased incidences of neoplasms of the mammary gland, oesophagus and thyroid were observed in female rats (National Toxicology Program, 1996; Dunnick *et al*., 1997).

Table 2. Neoplastic and related lesions in rats treated with 2,2-bis(bromomethyl)propane-1,3-diol in the diet for three months (stop-exposure) or two years

Tumour site/type	Number of animals with tumours				
Dose (mg/kg diet):	0	2500	5000	10 000	20 000 stop-exposure
Males					
Skin					
All skin tumours (benign and malignant)	4/51	6/53	14/51**	24/55**	21/59**
Subcutaneous tissue					
Fibroma	2/51	8/53*	11/51**	15/55**	7/60
Fibrosarcomas/sarcomas	0/51	1/53	2/51	3/55	3/60
Fibromas and sarcomas combined	2/51	9/53*	13/51**	16/55**	10/59**
Mammary gland					
Fibroadenomas	0/51	4/53*	6/51**	6/55**	5/60**
Zymbal gland					
Adenomas	0/51	0/53	1/51	3/55	2/60
Carcinomas	2/51	1/53	3/51	2/55	15/60**
Adenomas and carcinomas combined	2/51	1/53	4/51	5/55	15/60**
Oral cavity					
Squamous-cell papilloma	0/51	4/53*	8/51**	10/55**	12/60**
Oesophagus					
Squamous-cell papilloma	0/51	0/53	1/51	5/55*	0/60
Forestomach					
Squamous-cell papilloma	0/51	0/53	0/51	1/55	5/60**
Small intestine					
Adenomas	0/51	0/53	0/51	0/55	1/60
Carcinomas	0/51	0/53	0/51	2/55	4/60
Adenomas and carcinomas combined	0/51	0/53	0/51	2/55	5/60*
Large intestine					
Adenomas	0/51	0/53	3/51	4/55	10/59*
Carcinomas	0/51	0/53	0/51	0/55	2/59
Adenomas and carcinomas combined	0/51	0/53	3/51	4/55	11/59**
Peritoneum					
Malignant mesotheliomas	0/51	3/53	8/51**	9/55**	26/60**
Lung					
Alveolar/bronchiolar adenomas	1/51	0/53	3/51	1/55	4/60
Alveolar/bronchiolar carcinomas	0/51	1/53	0/51	3/55*	3/60
Alveolar/bronchiolar adenomas and carcinomas combined	1/51	1/53	3/51	4/55**	7/60**
Thyroid					
Follicular-cell adenomas	0/51	1/53	2/51	2/55	7/59*
Follicular-cell carcinomas	0/51	1/53	4/51*	1/55	2/59
Adenomas and carcinomas combined	0/51	2/53	6/51*	3/55	9/59**

Table 2 (contd)

Tumour site/type	Number of animals with tumours				
Dose (mg/kg diet):	0	2500	5000	10 000	20 000 stop-exposure
Females					
Mammary gland					
Fibroadenomas	25/50	45/51**	46/53**	45/52**	
Oesophagus					
Squamous-cell papilloma	0/50	0/51	1/53	10/52**	
Thyroid					
Follicular-cell adenomas	0/50	0/51	2/53	3/52	
Follicular-cell carcinomas	0/50	0/51	0/53	1/52	
Adenomas and carcinomas combined	0/50	0/51	2/53	4/52**	

From National Toxicology Program (1996); Dunnick *et al*. (1997)
* $p < 0.05$, logistic regression test
** $p < 0.01$, logistic regression test

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

4.1 Absorption, distribution, metabolism and excretion

No data were available to the Working Group.

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

The acute oral LD_{50} of 2,2-bis(bromomethyl)propane-1,3-diol in male rats was reported to be 3458 mg/kg bw (95% CI, 2810–4257 mg/kg) (Keyes *et al*., 1980).

Thirteen-week toxicity studies of 2,2-bis(bromomethyl)propane-1,3-diol were conducted in male and female Fischer 344/N rats and $B6C3F_1$ mice to determine target organ toxicity. 2,2-Bis(bromomethyl)propane-1,3-diol (technical grade, 78.6% pure) was administered by gavage in corn oil for five days per week to rats, 6–7 weeks of age, at doses of 0, 50, 100, 200, 400 or 800 mg/kg bw and to mice, 6–9 weeks of age,

at doses of 0, 25, 50, 100, 200 or 400 mg/kg bw, or in the feed at concentrations of 0, 1250, 2500, 5000, 10 000 or 20 000 ppm for rats and 0, 625, 1250, 2500, 5000 or 10 000 ppm for mice. In both studies, the kidney (papillary degeneration and necrosis) and urinary bladder (hyperplasia of the transitional-cell epithelium) were target organs, with mice being more sensitive than rats. Male rats and mice were more sensitive than females to the development of renal papillary degeneration or necrosis. At similar dose levels, on a mg/kg bw basis, treatment-related lesions in rats were similar in the gavage and feed studies. Lesions developed at a slightly lower dose level in mice treated by gavage than in those given the chemical in the diet (Elwell *et al.*, 1989; National Toxicology Program, 1996).

In a two-year study, male and female Fischer 344/N rats and B6C3F_1 mice received 0, 2500, 5000 or 10 000 ppm [average daily doses, 0, 100, 200 and 430 mg/kg bw for males and 115, 230 or 460 mg/kg bw for females] and 0, 312, 625 or 1250 ppm [average daily doses, 0, 35, 70 or 140 mg/kg bw for males and 40, 80 or 170 mg/kg bw for females] 2,2-bis(bromomethyl)propane-1,3-diol (purity, 78.6%; FR-1138®) in the diet, respectively. Non-neoplastic effects observed in the kidney of rats included papillary degeneration, increases in the incidences of hyperplasia of the renal papilla epithelium, hyperplasia of the transitional epithelium lining the renal pelvis and focal renal tubule atrophy in male rats. In male rats, transitional-cell hyperplasia of the urinary bladder was also present. In female mice, the incidence of alveolar epithelial hyperplasia was greater than in the control group (National Toxicology Program, 1996).

Groups of male and female Sprague-Dawley SPF-derived rats, seven to eight weeks of age, were placed on a lifetime diet supplying 0, 5 or 100 mg/kg bw per day FR-1138® (containing 80% 2,2-bis(bromomethyl)propane-1,3-diol). No changes in haematological parameters, urinary parameters or blood urea nitrogen, serum glutamic pyruvic transaminase and serum alkaline phosphatase levels were observed. Rats ingesting the dietary level of 5 mg/kg FR-1138 per day had no adverse effects related to the treatment. At the higher dose, evidence of toxicity included degenerative changes in the liver (increased centrilobular homogeneity of the hepatocellular cytoplasm), eye (bilateral diffuse opacity of the lenses) and increased incidence of thyroid retention cyst formation (Keyes *et al.*, 1980).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Treinen *et al.* (1989) investigated 2,2-bis(bromomethyl)propane-1,3-diol (purity, 87.3%) in a continuous breeding study by administration in the feed to Swiss CD-1 mice at levels of 0, 0.1, 0.2 and 0.4% (daily doses estimated to be 0, 141, 174 and 589 mg/kg

bw, respectively) to 20 male/female pairs per group. A control group of 40 breeding pairs was included. Dosing started seven days before and continued during a 98-day cohabitation period. At the end of the 98-day cohabitation period, the males were removed, allowing the dams to deliver and rear the final litter, while dosing continued. From weaning at postnatal day 21, these F_1 litters received 2,2-bis(bromomethyl)propane-1,3-diol at parental doses until mated with similarly treated non-siblings at 74 ± 10 days of age (20 breeding pairs per group). In addition, cross-over mating was performed on the parental (F_0) animals from the 0.4% dose level (20 breeding pairs per group). During the cohabitation period, postpartum body weights of the dams as well as weight gain in both males and females were significantly reduced at the two highest dose levels. In the same groups, a dose-dependent decrease in live pup weight, adjusted for average litter size, was observed. In the 0.4% dose group, the number of litters per pair and the number of live pups born per litter were reduced. In the F_1 generation, weight gain was significantly reduced at the two highest dose levels in males and at the highest dose level in females. Also, the number of live pups born per litter and adjusted live pup weight was significantly decreased in the high-dose group compared with controls. Cross-breeding of the F_0 exposed (0.4%) males and females with F_0 controls after 11 weeks of dosing resulted in a reduction of the number of live pups per litter and of pup weight only when exposed females (0.4%) were mated with control males. In addition, fertility was lower in exposed females, a parameter not affected in other parts of the study. The reduction in body weights of high-dose males was paralleled by reduction of the weight of reproductive organs, with spermatozoal parameters showing no sign of toxicity (Morrissey *et al*., 1989; Treinen *et al*., 1989). Differential counting of follicles was performed in ovaries originating from the cross-bred F_0 females (controls and 0.4%) and the F_1 females (all dose levels) (ovaries from 10 mice per group). Numbers of follicles were reduced in females exposed to 0.2 or 0.4% 2,2-bis(bromomethyl)-1,3-propanediol (Bolon *et al*., 1997).

Effects on the weight of male reproductive organs, epididymal spermatozoal parameters and estrous cyclicity were addressed after dietary administration of 2,2-bis-(bromomethyl)propane-1,3-diol for 13 weeks to groups of 10 males and 10 females per dose level. Dose levels were 0, 2500, 5000 and 10 000 ppm in $B6C3F_1$ mice (corresponding to estimated daily doses in males of 0, 500, 1300 and 3000 mg/kg bw and 0, 600, 1200 and 2900 mg/kg bw in females). In Fischer 344/N rats, doses were 0, 5000, 10 000 and 20 000 ppm (corresponding to estimated daily doses in males of 0, 400, 800 and 1700 mg/kg bw and 0, 400, 800 and 1630 mg/kg bw in females). In both species, mean body weight was depressed at doses of 5000 ppm and above, and was parallelled by reduced weight of the male reproductive organs, to a greater extent in mice than in rats (National Toxicology Program, 1996). In several studies, as in the National Toxicology Program continuous breeding study, no depression of reproductive organ weight was observed without concurrent reduction of body weight in mice and rats. Estrous cyclicity and spermatozoal parameters were not affected (Morrissey *et al*., 1989; Treinen *et al*., 1989; National Toxicology Program, 1996).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 3 for references)

While two studies of the bacterial mutagenicity of 2,2-bis(bromomethyl)propane-1,3-diol gave negative results, a positive result was obtained in *Salmonella typhimurium* TA100 when liver S9 from Aroclor-induced male Syrian hamsters was used at a concentration of 30% (v/v). In other strains of *S. typhimurium* and in experiments with rat liver S9, no mutagenic activity was detected.

2,2-Bis(bromomethyl)propane-1,3-diol caused a dose-related increase in chromosomal aberrations in Chinese hamster ovary cells, but only at doses that caused significant cytotoxicity; a majority of the breaks were located in the heterochromatic region of the long arm of chromosome X, but the reasons for this are unclear. Induction of sister chromatid exchanges in Chinese hamster ovary cells was judged to be equivocal.

2,2-Bis(bromomethyl)propane-1,3-diol caused significant increases in micronucleated normochromatic erythrocytes in peripheral blood samples from male and female mice exposed for 13 weeks via the diet, whereas in tests for micronucleus formation in mouse bone marrow, results were positive for females but inconsistent for males [routes of administration were different].

4.5 Mechanistic considerations

The in-vitro mutagenicity of 2,2-bis(bromomethyl)propane-1,3-diol was dependent on the presence of a metabolic activation system.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

2,2-Bis(bromomethyl)propane-1,3-diol is a flame retardant used in polyester resins and polyurethane foams. No data on human exposure to this substance were available.

5.2 Human carcinogenicity data

No data were available to the Working Group.

Table 3. Genetic and related effects of 2,2-bis(bromomethyl)propane-1,3-diol

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–[c]	10 000 µg/plate	Mortelmans *et al.* (1986)
Salmonella typhimurium TA100, reverse mutation	–	+[d]	1000 µg/plate	Zeiger *et al.* (1992)
Salmonella typhimurium TA98, reverse mutation	–	–	6666 µg/plate	Zeiger *et al.* (1992)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	?	1200	Galloway *et al.* (1987)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	+	800	Galloway *et al.* (1987)
Micronucleus formation, male B6C3F$_1$ mouse bone-marrow cells *in vivo*	?		400 po × 1	National Toxicology Program (1996)
Micronucleus formation, male and female B6C3F$_1$ mouse bone-marrow cells *in vivo*	+		150 ip × 1	National Toxicology Program (1996)
Micronucleus formation, male and female B6C3F$_1$ mouse peripheral blood erythrocytes *in vivo*	+		2500 ppm feed; 13 w	National Toxicology Program (1996)

[a] +, positive; (+), weak positive; –, negative; ?, inconclusive
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; po, oral; ip, intraperitoneal; w, week
[c] Aroclor-induced S9 (10%), male Sprague-Dawley rat or male Syrian hamster
[d] Aroclor-induced S9 (30%), male Syrian hamster; negative with 30% S9 from rat liver

5.3 Animal carcinogenicity data

2,2-Bis(bromomethyl)propane-1,3-diol was tested for carcinogenicity as a commercial mixture (FR-1138®) containing 80% of the parent compound in one experiment in mice and in two experiments in rats by oral administration in the diet. In mice, it increased the incidence of tumours of the Harderian gland, forestomach and lung in both males and females and of subcutaneous sarcomas in females. In one study in male rats, it increased the incidences of tumours of the skin, subcutaneous tissue, mammary gland, Zymbal gland, oral cavity, oesophagus, forestomach, small and large intestine, peritoneum, lung and thyroid. In female rats the incidences of oesophageal, mammary gland and thyroid follicular tumours were increased.

5.4 Other relevant data

No data on the metabolism of 2,2-bis(bromomethyl)propane-1,3-diol were available.

Histopathological changes were observed in the kidney and the urinary bladder of rats and mice administered 2,2-bis(bromomethyl)propane-1,3-diol for 13 weeks.

No data on reproductive and developmental effects in humans were available.

No effects were observed, after 13 weeks' exposure, on sperm parameters or vaginal cytology in mice or rats. However, in a mouse continuous breeding study, exposure to 2,2-bis(bromomethyl)propane-1,3-diol in feed caused a female-specific decrease in reproductive capacity.

2,2-Bis(bromomethyl)propane-1,3-diol was mutagenic in only one of several bacterial strains tested, and only with metabolic activation. In cultured mammalian cells, it was only weakly active in tests for chromosomal aberrations and sister chromatid exchanges. Micronucleus formation, indicative of chromosomal damage, was induced in cells from mice exposed to 2,2-bis(bromomethyl)propane-1,3-diol *in vivo*.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of 2,2-bis(bromomethyl)-propane-1,3-diol were available.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2,2-bis(bromomethyl)propane-1,3-diol.

Overall evaluation

2,2-Bis(bromomethyl)propane-1,3-diol is *possibly carcinogenic to humans (Group 2B)*.

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GLYCIDOL

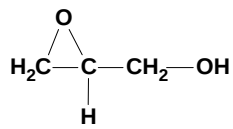
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 556-52-5
Deleted CAS Reg. Nos: 61915-27-3; 98913-54-3
Chem. Abstr. Name: Oxiranemethanol
IUPAC Systematic Name: 2,3-Epoxypropan-1-ol
Synonyms: Allyl alcohol oxide; epihydrin alcohol; 1,2-epoxy-3-hydroxypropane; 2,3-epoxy-1-propanol; (±)-2,3-epoxy-1-propanol; glycide; (±)-glycidol; (RS)-glycidol; dl-glycidol; glycidyl alcohol; hydroxy-1,2-epoxypropane; 1-hydroxy-2,3-epoxypropane; 2-(hydroxymethyl)oxirane; 3-hydroxypropylene oxide; oxiranylmethanol; racemic glycidol

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_3H_6O_2$ Relative molecular mass: 74.08

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless, odourless liquid (Sienel *et al*., 1987)
(*b*) *Boiling-point*: 162 °C (decomposes) (Verschueren, 1996)
(*c*) *Melting-point*: –54 °C (Verschueren, 1996)
(*d*) *Density*: 1.143 g/cm^3 at 25 °C (Lide & Milne, 1996)
(*e*) *Spectroscopy data*: Infrared (prism [15765]; grating [28381]), nuclear magnetic resonance (proton [18790]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980; Lide & Milne, 1996)

(*f*) *Solubility*: Miscible in all proportions in water, alcohols, ketones, esters, ethers and aromatics; almost insoluble in aliphatic hydrocarbons (Sienel *et al*., 1987)
(*g*) *Volatility*: Vapour pressure, 120 Pa at 25 °C (American Conference of Governmental Industrial Hygienists, 1999); relative vapour density (air = 1), 2.15 (Verschueren, 1996)
(*h*) *Stability*: Flash-point, 71 °C (Sienel *et al*., 1987); reacts vigorously with strong caustic soda, strong sulfuric acid and with anhydrous metal halides, such as stannic and ferric chlorides (Dixie Chemical Co., 1995)
(*i*) *Octanol/water partition coefficient (P)*: log P, –0.95 (Hansch *et al.*, 1995)
(*j*) *Conversion factor*[1]: $mg/m^3 = 3.03 \times ppm$

1.1.4 *Technical products and impurities*

Glycidol is commercially available with a minimum purity of 95% and a maximum water content of 1% (Dixie Chemical Co., 1999).

Trade names for glycidol include: Epiol OH.

1.1.5 *Analysis*

Glycidol can be determined in workplace air by adsorbing the air sample on charcoal, desorbing with tetrahydrofuran and analysing by gas chromatography with flame ionization detection (Eller, 1994).

1.2 Production

Glycidol is commercially produced by two methods: (1) epoxidation of allyl alcohol with hydrogen peroxide and a catalyst (tungsten or vanadium); and (2) reaction of epichlorohydrin with caustic (Grigor'ev *et al.*, 1979; Yoshida & Koyama, 1992; Richey, 1993; Hutchings *et al.*, 1995).

Information available in 1999 indicated that glycidol was manufactured by two companies in Japan, and one company each in Germany and the United States (Chemical Information Services, 1999).

1.3 Use

In 1956, glycidol was only used for research purposes (Hine *et al*., 1956), but by 1978 it was used in the preparation of glycerol, glycidyl ethers, esters and amines in the pharmaceutical industry (Proctor & Hughes, 1978) and as a sterilant in pharmaceuticals (Ivashkiv & Dunham, 1973).

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[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

Glycidol has become an important intermediate for the production of functional epoxides. For example, reaction of phosgene with glycidol yields 2,3-epoxypropyl chloroformate. Reaction of glycidol with isocyanates affords the commercially important glycidyl urethanes (Sienel *et al*., 1987). It is used as an intermediate in the production of pharmaceuticals, as an additive for synthetic hydraulic fluids and as a reactive diluent in some epoxy resin systems (Hooper *et al*., 1992; American Conference of Governmental Industrial Hygienists, 1999). It is a stabilizer for natural oils and vinyl polymers, a dye-levelling agent and a demulsifier (American Conference of Governmental Industrial Hygienists, 1986).

1.4 Occurrence

1.4.1 *Natural occurrence*

Glycidol is not known to occur as a natural product.

1.4.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1999), as many as 4900 workers in the United States were potentially exposed to glycidol (see General Remarks). National estimates of workers potentially exposed in other countries were not available.

1.4.3 *Environmental occurrence*

Production of glycidol and its broad applications as an intermediate, as a reactive diluent in epoxy resins and as a stabilizer and a sterilant may result in its release into the environment through various waste streams (Ivashkiv & Dunham, 1973; Kaplan *et al*., 1982; Nomeir *et al*., 1995; Department of Health and Human Services, 1999).

1.5 Regulations and guidelines

Occupational exposure limits and guidelines for glycidol are presented in Table 1.

2. Studies of Cancer in Humans

No data were available to the Working Group.

Table 1. Occupational exposure limits and guidelines for glycidol[a]

Country	Year	Concentration (mg/m^3)	Interpretation[b]
Australia	1993	75	TWA
Belgium	1993	75	TWA
Denmark	1993	1	STEL
Finland	1998	150 (sk)	TWA
		225	STEL
France	1993	75	TWA
Germany	1999	150	TWA
Ireland	1997	75	TWA
Netherlands	1997	150	TWA
Philippines	1993	150	TWA
Russian Federation	1993	5	STEL
Switzerland	1993	75	TWA
		150	STEL
Turkey	1993	150	TWA
United States			
ACGIH	1999	6.1 (A3)[c]	TWA
NIOSH	1999	75	TWA
OSHA	1999	150	TWA

[a] From Finnish Ministry of Social Affairs and Health (1998); American Conference of Governmental Industrial Hygienists (ACGIH) (1999); Deutsche Forschungsgemeinschaft (1999); Occupational Safety and Health Administration (OSHA) (1999)
[b] TWA, time-weighted average; STEL, short-term exposure limit; A3, confirmed animal carcinogen with unknown relevance to humans; sk, skin notation
[c] These countries follow the recommendations of the ACGIH threshold limit values: Bulgaria, Colombia, Jordan, Republic of Korea, New Zealand, Singapore and Viet Nam

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female $B6C3F_1$ mice, nine weeks of age, were administered 0, 25 or 50 mg/kg bw of glycidol (94% purity, with the primary impurity, as determined by gas chromatography, being diglycidyl ether at a concentration of 2.8%, and 3-methoxy-1,2-propanediol (1.2%), 2,6-dimethanol-1,4-dioxane (1.1%), 3-chloro-1,2-propanediol (0.4%) and methanol (0.1%) as lesser impurities) in distilled water by gavage on five days per week for 103 weeks. The survival of female mice at the high dose was significantly lower after week 101 than in the controls. As shown in Table 2, there was a significantly increased incidence of Harderian gland adenomas

Table 2. Incidence of primary tumours in B6C3F$_1$ mice exposed to glycidol

Tumour site	Animals with tumours					
	Males			Females		
	0 mg/kg bw	25 mg/kg bw	50 mg/kg bw	0 mg/kg bw	25 mg/kg bw	50 mg/kg bw
Harderian gland[a]	8/46	12/41	22/44***	4/46	11/43*	17/43***
Forestomach[b]	0/50	2/50	9/50***	3/50	5/50	4/50
Liver[c]	24/50	31/50	35/50**	9/50	7/50	14/50
Mammary gland[d]				1/50	5/50	15/50***
Lung[e]	13/50	11/50	21/50*	3/50	4/50	5/50
Skin[b]	0/50	0/50	4/50*	–	–	–
Subcutis[f]	11/50	3/50	4/50	0/50	3/50	9/50***
Uterus[g]	–	–	–	0/50	3/50	3/50

From Irwin *et al.* (1996)
bw, body weight
[a] Adenomas and adenocarcinomas
[b] Squamous-cell papillomas
[c] Hepatocellular adenomas and carcinomas
[d] Adenocarcinomas
[e] Alveolar/bronchiolar adenomas or carcinomas
[f] Sarcomas and fibrosarcomas
[g] Carcinomas and adenocarcinomas
* $p \leq 0.05$; Fisher's exact test or incidental tumour test
** $p \leq 0.01$; Fisher's exact test
*** $p \leq 0.001$; Fisher's exact test

and adenocarcinomas combined in the high-dose males and in the high- and mid-dose females, and of Harderian gland adenocarcinomas in the high-dose males. In male mice only, the incidences of adenomas and carcinomas of the liver, squamous-cell papillomas of the forestomach and skin and alveolar/bronchiolar adenomas of the lung were significantly increased at the high dose; in females only, the incidences of mammary gland adenocarcinomas and of subcutaneous sarcomas and fibrosarcomas combined were significantly increased at the high dose. There was also a slight increase in uterine glandular carcinomas in female mice (National Toxicology Program, 1990; Irwin *et al*., 1996).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344 rats, eight weeks of age, were administered 0, 37.5 or 75 mg/kg bw of glycidol (purity, 94%, with the main impurities being those listed in Section 3.1.1) in distilled water by gavage on five days per week for 103 weeks. Survival of rats was significantly lower in the treated groups in both males and females than in the control groups, with the mean survival being 92, 82 and 66 weeks for the control, mid- and high-dose males, respectively, and 97, 85 and 78 weeks for the female dose groups. As shown in Table 3, there was a statistically significant increase in the incidence of mesotheliomas of the tunica vaginalis/peritoneum in males at both 37.5 and 75 mg/kg bw. There was a statistically significant increase in the incidence of fibroadenoma and adenocarcinoma of the mammary gland in female rats, and of mammary fibroadenoma in male rats at both doses in each case. The incidences in the forestomach of squamous-cell papilloma and of squamous papilloma or carcinoma combined were significantly increased in female rats at both doses and in male rats at the high dose. Gliomas of the brain were significantly increased in both sexes at the high dose. Other tumour types with increased incidence included squamous-cell papillomas or carcinomas of the mouth or tongue, adenomas or carcinomas of the clitoral gland and leukaemia in female rats; and Zymbal gland carcinomas, thyroid follicular-cell adenomas or carcinomas, skin tumours and adenomatous polyps or adenocarcinomas of the intestine combined in male rats (National Toxicology Program, 1990; Irwin *et al*., 1996).

3.1.3 *Hamster*

Groups of 20 male and 20 female Syrian golden hamsters, 10 weeks of age, were administered 0.2 mL glycidol solution (96% purity with ‘varying amounts of polymer’) in a 1:1 mixture of ethyl acetate and corn oil, representing a dose of 12 mg glycidol (approximately 100 mg/kg bw) by gavage twice a week for 60 weeks. This was calculated to amount to a total dose of 1.45 g per animal. Groups of 12 male and 12 female hamsters given 0.2 mL ethyl acetate:corn oil for 90 weeks comprised the controls. Survival was similar among the groups, with the median week of death being

Table 3. Incidence of primary tumours in Fischer 344/N rats exposed to glycidol

Site/tumour	Animal with tumours[a]					
	Males			Females		
	0 mg/kg bw	37.5 mg/kg bw	75 mg/kg bw	0 mg/kg bw	37.5 mg/kg bw	75 mg/kg bw
Tunica vaginalis/peritoneum[b]	3/49	34/50***	39/47***	–	–	–
Brain[c]	0/46	5/50*	6/30**	0/49	4/46*	4/46**
Clitoral gland[d]	–	–	–	5/49	9/47	12/45*
Forestomach[e]	1/46	2/50	6/32*	0/47	4/38*	11/30***
Haematopoietic system[f]	25/50	33/50	21/50	13/49	14/44	20/41*
Intestine[g]	0/47	1/50	4/37*	–	–	–
Mammary gland[h]	3/45	8/39	7/17**	14/50	34/50***	37/50***
Mouth/tongue[i]	3/50	2/50	5/50	1/46	3/37	7/26**
Skin[j]	0/45	5/41*	4/18*	–	–	–
Thyroid gland[k]	1/46	4/42	6/19**	0/50	1/50	3/49
Zymbal's gland[l]	1/49	3/50	6/48*	–	–	–

From Irwin *et al.* (1996)

[a] Effective numbers (number of animals with tumours among the number of animals surviving at the appearance of the first tumour)

[b] Mesotheliomas

[c] Gliomas

[d] Adenomas and carcinomas

[e] Squamous-cell papillomas and carcinomas

[f] Leukaemia

[g] Adenomatous polyps and adenocarcinomas

[h] Fibroadenomas and adenocarcinomas

[i] Sebaceous gland adenomas and carcinomas or basal-cell tumours

[j] Follicular-cell adenomas and carcinomas

[k] Carcinomas

* $p \leq 0.05$; Fisher's exact test or incidental tumour test

** $p \leq 0.01$; Fisher's exact test

*** $p \leq 0.001$; Fisher's exact test

97, 92, 84 and 84 for control males, treated males, control females and treated females, respectively. There was no statistically significant increase in the incidence of tumours in any target organ. However, 2/19 males and 4/20 females had haemangiosarcomas of the spleen compared with 0/12 male and 0/12 female controls (Lijinsky & Kovatch, 1992).

3.2 Skin application

3.2.1 *Mouse*

A group of 20 female ICR/Ha Swiss mice, eight weeks of age, was administered glycidol (commercial grade purified by distillation under vacuum) by skin painting onto the dorsal skin three times per week for 520 days at a concentration of 5% in acetone. Control groups of 100 female mice of the same strain each included three 100% acetone-treatment and three no-treatment groups, with the vehicle administered in the same way. Survival was similar between groups. Under this regimen, glycidol did not induce skin damage or skin tumours (Van Duuren *et al*., 1967).

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Glycidol is rapidly hydrolysed to glycerol (97.2%) and α-chlorohydrin (3-chloro-1,2-propanediol, 2.8%) in 0.1 M hydrochloric acid, with a half-life of 10 min. At pH 7 or 8, glycidol readily reacts with glutathione to form *S*-(2,3-dihydroxypropyl)glutathione (Jones, 1975).

Approximately 87–92% of 37.5 or 75 mg/kg body weight (bw) orally administered glycidol is absorbed from the gastrointestinal tract of male Fischer 344 rats. Seven to eight per cent of the dose remained in tissues 72 h following administration. The highest concentrations of radioactivity were observed in blood cells, thyroid, liver, kidney and spleen (Nomeir *et al.*, 1995).

S-(2,3-Dihydroxypropyl)glutathione, *S*-(2,3-dihydroxypropyl)cysteine and β-chlorolactic acid are the major metabolites isolated from rat urine after intraperitoneal administration of glycidol. The generation of β-chlorolactic acid is presumably a result of initial formation of α-chlorohydrin, with subsequent oxidation by alcohol and aldehyde

dehydrogenases. Glycidol is hydrolysed to glycerol by rat liver microsomal preparations (Jones, 1975; Jones & O'Brien, 1980; Patel *et al.*, 1983).

A single dose by gavage of 500 μL/kg [560 mg/kg] bw glycidol to male Wistar rats led to significant decreases in hepatic glutathione content between 30 min and 12 h after treatment. Recovery was complete by 24 h. The decrease in glutathione content 2 h after dosing with 750 μL/kg [840 mg/kg] bw was no greater than that observed after dosing with 150 μL/kg [168 mg/kg] bw (Montaldo *et al.*, 1984).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

No histopathological or serum enzyme signs of liver damage were observed in male Wistar rats dosed by gavage with up to 750 μL/kg [840 mg/kg] bw glycidol (Montaldo *et al.*, 1984).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Groups of 30–37 pregnant CD-1 mice were treated with glycidol by gavage at dose levels of 0, 100, 150 or 200 mg/kg bw per day on days 6–15 of gestation, and then killed on day 18 of gestation. Five of the 30 dams died in the highest-dose group. There was no reduction in the total number of implantations and no increase in the numbers of resorptions or fetal deaths per pregnancy, or morphological variations that could be directly attributed to the treatment. In the highest-dose group, one litter consisted of 15 stunted fetuses, among which six had cleft palates. However, all 15 stunted fetuses were attributable to a single dam (Marks *et al*., 1982).

Groups of pregnant Sprague-Dawley rats [numbers not specified] were laparotomized on day 13 of gestation and the embryos of one uterine horn were given intra-amniotic injections of glycidol in 0.9% sodium chloride at dose levels of 10, 100 and 1000 μg per fetus. The contrauterine horn embryos received intra-amniotic injections of 0.9% sodium chloride. The dams were killed on day 20 of gestation. Throughout the treated groups, there was an approximately 50% resorption frequency. No malformed fetuses were observed in the saline-treated uterine horns or in the glycidol-treated horns

at the lower doses, but 44% of the surviving fetuses treated with 1000 μg glycidol were malformed. The commonest malformations were of the forelimbs (39%), hindlimbs (22%) and pinnae (11%) (Slott & Hales, 1985).

Adult male and female mice [strain not specified] were mated for 30 min and then groups of 23–31 females were treated with glycidol [route not stated] at dose levels of 0 or 250 mg/kg bw. The glycidol treatment was given at 1, 6, 9 or 25 h after mating and the mice were killed on gestational day 17. Resorptions were increased by glycidol treatment, from 3.2% in the controls to 11.6%, 15.0%, 10.6% and 7.9% in the 1-, 6-, 9- and 25-h treatment time groups, respectively. Late deaths and anomalies were also increased in the 1- and 6-h treatment time groups (Rutledge *et al*., 1992).

Groups of 34 female (SEC × C57BL6)F_1 mice were injected intraperitoneally once with 0 or 300 mg/kg bw glycidol and then mated the following morning with untreated (C3H/R1 × C57BL10)F_1 males. Newborn mice were looked for in the breeding cages beginning 18 days after pairing, and were counted and killed. This procedure was continued through 17 breeding intervals (approximately 347 days). There was no significant difference in the numbers of offspring per female between the treated (143.4) and control (147.8) groups (Bishop *et al*., 1997).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 4 for references)

The genetic toxicity of glycidol has been reviewed (Ehrenberg & Hussain, 1981).

Glycidol gave a positive response in assays of prophage induction and SOS repair in *Escherichia coli*. Results were uniformly positive in several *Salmonella typhimurium* reverse mutation assays and in two fungal mutation assays. The sex-linked recessive lethal mutation assay and the heritable translocation test in *Drosophila melanogaster* also gave positive results.

In mammalian assays, glycidol has been tested in human lymphocytes and Chinese hamster cells *in vitro* for induction of chromosomal aberrations and sister chromatid exchanges. It was also tested *in vivo* in the mouse micronucleus assay. All test results were positive, as were those of gene mutation assays using Chinese hamster V79 cells and mouse lymphoma L5178Y cells. An in-vivo assay to detect chromosomal aberrations in mouse bone-marrow cells gave negative results.

The only mammalian assay to give no response without exogenous metabolism was an unscheduled DNA synthesis test using human cells. This test did, however, give a positive response in the presence of S9, which was also in contrast to the majority of the tests, in which the addition of an exogenous metabolizing system reduced the activity of the compound.

Table 4. Genetic and related effects of glycidol

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Prophage induction, SOS repair test, DNA strand breaks, cross-links or related damage	–	NT	500 µg/plate	Mamber *et al*. (1984)
Escherichia coli PQ37, SOS chromotest	+	NT	244.5	von der Hude *et al*. (1990)
Escherichia coli pol A/W3110-p3478, differential toxicity (liquid suspension test)	+	NT	430 µg/well	McCarroll *et al*. (1981)
Escherichia coli WP2/WP100 *rec* assay, differential toxicity	+	NT	54 µg/well	McCarroll *et al*. (1981)
Escherichia coli WP2/WP100 *rec* assay, differential toxicity	+	NT	10 000 µg/plate	Mamber *et al*. (1984)
Salmonella typhimurium TA100, reverse mutation	+	NT	100 µg/plate	Wade *et al*. (1979)
Salmonella typhimurium TA100, reverse mutation	+	+	61.7 µg/plate	Thompson *et al*. (1981)
Salmonella typhimurium TA100, reverse mutation	+	+	33 µg/plate	Canter *et al*. (1986)
Salmonella typhimurium TA100, reverse mutation	+	NT	25 µg/plate	Claxton *et al*. (1991)
Salmonella typhimurium TA1535, reverse mutation	+	+	20.6 µg/plate	Thompson *et al*. (1981)
Salmonella typhimurium TA1535, reverse mutation	+	+	500 µg/plate	Mamber *et al*. (1984)
Salmonella typhimurium TA1535, reverse mutation	+	+	3 µg/plate	Canter *et al*. (1986)
Salmonella typhimurium TA1537, reverse mutation	(+)	+	1670 µg/plate	National Toxicology Program (1990)
Salmonella typhimurium TA98, reverse mutation (spot test)	–	NT	10 000 µg/plate	Wade *et al*. (1979)
Salmonella typhimurium TA98, reverse mutation	+	+	3333 µg/plate	National Toxicology Program (1990)
Salmonella typhimurium TA97, reverse mutation	+	+	333 µg/plate	National Toxicology Program (1990)
Escherichia coli (Sd-4), reverse mutation	+	NT	740	Hussain (1984)
Klebsiella pneumoniae, forward mutation	+	NT	14.8	Voogd *et al*. (1981)
Schizosaccharomyces pombe, forward mutation	+	+	74	Migliore *et al*. (1982)
Neurospora crassa, reverse mutation	+	NT	37 000[c] (15 min)	Kolmark & Giles (1955)
Drosophila melanogaster, sex-linked recessive lethal mutations	+		1230 in feed	Foureman *et al*. (1994)

Table 4 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Drosophila melanogaster, heritable translocation test	+		1230 in feed	Foureman *et al*. (1994)
Gene mutation, Chinese hamster lung V79 cells, 6-thioguanine resistance *in vitro*	+	NT	0.15	Smith *et al*. (1990)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	+	+	8	Thompson *et al*. (1981)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	+	NT	1.43	National Toxicology Program (1990)
Sister chromatid exchange, Chinese hamster cells *in vitro*	+	+	1.11	National Toxicology Program (1990)
Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	+	NT	92.6	von der Hude *et al*. (1991)
Chromosomal aberrations, Chinese hamster cells *in vitro*	+	+	12.5	National Toxicology Program (1990)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	3.7	Norppa *et al*. (1981)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	29.6	Norppa *et al*. (1981)
Micronucleus formation, B6C3F$_1$ mice *in vivo*	+		150 ip × 2	National Toxicology Program (1990)
Chromosomal aberrations, mouse bone-marrow cells *in vivo*	–		226 po × 5	Thompson & Hiles (1991)
Chromosomal aberrations, mouse bone-marrow cells *in vivo*	–		145 ip × 5	Thompson & Hiles (1991)

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

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw/day; *Drosophila* tests, ppm; po, oral; ip, intraperitoneal

[c] One dose tested; time-dependent response

Glycidol has been demonstrated to alkylate DNA in a number of in-vitro studies (Hemminki, 1979, Hemminki *et al*., 1980; Hemminki, 1983; Djuric & Sinsheimer, 1984a,b; Djuric *et al*., 1986; Segal *et al*., 1990).

4.5 Mechanistic considerations

Glycidol possesses a reactive epoxide moiety. This is likely to be responsible for the genotoxic activity of the compound without a requirement for metabolic activation.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Glycidol is an epoxide used as a chemical intermediate in the production of functional epoxides, glycidyl urethanes, pharmaceuticals and other products. It is also used as a reactive diluent in epoxy resin systems and as a sterilant. Occupational exposure may occur during its production and use. No data were available on environmental exposure to glycidol.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Glycidol has been tested by oral administration in one study in mice, in one study in rats and in one study in hamsters. It was also tested by skin application in one study in mice. After oral administration to mice, it produced increases in tumours of the Harderian gland in both males and females, of the forestomach, lung, liver and skin in males, and of the mammary gland and subcutaneous tissue in females. In rats, it produced increases in the incidence of gliomas of the brain and forestomach tumours in both males and females. Mesotheliomas of the tunica vaginalis/peritoneum, as well as tumours of the intestine, skin, thyroid gland and Zymbal gland were increased in males. Tumours of the clitoral gland, mammary gland and oral mucosa as well as leukaemia were increased in females.

In hamsters, there was a marginal increase in the incidence of splenic haemangiosarcomas after oral administration.

No skin tumours were observed in mice after skin application.

5.4 Other relevant data

Glycidol is an alkylating agent which reacts readily with glutathione. It causes a decrease in glutathione content in rat liver, probably reflecting its binding to glutathione. In rats, it is metabolized to oxidative and glutathione-derived products. No toxicokinetic data on humans were available.

No data on developmental and reproductive effects in humans were available to the Working Group.

No effects on fertility or development were observed in mice given intraperitoneal injections of glycidol 24 h before mating or orally during organogenesis. In contrast, when a single dose of glycidol was administered to female mice within 25 h after mating, the numbers of fetal deaths and anomalies were increased. Intra-amniotic injection of glycidol on day 13 of gestation in rats increased the frequency of resorptions and, at high doses, limb malformations.

Glycidol has been shown to be genotoxic using assays covering a wide range of end-points. *In vitro*, it did not require metabolic activation to elicit positive responses.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of glycidol were available.

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

Overall evaluation

Glycidol is *probably carcinogenic to humans (Group 2A)*.

In making the overall evaluation, the Working Group took into consideration that glycidol is a direct-acting alkylating agent that is mutagenic in a wide range of in-vivo and in-vitro test systems.

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NITROMETHANE

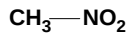
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 75-52-5
Chem. Abstr. Name: Nitromethane
IUPAC Systematic Name: Nitromethane
Synonyms: Nitrocarbol

1.1.2 *Structural and molecular formulae and relative molecular mass*



CH_3NO_2 Relative molecular mass: 61.04

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless, oily liquid with a moderately strong, somewhat disagreeable odour (Budavari, 1998)
(*b*) *Boiling-point*: 101.1 °C (Lide & Milne, 1996)
(*c*) *Melting-point*: –28.5 °C (Lide & Milne, 1996)
(*d*) *Density*: 1.1371 g/cm^3 at 20 °C (Lide & Milne, 1996)
(*e*) *Spectroscopy data*: Infrared (grating [25]), Raman [296], ultraviolet [29], nuclear magnetic resonance (proton [9146], C-13[4002]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980; Lide & Milne, 1996)
(*f*) *Solubility*: Slightly soluble in water (95 mL/L at 20 °C; Budavari, 1998) acetone, alkali (bases), carbon tetrachloride, diethyl ether and ethanol (Lide & Milne, 1996; Verschueren, 1996; American Conference of Governmental Industrial Hygienists, 1999)

(*g*) *Volatility*: Vapour pressure, 3.7 kPa at 20 °C; relative vapour density (air = 1), 2.11; flash-point, 35 °C (closed-cup) (Verschueren, 1996; American Conference of Governmental Industrial Hygienists, 1999)
(*h*) *Stability*: Lower explosive limit in air, 7.3% by volume; sensitive to adiabatic compression (Angus Chemical Co., 1998); forms an explosive sodium salt which bursts into flame on contact with water (Budavari, 1998)
(*i*) *Octanol/water partition coefficient (P)*: log P, –0.35 (Hansch *et al*., 1995)
(*j*) *Conversion factor*[1]: $mg/m^3 = 2.50 \times ppm$

1.1.4 *Technical products and impurities*

Nitromethane is commercially available with the following specifications (by weight): purity, 98.0% min.; total nitroparaffins, 99.0% min.; acidity (as acetic acid), 0.1% max.; and water, 0.1% max. Nitromethane can be made less sensitive to detonation by shock by the addition of compounds such as alcohols, hydrocarbons, esters and ketones. These desensitizers, with the minimum content by weight that must be present in the mixtures, are: cyclohexanone (25%), 1,4-dioxane (35%), 1,2-butylene oxide (40%), methanol (45%), 2-nitropropane (47%), 1-nitropropane (48%) or methyl chloroform (50%) (Angus Chemical Co., 1998).

1.1.5 *Analysis*

Nitromethane can be determined in workplace air by adsorbing the air sample on Chromosorb, desorbing with ethyl acetate and analysing by gas chromatography with nitrogen-specific detection (method 2527) (Eller, 1994).

1.2 Production

Nitromethane was first prepared in 1872 by Kolbe, and is produced commercially by high-temperature vapour-phase nitration of propane. The process, which uses nitric acid as the nitrating agent, is based on a free-radical reaction in which the active species is the NO_2 radical (Markofsky, 1991; Angus Chemical Co., 1998).

Information available in 1999 indicated that nitromethane was manufactured by four companies in China, two companies in India and one company each in Germany, Spain and the United States (Chemical Information Services, 1999).

[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

1.3 Use

One of the most important direct uses for nitromethane is in the stabilization of halogenated hydrocarbons. For example, small amounts of nitromethane are widely used in industry to form stable non-corrosive mixtures with 1,1,1-trichloroethane for vapour degreasing, dry cleaning and for cleaning semiconductors and lenses. It is also used to stabilize the halogenated propellants for aerosols and to inhibit corrosion on the interiors of tin-plated steel cans containing water-based aerosol formulations (Markofsky, 1991; Angus Chemical Co., 1998).

Nitromethane is frequently used as a polar solvent for cellulose esters (Lundberg, 1989) and for cyanoacrylate adhesives and acrylic coatings. It is also used for cleaning electronic circuit boards. Nitromethane alone, and in mixtures with methanol and other nitroparaffins, is used as a fuel by professional drag racers and hobbyists. The explosives industry uses nitromethane in a binary explosive formulation and in shaped charges (Markofsky, 1991; Angus Chemical Co., 1998).

Nitromethane is used as a metal stabilizer for various chlorinated and fluorinated hydrocarbon solvents. The primary role of the nitromethane is to complex metal salts from the solvent–metal corrosion reaction (Archer, 1996).

1.4 Occurrence

1.4.1 *Natural occurrence*

Nitromethane is not known to occur as a natural product.

1.4.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1999) as many as 135 000 workers in the United States were potentially exposed to nitromethane (see General Remarks).

1.4.3 *Environmental occurrence*

The production of nitromethane and its use as a solvent, fuel additive, stabilizer for halogenated alkanes, and intermediate may result in the release of nitromethane into the environment, principally into the atmosphere. Human exposure to nitromethane may additionally occur via dermal contact and accidental ingestion of methanol–nitromethane fuel mixtures (Kaiffer *et al*., 1972; Sandyk & Gillman, 1984; Dayal *et al*., 1989; De Leacy *et al*., 1989; Lundberg, 1989; National Toxicology Program, 1997; Mullins & Hammett-Stabler, 1998).

The concentration of nitromethane in automobile exhaust using nine hydrocarbon test fuels under simulated driving conditions ranged from < 0.8 to 5.0 ppm (Seizinger & Dimitriades, 1972).

Nitromethane has been found in one of twelve samples of mother's milk (Pellizzari *et al*., 1982).

1.5 Regulations and guidelines

Occupational exposure limits and guidelines for nitromethane are presented in Table 1.

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 50 male and 50 female B6C3F$_1$ mice, seven weeks of age, were exposed by inhalation to 0, 188, 375 or 750 ppm [0, 470, 938 or 1875 mg/m^3] nitromethane (purity, 98%, with 0.25% nitroethane and 0.03% 2-nitropropane as contaminants) for 6 h plus T_{90} (12 min) per day on five days per week for 103 weeks [T_{90} is the time to achieve 90% of the target concentration]. The high dose was estimated to be the maximal tolerated dose. The average age of mice at necropsy was 111–112 weeks. The mean survival was 681, 700, 674 and 687 days among males and 662, 663, 673 and 695 days among females in the respective dose groups. As summarized in Table 2, statistically significant increases in the incidence of Harderian gland tumours and of alveolar/bronchiolar tumours in males and females and of hepatocellular adenomas in females were observed (National Toxicology Program, 1997).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344/N rats, seven weeks of age, were exposed by inhalation to concentrations of 0, 94, 188 or 375 ppm [0, 135, 470 or 938 mg/m^3] nitromethane (purity, 98%, with 0.25% nitroethane and 0.03% 2-nitropropane as contaminants) for 6 h plus T_{90} (12 min) per day on five days per week for 103 weeks. The high dose was estimated to be the maximal tolerated dose. The average age of rats at necropsy was 111 weeks. The mean survival was 642, 631, 646 and 640 days among males and 683, 653, 679 and 670 days among females in the respective dose groups. The incidences of mammary gland fibroadenomas were increased in females

Table 1. Occupational exposure limits and guidelines for nitromethane[a]

Country	Year	Concentration (mg/ m^3)	Interpretation[b]
Denmark	1993	250	TWA
Finland	1993	250	TWA
		375	STEL
France	1993	250	TWA
Germany	1998	250	TWA
Ireland	1997	250	TWA
		375	STEL
Netherlands	1997	50	TWA
Philippines	1993	250	TWA
Poland	1998	30	TWA
		240	STEL
Switzerland	1993	250	TWA
Turkey	1993	250	TWA
United Kingdom	1997	250	TWA
		375	STEL
United States			
ACGIH	1999	50	TWA
OSHA	1999	250	TWA

[a] From Finnish Ministry of Social Affairs and Health (1998); Occupational Safety and Health Administration (OSHA) (1999); American Conference of Governmental Industrial Hygienists (ACGIH) (1999); National Library of Medicine (1999)

[b] TWA, time-weighted average; STEL, short-term exposure limit

[c] These countries follow the recommendations of the ACGIH threshold limit values: Bulgaria, Colombia, Jordan, Republic of Korea, New Zealand, Singapore and Viet Nam

(19/50, 21/50, 33/50 ($p < 0.001$, logistic regression test), and 36/50 ($p < 0.001$, logistic regression test)), as were those of mammary gland carcinomas (2/50, 7/50, 1/50 and 11/50 ($p < 0.05$, logistic regression test)) in the control, low-, mid- and high-dose groups, respectively (National Toxicology Program, 1997).

Groups of 40 male and 40 female BLU:(LE)BR Long-Evans rats [age unspecified] were exposed by inhalation to 0, 100 or 200 ppm [0, 250 or 500 mg/m^3] nitromethane (purity, 96.26%, with 2.79% nitroethane and 0.62% 2-nitropropane as contaminants) for 7 h per day on five days per week for two years. There was no difference in body weight gain in males, but body weight gain in females exposed to 100 or 200 ppm was slightly less than that of controls. The numbers of survivors at the end of the experiment were 25, 23 and 25 (males) and 30, 29 and 24 (females) in the control, low- and high-dose

Table 2. Incidence of tumours in B6C3F$_1$ mice exposed by inhalation to nitromethane

	Number of animals with tumours			
	0 ppm	188 ppm	375 ppm	750 ppm
Males				
Harderian gland adenoma	9/50	10/50	19/50*	32/50**
Harderian gland carcinoma	1/50	1/50	6/50	5/50
Harderian gland adenoma or carcinoma	10/50	11/50	25/50**	37/50**
Alveolar/bronchiolar adenoma	11/50	10/50	9/50	12/50
Alveolar/bronchiolar carcinoma	2/50	3/50	3/50	11/50**
Alveolar/bronchiolar adenoma or carcinoma	13/50	13/50	12/50	20/50
Females				
Harderian gland adenoma	5/50	7/50	16/50**	19/50**
Harderian gland carcinoma	1/50	2/50	4/50	3/50
Harderian gland adenoma or carcinoma	6/50	9/50	20/50**	21/50**
Hepatocellular adenoma	14/50	24/49**	17/49	35/50**
Hepatocellular carcinoma	10/50	14/49	8/49	12/50
Hepatocellular adenoma or carcinoma	19/50	34/49**	22/49	40/50**
Alveolar/bronchiolar adenoma	3/50	3/50	2/49	9/50
Alveolar/bronchiolar carcinoma	0/50	3/50	5/49*	3/50
Alveolar/bronchiolar adenoma or carcinoma	3/50	6/50	6/49	12/50*

From National Toxicology Program (1997)
* $p \leq 0.05$, logistic regression test
* $p \leq 0.01$, logistic regression test

groups, respectively. There was no significant increase in the incidence of tumours related to nitromethane (Griffin *et al.*, 1996).

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Nitromethane was administered to Wistar rats [sex not specified] by a single lethal intraperitoneal injection of 1.5 g/kg body weight (bw), by eight injections of 0.11 g/kg

bw over two weeks, or by inhalation of a lethal concentration of 33 g/m^3 for about 6 h. In all cases no methaemoglobin was detected in the blood and low concentrations of nitrite were found in the heart, lungs, kidney and spleen, but not in the liver. After the inhalation study nitromethane was detected only in the liver (Dequidt *et al.*, 1973).

Formaldehyde generated from nitromethane was found only in trace amounts after incubation with microsomes from from Fischer 344 rat liver, but none was found after incubation with rat nasal microsomes (Dahl & Hadley, 1983). Nitromethane inhibited rabbit liver cytochrome P450 activity, apparently competing for the same ferrohaemochrome-binding sites as carbon monoxide (Wade *et al.*, 1977).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Nitromethane was administered intraperitoneally (200 mg/kg bw) to male Wistar rats (three months of age) as a 10% solution in olive oil. The effects of nitromethane in the liver were detected only 48 h after administration and included a decrease in NADPH-cytochrome c reductase activity with proliferation of the smooth endoplasmic reticulum. Nitromethane also caused an increase in brain acid proteinase (4 h after injection) and acetylcholine esterase activities (4, 24 and 48 h after injection) (Zitting *et al.*, 1982).

BALB/c male mice (19–25 g) received a single intraperitoneal injection of 4.5, 6.7 or 9.0 mmol/kg bw nitromethane in a volume of 0.2 mL saline. Control mice were injected with the same volume of 0.9% sodium chloride. Mice were killed 24, 48, 72 or 96 h after treatment. Blood was obtained by cardiac puncture and plasma was analysed for changes in sorbitol dehydrogenase, alanine aminotransferase and aspartate aminotransferase activity as measures of liver damage. Sections from three different liver lobes were processed and stained with haematoxylin and eosin for histopathological analysis. There were no significant changes in any of the enzymes measured or significant abnormalities in the livers of mice following nitromethane administration, demonstrating a lack of hepatotoxicity (Dayal *et al.*, 1989).

Nitromethane has been shown to produce histidinaemia in rats. Inbred weanling male Sprague-Dawley rats given subcutaneous injections of nitromethane (1.2 mol/L, 0.4 mL/100 g bw) every other day for one, three, six, 12 and 18 days. The histidine concentration in tissues increased gradually to reach a plateau after six days of treatment and after 18 days, levels were increased 4.7-fold in plasma, 2.7-fold in brain, 3.0-fold in liver and 1.7-fold in kidney (Lee & Wang, 1975). In the same strain of rats injected subcutaneously with nitromethane (1.8 mol/L, 0.8 mL/100 g bw) every day for six days, 61% of the rats had paralysis of the limbs and 15% had occasional

seizures. Liver weights and liver total protein did not change with treatment with nitromethane. Hepatic histidase activity decreased significantly in the nitromethane-treated rats compared with controls, with approximately a 3–3.5-fold corresponding increase in histidine concentration in plasma, liver and brain. No significant change in serotonin content of the various areas of the brain or in free amino acid concentration in plasma was detected. These results are consistent with nitromethane being a histidase inhibitor (Douay & Kamoun, 1980). In male Wistar rats (30 days of age), nitromethane (730 mg/kg bw) injected intraperitoneally three times over a 24-h period caused a 90% inhibition of histidase activity and higher serum histidine levels compared with controls. A consistently lower locomotor activity was observed in these histidinaemic rats compared with controls (Dutra-Filho *et al*., 1989).

Male and female Fischer 344/N rats and B6C3F$_1$ mice (seven weeks of age) were exposed to 0, 94, 188, 375, 750 or 1500 ppm [0, 235, 470, 938, 1875 or 3750 mg/m^3] nitromethane by inhalation for 6 h per day on five days per week over a 16-day period for a total of 12 exposure days. The mean body weight gain of male rats exposed to 1500 ppm [3750 mg/m^3] nitromethane only was slightly but significantly decreased. There was increased preening, rapid breathing, and hyperactivity early in the study and hypoactivity and loss of coordination in the hindlimbs near the end of the study in rats of both sexes. Exposure to nitromethane caused a concentration-related increase in the absolute and relative liver weights and minimal to mild degeneration of the olfactory epithelium in the nose of rats and mice. In nitromethane-exposed male and female rats, there was sciatic nerve degeneration. Concentrations of 750 or 1500 ppm [1875 or 3750 mg/m^3] nitromethane resulted in reduced myelin around sciatic axons in rats (National Toxicology Program, 1997).

Male and female Fischer 344/N rats and B6C3F$_1$ mice (six weeks of age) were exposed by inhalation to 0, 94, 188, 375, 750 or 1500 ppm [0, 235, 470, 938, 1875 or 3750 mg/m^3] nitromethane for 6 h per day on five days per week for 13 weeks to evaluate the cumulative toxic effects of repeated exposure to nitromethane and to determine the appropriate exposure concentrations to be used in a two-year study. Additional groups of rats were designated for clinical pathology evaluation on days 3 and 23. Neurobehavioural tests were carried out on all core study rats during week 11 of the study. Body weight and body weight gain were significantly less in male rats exposed to 1500 ppm [3750 mg/m^3] nitromethane than in the control group. Clinical findings included hindlimb paralysis in rats exposed to 750 and 1500 ppm [1875 and 3750 mg/m^3] nitromethane. Nitromethane caused exposure-related microcytic, responsive anaemia in male and female rats. Evidence that a haemolytic process occurred in exposed rats included the presence of schistocytes, Heinz bodies and spherocytes and increased mean cell haemoglobin and methaemoglobin concentration. On exposure day 23, there was a transient decrease in serum levels of triiodothyronine, and of total and free thyroxine in male and female rats exposed to nitromethane. Nitromethane exposure also caused minimal to mild hyperplasia of the bone marrow. Both rats and mice exposed to nitromethane had olfactory epithelial degeneration and

respiratory epithelial hyaline droplets. Goblet-cell hyperplasia occurred in male and female rats. Mild degeneration of the sciatic nerve and the lumbar spinal cord was also observed in male and female rats exposed to 375 ppm [938 mg/m^3] nitromethane. Forelimb and hindlimb grip strengths decreased in rats exposed to the highest concentration of nitromethane compared with controls. Both male and female mice in the 1500-ppm exposure group had minimal extramedullary haematopoiesis of the spleen (National Toxicology Program, 1997).

In a six-month inhalation study, New Zealand White rabbits and Sprague-Dawley rats were exposed by inhalation to 0, 98 or 745 ppm [0, 245 or 1860 mg/m^3] nitromethane for 7 h per day on five days per week for six months. Decreased body weight gain in rats was seen after eight weeks of exposure to 745 ppm. The most notable response in rabbits was an effect on the thyroid: increased thyroid weight and decreased serum thyroxine levels. There were no exposure-related gross or microscopic lesions in either rats or rabbits exposed to 98 or 745 ppm (Lewis *et al.*, 1979).

Male and female Long-Evans (BLU:(LE)BR) rats were exposed by inhalation to 0, 100 or 200 ppm [0, 250 or 500 mg/m^3] nitromethane for 7 h per day on five days per week for two years. Serum chemistry and haematology measurements were not found to be significantly different in nitromethane-exposed rats compared with rats exposed to room air. Body weights of exposed female rats were slightly lower than those of control rats. Tissues weights, however, were unaffected by chronic exposure to nitromethane. Non-neoplastic lesions were not related to nitromethane exposure but in most cases were similar to those found in populations of ageing laboratory rats (Griffin *et al.*, 1996).

In a two-year inhalation study, male and female Fischer 344/N rats and B6C3F$_1$ mice (six weeks of age) were exposed to 0, 94, 188 or 375 ppm [0, 235, 470 or 938 mg/m^3] and 0, 188, 375 or 750 ppm [0, 470, 938 or 1875 mg/m^3] nitromethane, respectively, for 6 h per day on five days per week for 103 weeks. Non-neoplastic lesions that developed with increased incidence included nasal lesions with degeneration and metaplasia of the olfactory epithelium and degeneration of the respiratory epithelium in male and female mice (National Toxicology Program, 1997).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

(*a*) *Developmental toxicity studies*

No data were available to the Working Group.

(*b*) *Reproductive toxicity studies*

In a 13-week inhalation study of nitromethane in male and female Fischer 344/N rats and B6C3F$_1$ mice exposed to 375, 750 or 1500 ppm [938, 1875 or 3750 mg/m^3] for 6 h per day on five days per week, a dose-related decrease in sperm motility was observed. The decrease was significant at doses of 750 and 1500 ppm in rats and at all dose levels in mice. In the 1500-ppm group, body weight as well as weight of cauda, epididymis and testis were decreased in rats. In female mice, estrous cycle length was dose-relatedly increased at all dose levels (National Toxicology Program, 1997).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 3 for references)

Nitromethane has given consistently negative results in bacterial mutagenicity assays. It also gave negative results in in-vitro mammalian tests for sister chromatid exchanges and chromosomal aberrations. It was not mutagenic in *Drosophila*. It did not induce micronuclei *in vitro* in Syrian hamster embryo cells or *in vivo* in mice. However, nitromethane did show a positive response at high concentration in a cell transformation assay in Syrian hamster embryo cells.

4.5 Mechanistic considerations

The results of short-term tests on nitromethane do not indicate that the compound has genotoxic activity.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Nitromethane is a volatile liquid that is added in small amounts to many halogenated solvents and aerosol propellants as a stabilizer. It is also used as a polar solvent for certain polymers and resins, in specialized fuels and in explosives. Exposures may occur from the use of solvents, propellants and fuels containing nitromethane.

5.2 Human carcinogenicity data

No data were available to the Working Group.

Table 3. Genetic and related effects of nitromethane

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA98, reverse mutation	–	NT	610 µg/plate	Chiu *et al.* (1978)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	3600 µg/plate	Gocke *et al.* (1981)
Salmonella typhimurium TA100, TA1535, TA98, reverse mutation	–	–	20 000 or 50 000 µg/plate	Löfroth *et al.* (1986)
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	10 000 µg/plate	Mortelmans *et al.* (1986)
Salmonella typhimurium TA100, TA98, TA102, reverse mutation	–	NT	12 200 µg/plate	Dayal *et al.* (1989)
Salmonella typhimurium TA100, TA98, reverse mutation	NT	–	6100 µg/plate	Dellarco & Prival (1989)
Drosophila melanogaster, sex-linked recessive lethal mutations, Basc test	–		7625 in feed	Gocke *et al.* (1981)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	–	4965	National Toxicology Program (1997)
Micronucleus test, Syrian hamster embryo cells *in vitro*	–		5000	Gibson *et al.* (1997)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	–	4980	National Toxicology Program (1997)
Cell transformation, Syrian hamster embryo cells *in vitro*	+		4000	Kerckaert *et al.* (1996)
Micronucleus formation, male and female NMRI mouse bone marrow *in vivo*	–		1830 ip × 2	Gocke *et al.* (1981)
Micronucleus formation, male and female B6C3F$_1$ mouse peripheral blood erythrocytes *in vivo*	–		1500 ppm by inh × 13 w	National Toxicology Program (1997)

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

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; inh, inhalation; w, week

5.3 Animal carcinogenicity data

Nitromethane was tested for carcinogenicity by inhalation in one experiment in mice and in two experiments in rats. In mice, it increased the incidence of Harderian gland and lung tumours in males and females as well as of hepatocellular adenomas in females. In one experiment in rats, nitromethane increased the incidence of benign and malignant mammary gland tumours in females, but produced no increase in the incidence of tumours in a second study in a different strain of rat.

5.4 Other relevant data

Nitromethane produces histidinaemia in rats by decreasing hepatic histidase activity, leading to increased tissue levels of histidine.

Neurological effects were observed in nitromethane-exposed rats.

Nitromethane caused mild degeneration of the olfactory epithelium of exposed rats and mice and microcytic anaemia with minimal to mild hyperplasia of the bone marrow in rats.

No data on reproductive or developmental effects in humans were available.

In rats and mice, dose-related decreases in sperm motility were found after inhalation of nitromethane. In females, estrous cycle length was increased in mice but not in similarly exposed rats.

Nitromethane gave negative results in all short-term tests for genetic effects, with the exception of a cell transformation assay in which it was positive at high concentration.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of nitromethane were available.

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

Overall evaluation

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

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PYRIDINE

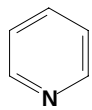
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 110-86-1
Deleted CAS Reg. Nos: 6999-00-4; 45410-39-7; 62301-32-0; 82005-06-9; 85404-19-9; 85404-20-2; 152758-95-7; 163392-20-9
Chem. Abstr. Name: Pyridine
IUPAC Systematic Name: Pyridine
Synonyms: Azabenzene; azine

1.1.2 *Structural and molecular formulae and relative molecular mass*



C_5H_5N Relative molecular mass: 79.10

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless liquid with a disagreeable odour (Budavari, 1998)
(*b*) *Boiling-point*: 115.2 °C (Lide & Milne, 1996)
(*c*) *Melting-point*: –41.6 °C (Lide & Milne, 1996)
(*d*) *Density*: 0.9819 g/cm^3 at 20 °C (Lide & Milne, 1996)
(*e*) *Spectroscopy data*: Infrared (prism [15]; grating [12]), ultraviolet [9], nuclear magnetic resonance (proton [10200, V96]; C-13 [1201]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980; Lide & Milne, 1996)
(*f*) *Solubility*: Miscible with water, acetone, benzene, chloroform, diethyl ether and ethanol (Lide & Milne, 1996)

(*g*) *Volatility*: Vapour pressure, 2.76 kPa at 25 °C (Lide & Milne, 1996); relative vapour density (air = 1), 2.73 (Verschueren, 1996)
(*h*) *Stability*: Highly flammable; flash point, 20 °C (closed cup) (Budavari, 1998); explosive limits, 12.4% (upper), 1.8% (lower) by volume in air (American Conference of Governmental Industrial Hygienists, 1999)
(*i*) *Octanol/water partition coefficient (P)*: log P, 0.60/0.65 (Hansch *et al*., 1995)
(*j*) *Conversion factor*[1]: $mg/m^3 = 3.24 \times ppm$

1.1.4 *Technical products and impurities*

Pyridine is commercially available in several grades for specific applications. Specifications vary according to country but are usually greater than 99.8% purity by gas chromatographic analysis (Scriven *et al*., 1996; Burdick & Jackson, 1997).

Trade names for pyridine include: CP 32.

1.1.5 *Analysis*

Selected methods for the analysis of pyridine in various matrices are given in Table 1.

1.2 Production

Pyridine was first synthesized in 1876 from acetylene and hydrogen cyanide (Shimizu *et al.*, 1993). A more plentiful source was found in coal tar, the condensate from coking ovens in the steel industry. Pyridine bases are found in the light-oil and middle-oil fractions from coal tar and comprise pyridine, the picolines and higher homologues. Pyridine has been produced commercially from coal-tar sources since the 1920s. During the 1950s, synthetic processes were developed to provide alternatives to isolation from coal-tar sources (Santodonato *et al.*, 1985; Scriven *et al.*, 1996). There are few selective commercial processes for preparing pyridine and its derivatives, and almost all manufacturing processes produce pyridine along with a series of alkylated pyridines in admixture. The reaction of aldehydes or ketones with ammonia is the most general synthetic reaction for the manufacture of pyridine bases and allows the preparation of various pyridine derivatives. Reaction of acetaldehyde and formaldehyde with ammonia is the most widely used method for pyridine production (Scriven *et al*., 1996).

Pyridine can also be prepared from cyclopentadiene by ammoxidation, or from 2-pentenenitrile by cyclization and dehydrogenation. Furfuryl alcohol or furfural reacts with ammonia in the gas phase to give pyridine (Scriven *et al*., 1996).

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[1] Calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

Table 1. Selected methods for the analysis of pyridine

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Adsorb (charcoal); desorb (dichloromethane)	GC/FID	0.02 mg/sample	Eller (1994) [Method 1613]
Water, soil, municipal waste	Add isotope-labelled analogue; extract with dichloromethane; dry over sodium sulfate; concentrate	GC/MS	5 µg/L	Environmental Protection Agency (1995) [Method 1665]
Solid waste matrices[b]	Solvent extraction or direct injection (with azeotropic distillation) into capillary GC column	GC/FID	9–21 µg/L (aqueous matrices); 0.08–0.20 mg/kg (solid matrices)	Environmental Protection Agency (1996a) [Method 8015B]
	Direct injection (with azeotropic distillation) into capillary GC column	GC/MS	4 µg/L	Environmental Protection Agency (1996b) [Method 8260B]

[a] Abbreviations: GC, gas chromatography; FID, flame ionization detection; MS, mass spectrometry
[b] Includes: groundwater, sludges, caustic and acid liquors, waste solvents, oily wastes, mousses, tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils and sediments

Information available in 1999 indicated that pyridine was manufactured by four companies in the United States, three companies in China, two companies each in Germany, India, Japan and United Kingdom, and one company each in Argentina, Belgium, France, Hungary, Israel, Italy, the Russian Federation, Spain, Taiwan and Ukraine (Chemical Information Services, 1999).

1.3 Use

Pyridine is widely used as a solvent in organic chemistry and in industrial practice. Pyridine is an effective, basic solvent that is relatively unreactive, which makes it a good acid scavenger. Pyridine is the solvent of choice for acylation and dehydrochlorination reactions. It is also used as a solvent for paint, rubber, pharmaceuticals, polycarbonate resins and textile water repellants. Large amounts of pyridine are used as an intermediate in the manufacture of substituted pyridines, piperidine, agrochemicals (herbicides: diquat, paraquat; insecticide: chlorpyrifos; fungicide: pyrithione), pharmaceuticals and other products (Santodonato *et al.*, 1985; Agency for Toxic Substances and Disease Registry, 1992; Shimizu *et al.*, 1993; Scriven *et al.*, 1996).

1.4 Occurrence

1.4.1 *Natural occurrence*

Pyridine is found among the volatile components of black tea (Vitzthum *et al.*, 1975) and in the leaves and roots of *Atropa belladonna* (Burdock, 1995).

1.4.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (NOES, 1999), as many as 43 000 workers in the United States were potentially exposed to pyridine. Occupational exposure may occur by inhalation and dermal contact during its production by synthesis or by treatment and distillation of crude coal-tar, during the processing of oil-shale and at coke-oven works. Exposure may also occur during its wide use as a chemical intermediate and/or solvent (Santodonato *et al.*, 1985; Agency for Toxic Substances and Disease Registry, 1992). Laboratory use appears to account for a large number of workers potentially exposed (Santodonato *et al.*, 1985; NOES, 1999).

Few data, especially recent data, are available on occupational exposure levels of pyridine. In 1978, the United States Pyridine Task Force of the Interagency Testing Committee reported that in United States workplaces where pyridine was manufactured or used as a chemical intermediate or as a solvent, workers were exposed to 8-h time-weighted average (TWA) pyridine concentrations ranging from 0.008 to 1.0 ppm [0.026 to 3.24 mg/m^3]. Technicians working in quality control and research and development laboratories of one of the pyridine manufacturers were exposed to TWA concentrations (measured over 6-h periods) of no more than 0.09 ppm [0.29 mg/m^3] (Santodonato *et al.*, 1985).

A few studies have evaluated exposure levels in industries where pyridine originates from coal or coal-tar or where coal-tar products are processed or used. In a pyridine production shop of a coal-tar manufacture in the former USSR, pyridine levels were 7.5–10 mg/m^3 and occasionally reached 20 mg/m^3 (Izmerov, 1984). Mašek (1981) measured airborne concentrations in various industries in former Czechoslovakia. Values ranged from 0.005 to 2.98 mg/m^3 [0.0015–0.92 ppm] in coking plants, from 0.005 to 0.135 mg/m^3 [0.0015–0.042 ppm] in blast furnaces and steel works and from 0.010 to 0.630 mg/m^3 [0.0031–0.195 ppm] in rolling mills and foundries. More recently, Bienik *et al.* (1993) reported personal exposure levels ranging from 0.002 to 0.7 mg/m^3 [0.00062–0.22 ppm] (24 workers) in a Polish carbochemical plant. A study in a United States laboratory conducting research on coal conversion technology found no level of pyridine greater than 0.2 ppm [0.65 mg/m^3] throughout the 1979–82 sampling period (Dreibelbis *et al.*, 1982).

Air samples were collected in the moulding and pouring departments of a United States iron foundry using a phenolic urethane binder. The two-day average level of pyridine, emitted as a breakdown product of 4-phenylpropylpyridine used as a binder

catalyst, was 5.9 ppm [19.1 mg/m^3] in the moulding area (Apol, 1982). Pyridine as a possible pyrolysis product was not detected (< 0.1 ppm) [0.32 mg/m^3] near a nylon injection-moulding operation of a United States electrical components production plant (Hartle & Erhenberg, 1985).

1.4.3 *Environmental occurrence*

The production of pyridine and its wide use as a solvent and as an intermediate in the synthesis of piperidine and a wide variety of drugs, insecticides and herbicides and chemicals used in rubber vulcanization may result in its release into the environment (Jori *et al*., 1983; Agency for Toxic Substances Disease Registry, 1992; Environmental Protection Agency, 1999). Pyridine occurs in the environment as a by-product of coal gasification (Stuermer *et al*., 1982).

Pyridine and its homologues are produced in coking procedures and present in the non-condensable gases. Pyridine is also present in coal-tar and as a component in creosote (Agency for Toxic Substances Disease Registry, 1992; Dutch Ministry of Social Affairs and Employment, 1993).

(*a*) *Air*

Pyridine has rarely been detected in ambient rural or urban air except in the vicinity of industrial or waste-treatment facilities (Hawthorne & Sievers, 1984; Shah & Heyerdahl, 1988; US Agency for Toxic Substances Disease Registry, 1992). Atmospheric emission of pyridine has been detected at a concentration of 13 μg/m^3 in the air in the vicinity of an oil-shale wastewater facility (Hawthorne & Sievers, 1984). Pyridine is released into the atmosphere as fugitive emissions from coal gasification and oil-shale processing facilities and from iron working and coking plants (Junk & Ford, 1980; National Toxicology Program, 1997).

According to the Environmental Protection Agency Toxics Release Inventory (TRI), air emissions of pyridine from 43 industrial facilities in 1997 were approximately 46 325 kg in the United States (Environmental Protection Agency, 1999).

(*b*) *Water*

Pyridine in water may partition to soils and sediments to an extent that depends on the pH of the water, and to a lesser extent, the organic carbon content of the soil (Agency for Toxic Substances Disease Registry, 1992).

Surface water discharges of pyridine from 43 industrial facilities in 1997 in the United States amounted to 247 kg; in addition, underground injection of pyridine amounted to 278 290 kg as reported in the Toxics Release Inventory (US Environmental Protection Agency, 1996c).

Pyridine has been found in both subsurface and groundwater as a result of industrial activities such as synthetic fuel production and chemical manufacturing (Sims & O'Loughlin, 1989). Pyridine is a component in the basic fraction of oil-shale

retort waters (Leenheer & Stuber, 1981) with typical concentrations of 20–100 mg/L (Zhu *et al*., 1988). Pyridine was detected at a concentration of about 5 mg/L in Australian oil-shale retort water (Dobson *et al*., 1985). It was detected in one of two oil-shale processing effluents at a concentration of 152 µg/L, but not in coal gasification plant effluents (Pellizzari *et al.*, 1979; cited in Agency for Toxic Substances Disease Registry, 1992). Pyridine was found at levels of 0.82, 49 and 53 ppb (µg/L) in groundwater samples from three wells near an underground coal gasification site in northeastern Wyoming (Stuermer *et al*., 1982).

(*c*) *Soil and sediments*

Pyridine (and its derivatives) are water-soluble and do not readily bind to organic constituents of soil and aquifer materials. They may, therefore, be transported through aquifer materials, sediments and soils and thus contaminate rivers and estuaries (Liu & Kuo, 1997).

Although pyridine releases to land from industrial sources in the United States totalled an estimated 510 kg in 1988 as reported in the Toxics Release Inventory (Environmental Protection Agency, 1996c), release of only 2 kg to land was reported in 1997 (Environmental Protection Agency, 1999).

(*d*) *Food*

Pyridine has been isolated in the volatile components from cooked beef (‘sukiyaki’) in Japan (Shibamoto *et al*., 1981), fried chicken in the United States (Tang *et al*., 1983), fried bacon (Ho *et al*., 1983), Beaufort cheese (Dumont & Adda, 1978), black tea aroma (Vitzthum *et al*., 1975) and coffee aroma (Aeschbacher *et al*., 1989).

(*e*) *Tobacco smoke*

Pyridine has been detected as a component of tobacco and marijuana smoke (Schmeltz & Hoffmann, 1977; Curvall *et al.*, 1984; Eatough *et al.*, 1989). It has been found in tobacco smoke at 21–32 µg per cigarette (IARC, 1986). In indoor air, pyridine has been detected at concentrations as high as 16 µg/m^3 in indoor air contaminated with cigarette smoke (Brunnemann *et al.*, 1991; cited in Agency for Toxic Substance Disease Registry, 1992). Otson *et al.* (1994) reported a pyridine level of 6 µg/m^3 in air in Canadian homes.

1.5 Regulations and guidelines

Occupational exposure limits and guidelines for pyridine are presented in Table 2.

The Food and Drug Administration (1999) permits the use of pyridine as a synthetic flavouring substance or adjuvant generally recognized as safe in foods in the United States.

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

Country	Year	Concentration (mg/ m^3)	Interpretation[b]
Australia	1993	15	TWA
Belgium	1993	15	TWA
Czech Republic	1993	5	TWA
		10	STEL
Denmark	1993	15	TWA
Egypt	1993	15	TWA
Finland	1998	15 (sk)	TWA
		30	STEL
France	1993	15	TWA
		30	STEL
Germany	1999	15	TWA
Hungary	1993	5 (sk)	TWA
		10	STEL
Ireland	1997	15	TWA
		30	STEL
Netherlands	1997	0.9	TWA
Philippines	1993	5	TWA
Poland	1998	5 (sk)	TWA
Russian Federation	1993	5	STEL
Sweden	1993	15	TWA
		30	STEL
Switzerland	1993	15	TWA
		30	STEL
Turkey	1993	15	TWA
United Kingdom	1997	15	TWA
		30	STEL
United States			
ACGIH	1999	15	TWA
NIOSH			
OSHA	1999	15	TWA
	1999	15	TWA

[a] From Finnish Ministry of Social Affairs and Health (1998); American Conference of Governmental Industrial Hygienists (ACGIH) (1999); Deutsche Forschungsgemeinschaft (1999); Occupational Safety and Health Administration (OSHA) (1999)

[b] TWA, time-weighted average; STEL, short-term exposure limit; sk, skin notation

[c] These countries follow the recommendations of the ACGIH threshold limit values: Bulgaria, Colombia, Jordan, Republic of Korea, New Zealand, Singapore and Viet Nam.

2. Studies of Cancer in Humans

Cohort study

A cohort of 729 male workers was set up at three plants manufacturing 4,4′-bipyridyl from pyridine in the north-west of England, including all employees working in 1983 at the time when the cohort was established and all past employees in the manufacturing process since 1961 (Paddle *et al*., 1991). The mortality was assessed up to the end of 1985; 3.4% of the cohort could not be traced. Reference rates from England and Wales were obtained and corrected upwards to account for higher mortality rates in the area. Overall, 75 deaths were observed versus 96.3 expected (standard mortality ratio [SMR], 0.8 [95% confidence interval (CI), 0.6–1.0]), including 29 cancer deaths versus 27.1 expected (SMR, 1.1 [95% CI, 0.7–1.5]). When a 10-year latency was imposed between the start of exposure and the start of follow-up, an excess of mortality from lung cancer was observed (SMR, 1.7 [95% CI, 0.9–3.1]), increasing to 2.1 after 15 years. Additional analysis by job, plant and categories of exposure to chemicals in a nested case–referent study did not identify any risk factor for lung cancer except for exposure to diethylene glycol dimethyl ether (diglyme). An examination of the exposure levels and time since exposure of the lung cancer cases did not support a causal interpretation. Data on the relationship between lung cancer and exposure to pyridine were not reported. [The Working Group noted that the precise list of chemicals investigated was not mentioned.]

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 50 male and 50 female B6C3F_1 mice, seven weeks of age, were exposed to pyridine (purity, 99.8%) in the drinking-water at concentrations of 0, 250, 500 or 1000 mg/L (ppm), equivalent to an average daily dose of 0, 35, 65 or 110 mg/kg bw, for males and 0, 125, 250 or 500 ppm [mg/L], equivalent to an average daily dose of 15, 25 or 70 mg/kg bw, for females for 104 weeks (males) or 105 weeks (females). Mean body weights of males were similar to those of the controls; mean body weights of 250- and 500-ppm females were lower than those of controls from weeks 89 and 73, respectively. Survival of exposed males and females was similar to that of the controls. Statistical analyses were carried out using the Poly-3 test. Hepatocellular adenomas occurred at an increased incidence in males: 29/50, 40/50 ($p = 0.003$), 34/49 and 39/50 ($p = 0.011$) in control, low-, mid- and high-dose mice, respectively. The incidence of hepatocellular carcinomas in males was: 15/50 control, 35/50 low-dose,

41/49 mid-dose and 40/50 high-dose mice, respectively ($p < 0.001$, pairwise comparisons for all treated groups). The incidence of hepatoblastomas in males was: 2/50, 18/50, 22/49 and 15/50 ($p < 0.001$, pairwise comparisons for all treated groups) in control, low-dose, mid-dose and high-dose mice, respectively. In female mice, the incidence of hepatocellular carcinomas was increased in a dose-related manner: 13/49 control, 23/50 low-dose, 33/50 ($p = 0.014$) mid-dose and 41/50 high-dose mice ($p < 0.001$). The incidence of hepatoblastomas was also significantly increased: 1/49 control, 2/50 low-dose, 9/50 ($p = 0.007$) mid-dose and 16/50 high-dose mice ($p < 0.001$) (National Toxicology Program, 1997).

3.1.2 *Rat*

Groups of 50 male and 50 female Fischer 344/N rats, eight weeks of age, were given drinking-water containing 0, 100, 200 or 400 ppm pyridine (purity, 99.8%) for 103 weeks (males) or 104 weeks (females). Average daily doses were 0, 7, 14 and 33 mg/kg bw. Mean body weights of 200- and 400-ppm males after weeks 73 and 6, respectively, and females after weeks 61 and 9, respectively, were less than those of controls [statistical significance not reported]. Survival of exposed males and females was not significantly different from that of controls. As shown in Table 3, the incidence of renal tubule adenomas in male rats was increased, whether single sections or single and multiple sections combined were evaluated (National Toxicology Program, 1997).

Table 3. Incidence of renal tubule tumours in male Fischer 344 rats fed pyridine in the diet

Dose	Number of animals			
	0	100 ppm	200 ppm	400 ppm
Single sections				
Renal tubule adenomas	1/50	0/48	2/50	6/49*
Renal tubule carcinomas	0/50	1/48	0/50	0/50
Step sections				
Renal tubule adenomas	1/50	3/48	5/50	9/49**
Single section and step sections combined				
Renal tubule adenomas	2/50	3/48	6/50	10/49**
Renal tubule carcinomas	0/50	1/48	0/50	0/49
Renal tubule adenomas and carcinomas	2/50	4/48	6/50	10/49**

From National Toxicology Program (1997)
* $p < 0.05$, Poly-3 test
** $p < 0.01$, Poly-3 test

In a concurrent experiment, groups of 50 male Wistar rats, seven weeks of age, were given drinking-water containing 0, 100, 200 or 400 ppm pyridine (purity, 99.8–0.6%), resulting in average daily doses of approximately 8, 17 or 36 mg/kg bw, for 104 weeks. Mean body weights of rats exposed to 100, 200 or 400 ppm were significantly lower than those of controls, beginning at weeks 69, 49 and 6, respectively. Survival of rats exposed to 200 or 400 ppm pyridine was significantly lower than that of the controls. The incidence of testicular adenoma in rats exposed to 400 ppm was significantly increased compared with controls: 5/50 controls, 6/49 low-dose, 4/49 mid-dose and 12/50 high-dose ($p = 0.012$, pairwise comparison). In contrast to the Fischer rats, renal tubule cell tumours were not observed at increased incidence, even after step sectioning (US National Toxicology Program, 1997). [The Working Group noted that the National Toxicology Program cited a historical control range for testicular adenomas in Wistar rats (0–22%) (Walsh & Poteracki, 1994), the upper limit of which was similar to the incidence in high-dose males seen in this study.]

3.2 Subcutaneous administration

Rat: Groups of 10, 20, 30 or 40 male and 10, 20, 30 or 40 female Fischer 344/N rats, four weeks of age, were injected subcutaneously with 0, 3, 10, 30 or 100 mg/kg bw pyridine (commercial product) in physiological saline twice a week for 52 weeks. The animals were observed for a further six months. Body weights were similar in treated and untreated animals. Distributed across all groups, only three animals died by 12 months and eight by 18 months. There was no increase in the incidence of tumours either at the injection site or at any other site (Mason *et al*., 1971).

3.3 Genetically modified mouse models

Groups of 15–20 hemizygous female Tg.AC mice (zeta-globin promoted v-Ha-*ras* on an FVB background), 14 weeks of age, were administered pyridine [purity not specified] in 200 μL acetone topically on shaved skin in the interscapular region five times per week at doses of 0, 1.5, 3.0 or 6.0 mg per mouse for 20 weeks. Concurrent negative control groups of 15 mice were treated with 200 μL acetone alone. A group of 15 animals was treated topically with 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (approximately 99% pure) dissolved in 200 μL acetone at a dose of 1.25 μg per mouse three times per week. Skin papillomas were observed at the end of the study in 1/15, 2/15, 0/14 and 1/20 in the untreated control, low-dose, mid-dose and high-dose animals, respectively. Skin papillomas were observed in 15/15 TPA-treated mice (Spalding *et al*., 2000).

Heterozygous male and female $p53^{+/-}$ mice (C57BL/6-Trp53(+/–)tm1Dol;N5), eight to 11 weeks of age, were given pyridine [purity not specified] in the drinking-water *ad libitum* for seven days per week for 26 weeks at doses of 0, 250, 500 or 1000 mg/L (ppm) for males and at 0, 125, 250 or 500 ppm for females. There was no increase in the incidence of tumours in any of the treated groups (Spalding *et al*., 2000).

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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

The fate of pyridine was examined in two healthy male subjects, who each received an oral dose of 3.4 mg [^{14}C]pyridine [approx. 0.01 mg/kg bw] in orange juice. A total of 65 and 68% of the ^{14}C-dose was recovered in the 0–24-h urine of the two subjects, respectively, and two metabolites were identified: pyridine *N*-oxide, which accounted for 32% of the dose, and *N*-methylpyridinium ion, accounting for 6 and 12% of the dose, respectively. Some 25% of the dose was not characterized (D'Souza *et al.*, 1980; Damani *et al.*, 1982).

4.1.2 *Experimental systems*

Pyridine is metabolized in animals by oxidation at the nitrogen atom, giving pyridine *N*-oxide, and at all carbon atoms of the ring, giving 2- and 4-pyridone and 3-hydroxypyridine. In addition, it undergoes *N*-methylation, yielding the quaternary ammonium ion *N*-methylpyridinium. The relative contributions of these pathways to the overall fate of pyridine were examined by Damani *et al*. (1982) who administered [^{14}C]pyridine intraperitoneally to rats, mice, guinea-pigs, hamsters, gerbils, rabbits and cats at a dose of 7 mg/kg bw. At least 50% of the administered ^{14}C was recovered in the urine of the animals and the amounts of the various specific metabolites differed markedly between species, as shown in Table 4. Only small amounts (0.4–5% of dose) of unchanged pyridine were found in most species, but cats excreted 14% and rabbits 25% of the dose in this form. The extent of *N*-oxidation varied widely between species, from 0.3% of dose in rats to 39% in hamsters. Pyridine *N*-oxide was not detected in rabbit urine. The excretion of *N*-methylpyridinium also varied between species, being lowest in gerbils (~2% of dose) and highest in cats (51% of dose). The major *C*-oxidation product was 4-pyridone, which ranged from 4% of the dose in hamsters to 19% in rabbits. 2-Pyridone and 3-hydroxypyridine were minor metabolites in all species, the former being absent from the metabolic profile in rabbits. Mice did not oxidize pyridine at any carbon atom of the ring. The occurrence of additional metabolic pathways is suggested by the excretion of unidentified products, accounting for up to 37% of the dose, in all species except guinea-pigs and cats (Damani *et al.*, 1982). These pathways include glucuronidation of 3-hydroxypyridine, previously observed in rabbits (Smith, 1953).

There is a dose-dependence in the metabolism of pyridine. At low doses of pyridine, *N*-methylation may be the preferred route of biotransformation, while at higher doses, such as 40 mg/kg bw, the extent of *N*-oxidation varied from some 10% in rats to 20–40%

Table 4. Species variations in the metabolic *C*- and *N*-oxidation and *N*-methylation of [^{14}C]pyridine in various laboratory animals *in vivo*

	Total ^{14}C recovery	% of dose in 0–24-h urine						
		Pyridine	*N*-Methyl-pyridinium[a]	2-Pyridone	3-Hydroxy-pyridine	4-Pyridone	Pyridine *N*-oxide[b]	Unknown(s)
Rat	48	2	4 (5)	1	2	10	0.5 (0.3)	28
Mouse	66	2	21 (12)	ND	ND	ND	5 (6)	37
Guinea-pig	66	5	31 (30)	2	2	18	9 (8)	0
Hamster	67	0	17 (26)	1	0.3	4	39 (37)	6
Gerbil	52	0.4	1 (2 and 3)	1	1	7	8 (10)	34
Rabbit[c]	77	25	13 (15)	0	4	19	0	17
Cat[d]	75	14	51 (40)	2	1	10	3	0
Human	66	ND	(6 and 12)	ND	ND	ND	(32)	~25

From Damani *et al.* (1982). Values obtained by high-performance liquid chromatography
ND, not determined
[a] Values in parentheses obtained by reverse isotope dilution
[b] Values in parentheses obtained by gas chromatography
[c] 0–72-h urine
[d] 0–48-h urine

in mice, hamsters, guinea-pigs, rabbits and ferrets (Damani *et al.*, 1982). In rats, the formation of *N*-methylpyridinium ion as a percentage of administered dose fell from 10 to 0.8% with increasing dose over the range 1–500 mg/kg bw (D'Souza *et al.*, 1980). The occurrence of *N*-methylation was similar whether pyridine was given orally or by intraperitoneal injection. In contrast, guinea-pigs excreted 31% of a dose as *N*-methylpyridinium independently of dose (either 1 or 7 mg/kg bw, as for rats): this was unaffected by the route of administration but the excretion decreased to 2% when the intraperitoneal dose was 500 mg/kg bw. The low *N*-methylation capacity of the rat was not enhanced by pre-treatment and dietary supplementation with DL-methionine, the precursor of the methyl donor *S*-adenosylmethionine.

D'Souza *et al*. (1980) examined the further metabolism of *N*-methylpyridinium. Rats and guinea-pigs given *N*-methyl[^{14}C]pyridinium by intraperitoneal injection excreted 53% and 85% respectively of the dose in the 0–24-h urine. In both species, > 95% of urinary ^{14}C was present as unchanged *N*-methylpyridinium.

4.2 Toxic effects

4.2.1 *Humans*

Ingestion of approximately 500 mg/kg bw pyridine by a 29-year-old man caused nausea, dizziness, abdominal pain and lung congestion followed by death after 43 h. Inhalation by workers of pyridine vapours at a concentration of about 125 ppm [405 mg/m^3] pyridine for 4 h per day for one to two weeks resulted in headache, dizziness, insomnia, nausea and anorexia (Jori *et al*., 1983).

4.2.2 *Experimental systems*

Pyridine citrate given in the diet (0.7–1.0%) to young adult male rats [strain not stated] resulted in the death of most of the animals within two or three weeks (Baxter, 1947). Clinical and histopathological examination revealed acute hepatic and renal injury, followed by regenerative changes, cirrhosis and chronic renal injury. Increasing the choline content of the diet at the same time that the pyridine level was also being increased caused a marked reduction in fatty degeneration and fibrosis of the liver without any significant reduction in the severity and extent of the acute necrosis.

In rats, the LD_{50} of pyridine after a single subcutaneous injection was estimated to be 866 mg/kg bw (Mason *et al.*, 1971). It is of interest to note that the intraperitoneal LD_{50} of *N*-methylpyridinium, a major metabolite in some species, is 0.22 g/kg bw in mice, compared with 1.2 g/kg bw for pyridine (D'Souza *et al.*, 1980). A dose of 100 mg/kg bw administered subcutaneously twice weekly for one year to male and female Fischer 344 rats led to significant retardation of weight gain (Mason *et al.*, 1971). Inhalation of 5 ppm [16 mg/m^3] pyridine for 6 h per day for four days caused olfactory epithelial lesions in male Fischer 344/N rats. These included vascular degeneration of sustentacular cells, focal, marked attenuation of the epithelium, loss of neurons and

the presence of intraepithelial luminal structures (Nikula & Lewis, 1994). In male Sprague-Dawley rats, a single intraperitoneal dose of pyridine (200 mg/mL saline; 1 mmol/kg bw) caused a significant increase in serum level of sorbitol dehydrogenase, indicating liver damage (Felten *et al.*, 1998).

In male New Zealand White rabbits, pyridine treatment (one intraperitoneal injection of 100 mg/kg bw daily for five days) resulted in increased hepatic microsomal content of cytochrome P450, with induction of several isoforms that exhibit elevated catalytic activities toward pyridine, *N*-nitrosodimethylamine, alcohols and aniline (Kaul & Novak, 1987). Kim *et al.* (1988) confirmed these findings, demonstrating the induction of a high-affinity isozyme [subsequently understood to be CYP2E1] responsible for the production of pyridine *N*-oxide. Treatment of male Sprague-Dawley rats with pyridine as a single intraperitoneal dose of 100 mg/kg bw resulted in moderate induction of hepatic CYP1A1, as judged by inspection of immunoblots (not quantified) (Kim *et al.*, 1991a). Similar results were obtained for renal CYP1A1 and CYP1A2 (Kim *et al.*, 1995). Combined treatment of male Sprague-Dawley rats with pyridine and acetone had over-additive (synergistic) inducing effects on CYP1A1 and CYP1A2 in the liver and CYP1A1 in the lung, where the effect was particularly great. Thus, lung CYP1A1 activity was increased 21-fold by exposure to 20 ppm [65 mg/m^3] pyridine vapour (5–6 h per day for 10 consecutive days), fivefold by oral intake of acetone (7.5% v/v solution in drinking water for 10 consecutive days) and 115.5-fold by the combined treatment (Iba *et al.*, 1993). Other isozymes inducible in rat liver (male Sprague-Dawley) by pyridine treatment are CYP2B1/2B2 and, most notably, CYP2E1 (Kim *et al.*, 1993), while in rabbit liver (male New Zealand White) an increase in CYP2E1 but no increase in CYP2B and only a marginal increase in CYP4B expression was observed (Kim *et al.*, 1991b). In SENCAR mouse skin, topical application of pyridine (300 or 500 mg/kg bw) resulted in increases in CYP1A1, CYP2B1 and CYP3A (Agarwal *et al.*, 1994).

In inhalation experiments in male Fischer 344/N rats, exposure to 5 ppm pyridine for four days (6 h per day) resulted in induction of hepatic CYP2E1 (Hotchkiss *et al.*, 1993). Induction of hepatic CYP2E1 was also observed in male Swiss albino mice and male Sprague-Dawley rats treated daily with 80 mg/kg bw pyridine (given intraperitoneally) for one to three days (Anari *et al.*, 1995). In pyridine-treated male Sprague-Dawley rats (one dose of 100 mg/kg bw, daily for four days), increased CYP2E1 protein was observed in the liver and kidney, while CYP2E1 mRNA was induced only in the kidney, indicating tissue-specific mechanisms of induction (Goasduff *et al.*, 1996). Another enzyme inducible by pyridine is carboxylesterase. Inhalation of 5 ppm pyridine for four days (6 h per day) resulted in increased carboxylesterase immunoreactivity in Bowman's glands and sustentacular cells of the nasal mucosa in male Fischer 344/N rats, 20 hours after the end of exposure (Nikula *et al.*, 1995). In primary cultures of hepatocytes from male Sprague-Dawley rats treated with 25 mM [2 g/L] pyridine for 24 h, the CYP2E1 protein level was increased by about ninefold, and that of CYP2B mRNA and protein by about 30-fold (Zangar *et al.*, 1995).

Acute pyridine treatment (single intraperitoneal dose of 200 mg/kg bw) increased the metabolism of 2-butanol twofold in Sprague-Dawley rat liver microsomes and threefold in rabbit (New Zealand White) liver microsomes (Page & Carlson, 1993). In liver microsomes from pyridine-treated (one intraperitoneal injection of 100 mg/kg bw, daily for four days) male Sprague-Dawley rats, increased oxidative biotransformation of the chlorofluorocarbon 1,2-dichloro-1,1,2-trifluoroethane was found the day after the last injection (Dekant *et al.*, 1995).

In male Sprague-Dawley rats, a single intraperitoneal treatment with pyridine (100 mg/kg bw) enhanced the metabolic activation of carbon tetrachloride 24 h later, leading to increased hepatic expression of the immediate early genes c-*fos* and c-*jun* (Gruebele et al., 1996). Similarly, pyridine treatment (a single intraperitoneal dose of 200 mg/kg bw) of male NSA and CD1 mice resulted in enhanced formation of styrene oxide (*S* enantiomer) from styrene in liver microsomes 18 h after dosing (Carlson, 1997). In agreement with these results, Gadberry *et al.* (1996) reported enhanced hepato- and pneumotoxicity of styrene in male non-Swiss albino mice treated with pyridine. Induction of CYP2E1, after a single intraperitoneal dose of pyridine (200 mg/kg bw) given to male Sprague-Dawley rats, was correlated with increases in *para*-nitrophenol hydroxylation, ethoxyresorufin deethylation and *N*-nitrosodimethylamine metabolism in lung and liver microsomes 24 h after dosing (Carlson & Day, 1992). Treatment of Sprague-Dawley rats by intraperitoneal injection with 100 mg/kg bw pyridine per day for three days led to a threefold increase in testicular microsomal CYP2E1 content and *para*-nitrophenol hydroxylation (Jiang *et al.*, 1998).

Male and female Fischer 344 rats and B6C3F1 mice and male Wistar rats were given drinking water containing 0, 50, 100, 250, 500 or 1000 ppm (mg/L) pyridine for 13 weeks (National Toxicology Program, 1997). Water consumption by female Fischer 344 rats and male Wistar rats exposed to 1000 ppm pyridine was lower than that of control rats. Evidence of anaemia was present in male and female Fischer 344 rats. Exposure to pyridine (500 or 1000 ppm) increased serum alanine aminotransferase and sorbitol dehydrogenase activities and the incidence of centrilobular degeneration, hypertrophy, chronic inflammation and pigmentation in all rats. Spontaneous nephropathy, common in ageing male rats, increased only in male Fischer 344 rats exposed to 500 or 1000 ppm pyridine. The incidence of granular casts and renal tubule hyaline degeneration was increased in male Fischer 344 rats exposed to 500 or 1000 ppm pyridine. Immunohistochemical staining for α_{2u}-globulin was positive in all male rats and negative in female Fischer 344 rats [actual data not reported]. In mice, no treatment-related clinical findings were observed.

Male and female Fischer 344 rats and male Wistar rats were given drinking water containing 0, 100, 200 or 400 ppm (mg/L) pyridine for 103–104 weeks (National Toxicology Program, 1997). Male $B6C3F_1$ mice were given drinking water containing 0, 250, 500 or 1000 ppm pyridine for 104 weeks and female $B6C3F_1$ mice 0, 125, 250 or 500 ppm pyridine for 105 weeks. In Fischer 344 rats, the incidence of renal tubule hyperplasia was increased in males exposed to 400 ppm pyridine compared with

controls; the severity of chronic progressive nephropathy was increased slightly in males with increasing exposure concentration, but statistical significance was not indicated; the incidence of mineralization of the stomach in the high-dose males was increased significantly compared with controls; increases in centrilobular cytomegaly and cytoplasmic vacuolization, and pigmentation in the liver were seen in male and females; periportal fibrosis was increased in the liver of males, with an increased incidence of bile duct hyperplasia in females. In male Wistar rats, liver lesions included an increase in the incidence of centrilobular degeneration and necrosis, fibrosis, periportal fibrosis, and pigmentation. Spontaneous, age-related chronic progressive nephropathy was moderately severe in control and exposed male Wistar rats and was considered to be the cause of the high mortality in this study. In mice, pyridine increased the incidence of haematopoietic cell proliferation in the spleen and follicular cell hyperplasia in the thyroid gland.

Incubation in 1–20 μM pyridine for 20 min inhibited norepinephrine-induced phasic and tonic contractions in the thoracic aorta, incubated as aortic rings, as well as the endothelium-denuded aorta of Wistar rats (Hsu & Lin-Shiau, 1995). These effects were related to inhibition of the calcium influx normally elicited by norepinephrine.

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Groups of 10 male and 10 female B6C3F_1 mice were exposed to 0, 250, 500 or 1000 ppm (mg/L) pyridine in the drinking-water for 13 weeks. Average daily doses were 0, 50, 85 and 160 mg/kg bw for males and 0, 60, 100 and 190 mg/kg bw for females. Spermatozoal motility was slightly, but significantly, decreased at all three dose levels tested. There were no significant differences in estrous cycle lengths in females (National Toxicology Program, 1997).

Groups of 10 male and 10 female Fischer 344 rats were exposed to 0, 250, 500 and 1000 ppm pyridine in the drinking-water for 13 weeks. Average daily doses were 0, 25, 55 and 90 mg/kg bw. At the highest dose level, a lower body weight was accompanied by reduced weight of epididymis and testes in males. In females, average estrous cycle length was significantly increased at the highest dose level (National Toxicology Program, 1997).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see Table 5 for references)

Ecotoxicological, including genetic toxicological, data have been reviewed by Jori *et al.* (1983) and the National Toxicology Program (1997).

Pyridine did not induce mutations in *Salmonella typhimurium* with or without metabolic activation, nor in mouse lymphoma L5178Y cells. It also gave negative results in chromosomal aberration assays in two Chinese hamster studies *in vitro* (one without an exogenous metabolic system only) and a mouse bone-marrow test *in vivo*, and in the sister chromatid exchange assay in Chinese hamster cells *in vitro*. The in-vivo mouse micronucleus test was also negative. The cell transformation assay with Syrian hamster embryo cells (without exogenous metabolism only) was negative.

Pyridine lowered the clastogenicity of benzene but not of benzo[*a*]pyrene or cyclophosphamide in the in-vivo mouse bone marrow micronucleus test after oral administration (Harper *et al.*, 1984; Harper & Legator, 1987).

Pyridine induced aneuploidy in *Saccharomyces cerevisiae*, when tested without metabolic activation only.

Three studies have been reported on the sex-linked recessive lethal mutation assay in *Drosophila melanogaster*. An equivocal result was obtained following feeding of pyridine, while a repeat test at a slightly higher concentration was negative; the injection method of treatment also gave a negative result. In another study, administration in the feed gave negative results, while injection gave positive results. The heritable translocation assay was negative in the same laboratory. A further set of experiments in the same laboratory produced negative results for the sex-linked recessive lethal test, although a lower dose for injection was used than in the other experiments.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Pyridine is an organic liquid of disagreeable odour, produced from coal-tar or by chemical synthesis. It is widely used as a solvent and intermediate in the production of piperidine, agricultural chemicals, drugs, dyestuffs, paints, rubber products, polycarbonate resins and textile water-repellents, as well as in laboratories. Occupational exposure may occur through inhalation and dermal contact during its production and its various uses as well as during the processing of oil-shale and at coke-oven works.

Table 5. Genetic and related effects of pyridine

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	1000 µg/plate	Haworth *et al*. (1983)
Saccharomyces cerevisiae, aneuploidy	+	NT	9000	Zimmerman *et al*. (1985)
Drosophila melanogaster, sex-linked recessive lethal mutations	?[c]		700 µg/mL; feed	Valencia *et al*. (1985)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		7000 µg/mL; inj	Valencia *et al*. (1985)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		500 µg/mL; feed	National Toxicology Program (2000)
Drosophila melanogaster, sex-linked recessive lethal mutations	+		4300 µg/mL; inj	National Toxicology Program (2000)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		730 µg/mL; feed	Foureman *et al*. (1994)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		500 µg/mL; inj	Foureman *et al*. (1994)
Drosophila melanogaster, heritable translocation test	–		4300 µg/mL; inj	Mason *et al*. (1992)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	–	–	5000	McGregor *et al*. (1988)
Sister chromatid exchange, Chinese hamster (Don) cells *in vitro*	–	NT	395	Abe & Sasaki (1977)
Sister chromatid exchange, Chinese hamster cells *in vitro*	–	–	5020	National Toxicology Program (2000)
Chromosomal aberrations, Chinese hamster (Don) cells *in vitro*	–	NT	395	Abe & Sasaki (1977)
Chromosomal aberrations, Chinese hamster cells *in vitro*	–	NT	4000	Ishidate & Odashima (1977)
Chromosomal aberrations, Chinese hamster cells *in vitro*	–	–	5000	National Toxicology Program (2000)

Table 5 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cell transformation, Syrian hamster embryo cells, clonal assay	–		5000	Kerckaert *et al*. (1996)
Micronucleus formation, B6C3F_1 mice *in vivo*	–		500 ip × 1	National Toxicology Program (2000)
Chromosomal aberrations, B6C3F_1 mouse bone-marrow cells *in vivo*	–		600 ip × 1	National Toxicology Program (2000)

[a] +, positive; –, negative; ?, inconclusive; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; *Drosophila* tests, ppm in feed; inj, injection
[c] Repeat test negative at 729 ppm

It is rarely detected in ambient air or drinking water but is frequently found in indoor air. It is present in cigarette smoke and in the volatile components of certain foodstuffs.

5.2 Human carcinogenicity data

One mortality study of workers at a 4,4′-bipyridyl manufacturing plant using pyridine as a starting material showed a small non-significant excess of lung cancer mortality. This excess could not be attributed to specific chemical exposures within the plant, and it was not clear if the risk associated with pyridine exposure was specifically assessed.

5.3 Animal carcinogenicity data

Pyridine was tested for carcinogenicity by oral administration in the drinking-water in one experiment in mice and in two experiments in rats and by subcutaneous injection in one experiment in rats. In male and female mice, it increased incidences of hepatocellular carcinomas and hepatoblastomas. In male Fischer 344 rats, it increased the incidence of renal tubule adenomas but not in male Wistar rats. No increase in tumour incidence at any site was observed in rats following subcutaneous injection of pyridine for one year and a subsequent observation period of six months.

In two studies with genetically modified mice, there was no treatment-related increase in the incidence of tumours.

5.4 Other relevant data

Pyridine is well absorbed from the gastrointestinal tract in mammals, and undergoes extensive metabolism by *C*- and *N*-oxidation and by *N*-methylation, giving the quaternary ion *N*-methylpyridinium.

In humans, acute pyridine intoxication affects the central nervous system, leading to dizziness, headache, nausea and anorexia. There is one case report of lethality after a high dose. Further symptoms include abdominal pain and pulmonary congestion. Pyridine was hepatotoxic in Fischer 344 and Wistar rats and caused an increase in granular casts and renal tubule hyaline degeneration in male Fischer 344 rats. Inhalation of pyridine can cause necrotic damage of the nasal epithelium. In rats and rabbits, pyridine is an inducer of CYP2E1 in the liver and kidney.

No data on reproductive and developmental effects in humans were available.

Exposure to pyridine in drinking-water led to reduction of sperm motility at all dose levels in mice and increased estrous cycle length at the highest dose level in rats.

Apart from positive responses in the sex-linked recessive lethal assay in *Drosophila melanogaster* and for aneuploidy in a fungal system, all tests, covering a range of end-points, for genetic toxicology of pyridine gave negative results.

5.5 Evaluation

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

There is *limited evidence* in experimental animals for the carcinogenicity of pyridine.

Overall evaluation

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

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SUMMARY OF FINAL EVALUATIONS

Agent	Degree of evidence of carcinogenicity		Overall evaluation of carcinogenicity to humans
	Human	Animal	
2,2-Bis(bromomethyl)propane-1,3-diol	I (ND)	S	2B
4-Chloro-*ortho*-toluidine	L	S	2A
5-Chloro-*ortho*-toluidine	I (ND)	L	3
Cinnamyl anthranilate	I (ND)	L	3
Coumarin	I (ND)	L	3
2,3-Dibromopropan-1-ol	I (ND)	S	2B
Diethanolamine	I	L	3
Di(2-ethylhexyl) adipate	I (ND)	L	3
Di(2-ethylhexyl) phthalate	I	S	3[a]
Ethylbenzene	I	S	2B
Glycidol	I (ND)	S	2A[a]
Nitromethane	I (ND)	S	2B
N-Nitrosodiethanolamine	I	S	2B
Pyridine	I	L	3
ortho-Toluidine	L	S	2A
Triethanolamine	I	I	3

S, sufficient evidence of carcinogenicity; L, limited evidence of carcinogenicity; I, inadequate evidence of carcinogenicity; ND, no data; group 2A, probably carcinogenic to humans; group 2B, possibly carcinogenic to humans; group 3, not classifiable as to its carcinogenicity to humans; for definitions of criteria for degrees of evidence and groups, see preamble, pp. 23–27.

[a] Other relevant data taken into consideration

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.

B

C

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M

Nitrofural	*7*, 171 (1974); *Suppl. 7*, 67 (1987); *50*, 195 (1990)
5-Nitro-2-furaldehyde semicarbazone (*see* Nitrofural)	
Nitrofurantoin	*50*, 211 (1990)
Nitrofurazone (*see* Nitrofural)	
1-[(5-Nitrofurfurylidene)amino]-2-imidazolidinone	*7*, 181 (1974); *Suppl. 7*, 67 (1987)
N-[4-(5-Nitro-2-furyl)-2-thiazolyl]acetamide	*1*, 181 (1972); *7*, 185 (1974); *Suppl. 7*, 67 (1987)
Nitrogen mustard	*9*, 193 (1975); *Suppl. 7*, 269 (1987)
Nitrogen mustard *N*-oxide	*9*, 209 (1975); *Suppl. 7*, 67 (1987)
Nitromethane	*77*, 487 (2000)
1-Nitronaphthalene	*46*, 291 (1989)
2-Nitronaphthalene	*46*, 303 (1989)
3-Nitroperylene	*46*, 313 (1989)
2-Nitro-*para*-phenylenediamine (*see* 1,4-Diamino-2-nitrobenzene)	
2-Nitropropane	*29*, 331 (1982); *Suppl. 7*, 67 (1987); *71*, 1079 (1999)
1-Nitropyrene	*33*, 209 (1984); *Suppl. 7*, 67 (1987); *46*, 321 (1989)
2-Nitropyrene	*46*, 359 (1989)
4-Nitropyrene	*46*, 367 (1989)
N-Nitrosatable drugs	*24*, 297 (1980) (*corr. 42*, 260)
N-Nitrosatable pesticides	*30*, 359 (1983)
N′-Nitrosoanabasine	*37*, 225 (1985); *Suppl. 7*, 67 (1987)
N′-Nitrosoanatabine	*37*, 233 (1985); *Suppl. 7*, 67 (1987)
N-Nitrosodi-*n*-butylamine	*4*, 197 (1974); *17*, 51 (1978); *Suppl. 7*, 67 (1987)
N-Nitrosodiethanolamine	*17*, 77 (1978); *Suppl. 7*, 67 (1987); *77*, 403 (2000)
N-Nitrosodiethylamine	*1*, 107 (1972) (*corr. 42*, 251); *17*, 83 (1978) (*corr. 42*, 257); *Suppl. 7*, 67 (1987)
N-Nitrosodimethylamine	*1*, 95 (1972); *17*, 125 (1978) (*corr. 42*, 257); *Suppl. 7*, 67 (1987)
N-Nitrosodiphenylamine	*27*, 213 (1982); *Suppl. 7*, 67 (1987)
para-Nitrosodiphenylamine	*27*, 227 (1982) (*corr. 42*, 261); *Suppl. 7*, 68 (1987)
N-Nitrosodi-*n*-propylamine	*17*, 177 (1978); *Suppl. 7*, 68 (1987)
N-Nitroso-*N*-ethylurea (*see N*-Ethyl-*N*-nitrosourea)	
N-Nitrosofolic acid	*17*, 217 (1978); *Suppl. 7*, 68 (1987)
N-Nitrosoguvacine	*37*, 263 (1985); *Suppl. 7*, 68 (1987)
N-Nitrosoguvacoline	*37*, 263 (1985); *Suppl. 7*, 68 (1987)
N-Nitrosohydroxyproline	*17*, 304 (1978); *Suppl. 7*, 68 (1987)
3-(*N*-Nitrosomethylamino)propionaldehyde	*37*, 263 (1985); *Suppl. 7*, 68 (1987)
3-(*N*-Nitrosomethylamino)propionitrile	*37*, 263 (1985); *Suppl. 7*, 68 (1987)
4-(*N*-Nitrosomethylamino)-4-(3-pyridyl)-1-butanal	*37*, 205 (1985); *Suppl. 7*, 68 (1987)
4-(*N*-Nitrosomethylamino)-1-(3-pyridyl)-1-butanone	*37*, 209 (1985); *Suppl. 7*, 68 (1987)
N-Nitrosomethylethylamine	*17*, 221 (1978); *Suppl. 7*, 68 (1987)
N-Nitroso-*N*-methylurea (*see N*-Methyl-*N*-nitrosourea)	
N-Nitroso-*N*-methylurethane (*see N*-Methyl-*N*-nitrosourethane)	
N-Nitrosomethylvinylamine	*17*, 257 (1978); *Suppl. 7*, 68 (1987)
N-Nitrosomorpholine	*17*, 263 (1978); *Suppl. 7*, 68 (1987)
N′-Nitrosonornicotine	*17*, 281 (1978); *37*, 241 (1985); *Suppl. 7*, 68 (1987)

O

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T

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