



IARC MONOGRAPHS

**SOME CHEMICALS PRESENT
IN INDUSTRIAL AND CONSUMER
PRODUCTS, FOOD
AND DRINKING-WATER**

VOLUME 101

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

International Agency for Research on Cancer



World Health
Organization



SOME CHEMICALS PRESENT IN INDUSTRIAL AND CONSUMER PRODUCTS, FOOD AND DRINKING-WATER

VOLUME 101

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 2011

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IARC MONOGRAPHS
ON THE EVALUATION
OF CARCINOGENIC RISKS
TO HUMANS

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, lifestyle 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 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 the Internet at <http://monographs.iarc.fr/>.

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NOTE TO THE READER

The term ‘carcinogenic risk’ in the *IARC Monographs* series is taken to mean that an agent is capable of causing cancer. The *Monographs* evaluate cancer hazards, despite the historical presence of the word ‘risks’ in the title.

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. Similarly, identification of cancer sites with *sufficient evidence* or *limited evidence* in humans should not be viewed as precluding the possibility that an agent may cause cancer at other sites.

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 Section of IARC Monographs, 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 Section of IARC Monographs, so that corrections can be reported in future volumes.

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⁶ Observer for the International Technical Caramel Association (ITCA). Dr Adamson consults for the American Beverage Association. His travel expenses and time as an observer for this meeting are paid by the ITCA, a trade association that represents corporations that produce and use caramel colouring in their products. Caramel colours may contain 4-methylimidazole.

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PREAMBLE

The Preamble to the *IARC Monographs* describes the objective and scope of the programme, the scientific principles and procedures used in developing a *Monograph*, the types of evidence considered and the scientific criteria that guide the evaluations. The Preamble should be consulted when reading a *Monograph* or list of evaluations.

A. GENERAL PRINCIPLES AND PROCEDURES

1. Background

Soon after IARC was established in 1965, it received frequent requests for advice on the carcinogenic risk of chemicals, including requests for lists of known and suspected human carcinogens. It was clear that it would not be a simple task to summarize adequately the complexity of the information that was available, and IARC began to consider means of obtaining international expert opinion on this topic. In 1970, the IARC Advisory Committee on Environmental Carcinogenesis recommended ‘...that a compendium on carcinogenic chemicals be prepared by experts. The biological activity and evaluation of practical importance to public health should be referenced and documented.’ The IARC Governing Council adopted a resolution concerning the role of IARC in providing government authorities with expert, independent, scientific opinion on environmental carcinogenesis. As one means to that end, the Governing Council recommended that IARC should prepare monographs on the evaluation of carcinogenic

risk of chemicals to man, which became the initial title of the series.

In the succeeding years, the scope of the programme broadened as *Monographs* were developed for groups of related chemicals, complex mixtures, occupational exposures, physical and biological agents and lifestyle factors. In 1988, the phrase ‘of chemicals’ was dropped from the title, which assumed its present form, *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*.

Through the *Monographs* programme, IARC seeks to identify the causes of human cancer. This is the first step in cancer prevention, which is needed as much today as when IARC was established. The global burden of cancer is high and continues to increase: the annual number of new cases was estimated at 10.1 million in 2000 and is expected to reach 15 million by 2020 ([Stewart & Kleihues, 2003](#)). With current trends in demographics and exposure, the cancer burden has been shifting from high-resource countries to low- and medium-resource countries. As a result of *Monographs* evaluations, national health agencies have been able, on scientific grounds, to take measures to reduce human exposure to carcinogens in the workplace and in the environment.

The criteria established in 1971 to evaluate carcinogenic risks to humans were adopted by the Working Groups whose deliberations resulted in the first 16 volumes of the *Monographs* series. Those criteria were subsequently updated by further ad hoc Advisory Groups ([IARC, 1977, 1978, 1979, 1982, 1983, 1987, 1988, 1991](#); [Vainio et al., 1992](#); [IARC, 2005, 2006](#)).

The Preamble is primarily a statement of scientific principles, rather than a specification of working procedures. The procedures through which a Working Group implements these principles are not specified in detail. They usually involve operations that have been established as being effective during previous *Monograph* meetings but remain, predominantly, the prerogative of each individual Working Group.

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* represent the first step in carcinogen risk assessment, which involves examination of all relevant information to assess the strength of the available evidence that an agent could alter the age-specific incidence of cancer in humans. The *Monographs* may also indicate where additional research efforts are needed, specifically when data immediately relevant to an evaluation are not available.

In this Preamble, the term ‘agent’ refers to any entity or circumstance that is subject to evaluation in a *Monograph*. As the scope of the programme has broadened, categories of agents now include specific chemicals, groups of related chemicals, complex mixtures, occupational or environmental exposures, cultural or behavioural practices, biological organisms and physical agents. This list of categories may expand as

causation of, and susceptibility to, malignant disease become more fully understood.

A cancer ‘hazard’ is an agent that is capable of causing cancer under some circumstances, while a cancer ‘risk’ is an estimate of the carcinogenic effects expected from exposure to a cancer hazard. The *Monographs* are an exercise in evaluating cancer hazards, despite the historical presence of the word ‘risks’ in the title. The distinction between hazard and risk is important, and the *Monographs* identify cancer hazards even when risks are very low at current exposure levels, because new uses or unforeseen exposures could engender risks that are significantly higher.

In the *Monographs*, an agent is termed ‘carcinogenic’ if it is capable of increasing the incidence of malignant neoplasms, reducing their latency, or increasing their severity or multiplicity. The induction of benign neoplasms may in some circumstances (see Part B, Section 3a) contribute to the judgement that the agent is carcinogenic. The terms ‘neoplasm’ and ‘tumour’ are used interchangeably.

The Preamble continues the previous usage of the phrase ‘strength of evidence’ as a matter of historical continuity, although it should be understood that *Monographs* evaluations consider studies that support a finding of a cancer hazard as well as studies that do not.

Some epidemiological and experimental studies indicate that different agents may act at different stages in the carcinogenic process, and several different mechanisms may be involved. The aim of the *Monographs* has been, from their inception, to evaluate evidence of carcinogenicity at any stage in the carcinogenesis process, independently of the underlying mechanisms. Information on mechanisms may, however, be used in making the overall evaluation ([IARC, 1991](#); [Vainio et al., 1992](#); [IARC, 2005, 2006](#); see also Part B, Sections 4 and 6). As mechanisms of carcinogenesis are elucidated, IARC convenes international scientific conferences to determine whether a broad-based consensus has emerged

on how specific mechanistic data can be used in an evaluation of human carcinogenicity. The results of such conferences are reported in IARC Scientific Publications, which, as long as they still reflect the current state of scientific knowledge, may guide subsequent Working Groups.

Although the *Monographs* have emphasized hazard identification, important issues may also involve dose–response assessment. In many cases, the same epidemiological and experimental studies used to evaluate a cancer hazard can also be used to estimate a dose–response relationship. A *Monograph* may undertake to estimate dose–response relationships within the range of the available epidemiological data, or it may compare the dose–response information from experimental and epidemiological studies. In some cases, a subsequent publication may be prepared by a separate Working Group with expertise in quantitative dose–response assessment.

The *Monographs* are used by national and international authorities to make risk assessments, formulate decisions concerning preventive measures, provide effective cancer control programmes and decide among alternative options for public health decisions. The evaluations of IARC Working Groups are scientific, qualitative judgements on the evidence for or against carcinogenicity provided by the available data. These evaluations represent only one part of the body of information on which public health decisions may be based. Public health options vary from one situation to another and from country to country and relate to many factors, including different socioeconomic and national priorities. Therefore, no recommendation is given with regard to regulation or legislation, which are the responsibility of individual governments or other international organizations.

3. Selection of agents for review

Agents are selected for review on the basis of two main criteria: (a) there is evidence of human

exposure and (b) there is some evidence or suspicion of carcinogenicity. Mixed exposures may occur in occupational and environmental settings and as a result of individual and cultural habits (such as tobacco 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. Ad hoc Advisory Groups convened by IARC in 1984, 1989, 1991, 1993, 1998 and 2003 made recommendations as to which agents should be evaluated in the *Monographs* series. Recent recommendations are available on the *Monographs* programme web site (<http://monographs.iarc.fr>). IARC may schedule other agents for review as it becomes aware of new scientific information or as national health agencies identify an urgent public health need related to cancer.

As significant new data become available on an agent for which a *Monograph* exists, a re-evaluation may be made at a subsequent meeting, and a new *Monograph* published. In some cases it may be appropriate to review only the data published since a prior evaluation. This can be useful for updating a database, reviewing new data to resolve a previously open question or identifying new tumour sites associated with a carcinogenic agent. Major changes in an evaluation (e.g. a new classification in Group 1 or a determination that a mechanism does not operate in humans, see Part B, Section 6) are more appropriately addressed by a full review.

4. Data for the *Monographs*

Each *Monograph* reviews all pertinent epidemiological studies and cancer bioassays in experimental animals. Those judged inadequate

or irrelevant to the evaluation may be cited but not summarized. If a group of similar studies is not reviewed, the reasons are indicated.

Mechanistic and other relevant data are also reviewed. A *Monograph* does not necessarily cite all the mechanistic literature concerning the agent being evaluated (see Part B, Section 4). Only those data considered by the Working Group to be relevant to making the evaluation are included.

With regard to epidemiological studies, cancer bioassays, and mechanistic and other relevant data, only reports that have been published or accepted for publication in the openly available scientific literature are reviewed. The same publication requirement applies to studies originating from IARC, including meta-analyses or pooled analyses commissioned by IARC in advance of a meeting (see Part B, Section 2c). Data from government agency reports that are publicly available are also considered. Exceptionally, doctoral theses and other material that are in their final form and publicly available may be reviewed.

Exposure data and other information on an agent under consideration are also reviewed. In the sections on chemical and physical properties, on analysis, on production and use and on occurrence, published and unpublished sources of information may be considered.

Inclusion of a study 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 each study description (see Part B). The reasons for not giving further consideration to an individual study also are indicated in the square brackets.

5. Meeting participants

Five categories of participant can be present at *Monograph* meetings.

(a) *The Working Group*

The Working Group is responsible for the critical reviews and evaluations that are developed during the meeting. The tasks of Working Group Members 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 mechanisms of carcinogenesis; and (vi) to make an overall evaluation of the carcinogenicity of the exposure to humans. Working Group Members generally have published significant research related to the carcinogenicity of the agents being reviewed, and IARC uses literature searches to identify most experts. Working Group Members are selected on the basis of (a) knowledge and experience and (b) absence of real or apparent conflicts of interests. Consideration is also given to demographic diversity and balance of scientific findings and views.

(b) *Invited Specialists*

Invited Specialists are experts who also have critical knowledge and experience but have a real or apparent conflict of interests. These experts are invited when necessary to assist in the Working Group by contributing their unique knowledge and experience during subgroup and plenary discussions. They may also contribute text on non-influential issues in the section on exposure, such as a general description of data on production and use (see Part B, Section 1). Invited Specialists do not serve as meeting chair or subgroup chair, draft text that pertains to the description or interpretation of cancer data, or participate in the evaluations.

(c) *Representatives of national and international health agencies*

Representatives of national and international health agencies often attend meetings because their agencies sponsor the programme or are interested in the subject of a meeting. Representatives do not serve as meeting chair or subgroup chair, draft any part of a *Monograph*, or participate in the evaluations.

(d) *Observers with relevant scientific credentials*

Observers with relevant scientific credentials may be admitted to a meeting by IARC in limited numbers. Attention will be given to achieving a balance of Observers from constituencies with differing perspectives. They are invited to observe the meeting and should not attempt to influence it. Observers do not serve as meeting chair or subgroup chair, draft any part of a *Monograph*, or participate in the evaluations. At the meeting, the meeting chair and subgroup chairs may grant Observers an opportunity to speak, generally after they have observed a discussion. Observers agree to respect the Guidelines for Observers at *IARC Monographs* meetings (available at <http://monographs.iarc.fr>).

(e) *The IARC Secretariat*

The IARC Secretariat consists of scientists who are designated by IARC and who have relevant expertise. They serve as rapporteurs and participate in all discussions. When requested by the meeting chair or subgroup chair, they may also draft text or prepare tables and analyses.

Before an invitation is extended, each potential participant, including the IARC Secretariat, completes the WHO Declaration of Interests to report financial interests, employment and consulting, and individual and institutional research support related to the subject of the meeting. IARC assesses these interests to determine

whether there is a conflict that warrants some limitation on participation. The declarations are updated and reviewed again at the opening of the meeting. Interests related to the subject of the meeting are disclosed to the meeting participants and in the published volume ([Cogliano et al., 2004](#)).

The names and principal affiliations of participants are available on the *Monographs* programme web site (<http://monographs.iarc.fr>) approximately two months before each meeting. It is not acceptable for Observers or third parties to contact other participants before a meeting or to lobby them at any time. Meeting participants are asked to report all such contacts to IARC ([Cogliano et al., 2005](#)).

All participants are listed, with their principal affiliations, at the beginning of each volume. 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.

6. Working procedures

A separate Working Group is responsible for developing each volume of *Monographs*. A volume contains one or more *Monographs*, which can cover either a single agent or several related agents. Approximately one year in advance of the meeting of a Working Group, the agents to be reviewed are announced on the *Monographs* programme web site (<http://monographs.iarc.fr>) and participants are selected by IARC staff in consultation with other experts. Subsequently, relevant biological and epidemiological data are collected by IARC from recognized sources of information on carcinogenesis, including data storage and retrieval systems such as PubMed. Meeting participants who are asked to prepare preliminary working papers for specific sections are expected to supplement the IARC literature searches with their own searches.

Industrial associations, labour unions and other knowledgeable organizations may be asked to provide input to the sections on production and use, although this involvement is not required as a general rule. Information on production and trade is obtained from governmental, trade and market research publications and, in some cases, by direct contact with industries. Separate production data on some agents may not be available for a variety of reasons (e.g. not collected or made public in all producing countries, production is small). 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 to prepare preliminary working papers. The working papers are compiled by IARC staff and sent, before the meeting, to Working Group Members and Invited Specialists for review.

The Working Group meets at IARC for seven to eight days to discuss and finalize the texts and to formulate the evaluations. The objectives of the meeting are peer review and consensus. During the first few days, four subgroups (covering exposure data, cancer in humans, cancer in experimental animals, and mechanistic and other relevant data) review the working papers, develop a joint subgroup draft and write summaries. Care is taken to ensure that each study summary is written or reviewed by someone not associated with the study being considered. During the last few days, the Working Group meets in plenary session to review the subgroup drafts and develop the evaluations. As a result, the entire volume is the joint product of the Working Group, and there are no individually authored sections.

IARC Working Groups strive to achieve a consensus evaluation. Consensus reflects broad agreement among Working Group Members, but

not necessarily unanimity. The chair may elect to poll Working Group Members to determine the diversity of scientific opinion on issues where consensus is not readily apparent.

After the meeting, the master copy is verified by consulting the original literature, edited and prepared for publication. The aim is to publish the volume within six months of the Working Group meeting. A summary of the outcome is available on the *Monographs* programme web site soon after the meeting.

B. SCIENTIFIC REVIEW AND EVALUATION

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 that directly impinges on its interpretation should be brought to the attention of the reader, a Working Group comment is given in square brackets.

The scope of the *IARC Monographs* programme has expanded beyond chemicals to include complex mixtures, occupational exposures, physical and biological agents, lifestyle factors and other potentially carcinogenic exposures. Over time, the structure of a *Monograph* has evolved to include the following sections:

- Exposure data
- Studies of cancer in humans
- Studies of cancer in experimental animals
- Mechanistic and other relevant data
- Summary
- Evaluation and rationale

In addition, a section of General Remarks at the front of the volume discusses the reasons the agents were scheduled for evaluation and some key issues the Working Group encountered during the meeting.

This part of the Preamble discusses the types of evidence considered and summarized in each section of a *Monograph*, followed by the scientific criteria that guide the evaluations.

1. Exposure data

Each *Monograph* includes general information on the agent: this information may vary substantially between agents and must be adapted accordingly. Also included is information on production and use (when appropriate), methods of analysis and detection, occurrence, and sources and routes of human occupational and environmental exposures. Depending on the agent, regulations and guidelines for use may be presented.

(a) *General information on the agent*

For chemical agents, sections on chemical and physical data are included: the Chemical Abstracts Service Registry Number, the latest primary name and the IUPAC systematic name are recorded; other synonyms are given, but the list is not necessarily comprehensive. Information on chemical and physical properties that are relevant to identification, occurrence and biological activity is included. 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.

For biological agents, taxonomy, structure and biology are described, and the degree of variability is indicated. Mode of replication, life cycle, target cells, persistence, latency, host

response and clinical disease other than cancer are also presented.

For physical agents that are forms of radiation, energy and range of the radiation are included. For foreign bodies, fibres and respirable particles, size range and relative dimensions are indicated.

For agents such as mixtures, drugs or lifestyle factors, a description of the agent, including its composition, is given.

Whenever appropriate, other information, such as historical perspectives or the description of an industry or habit, may be included.

(b) *Analysis and detection*

An overview of methods of analysis and detection of the agent is presented, including their sensitivity, specificity and reproducibility. Methods widely used for regulatory purposes are emphasized. Methods for monitoring human exposure are also given. No critical evaluation or recommendation of any method is meant or implied.

(c) *Production and use*

The dates of first synthesis and of first commercial production of a chemical, mixture or other agent are provided when available; for agents that 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 when available. 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.

The countries where companies report production of the agent, and the number of companies in each country, are identified. Available data on production, international trade and uses are

obtained for representative regions. 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.

(d) *Occurrence and exposure*

Information on the occurrence of an agent in the environment is obtained from data derived from the monitoring and surveillance of levels in occupational environments, air, water, soil, plants, foods and animal and human tissues. When available, data on the generation, persistence and bioaccumulation of the agent are also included. Such data may be available from national databases.

Data 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 reported. Information is presented on the range of human exposure, including occupational and environmental exposures. This includes relevant findings from both developed and developing countries. Some of these data are not distributed widely and may be available from government reports and other sources. In the case of mixtures, industries, occupations or processes, information is given about all agents known to be 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 date and place. For biological agents, the epidemiology of infection is described.

(e) *Regulations and guidelines*

Statements concerning regulations and guidelines (e.g. occupational exposure limits, maximal levels permitted in foods and water, pesticide registrations) are included, 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 vaccination and therapy, are described.

2. Studies of cancer in humans

This section includes all pertinent epidemiological studies (see Part A, Section 4). Studies of biomarkers are included when they are relevant to an evaluation of carcinogenicity to humans.

(a) *Types of study considered*

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

Cohort and case–control studies relate individual exposures under study to the occurrence of cancer in individuals and provide an estimate of effect (such as relative risk) as the main measure of association. Intervention studies may provide strong evidence for making causal inferences, as exemplified by cessation of smoking and the subsequent decrease in risk for lung cancer.

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 under study. In correlation studies, individual exposure is not documented, which renders this kind of study more prone to confounding. In some circumstances, however, correlation studies may be more informative than analytical study designs (see, for example, the *Monograph on arsenic in drinking-water*; [IARC, 2004](#)).

In some instances, case reports and case series have provided important information about the carcinogenicity of an agent. These types of study 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 and case series 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 that surround the interpretation of case reports, case series 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, these types of study may add materially to the judgement that a causal relationship exists.

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

(b) Quality of studies considered

It is necessary to take into account the possible roles of bias, confounding and chance in the interpretation of epidemiological studies. Bias is the effect of factors in study design or execution that lead erroneously to a stronger or weaker association than in fact exists between an

agent and disease. Confounding is a form of bias that occurs when 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. The role of chance is related to biological variability and the influence of sample size on the precision of estimates of effect.

In evaluating the extent to which these factors have been minimized in an individual study, consideration is given to several aspects of design and analysis as described in the report of the study. For example, when suspicion of carcinogenicity arises largely from a single small study, careful consideration is given when interpreting subsequent studies that included these data in an enlarged population. 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.

First, 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.

Second, the authors should have taken into account — in the study design and analysis — 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 or may not be more appropriate than those with national rates. Internal comparisons of frequency of disease among individuals at different levels of exposure are also desirable in cohort studies, since they minimize the potential for

confounding related to the difference in risk factors between an external reference group and the study population.

Third, 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. These methods have been reviewed for case–control studies ([Breslow & Day, 1980](#)) and for cohort studies ([Breslow & Day, 1987](#)).

(c) *Meta-analyses and pooled analyses*

Independent epidemiological studies of the same agent may lead to results that are difficult to interpret. Combined analyses of data from multiple studies are a means of resolving this ambiguity, and well conducted analyses can be considered. There are two types of combined analysis. The first involves combining summary statistics such as relative risks from individual studies (meta-analysis) and the second involves a pooled analysis of the raw data from the individual studies (pooled analysis) ([Greenland, 1998](#)).

The advantages of combined analyses are increased precision due to increased sample size and the opportunity to explore potential confounders, interactions and modifying effects

that may explain heterogeneity among studies in more detail. A disadvantage of combined analyses is the possible lack of compatibility of data from various studies due to differences in subject recruitment, procedures of data collection, methods of measurement and effects of unmeasured co-variates that may differ among studies. Despite these limitations, well conducted combined analyses may provide a firmer basis than individual studies for drawing conclusions about the potential carcinogenicity of agents.

IARC may commission a meta-analysis or pooled analysis that is pertinent to a particular *Monograph* (see Part A, Section 4). Additionally, as a means of gaining insight from the results of multiple individual studies, ad hoc calculations that combine data from different studies may be conducted by the Working Group during the course of a *Monograph* meeting. The results of such original calculations, which would be specified in the text by presentation in square brackets, might involve updates of previously conducted analyses that incorporate the results of more recent studies or de-novo analyses. Irrespective of the source of data for the meta-analyses and pooled analyses, it is important that the same criteria for data quality be applied as those that would be applied to individual studies and to ensure also that sources of heterogeneity between studies be taken into account.

(d) *Temporal effects*

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, peak exposure (when appropriate) and time since cessation of exposure, are reviewed and summarized when available. Analyses of temporal relationships may be useful in making causal inferences. In addition, 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 mechanisms of carcinogenesis.

(e) *Use of biomarkers in epidemiological studies*

Biomarkers indicate molecular, cellular or other biological changes and are increasingly used in epidemiological studies for various purposes (IARC, 1991; Vainio *et al.*, 1992; Toniolo *et al.*, 1997; Vineis *et al.*, 1999; Buffler *et al.*, 2004). These may include evidence of exposure, of early effects, of cellular, tissue or organism responses, of individual susceptibility or host responses, and inference of a mechanism (see Part B, Section 4b). This is a rapidly evolving field that encompasses developments in genomics, epigenomics and other emerging technologies.

Molecular epidemiological data that identify associations between genetic polymorphisms and interindividual differences in susceptibility to the agent(s) being evaluated may contribute to the identification of carcinogenic hazards to humans. If the polymorphism has been demonstrated experimentally to modify the functional activity of the gene product in a manner that is consistent with increased susceptibility, these data may be useful in making causal inferences. Similarly, molecular epidemiological studies that measure cell functions, enzymes or metabolites that are thought to be the basis of susceptibility may provide evidence that reinforces biological plausibility. It should be noted, however, that when data on genetic susceptibility originate from multiple comparisons that arise from subgroup analyses, this can generate false-positive results and inconsistencies across studies, and such data therefore require careful evaluation. If the known phenotype of a genetic polymorphism can explain the carcinogenic mechanism of the agent being evaluated, data on this phenotype may be useful in making causal inferences.

(f) *Criteria for causality*

After the quality of individual epidemiological studies of cancer has been summarized and assessed, a judgement is made concerning the strength of evidence that the agent in question is carcinogenic to humans. In making its judgement, the Working Group considers several criteria for causality (Hill, 1965). A strong association (e.g. a large relative risk) is more likely to indicate causality than a weak association, although it is recognized that estimates of effect of small magnitude do not imply lack of causality and may be important if the disease or exposure is common. Associations that are replicated in several studies of the same design or that use 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 exposure), and results of studies that are judged to be of high quality are given more weight than those of studies that are judged to be methodologically less sound.

If the risk increases with the exposure, this is considered to be a strong indication of causality, although the absence of a graded response is not necessarily evidence against a causal relationship. The 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.

Several scenarios may increase confidence in a causal relationship. On the one hand, an agent may be specific in causing tumours at one site or of one morphological type. On the other, carcinogenicity may be evident through the causation of multiple tumour types. Temporality, precision of estimates of effect, biological plausibility and coherence of the overall database are considered. Data on biomarkers may be employed in

an assessment of the biological plausibility of epidemiological observations.

Although rarely available, results from randomized trials that show different rates of cancer 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, a judgement may be made that, in the aggregate, they show evidence of lack of carcinogenicity. Such a judgement requires first that the studies 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 (a) be consistent with an estimate of effect of unity for any observed level of exposure, (b) when considered together, provide a pooled estimate of relative risk that is at or near to unity, and (c) have 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 that the relative risk of cancer increases with increasing level of exposure. It is important to note that evidence of lack of carcinogenicity obtained from several epidemiological studies can apply only to the type(s) of cancer studied, to the dose levels reported, and to the intervals between first exposure and disease onset observed in these studies. Experience with human cancer indicates that the period from first exposure to the development of clinical cancer is sometimes longer than 20 years; latent periods substantially shorter than 30 years cannot provide evidence for lack of carcinogenicity.

3. Studies of cancer in experimental animals

All known human carcinogens that have been studied adequately for carcinogenicity in experimental animals have produced positive results in one or more animal species ([Wilbourn *et al.*, 1986](#); [Tomatis *et al.*, 1989](#)). For several agents (e.g. aflatoxins, diethylstilbestrol, solar radiation, 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 that cause cancer in experimental animals also cause cancer in humans, it is biologically plausible that agents for which there is *sufficient evidence of carcinogenicity* in experimental animals (see Part B, Section 6b) also present a carcinogenic hazard to humans. Accordingly, in the absence of additional scientific information, these agents are considered to pose a carcinogenic hazard to humans. Examples of additional scientific information are data that demonstrate that a given agent causes cancer in animals through a species-specific mechanism that does not operate in humans or data that demonstrate that the mechanism in experimental animals also operates in humans (see Part B, Section 6).

Consideration is given to all available long-term studies of cancer in experimental animals with the agent under review (see Part A, Section 4). In all experimental settings, the nature and extent of impurities or contaminants present in the agent being evaluated are given when available. Animal species, strain (including genetic background where applicable), sex, numbers per group, age at start of treatment, route of exposure, dose levels, duration of exposure, survival and information on tumours (incidence, latency, severity or multiplicity of neoplasms or preneoplastic lesions) are reported. Those studies in experimental animals that are judged to be irrelevant to the evaluation or judged to be inadequate

(e.g. too short a duration, too few animals, poor survival; see below) may be omitted. Guidelines for conducting long-term carcinogenicity experiments have been published (e.g. [OECD, 2002](#)).

Other studies considered may include: experiments in which the agent was administered in the presence of factors that modify carcinogenic effects (e.g. initiation–promotion studies, co-carcinogenicity studies and studies in genetically modified animals); studies in which the end-point was not cancer but a defined precancerous lesion; experiments on the carcinogenicity of known metabolites and derivatives; and studies of cancer in non-laboratory animals (e.g. livestock and companion animals) exposed to the agent.

For studies of mixtures, consideration is given to the possibility that changes in the physicochemical properties of the individual substances may occur during collection, storage, extraction, concentration and delivery. Another consideration is that chemical and toxicological interactions of components in a mixture may alter dose–response relationships. The relevance to human exposure of the test mixture administered in the animal experiment is also assessed. This may involve consideration of the following aspects of the mixture tested: (i) physical and chemical characteristics, (ii) identified constituents that may indicate the presence of a class of substances and (iii) the results of genetic toxicity and related tests.

The relevance of results obtained with an agent that is analogous (e.g. similar in structure or of a similar virus genus) to that being evaluated is also considered. Such results may provide biological and mechanistic information that is relevant to the understanding of the process of carcinogenesis in humans and may strengthen the biological plausibility that the agent being evaluated is carcinogenic to humans (see Part B, Section 2f).

(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, schedule and duration of exposure, species, strain (including genetic background where applicable), sex, age and 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.

Considerations of importance 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 monitored adequately, particularly in inhalation experiments; (iii) whether the doses, duration of treatment and route of exposure were appropriate; (iv) whether the survival of treated animals was similar to that of controls; (v) whether there were adequate numbers of animals per group; (vi) whether both male and female animals were used; (vii) whether animals were allocated randomly to groups; (viii) whether the duration of observation was adequate; and (ix) whether the data were reported and analysed adequately.

When benign tumours (a) occur together with and originate from the same cell type as malignant tumours in an organ or tissue in a particular study and (b) appear to represent a stage in the progression to malignancy, they are usually combined in the assessment of tumour incidence ([Huff *et al.*, 1989](#)). The occurrence of lesions presumed to be preneoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed. If an agent induces only benign neoplasms that appear to be end-points that do not readily undergo

transition to malignancy, the agent should nevertheless be suspected of being carcinogenic and requires further investigation.

(b) Quantitative aspects

The probability that tumours will occur may depend on the species, sex, strain, genetic background and age of the animal, and on the dose, route, timing and duration of the exposure. Evidence of an increased incidence of neoplasms with increasing levels 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. Mechanisms such as induction of DNA damage or inhibition of repair, altered cell division and cell death rates and changes in intercellular communication are important determinants of dose–response relationships for some carcinogens. Since many chemicals require metabolic activation before being converted to their reactive intermediates, both metabolic and toxicokinetic 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 ([Hoel et al., 1983](#); [Gart et al., 1986](#)), as could saturation of processes such as DNA repair. The dose–response relationship can also be affected by differences in survival among the treatment groups.

(c) Statistical analyses

Factors considered include the adequacy of the information given for each treatment group: (i) number of animals studied and number examined histologically, (ii) 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](#); [Portier & Bailer, 1989](#); [Bieler & Williams, 1993](#)). The choice of the most appropriate statistical method requires consideration of whether or not there are differences in survival among the treatment groups; for example, reduced survival because of non-tumour-related mortality can preclude the occurrence of tumours later in life. When detailed information on survival is not available, comparisons of the proportions of tumour-bearing animals among the effective number of animals (alive at the time the first tumour was discovered) can be useful when significant differences in survival occur before tumours appear. The lethality of the tumour also requires consideration: for rapidly fatal tumours, the time of death provides an indication of the time of tumour onset and can be assessed using life-table methods; non-fatal or incidental tumours that do not affect survival can be assessed using methods such as the Mantel-Haenzel test for changes in tumour prevalence. Because tumour lethality is often difficult to determine, methods such as the Poly-K test that do not require such information can also be used. When results are available on the number and size of tumours seen in experimental animals (e.g. papillomas on mouse skin, liver tumours observed through nuclear magnetic resonance tomography), other more complicated statistical procedures may be needed ([Sherman et al., 1994](#); [Dunson et al., 2003](#)).

Formal statistical methods have been developed to incorporate historical control data into the analysis of data from a given experiment. These methods assign an appropriate weight to historical and concurrent controls on the basis of the extent of between-study and within-study variability: less weight is given to historical controls when they show a high degree of variability, and greater weight when they show little variability. It is generally not appropriate to discount a tumour response that is significantly increased compared with concurrent controls by arguing that it falls within the range of historical controls,

particularly when historical controls show high between-study variability and are, thus, of little relevance to the current experiment. In analysing results for uncommon tumours, however, the analysis may be improved by considering historical control data, particularly when between-study variability is low. Historical controls should be selected to resemble the concurrent controls as closely as possible with respect to species, gender and strain, as well as other factors such as basal diet and general laboratory environment, which may affect tumour-response rates in control animals ([Haseman et al., 1984](#); [Fung et al., 1996](#); [Greim et al., 2003](#)).

Although meta-analyses and combined analyses are conducted less frequently for animal experiments than for epidemiological studies due to differences in animal strains, they can be useful aids in interpreting animal data when the experimental protocols are sufficiently similar.

4. Mechanistic and other relevant data

Mechanistic and other relevant data may provide evidence of carcinogenicity and also help in assessing the relevance and importance of findings of cancer in animals and in humans. The nature of the mechanistic and other relevant data depends on the biological activity of the agent being considered. The Working Group considers representative studies to give a concise description of the relevant data and issues that they consider to be important; thus, not every available study is cited. Relevant topics may include toxicokinetics, mechanisms of carcinogenesis, susceptible individuals, populations and life-stages, other relevant data and other adverse effects. When data on biomarkers are informative about the mechanisms of carcinogenesis, they are included in this section.

These topics are not mutually exclusive; thus, the same studies may be discussed in more than

one subsection. For example, a mutation in a gene that codes for an enzyme that metabolizes the agent under study could be discussed in the subsections on toxicokinetics, mechanisms and individual susceptibility if it also exists as an inherited polymorphism.

(a) *Toxicokinetic data*

Toxicokinetics refers to the absorption, distribution, metabolism and elimination of agents in humans, experimental animals and, where relevant, cellular systems. Examples of kinetic factors that may affect dose-response relationships include uptake, deposition, biopersistence and half-life in tissues, protein binding, metabolic activation and detoxification. Studies that indicate the metabolic fate of the agent in humans and in experimental animals are summarized briefly, and comparisons of data from humans and animals are made when possible. Comparative information on the relationship between exposure and the dose that reaches the target site may be important for the extrapolation of hazards between species and in clarifying the role of in-vitro findings.

(b) *Data on mechanisms of carcinogenesis*

To provide focus, the Working Group attempts to identify the possible mechanisms by which the agent may increase the risk of cancer. For each possible mechanism, a representative selection of key data from humans and experimental systems is summarized. Attention is given to gaps in the data and to data that suggests that more than one mechanism may be operating. The relevance of the mechanism to humans is discussed, in particular, when mechanistic data are derived from experimental model systems. Changes in the affected organs, tissues or cells can be divided into three non-exclusive levels as described below.

(i) Changes in physiology

Physiological changes refer to exposure-related modifications to the physiology and/or response of cells, tissues and organs. Examples of potentially adverse physiological changes include mitogenesis, compensatory cell division, escape from apoptosis and/or senescence, presence of inflammation, hyperplasia, metaplasia and/or preneoplasia, angiogenesis, alterations in cellular adhesion, changes in steroidal hormones and changes in immune surveillance.

(ii) Functional changes at the cellular level

Functional changes refer to exposure-related alterations in the signalling pathways used by cells to manage critical processes that are related to increased risk for cancer. Examples of functional changes include modified activities of enzymes involved in the metabolism of xenobiotics, alterations in the expression of key genes that regulate DNA repair, alterations in cyclin-dependent kinases that govern cell cycle progression, changes in the patterns of post-translational modifications of proteins, changes in regulatory factors that alter apoptotic rates, changes in the secretion of factors related to the stimulation of DNA replication and transcription and changes in gap-junction-mediated intercellular communication.

(iii) Changes at the molecular level

Molecular changes refer to exposure-related changes in key cellular structures at the molecular level, including, in particular, genotoxicity. Examples of molecular changes include formation of DNA adducts and DNA strand breaks, mutations in genes, chromosomal aberrations, aneuploidy and changes in DNA methylation patterns. Greater emphasis is given to irreversible effects.

The use of mechanistic data in the identification of a carcinogenic hazard is specific to the mechanism being addressed and is not readily

described for every possible level and mechanism discussed above.

Genotoxicity data are discussed here to illustrate the key issues involved in the evaluation of mechanistic data.

Tests for genetic and related effects are described in view of the relevance of gene mutation and chromosomal aberration/aneuploidy to carcinogenesis ([Vainio et al., 1992](#); [McGregor et al., 1999](#)). The adequacy of the reporting of sample characterization is considered and, when necessary, commented upon; with regard to complex mixtures, such comments are similar to those described for animal carcinogenicity tests. The available data are interpreted critically according to the end-points detected, which may include DNA damage, gene mutation, sister chromatid exchange, micronucleus formation, chromosomal aberrations and aneuploidy. The concentrations employed are given, and mention is made of whether the use of an exogenous metabolic system in vitro affected the test result. These data are listed in tabular form by phylogenetic classification.

Positive results in tests using prokaryotes, lower eukaryotes, insects, plants and cultured mammalian cells suggest that genetic and related effects could occur in mammals. Results from such tests may also give information on the types of genetic effect produced and on the involvement of metabolic activation. Some end-points described are clearly genetic in nature (e.g. gene mutations), while others are associated with genetic effects (e.g. unscheduled DNA synthesis). In-vitro tests for tumour promotion, cell transformation and gap-junction intercellular communication 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. Critical appraisals of these tests have been published ([Montesano et al., 1986](#); [McGregor et al., 1999](#)).

Genetic or other activity manifest in humans and experimental mammals is regarded to be of

greater relevance than that in other organisms. The demonstration that an agent can induce gene and chromosomal mutations in mammals *in vivo* indicates that it may have carcinogenic activity. 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 that rules out the carcinogenicity of agents that act through other mechanisms (e.g. receptor-mediated effects, cellular toxicity with regenerative cell division, peroxisome proliferation) ([Vainio et al., 1992](#)). Factors that may give misleading results in short-term tests have been discussed in detail elsewhere ([Montesano et al., 1986](#); [McGregor et al., 1999](#)).

When there is evidence that an agent acts by a specific mechanism that does not involve genotoxicity (e.g. hormonal dysregulation, immune suppression, and formation of calculi and other deposits that cause chronic irritation), that evidence is presented and reviewed critically in the context of rigorous criteria for the operation of that mechanism in carcinogenesis (e.g. [Capen et al., 1999](#)).

For biological agents such as viruses, bacteria and parasites, other data relevant to carcinogenicity may include descriptions of the pathology of infection, integration and expression of viruses, and genetic alterations seen in human tumours. Other observations that might comprise cellular and tissue responses to infection, immune response and the presence of tumour markers are also considered.

For physical agents that are forms of radiation, other data relevant to carcinogenicity may include descriptions of damaging effects at the physiological, cellular and molecular level, as for chemical agents, and descriptions of how these effects occur. 'Physical agents' may also be considered to comprise foreign bodies, such as

surgical implants of various kinds, and poorly soluble fibres, dusts and particles of various sizes, the pathogenic effects of which are a result of their physical presence in tissues or body cavities. Other relevant data for such materials may include characterization of cellular, tissue and physiological reactions to these materials and descriptions of pathological conditions other than neoplasia with which they may be associated.

(c) *Other data relevant to mechanisms*

A description is provided of any structure-activity relationships that may be relevant to an evaluation of the carcinogenicity of an agent, the toxicological implications of the physical and chemical properties, and any other data relevant to the evaluation that are not included elsewhere.

High-output data, such as those derived from gene expression microarrays, and high-throughput data, such as those that result from testing hundreds of agents for a single end-point, pose a unique problem for the use of mechanistic data in the evaluation of a carcinogenic hazard. In the case of high-output data, there is the possibility to overinterpret changes in individual end-points (e.g. changes in expression in one gene) without considering the consistency of that finding in the broader context of the other end-points (e.g. other genes with linked transcriptional control). High-output data can be used in assessing mechanisms, but all end-points measured in a single experiment need to be considered in the proper context. For high-throughput data, where the number of observations far exceeds the number of end-points measured, their utility for identifying common mechanisms across multiple agents is enhanced. These data can be used to identify mechanisms that not only seem plausible, but also have a consistent pattern of carcinogenic response across entire classes of related compounds.

(d) *Susceptibility data*

Individuals, populations and life-stages may have greater or lesser susceptibility to an agent, based on toxicokinetics, mechanisms of carcinogenesis and other factors. Examples of host and genetic factors that affect individual susceptibility include sex, genetic polymorphisms of genes involved in the metabolism of the agent under evaluation, differences in metabolic capacity due to life-stage or the presence of disease, differences in DNA repair capacity, competition for or alteration of metabolic capacity by medications or other chemical exposures, pre-existing hormonal imbalance that is exacerbated by a chemical exposure, a suppressed immune system, periods of higher-than-usual tissue growth or regeneration and genetic polymorphisms that lead to differences in behaviour (e.g. addiction). Such data can substantially increase the strength of the evidence from epidemiological data and enhance the linkage of in-vivo and in-vitro laboratory studies to humans.

(e) *Data on other adverse effects*

Data on acute, subchronic and chronic adverse effects relevant to the cancer evaluation are summarized. Adverse effects that confirm distribution and biological effects at the sites of tumour development, or alterations in physiology that could lead to tumour development, are emphasized. Effects on reproduction, embryonic and fetal survival and development are summarized briefly. The adequacy of epidemiological studies of reproductive outcome and genetic and related effects in humans is judged by the same criteria as those applied to epidemiological studies of cancer, but fewer details are given.

5. Summary

This section is a summary of data presented in the preceding sections. Summaries can be

found on the *Monographs* programme web site (<http://monographs.iarc.fr>).

(a) *Exposure data*

Data are summarized, as appropriate, on the basis of elements such as production, use, occurrence and exposure levels in the workplace and environment and measurements in human tissues and body fluids. Quantitative data and time trends are given to compare exposures in different occupations and environmental settings. Exposure to biological agents is described in terms of transmission, prevalence and persistence of infection.

(b) *Cancer in humans*

Results of epidemiological studies pertinent to an assessment of human carcinogenicity are summarized. When relevant, case reports and correlation studies are also summarized. The target organ(s) or tissue(s) in which an increase in cancer was observed is identified. Dose–response and other quantitative data may be summarized when available.

(c) *Cancer in experimental animals*

Data relevant to an evaluation of carcinogenicity in animals are summarized. For each animal species, study design and route of administration, it is stated whether an increased incidence, reduced latency, or increased severity or multiplicity of neoplasms or preneoplastic lesions were observed, and the tumour sites are indicated. If the agent produced tumours after prenatal exposure or in single-dose experiments, this is also mentioned. Negative findings, inverse relationships, dose–response and other quantitative data are also summarized.

(d) *Mechanistic and other relevant data*

Data relevant to the toxicokinetics (absorption, distribution, metabolism, elimination) and

the possible mechanism(s) of carcinogenesis (e.g. genetic toxicity, epigenetic effects) are summarized. In addition, information on susceptible individuals, populations and life-stages is summarized. This section also reports on other toxic effects, including reproductive and developmental effects, as well as additional relevant data that are considered to be important.

6. Evaluation and rationale

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

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 to a higher or lower category than a strict interpretation of these criteria would indicate.

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). A classification may change as new information becomes available.

An evaluation of the degree of evidence 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 the degree of evidence.

(a) Carcinogenicity in humans

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 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. A statement that there is *sufficient evidence* is followed by a separate sentence that identifies the target organ(s) or tissue(s) where an increased risk of cancer was observed in humans. Identification of a specific target organ or tissue does not preclude the possibility that the agent may cause cancer at other sites.

Limited evidence of carcinogenicity: A positive association has been observed between exposure to the agent 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 humans are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent and any studied cancer at any observed level of exposure. The results from these studies alone or combined should have narrow confidence intervals with an upper limit close to the null value (e.g. a relative risk of 1.0). Bias and confounding should be ruled out with reasonable confidence, and the studies should have an adequate length of follow-up. 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.

When the available epidemiological studies pertain to a mixture, process, occupation or industry, the Working Group seeks to identify the specific agent 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.

(b) *Carcinogenicity in experimental animals*

Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multi-stage process of carcinogenesis should be considered in evaluating the degree of evidence of 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 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) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well conducted study, ideally conducted under Good Laboratory Practices, can also provide *sufficient evidence*.

A single study in one species and sex 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, or when there are strong findings of tumours at multiple sites.

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; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

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 is not carcinogenic. A conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the species, tumour sites, age at exposure, and conditions and levels of exposure studied.

(c) *Mechanistic and other relevant data*

Mechanistic and other evidence judged to be relevant to an evaluation of carcinogenicity and of sufficient importance to affect the overall evaluation is highlighted. This may include data on preneoplastic lesions, tumour pathology, genetic and related effects, structure–activity relationships, metabolism and toxicokinetics,

physicochemical parameters and analogous biological agents.

The strength of the evidence that any carcinogenic effect observed is due to a particular mechanism is evaluated, using terms such as ‘weak’, ‘moderate’ or ‘strong’. The Working Group then assesses whether that particular mechanism is likely to be operative in humans. The strongest indications that a particular mechanism operates in humans derive 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.

The conclusion that a mechanism operates in experimental animals is strengthened by findings of consistent results in different experimental systems, by the demonstration of biological plausibility and by coherence of the overall database. Strong support can be obtained from studies that challenge the hypothesized mechanism experimentally, by demonstrating that the suppression of key mechanistic processes leads to the suppression of tumour development. The Working Group considers whether multiple mechanisms might contribute to tumour development, whether different mechanisms might operate in different dose ranges, whether separate mechanisms might operate in humans and experimental animals and whether a unique mechanism might operate in a susceptible group. The possible contribution of alternative mechanisms must be considered before concluding that tumours observed in experimental animals are not relevant to humans. An uneven level of experimental support for different mechanisms may reflect that disproportionate resources

have been focused on investigating a favoured mechanism.

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.

(d) Overall evaluation

Finally, the body of evidence is considered as a whole, to reach an overall evaluation of the carcinogenicity of the agent to humans.

An evaluation may be made for a group of agents that have been evaluated by the Working Group. In addition, when supporting data indicate that other related agents, for which there is no direct evidence of their 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 agents if the strength of the evidence warrants it.

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

Group 1: The agent is carcinogenic to humans.

This category is used when there is *sufficient evidence of carcinogenicity* in humans. Exceptionally, an agent 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 acts through a relevant mechanism of carcinogenicity.

Group 2.

This category includes agents 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 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 mechanistic and other relevant data. The terms *probably carcinogenic* and *possibly carcinogenic* have no quantitative significance and are used simply as descriptors of different levels of evidence of human carcinogenicity, with *probably carcinogenic* signifying a higher level of evidence than *possibly carcinogenic*.

Group 2A: The agent is 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 may be classified in this category when there is *inadequate evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Exceptionally, an agent may be classified in this category solely on the basis of *limited evidence of carcinogenicity* in humans. An agent may be assigned to this category if it clearly belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified in Group 1 or Group 2A.

Group 2B: The agent is possibly carcinogenic to humans.

This category is used for agents 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 for which there is *inadequate evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals together with supporting evidence from mechanistic and other relevant data may be placed in this group. An agent may be classified in this category solely on the basis of strong evidence from mechanistic and other relevant data.

Group 3: The agent is not classifiable as to its carcinogenicity to humans.

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

Exceptionally, agents 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 that do not fall into any other group are also placed in this category.

An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that further research is needed, especially when exposures are widespread or the cancer data are consistent with differing interpretations.

Group 4: The agent is probably not carcinogenic to humans.

This category is used for agents for which there is *evidence suggesting lack of carcinogenicity*

in humans and in experimental animals. In some instances, agents 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 mechanistic and other relevant data, may be classified in this group.

(e) Rationale

The reasoning that the Working Group used to reach its evaluation is presented and discussed. This section integrates the major findings from studies of cancer in humans, studies of cancer in experimental animals, and mechanistic and other relevant data. It includes concise statements of the principal line(s) of argument that emerged, the conclusions of the Working Group on the strength of the evidence for each group of studies, citations to indicate which studies were pivotal to these conclusions, and an explanation of the reasoning of the Working Group in weighing data and making evaluations. When there are significant differences of scientific interpretation among Working Group Members, a brief summary of the alternative interpretations is provided, together with their scientific rationale and an indication of the relative degree of support for each alternative.

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GENERAL REMARKS

This hundred-and-first volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of some chemicals present in industrial and consumer products, food contaminants and flavourings, and water chlorination by-products. Among these, dibromoacetonitrile (Volume 71), 2-nitrotoluene (Volume 65), diethanolamine and di(2-ethylhexyl) phthalate (Volume 77) had been evaluated previously. A summary of the findings of this volume appears in *The Lancet Oncology* ([Grosse et al., 2011](#)).

Assessment, quantification and relative contributions of sources of exposure

Human exposures to potentially carcinogenic agents may result from complex mixtures in the air, water, food and certain occupations, which make the assessment of their risk for cancer difficult, as exemplified by several agents evaluated in this volume.

Most of the agents reviewed in this volume of *IARC Monographs* do not have a single source and humans may be exposed occupationally, or through food, drinking-water and the environment. Quantitative determination of the most important sources of human exposure is relevant, and has been attempted by the Working Group when valid information was available in the scientific literature. For a few agents, however, quantitative information on many — if not all — sources of exposure was lacking or inconclusive. An example is methyleugenol, for which the daily estimates of dietary exposure from a given source (e.g. basil) varied over three orders of magnitude from the microgram to the milligram per kilogram of body weight range, and thus complicated relative comparisons with other sources.

Several of the substances reviewed in this volume may be contained in foods and beverages. They may either occur naturally (e.g. methyleugenol, 2,4-hexadienal, methyl isobutyl ketone or benzophenone), be formed as a contaminant during food processing (e.g. 3-monochloro-1,2-propanediol, 1,3-dichloro-2-propanol or 2,4-hexadienal), migrate from packaging materials (e.g. di(2-ethylhexyl) phthalate or benzophenone) or be added directly (e.g. as flavouring compounds such as methyleugenol, 2,4-hexadienal, methyl isobutyl ketone or benzophenone) or occur indirectly as natural constituents of or contaminants in food additives (e.g. 4-methylimidazole in caramel colouring). The Working Group noted either the general absence of data on occurrence in food (e.g. 2-methylimidazole) or the availability of only comparatively old data (e.g. 4-methylimidazole). For some agents, large sets of data from food surveys are available (e.g. 3-monochloro-1,2-propanediol and

1,3-dichloro-2-propanol) but these may not reflect the current situation of the market, because the surveys were often conducted before regulatory limits came into force or before measures to mitigate exposure were implemented by industry.

Epidemiology of complex exposures

In this volume of *IARC Monographs*, the evaluation of the risk for cancer attributed to specific agents was limited by the paucity of epidemiological studies specific for those chemicals. One study was identified that evaluated urinary metabolites of di(2-ethylhexyl) phthalate, but was limited by its cross-sectional design: exposure was assessed at the time of disease. Prospective studies linking biomarkers of exposure to di(2-ethylhexyl) phthalate with cancer risk may be feasible in view of current efforts to collect population data. For other substances (e.g. 2-nitrotoluene, anthraquinone, diethanolamine, bromochloroacetic acid, dibromoacetic acid and dibromoacetonitrile), studies of workers or people exposed to the substances were available but were limited by a lack of a chemical-specific exposure assessment or by the fact that exposure was to a mixture of chemicals. For example, workers exposed to 2-nitrotoluene are exposed to other carcinogens, such as *ortho*-toluidine, or mutagens, such as tri- and dinitrotoluenes. The use of mechanistic data and molecular epidemiological approaches should help in the evaluation of complex mixtures, and to disentangle the effects of cancer risks from a specific substance when people are exposed to that substance in conjunction with known or suspected carcinogens.

Metalworking fluids

This volume of *IARC Monographs* evaluated the carcinogenicity of diethanolamine (IARC Group 2B) and coconut oil diethanolamine condensate (IARC Group 2B). These constituents of metalworking fluids, which are used to lubricate, cool and flush chips in the machining, cutting and grinding of metal to manufacture items such as engine blocks, gears, transmissions, chassis parts and other metal products. The four major categories of metalworking fluids are: straight oils (generally mineral oils), soluble oils, semi-synthetic (straight oils diluted with water and additives) and synthetic (additives and water without oils). Ethanolamines — either diethanolamine or triethanolamine — are commonly added to soluble, semi-synthetic or synthetic metalworking fluids. Diethanolamine may also be present as an unintended impurity of intended triethanolamine or fatty-acid diethanolamide additives. Workers are exposed by inhalation of aerosols or skin contact. Since the mid-1940s, water-based fluids have been increasingly used in high-production operations, such as those in the automobile industry. The proportion of water-based fluids that contain diethanolamine and that of processes that used water-based fluids over time cannot be estimated precisely from the available literature. However, it appears that most workers in modern machining plants probably had some exposure to diethanolamine ([NIOSH, 1998](#)).

The carcinogenicity of diethanolamine and the epidemiological literature on metalworking fluids have been reviewed previously ([IARC, 2000](#)), when a few cohort studies of workers exposed to water-based fluids were identified. The most prominent was a large cohort study of workers in the automobile

parts manufacturing industry in the United States of America from which several nested studies were conducted ([Eisen et al., 1992](#)). The current Working Group identified an update of this cohort ([Eisen et al., 2001](#)) from which another series of substudies was conducted, as well as an update of another cohort of automobile industry workers ([Kazerouni et al., 2000](#)) and a population-based case-control study ([Colt et al., 2011](#)). Associations with an increased incidence of cancer at various tumour sites were noted in the majority of these studies ([Mirer, 2003, 2010](#); [Savitz, 2003](#)).

In this volume of *IARC Monographs* and the previous *Monograph* ([IARC, 2000](#)), the Working Groups concluded that evidence from this body of literature could not be used for a specific evaluation of the carcinogenicity of diethanolamine because of the potential presence of other agents such as *N*-nitrosodiethanolamine (*IARC Group 2B*), poorly refined mineral oils and chlorinated paraffins. The Working Group did not evaluate the carcinogenicity of metalworking fluids as an exposure circumstance. The Advisory Group meeting that discussed future priorities for the *IARC Monographs Programme* ([IARC, 2008](#)) recommended that the evaluation of metalworking fluids should have medium priority.

Disinfection by-products

Various disinfection by-products, some of which are evaluated in this volume of *IARC Monographs*, are also encountered almost exclusively through the drinking of, and swimming, showering and bathing in disinfected water.

Disinfection of drinking-water is one of the major public health interventions in history, and has reduced mortality from waterborne infectious diseases. Disinfection of drinking-water and water used in swimming pools is a necessity. However, disinfectants are highly reactive chemicals that, besides inactivating microorganisms, react with organic matter to form undesired by-products. Chlorine, in the form of chlorine gas or hypochlorite solution, has been the most widely used disinfectant worldwide. By-products of chlorination, and specifically trihalomethanes, were first detected in the early 1970s. Four trihalomethanes (chloroform [*IARC Group 2B*], bromodichloromethane [*IARC Group 2B*], dibromochloromethane [*IARC Group 3*] and bromoform [*IARC Group 3*]), together with nine bromine- and chlorine-based haloacetic acids, are the main by-products of chlorination on a weight basis. The chlorine-bromine speciation depends on the bromine content of the raw water. Trihalomethanes and haloacetic acids are regulated in the European Union, the USA and other countries.

Chlorine is being progressively replaced by alternative disinfectants to meet these regulations. Although the use of alternatives, including ozone, chlorine dioxide and chloramines, reduces the formation of the regulated trihalomethanes and haloacetic acids, other disinfection by-products are formed. For example, when raw water contains bromine, bromate is formed by ozonation. Chlorate and chlorite are by-products of disinfection with chlorine dioxide, and the use of chloramines may result in the formation of iodinated trihalomethanes and nitrogenated by-products, such as *N*-nitrosodimethylamine (*IARC Group 2A*). The potential health impact of these alternative disinfection by-products is mainly unknown.

No epidemiological studies have evaluated exposure specifically to bromochloroacetic acid, dibromoacetic acid or dibromoacetonitrile, the three disinfection by-products evaluated in this volume.

Human exposure to these chemicals always occurs in mixtures; other disinfection by-products in chlorinated drinking-water and chlorinated swimming pool water include more than 700 chemicals. The physical properties of these chemicals differ and determine their routes of exposure. Inhalation of volatile disinfection by-products, such as trihalomethanes, occurs in showers and swimming pools. Dermal absorption of trihalomethanes and other skin-permeable compounds may occur in showers, baths and swimming pools. However, ingestion is the main pathway of exposure for other disinfection by-products, such as haloacetic acids, because of their low volatility and skin permeability. Due to the complexity of the mixtures and exposure scenarios, future evaluations of the effects of disinfection by-products in humans should reflect actual exposure situations rather than isolating individual chemicals.

Several studies have been conducted during the last three decades to evaluate the risk for cancer associated with disinfection by-products. Because the characteristics of raw water and the types of treatment used are the main determinants of the concentration of disinfection by-products formed, many epidemiological studies have used such variables as surrogates of exposure to these compounds (e.g. chlorinated surface versus non-chlorinated water). The most recent studies quantified exposure to trihalomethanes as a proxy for the whole mixture. These have been reviewed in the Introduction to the *Monographs* on Bromochloroacetic acid, Dibromoacetic acid and Dibromoacetonitrile. However, evidence in humans is insufficient to attribute the observed effects to a single agent. Other disinfection by-products evaluated by the *IARC Monographs* programme are shown in [Table 1](#).

The use of biomarkers has been limited in epidemiological studies of cancer that evaluated the effects of exposure to disinfection by-products. These compounds do not accumulate in the body, and no biomarkers of internal dose that reflect long-term exposure have been identified. Studies that evaluate molecular markers of genetic susceptibility may help to disentangle the mechanisms and effects of specific disinfection by-products.

Using data on genotypes and other molecular analyses, some of the components of complex mixtures may be identified as a contributing factor in the causation of cancers associated with exposure to these compounds. For example, in a case–control study of urinary bladder cancer in Spain, [Cantor *et al.* \(2010\)](#) showed that the risk for bladder cancer increased sixfold after exposure to drinking-water, primarily through showering, bathing and swimming, among people who had both the glutathione *S*-transferase T-1 genotype, which metabolizes brominated trihalomethanes to mutagens, and a particular single-nucleotide polymorphism in glutathione *S*-transferase Z genotype, which metabolizes haloacetic acids. This type of molecular epidemiology pointed to brominated trihalomethanes and haloacetic acids as critical components with a potential causative role in bladder cancer associated with exposure to drinking-water.

The interpretation of the occurrence of rare tumours

The Working Group noted that several of the chemicals evaluated in this volume of *IARC Monographs* induced several unusual (rare) neoplasms in animal studies, including: hepatoblastomas in mice, histiocytic sarcomas in mice, mammary gland tumours in male rats, neuroendocrine tumours of the glandular stomach in mice and rats, urinary bladder tumours in rats, large intestinal tumours in mice and rats, glandular stomach adenomas in male rats, squamous-cell papillomas and

Table 1 Evaluations of chlorinated drinking-water, some chemicals used in the chlorination of drinking-water and some chlorination by-products, from this and previous IARC Monographs volumes

Agent	Degree of evidence of carcinogenicity		Overall evaluation of carcinogenicity to humans	IARC Monographs volume (year)
	Humans	Experimental animals		
Chlorinated drinking-water	I	I	3	Volume 52 (1991)
<i>Some chemicals used in the disinfection of drinking-water</i>				
Sodium chlorite	ND	I	3	Volume 52 (1991)
Hypochlorite salts	ND	I	3	Volume 52 (1991)
Chloramine	I	I	3	Volume 84 (2004)
<i>Disinfection by-products</i>				
<i>Trihalomethanes</i>				
Chloroform	I	S	2B	Volume 73 (1999)
Bromodichloromethane	I	S	2B	Volume 52 (1991)
Dibromochloromethane	I	L	3	Volume 52 (1991)
Bromoform	I	L	3	Volume 52 (1991)
<i>Haloacetic acids</i>				
Dichloroacetic acid	I	S	2B	Volume 84 (2004)
Trichloroacetic acid	I	L	3	Volume 84 (2004)
Bromochloroacetic acid	I	S	2B	Volume 101 (this volume)
Dibromoacetic acid	I	S	2B	Volume 101 (this volume)
<i>Halogenated acetonitriles</i>				
Bromochloroacetonitrile	ND	I	3	Volume 52 (1991)
Chloroacetonitrile	ND	I	3	Volume 52 (1991)
Dibromoacetonitrile	ND	I	3	Volume 52 (1991)
Dichloroacetonitrile	ND	I	3	Volume 52 (1991)
Trichloroacetonitrile	ND	I	3	Volume 52 (1991)
Dibromoacetonitrile	ND	S	2B	Volume 101 (this volume)
Chloral hydrate	I	L	3	Volume 84 (2004)
3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX)	I	L	2B*	Volume 84 (2004)
Potassium bromate	I	S	2B	Volume 73 (1999)

*Other relevant data were used to upgrade the overall evaluation.

I, inadequate evidence; L, limited evidence; ND, no data; S, sufficient evidence; for definition of the degrees of evidence and evaluations, see the Preamble

carcinomas of the tongue and oral cavity in rats, squamous-cell carcinomas of the tongue in male mice, squamous-cell papillomas and carcinomas of the forestomach in mice and rats, mesotheliomas in male rats, renal mesenchymal tumours in female rats, and adenomas of the respiratory epithelium of the nose in rats.

Several chemicals evaluated in this volume induce the above relatively rare neoplasms and are also mutagenic. Although each evaluation is for a specific chemical, there were similarities in the mutagenicity and carcinogenicity profiles among several members of the group that are structurally related, such as bromochloroacetic acid and dibromoacetic acid, which both induce hepatoblastomas in mice and mesotheliomas in rats. The consistency of these rare tumours lends biological plausibility to the findings and raises concerns of potential human carcinogenicity. To dismiss the occurrence of rare tumours would seem to be unwise. This observation supports the criteria in the Preamble that “a single study in one species and sex 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, or when there are strong findings of tumours at multiple sites.”

The IARC criteria regarding *sufficient evidence of carcinogenicity* in experimental animals may, under some circumstances, require a judgement call on the “appropriate combination of benign and malignant neoplasms” when an increase in the incidence of malignant tumours only is not observed. The Working Group felt that the rarity of the incidence of the tumour may also be taken into account, meaning that a rare neoplasm observed as the combination of some benign and a few malignant tumours could demonstrate the progression to cancer.

Dose differences between rodent studies and human exposures

For several chemicals considered in this volume, data showed that the chemical in question induced tumours in rodents at quite high doses; however, biomonitoring or feeding studies in humans showed that the typical or maximum doses to which humans are exposed are considerably lower than those used in rodent studies. For example, such analyses showed that the lowest dose of methyleugenol used in rodent studies that induced tumours is 10 000 times higher than the average or highest dose observed in humans in biomonitoring or feeding studies. Thus, the question arises whether the doses to which humans are exposed are sufficient to present a risk for cancer. An additional complication is that, for some agents, there was little evidence of mutagenicity. Thus, do low doses of non-genotoxic rodent carcinogens induce tumours in humans? The Working Group considered this to be an issue of potency or risk and not relevant to hazard identification. Thus, despite the differences in exposure, a chemical could be evaluated as a cancer-causing agent at some level in humans, although the actual risk might be quite low.

Single mode-of-action hypotheses

Several chemicals evaluated in this volume induced tumours in experimental animals that were associated with α 2u-globulin nephropathy, activation by pulmonary cytochrome P450 2F or peroxisome proliferation. However, advances in molecular biology have revealed that, subsequent to exposures, alterations in multiple processes lead to environmental or occupational carcinogenesis.

The potential roles of various cellular and molecular activities were considered in the evaluations of mechanistic events that underlie the carcinogenicity of the agents reviewed. For chemicals that elicit genotoxicity, this response cannot be excluded as a major contributing factor to the cancer outcome. For some chemicals, commonly used activation systems may not be adequate to detect genotoxic effects, e.g. the need for nitroreductase activity to convert 2-nitrotoluene to a mutagenic intermediate. For chemicals that do not show genotoxicity in a large battery of screening assays, it is frequently noted that multiple signalling pathways contribute to an altered phenotype. Single mode-of-action hypotheses require extensive testing, because a narrow focus on single pathways may result in oversimplified interpretations of the complex multiple pathways that lead to tumour induction.

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ANTHRAQUINONE

1. Exposure Data

1.1 Chemical and physical data

From [HSDB \(2010\)](#), [IPCS-CEC \(2005\)](#), and [NTP \(2005\)](#)

1.1.1 Nomenclature

Chem. Abstr. Services Reg. No.: 84-65-1

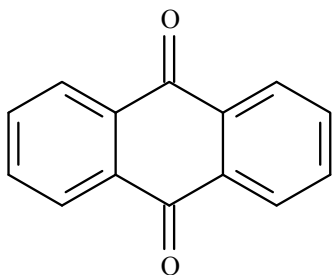
Chem. Abstr. Name: Anthraquinone;
9,10-anthraquinone

Synonyms: Anthracene, 9,10-dihydro-
9,10-dioxo-; anthradione; 9,10-anthra-
cenedione; bis-alkylamino
anthraquinone; 9,10-dioxoanthracene;
9,10-dihydro-9,10-dioxoanthracene.

RTECS No.: CB4725000

EINECS No.: 201-549-0

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{14}H_8O_2$

Relative molecular mass: 208.21

1.1.3 Chemical and physical properties of the pure substance

Description: Light yellow crystals

Boiling-point: 380 °C

Melting-point: 286 °C

Vapour pressure: 1.16×10^{-7} mm Hg at
25 °C

Solubility in water: 1.35 mg/L at 25 °C

Density: 1.44 g/cm³ at 20 °C

Flash-point: 185 °C

Relative vapour density (air = 1): 7.16

Auto-ignition temperature: 650 °C

Octanol/water partition coefficient: log K_{ow} ,
3.39

Henry's law constant: 2.35×10^{-8} atm.m³/
mol at 25 °C (estimated)

1.1.4 Technical products and impurities

No data were available to the Working Group.

1.1.5 Analysis

A series of methods to measure anthraquinone in different media has been reported ([HSDB, 2010](#); [Table 1.1](#)).

1.2 Production and use

1.2.1 Production

According to [HSDB \(2010\)](#), at least six methods are available for the manufacture of anthraquinone: (a) oxidation of naphthalene to

Table 1.1 Selected methods of analysis of anthraquinone

Medium	Method	Detection limit	Recovery
Seeds, crops and soil	Electron-capture detector	0.05 ppm	94–100%
Soils and sediment	GC/MS	29.7 µg/kg	NR
Rainwater	GC/MS	0.05–0.10 ng/L	NR
Filtered wastewater and natural water	GC/MS	0.11 µg/L	NR
Airborne particulate matter	Capillary GC and GC/MS	NR	NR
Fish tissue	Capillary GC and GC/MS	0.2 ppb	72%

GC, gas chromatography; MS, mass spectrometry; NR, not reported

Adapted from [HSDB \(2010\)](#)

naphthaquinone, which is then condensed with butadiene to yield tetrahydroanthraquinone, then dehydrogenated to produce anthraquinone; (b) industrial preparation from phthalic anhydride and benzene; (c) oxidation of anthracene with concentrated nitric acid; (d) dimerization of styrene to 1-methyl-3-phenylindane using phosphoric acid as a catalyst, followed by catalytic vapour-phase oxidation to anthraquinone; (e) oxidation of anthracene with chromic acid in 48% sulfuric acid or oxidation with air in the vapour phase; and (f) condensation of 1,4-naphthoquinone with butadiene.

In the United States of America, anthraquinone is listed as a chemical with a high production volume. As from 1986, between 250–500 tonnes per year were either produced or imported there. A major increase was then reported in 1998 and 2002 when volumes reached 5000–25 000 tonnes per year ([HSDB, 2010](#)).

In the People's Republic of China, production reached 37 500 tonnes in 2008 due to continuously increasing demand ([CRI, 2011](#)).

1.2.2 Use

Anthraquinone is an important and widely used raw material for the manufacture of vat dyes, which are a class of water-insoluble dyes that can easily be reduced to a water-soluble and usually colourless leuco form that readily impregnates fibres and textiles. Their principal properties are brightness and good fastness. Anthraquinone

is also used as a seed dressing or in seed treatments. Other major uses are as a pesticide, as a bird repellent (especially for geese), and as an additive in chemical alkaline pulp processes in the paper and pulp industry ([HSDB, 2010](#)).

1.3 Occurrence and exposure

1.3.1 Natural occurrence

Natural pigments that are derivatives of anthraquinone are found in plants (e.g. aloe latex, senna and rhubarb), fungi, lichens and some insects ([HSDB, 2010](#)).

1.3.2 Occupational exposure

Occupational exposure to anthraquinone can occur during its production, its use in the manufacture of other chemicals or its direct use. Workers in transport-related industries are also potentially exposed to anthraquinone during its release from diesel and gasoline engine vehicles (see [Table 1.2](#)).

The National Occupational Exposure Survey, conducted from 1981 to 1983, estimated that 6187 workers were potentially exposed to anthraquinone in the USA, mostly in the printing and publishing industry (5475 workers), but also in photographic processing machine operations, in the air transport industry, and in geology and geodesy ([NIOSH, 1990](#)).

Table 1.2 Environmental occurrence of anthraquinone from combustion sources

Source	Sample	Concentration or release rates	Reference
Diesel engine automobiles	Particulate emissions	NR	Yu & Hites (1981) , Choudhury (1982)
Diesel engine automobiles	Particulate matter, 5 samples	47.7 µg/g	Layshock et al. (2010)
	Diesel extract, 3 samples	5.23 µg/g	
Diesel and gasoline vehicles, Japan	Particulate emissions from exhaust pipes	1.3–52 µg/g	Oda et al. (1998)
Diesel vehicles	Particulate matter	18–43 µg/g	Jakober et al. (2007)
		40.4 µg/g	Cho et al. (2004)^a
		58 µg/g	Valavanidis et al. (2006)^a
		34 µg/g	Zielinska et al. (2004)^a
Diesel vehicles	Emission rates	15.46 µg/km	Sidhu et al. (2005)
Car with a catalyst	Emission rates	4.4 µg/km	Rogge et al. (1993)
Car without a catalyst car		24.3 µg/km	
Heavy-duty diesel trucks		23.5 µg/km	
Heavy-duty diesel trucks	Emission rates	21–27 µg/L of fuel consumed	Jakober et al. (2007)
Vehicle-related	Tyre wear particles	ND	Tong et al. (1984)^b
	Brake lining	0.31 µg/g	
	Road dust particles	0.41 µg/g	
Small craft gas turbine engine	Particulate emissions	0.06–58.49 ng/m ³	Robertson et al. (1980)^b
Burning cereal straw	Organic extracts of emissions	995 µg/kg fuel	Ramdahl & Becher (1982)^b
Domestic waste uncontrolled burning ^c	Open-air burn sample		Sidhu et al. (2005)
	Concentration	1.72 ng/µL	
	Emission rate	0.28 mg/kg	
Forest litter ^d , Amazon	Smoke particulate matter	2.8 µg/m ³	Radzibinas et al. (1995)^b
Residential oil burner	Particulate samples	NR	Leary et al. (1987)^b
Municipal waste incinerators (4)	Extracts of air samples (2/4 samples)	2.9–9.0 µg/mL	James et al. (1985)^b
Municipal waste incinerators, Japan	Fly ash		Eiceman et al. (1979)
	2/2 samples	NR	
Ontario, Canada	1/2 samples	NR	
The Netherlands	1 sample	ND	
Municipal solid waste incinerator, Japan	Fly ash	NR	Akimoto et al. (1997)

^a Cited by [Jakober et al. \(2007\)](#)

^b Cited by [HSDB \(2010\)](#)

^c Experimental burn of household garbage

^d Controlled burn

ND, not detected; NR, not reported

Table 1.3 Environmental occurrence of anthraquinone from industrial sources

Source	Sample	Concentration	Reference
Near a chemical factory, former Czechoslovakia	Mosses, 2/6 samples	0.176–4.95 µg/g	Holoubek et al. (1991)^a
	Needles, 3/6 samples	0.460–1.92 µg/g	
	Earthworm, 2/5 samples	0.473–4.72 µg/g	
	Air, 1/8 samples	44.5 ng/m ³	
Dye manufacturing plant	Raw wastewater	49–110 µg/L (49–110 ppb)	Games & Hites (1977)^a
	Final effluent	ND	
Timber production or organic and plastics production	Industrial wastewater, 2/79 samples	NR	Burse & Pellizzari (1982)^a
Wood preserving plant (abandoned)	Groundwater	132 mg/L	Middaugh et al. (1991)^a
	Stream water that flowed through site	2 µg/L	
Coal tar creosote waste site, Germany	Soil, 2 samples	2 and 20 µg/g	Meyer et al. (1999)^a

^a Cited by [HSDB \(2010\)](#)

ND, not detected; NR, not reported

Although no data on the number of workers exposed to anthraquinone were available from occupational surveys, a series of studies on health effects at a manufacturing plant in New Jersey, USA, reported that 842 workers were involved the production of anthraquinone dyes and intermediates ([Delzell et al., 1989](#); [Sathiakumar & Delzell, 2000](#)); however, neither the number of workers specifically exposed to anthraquinone nor their exposure levels were provided.

Anthraquinone was detected in air samples (297 ng/m³) in a potroom where Söderberg electrodes were used for aluminium reduction ([Thrane & Stray, 1986](#)). [Wei et al. \(2010\)](#) measured personal exposure (as ambient particles) to anthraquinone and polycyclic aromatic hydrocarbons (PAHs) of two nonsmoking security guards at a kerbside gate on a busy road (8000–10 000 vehicles per day). The mean concentration of anthraquinone was 63.2 ng/m³ (25th percentile, 26.1; 75th percentile, 86.8 ng/m³; 58 samples). The major source of the organic carbons detected in the personal monitors was gasoline engines.

Anthraquinone is used as a catalyst in the pulp industry to improve delignification of wood and increase pulp yield. A study designed to develop analytical procedures for the detection

of anthraquinone in pulp process liquors found that the concentrations (mg/L) of anthraquinone were 0.04–0.66 in filtrates from bleaching, 0.13–0.75 in wash liquors, 0.5–11.5 in alkaline pulp liquors and 3.0–170 in black liquors ([Nelson & Cietek, 1983](#)).

1.3.3 Environmental occurrence

Anthraquinone is ubiquitous in the environment, and has been detected in the air, water (including surface, ground- and drinking-water), soil, plants, fish/seafood and animal tissue (see [Table 1.3](#), [Table 1.4](#), and [Table 1.5](#)). The major sources of environmental exposure are both natural and anthropogenic. Anthraquinone and other oxygenated PAHs are formed from direct combustion processes (see [Table 1.2](#)) or the degradation of PAHs by atmospheric oxidants ([Layshock et al., 2010](#)). Specifically, anthraquinone is formed from anthracene through photolytic and biodegradation processes ([HSDB, 2006](#)). The levels of oxygenated PAHs in the soil and air have increased in recent years ([Layshock et al., 2010](#)). Moreover, anthraquinone may be released directly into the environment through its use as a bird repellent or via various wastestreams

through its use as an additive in the soda and kraft chemical alkaline pulp processes in the paper and pulp industry, and in the production of various dyes ([HSDB, 2006](#)).

(a) *Release/effluent*

[Table 1.2](#) summarizes studies of environmental exposure to anthraquinone from combustion sources, many of which detected anthraquinone in particulate matter from vehicles with diesel or gasoline engines at concentrations ranging up to 58 µg/g. Estimated emission rates of anthraquinone in diesel emission particles were reported to be 24.88 µg/mile [15.46 µg/km]. Another study reported emissions rates from exhaust pipes of various vehicles ranging from 4.4 µg/km for cars with a catalyst and 24.3 µg/km for cars without a catalyst to 23.5 µg/km for heavy-duty diesel trucks. [Jakober et al. \(2007\)](#) reported emission rates of 21–27 µg anthraquinone/L of fuel consumed from heavy-duty diesel vehicles. Anthraquinone is also released as particles from the combustion of plants, fuel or waste, and has been detected in municipal waste incinerators (fly ash or air samples) in Japan and Canada. The emission rate for a sample from an open-air domestic waste incinerator was 0.28 mg/kg.

Anthraquinone has also been detected in the environment near industrial or abandoned sites ([Table 1.3](#)). It was found in earthworms, mosses and ambient air near a chemical factory in former Czechoslovakia; in raw waste water at a dye manufacturing plant; in industrial wastewater from timber production or organic and plastics production; in groundwater and stream water from an abandoned wood preserving plant; and in soil samples from coal-tar creosote waste sites. Coal may be another source of exposure to anthraquinone, which was detected (0.7 µg/L) in an extract of model coal piles (Texas lignite) leached with distilled water under simulated rainfall conditions ([Stahl et al., 1984](#), cited in [HSDB, 2006](#)).

(b) *Ambient air*

[HSDB \(2006\)](#) reviewed information on and calculated parameters related to the environmental fate of anthraquinone in ambient air, water and soil. When released into the air, it is expected to remain in the vapour and particulate phases. [Albinet et al. \(2008\)](#) reported that the fraction of oxygenated PAHs in the particulate phase in the French alpine valleys mainly comprised the heaviest compounds. However, most studies have measured anthraquinone in particles. [Leotz-Gartzandia et al. \(2000\)](#) found higher levels of anthraquinone in the particulate phase than in the gaseous phase in samples of air from near a motorway in France. Particle-phase anthraquinone can be removed by wet or dry deposition ([HSDB, 2006](#)), and has been found in precipitations (see [Table 1.5](#)). Vapour-phase anthraquinone is degraded in the atmosphere by a reaction with photochemically produced hydroxyl radicals, and has an estimated atmospheric half-life of 11 days. The presence of sunlight may accelerate the degradation of anthraquinone by ozone in the atmosphere ([HSDB, 2006](#)).

Anthraquinone has been detected in ambient air (usually in particulate matter) near roadways, and in urban, suburban and rural areas (see [Table 1.4](#)). In general, levels are higher in the winter than in the summer, and in urban areas than in rural areas. However, a study in Algeria found higher levels in the summer, which the authors presumed were due to increased generation of ozone and hydrogen radicals by strong solar radiation ([Yassaa et al., 2001](#)). [Albinet et al. \(2007\)](#) reported that anthraquinone was the most abundant oxygenated PAH detected in the Marseilles area of France, and accounted for 20% of total oxygenated PAHs. Gasoline engines were an important source of this exposure.

Table 1.4 Environmental occurrence of anthraquinone in ambient air

Source	Sample	Concentration	Reference
Roads			
Freeway tunnel, Japan	Air samples	52 µg/g (extract mass)	Oda et al. (1998)^a
Freeway tunnel, Japan	Air samples – 5 sites	29–56 ng/m ³	Oda et al. (2001)
	Dust guardrails – 5 sites	9.2 (6.3–14) ng/m ^{3b}	
Urban/suburban			
Barcelona, Spain	<i>Organic extracts from airborne particulates</i>		Bayona et al. (1994)^a
	Spring	0.009 ng/m ³	
	Summer	ND	
	Autumn	0.026 ng/m ³	
Barcelona, Spain	<i>Aerosol samples</i>		Galceran & Moyano (1993)^a
	Winter	0.021 ng/m ³	
	Summer	0.082 ng/m ³	
	Winter	0.075 ng/m ³	
Duisburg, Germany	Particulate matter	0.22–1.89 ng/m ³	Koenig et al. (1983)^a
Munich, Germany	Particulate matter	0.96 (0.16–1.85) ng/m ^{3b}	Schnelle-Kreis (2001)^c
Augsburg, Germany	Urban particulate matter	0.39 (0.11–0.58) ng/m ^{3b}	Sklorz et al. (2007)
Chamonix Valley, French Alps, 2002–03	<i>Air particulates</i>		Albinet et al. (2008)
	<i>Suburban (7 samples)</i>		
	Winter	1.42 ng/m ³	
	Summer	1.59 ng/m ³	
	<i>Traffic (14 samples)</i>		
	Winter	3.60 ng/m ³	
Summer	0.97 ng/m ³		
Maurienne Valley, French Alps, 2002–03	<i>Air particulates</i>		Albinet et al. (2008)
	<i>Suburban (7 samples^d)</i>		
	Winter	2.76 ng/m ³	
	Summer	0.34 ng/m ³	
Paris, France	Air particulates	0.070 ng/m ³	Nicol et al. (2001)
Paris, France	Near motorway		Leotz-Gartziandia et al. (2000)
	Particles	~22 ng/m ^{3e}	
	Gas	~2 ng/m ^{3e}	

Table 1.4 (continued)

Source	Sample	Concentration	Reference
Marseilles area, France	<i>Air particulates</i>		Albinet et al. (2007)
	Urban	1.40 (0.378–2.57) ng/m ^{3b}	
	Suburban	0.77 (0.073–2.79) ng/m ^{3b}	
England, United Kingdom	Air particulates	0.210 ng/m ³	Kelly et al. (1993)^f
Santiago, Chile, 2000	<i>Particulate matter</i>		María del Rosario Sienra (2006)
	<i>Providencia</i>		
	Winter	1.58 ng/m ³	
	Spring	0.56 ng/m ³	
	<i>Las Condes</i>		
	Winter	0.67 ng/m ³	
	Spring	0.38 ng/m ³	
Toronto, Canada	Ambient air levels	0.0009 –0.0013 ng/m ³	Harkov (1986)^a
California and Louisiana, USA	Air particulates, 2/7 sites	NR	Kolber et al. (1982)^a
Portland, OR, USA	Gas phase	2.5 ng/m ³	Ligocki & Pankow (1989)^a
February to April 1984 and February to April 1985	Particulate phase	0.59 ng/m ³	
St Louis, MO, USA	Air particles	NR	Ramdahl & Becher (1982)^a
Los Angeles, CA, USA, 1993	Ambient air/smog	0.3 ng/m ³	Fraser et al. (2000)^a
Southern California, USA, 1995	Air particulate (12 sites)	0.011–0.22 ng/m ³	Manchester-Neesvig et al. (2003)^a
Washington DC, USA	Urban dust, 3 samples ^g	1.60 µg/g	Layshock et al. (2010)
Washington DC, USA	Urban dust ^h	2.24 µg/g	Albinet et al. (2006)
		0.220 µg/g	Fernandez & Bayona (1992)
		2.70 µg/g	Durant et al. (1998)ⁱ
		2.03 µg/g	Cho et al. (2004)ⁱ
Algiers, Algeria	<i>Particles</i>		Yassaa et al. (2001)
	<i>Downtown</i>		
	Winter	1.0 ng/m ³	
	Summer	6.2 ng/m ³	
	<i>Landfill</i>		
	Winter	0.1 ng/m ³	
	Summer	1.5 ng/m ³	
Rural			
Chacaltaya, Bolivia	Air, 2 samples, 1975	0.064–0.065 ng/ m ³	Cautreels et al. (1977)
Antwerp, Belgium	Air, 4 samples, 1976	0.57–1.0 ng/ m ³	Cautreels et al. (1977)
Japan	Air sample	2.8 µg/g (total weight mass)	Oda et al. (1998)^a

Table 1.4 (continued)

Source	Sample	Concentration	Reference
Chamonix Valley, French Alps, 2002–03	<i>Air particulates</i>		Albinet et al. (2008)
	<i>Altitude</i>		
	Winter	0.15 ng/m ³	
	Summer	0.05 ng/m ³	
	<i>Rural</i>		
	Winter	0.57 ng/m ³	
Maurienne Valley, French Alps, 2002–03	<i>Air particulates</i>		Albinet et al. (2008)
	<i>Tigny, 14 samples</i>		
	Winter	1.77 ng/m ³	
	Summer	0.47 ng/m ³	
	<i>Solières, 14 samples</i>		
	Winter	2.36 ng/m ³	
	Summer	0.13 ng/m ³	

^a Cited by [HSDB \(2010\)](#)

^b Mean and range

^c Cited by [María del Rosario Sienna \(2006\)](#)

^d Modane site, no winter samples were available for the other suburban site, Orelle; summer samples at Orelle (0.37 ng/m³) were comparable with Modane site

^e Estimated from graph

^f Cited by [Nicol et al. \(2001\)](#)

^g Sample SRM 1649b

^h Sample SRM 1649a sample collected in 1970s, authors used different chromatography methods, as cited by [Albinet et al. \(2006\)](#)

ⁱ As cited by [Albinet et al. \(2006\)](#)

ND, not detected; NR, not reported

Table 1.5 Environmental occurrence of anthraquinone in water and soil

Location or source	Source/sample	Concentration	Reference
Surface water			
Rhine river	Surface water	NR	Meijers & van der Leer (1976)^a
Baltic sea	Surface and deep water – 3 sites	NR	Ehrhardt et al. (1982)^a
Iowa, USA	Stream water	0.066 µg/L (max)	Kolpin et al. (2004)^a
Drinking-water			
Kitakyushu, Japan	Drinking-water – tap	5.2 ng/L	Akiyama et al. (1980)
Tsukuba, Japan	Drinking-water – tap	NR	Shiraishi et al. (1985)^a
Athens, GA, USA	Drinking-water – tap	20–100 ng/L	Thruston (1978)^a
Ottawa, Canada	Drinking-water supply	1.8–2.4 ng/L	Benoit et al. (1979a)^a
Great Lakes, Canada	<i>Drinking-water – 12 municipal water supplies</i>		Williams et al. (1982)
	Winter	ND–63.5 ng/L	
	Summer	0.2–72 ng/L	
Unspecified	Drinking-water treatment plants, 6 sites, June to October	0.6–2.1 ng/L	Benoit et al. (1979b)^a
Precipitation			
Oregon, USA	<i>Rain, 8/9 storms</i>		Pankow et al. (1984)^a
	Spring	2.2–16 ng/L	
	Autumn	18–74 ng/L	
Portland, OR, USA	Rain, 7/7 storms, February to April 1984	1.5–3.6 ng/m ³	Ligocki et al. (1985)^a
Norway	Precipitation	NR	Lunde (1976)^a
Soil and sediment			
Roadside (traffic pollution), Czech Republic	Soil	NR	Zdráhal et al. (2000)
Tunnel roadway	Soil, 5 sites	1.2 ^b (0.2–2.1) µg/g soil	Oda et al. (2001)
Sewage area, Marseilles, France	Marine sediments, 9/10 sites	2–400 ng/g	Milano & Vernet (1988)^a
Dokai Bay, Japan	Marine sediment	NR	Terashi et al. (1993)^a
USA, 20 river basins, 1992–95	22.2% of 536 sediment samples	Highest, 2 100 µg/kg; 50th percentile, < 50 µg/kg	Lopes & Furlong (2001)^a
New York Bay and Newark Bay, USA	<i>Marine sediments</i>		Layshock et al. (2010)
	Clean-up scheme 3 (2 samples)	1.70 mg/kg	
	Clean-up scheme 4 (3 samples)	1.53 mg/kg	

^a Cited by [HSDB \(2010\)](#)

^b Mean

ND, not detected; NR, not reported.

(c) *Water and soil*

Anthraquinone that is released into water is expected to adsorb onto suspended solids and sediment. Experimental studies have shown that the majority of the anthraquinone added was degraded within 3 days in both surface water (82%) and groundwater (91%) (reviewed by [HSDB, 2006](#)). Natural bacterial populations in groundwater and activated sludge were also shown to degrade anthraquinone (range, 50–100%) in experiments that lasted between 5 days and 3 weeks. Anthraquinone may also be removed through photolysis by sunlight, and its direct photolysis half-life is about 9 minutes in aqueous solution. It is not sensitive to aqueous environmental hydrolysis, and volatilization is not expected to be an important factor in its removal ([HSDB, 2006](#)).

Studies that evaluated levels of anthraquinone in water are reported in [Table 1.5](#). It has been detected in groundwater from industrial sites (see [Table 1.3](#)), surface water and drinking-water (at concentrations up to 100 ng/L) in Japan, the USA and Canada ([Table 1.5](#)), and also in precipitations in the USA and Norway. Although its estimated bioconcentration factor is low (12; [HSDB, 2006](#)), anthraquinone has been detected at a concentration of 42 ng/g wet tissue (42 ppb) in bullhead catfish fish from the Black River in Ohio, USA ([Vassilaros et al., 1982](#)), and in the tissue (180.8 µg/kg) of mussels from the Guanabara Bay in Brazil ([Layshock et al., 2010](#)).

In soil, anthraquinone is predicted to be slightly mobile or immobile based on its estimated soil absorption coefficients of 2755–17 416 that were determined using reference European soils ([Gawlik et al., 1998](#)). Similar to observations in water, volatilization of anthraquinone from moist or dry soil is not expected ([HSDB, 2006](#)). Biodegradation also appears to be the most important factor that influences the removal of anthraquinone from soil; 67% of the anthraquinone added was biodegraded in a mixed soil

population within 12 weeks. Other studies have reported half-lives in different soils of 3–10 days, and a study that used a mixed bacterial population found that 6.5% of the initial concentration of anthraquinone remained in the soil after 3 days (reviewed by [HSDB, 2006](#)).

Anthraquinone has been detected in the soil from roadways and in marine sediments from areas near sewage plants in France, and in river basins and bays in the USA (see [Table 1.5](#)). [McKinney et al. \(1999\)](#) proposed that the ratio of anthracene to anthraquinone in marine sediments could be used as an environmental marker of the source of contamination. They measured the concentrations of anthracene and anthraquinone in several samples of coastal marine sediments from four urban harbour sites in New England (USA) and two remote sites (Long Island Sound in New York, and the Slocums River, Massachusetts). The ratio of anthracene to anthraquinone was less than 1 (0.317–0.772) at the urban sites, suggesting that the source of the exposure was predominantly discharge, whereas the ratio at remote sites was greater than 1 (2.45–2.81), suggesting that the source was primarily atmospheric deposition. They also evaluated the oxidation of anthracene and reported that the compound was stable and did not rapidly undergo oxidation under normal conditions found in the marine environment, although, under extreme conditions, it could be photo-oxidized by exposure to ultraviolet radiation.

1.3.4 *Other occurrence*

Anthraquinone has been detected in fish, mussel tissue and plants ([HSDB, 2006](#)). Exposure to anthraquinone from food stuffs can also occur through its leaching from packaging. An experimental study ([Louch, 2008](#)) that evaluated the migration of anthraquinone from an unbleached kraft linerboard sample (representing a pizza delivery box) found that the mean level in the

baked pizza crust was 196.1 ng, indicating a 3.6% migration of anthraquinone.

1.4 Regulations and guidelines

According to European Union (EU) Commission directive 2007/565/EC, anthraquinone has been phased out as a repellent and attractant since 22 August 2008 in EU Member States ([ESIS, 2010](#)).

In the USA, anthraquinone has been accepted by the Environmental Protection Agency as a bird repellent for use near airports since 1998 ([US EPA, 1998](#)).

2. Cancer in Humans

No studies of human cancer were identified that evaluated exposure to anthraquinone *per se*; however, a series of publications on dye and resin workers in the USA, who were exposed to anthraquinone, was available. These workers were potentially exposed to anthraquinone during its production or its use to manufacture anthraquinone intermediates. Effect estimates were reported for subjects who worked in anthraquinone production areas, but they were also exposed to other chemicals, and effects specific for exposure to anthraquinone were not analysed. A study of substituted anthraquinone dyestuff workers in Scotland (United Kingdom) was also available; however, it was unclear whether anthraquinone was used to produce the intermediates in this study ([Gardiner et al., 1982](#)), which was therefore not reviewed by the Working Group. The main findings of the epidemiological studies of anthraquinone dye workers and cancer risk are summarized in [Table 2.1](#).

2.1 Cohort and nested case–control studies

2.1.1 USA

(a) Background

Delzell and colleagues evaluated mortality among manufacturing workers at a dye and resin plant in New Jersey. The study was initiated because of reported cases of central nervous system neoplasms. The findings were reported in a series of publications, including an analysis of mortality for the initial cohort as of 1985 ([Delzell et al., 1989](#)), two nested case–control analyses — one of central nervous system neoplasms and the other of lung cancer ([Barbone et al., 1992, 1994](#)) — that included both deaths and incident cases, and an analysis of mortality for an expanded cohort followed until 1996 ([Sathiakumar & Delzell, 2000](#)).

The plant comprised three major production areas: (1) South dyes, where anthraquinone dyes and intermediates were produced; (2) North dyes, where azo dyes and intermediates were produced; and (3) plastics and additives (P&A), where various resins and additives for resins were produced. This section focuses on data and findings for workers in the anthraquinone dye area. Production of anthraquinone ceased in 1980, production of anthraquinone dye intermediates and dye synthesis ceased in 1983 and the plant closed in 1996. Production of epichlorohydrin (Group 2A, [IARC, 2000](#)), another chemical produced in the anthraquinone dye area that has been associated with an increased risk of lung cancer, was only carried out for 5 years (1961–65) but potential exposure to epichlorohydrin occurred during the production of epoxy resins in the P&A production area. [Table 2.2](#) lists the processes and the associated raw materials or intermediates in the anthraquinone dye area (South dyes area) that could potentially confound the association between exposure to anthraquinone and the risk of lung cancer.

Table 2.1 Cohort studies of anthraquinone dye workers^a

Reference, study location and period	Total No. of subjects	Follow-up period	Exposure assessment	Organ site	Exposure categories	No. of cases/deaths	Relative risk (RR) (95%CI)	Covariates	Comments	
Sathiakumar & Delzell (2000) , New Jersey, USA, 1952–96	3266 dye and resin manufacturing workers (2 859 men, 407 women)	1952–96	Occupational history (job title, work area, and duration) from plant records	All deaths Lung	Overall	728	SMR 0.90 (0.83–0.97)	Age Age, calendar time and other work area	Local reference Elevated risk for lung cancer was also observed among maintenance workers and elevated risks for other cancers (e.g. bladder, CNS and stomach) were observed among workers employed in the other production areas. [Overlaps with Delzell et al. (1989) and Barbone et al. (1992, 1994)]	
					South dyes: White men					
					Total	32	1.68 (1.15–2.37)			
					Yr since hire/yr worked					
					< 20/ < 5	3	1.06 (0.22–3.10)			
					< 20/ > 5	5	1.79 (0.58–4.18)			
					> 20/ < 5	13	2.42 (1.29–4.14)			
					> 20/ > 5	11	1.37 (0.68–2.45)			
					RR					
					Never	48	1.0 (ref.)			
					Ever	32	1.7 (1.1–2.6)			
					Yr since hire/yr worked					
					< 20/ < 5	3	0.9 (0.3–3.0)			
					< 20/ > 5	5	1.7 (0.7–4.6)			
> 20/ < 5	13	2.4 (1.2–4.5)								
> 20/ > 5	11	1.5 (0.7–2.9)								

Table 2.1 (continued)

Reference, study location and period	Total No. of subjects	Follow-up period	Exposure assessment	Organ site	Exposure categories	No. of cases/deaths	Relative risk (RR) (95%CI)	Covariates	Comments	
Barbone et al. (1992) New Jersey, USA, before 1988	Nested case-control study*; 51 white men who developed lung tumours from the dye and resin manufacturing workers cohort ^a		Work history obtained from plant personal records; information on potential confounders obtained by interviews (subjects or next of kin) and plant medical records	Lung	Work area or building	21	OR	Cigarette smoking; outside employment was considered but was not a confounder	In a separate analysis considering possible exposure to asbestos (mainly a concern among pipe-cleaners), a non-significant association was observed with risk of lung cancer. Risk of lung cancer was also found to be associated with exposure to ECH when restricted to concentrations in the low cumulative exposure category. * 102 controls (2 per case) were matched on yr of birth, and employment status at the date of diagnosis (for living lung cancer cases), and who were not known to have died before the date of death or diagnosis of the case. [Overlaps with Delzell et al., 1989 ; Barbone et al., 1994 ; Sathiakumar & Delzell, 2000]	
					AQ dyes and ECH production > 10 or more yr since first employment		2.4 (1.1–5.2)			
					AQ dye and ECH area		4.6 (0.9–23)			
					AQ production		6			12 (1.4–99)
					AQ intermediate dye production		8			1.8 (0.6–5.1)
AQ dye synthesis	8	1.2 (0.5–2.9)								
AQ dye standardization	8	3.3 (1.0–11)								

Table 2.1 (continued)

Reference, study location and period	Total No. of subjects	Follow-up period	Exposure assessment	Organ site	Exposure categories	No. of cases/deaths	Relative risk (RR) (95%CI)	Covariates	Comments
Barbone et al. (1994) New Jersey, USA, before 1988	Nested case-control study*; 11 white men who developed CNS tumours from the dye and resin manufacturing workers cohort ^a	Work history obtained from plant personal records; information on potential confounders obtained by interviews (subjects or next of kin) and plant medical records	CNS	Work area, process, line, duty	AQ dyes	3	∞ (1.7–∞)	Unadjusted [cigarette smoking, outside employment, head radiation, head trauma, history of epilepsy and use of antiepileptic drugs were considered, but were not confounders]	All 3 cases in the AQ work area had an induction time of 20 yr or more. Routine exposure to ECH was also associated with CNS tumours (OR, 4.2; 95%CI: 0.7–26; 4 exposed cases); some of the exposed cases occurred among workers who worked in AQ intermediate dyes. * 44 controls (4 per case) matched by yr of birth and who had not died before the date of diagnosis or death for living cases. Matching criterion was employment as of date of diagnosis. [Overlaps with Delzell et al., 1989 ; Barbone et al., 1992 ; Sathiakumar & Delzell, 2000]
					intermediate dyes	3	∞ (1.7–∞)		
					Production	1	NR		
					Laboratory	1	0.3 (0.1–3.2)		
					Other AQ dyes	1	0.3 (0.1–3.9)		
					Production	1	1.0 (0.1–13)		
Laboratory	1	0.0 (0.0–4.6)							
Maintenance	0								

^a Findings for the early update of the cohort and effect estimates for workers in production areas other than anthraquinone dye area not included

AQ, anthraquinone; CI, confidence interval; CNS, central nervous system; ECH, epichlorohydrin; NR, not reported; OR, odds ratio; SMR, standardized mortality ratio; yr, year or years

Table 2.2 Selected raw materials or intermediates used in different processes associated with anthraquinone dyes

Processes or lines	Selected raw materials or intermediates
AQ production (South dyes)	Anthracene, vanadium pentoxide
AQ intermediate dyes (including AQ sulfonate, amino-AQ other substituted AQs)	AQ, sulfuric acid, mercury, AQ sulfonates, ammonia, arsenic acid, <i>m</i> -nitrobenzene sulfonic acid, methanol,
AQ dye synthesis	AQ intermediates, aniline, substituted anilines, benzene, nitrobenzene, chlorobenzene, chlorotoluenes, pyridine, alcohols, tetrachloroethylene
AQ dye standardization (final formulation – mixing, milling, drying)	Dye dusts, 2,4,5-trichlorophenol
Epichlorohydrin production	Allyl chloride, chloride lime

AQ, anthraquinone

From [Delzell *et al.* \(1989\)](#), [Sathiakumar & Delzell \(2000\)](#)

No data on exposure levels were available. [The Working Group noted that the major limitation of these studies was that they did not assess exposure to specific chemicals; risk estimates were calculated for employment in the various production areas or for different processes. These studies also had limited statistical power to detect effects for specific cancers because of the small numbers of exposed cases.] [Table 2.1](#) reports the findings (overall and those for employment in anthraquinone production areas) from the latest update of the cohort and the two nested case-control studies.

(b) Cohort study: 1986 follow-up

The initial retrospective cohort included all men (2642) who were employed at this plant for at least 6 months from 1 January 1952 (opening of the plant) until 1 January 1985 ([Delzell *et al.*, 1989](#)), and follow-up was from 1 July 1952 until 31 December 1985. Subjects were classified into work areas using work history information and standardized mortality ratios (SMRs) were calculated using national rates. Excesses of lung cancer and central nervous system tumours were found in certain subgroups of workers and the associations were evaluated in more detail in two nested case-control studies.

(c) Nested case-control study of cancer of the lung

A nested case-control analysis of lung cancer was conducted among the dye and resin workers ([Barbone *et al.*, 1992](#)). The cases comprised 51 (47 decedent and 4 living) male white workers who developed lung cancer before 1 October 1988. Two controls per case (102) were selected from the cohort, matched on year of birth and employment status at the date of diagnosis (for living lung cancer cases), and were not known to have died before the date of death or diagnosis of the case. Workers were assigned to one of the three production areas (see above) and processes within the production areas (processes that involved anthraquinone are described in [Table 2.2](#)), based on personnel records. Employees in each production area/process were also classified by duties — production, laboratory or maintenance — for each of the production areas. In addition to the production categories, workers could also be assigned to central laboratories and central maintenance for activities that were not carried out in one of the production areas or services. Cumulative potential exposure to epichlorohydrin and asbestos was calculated for each subject by multiplying each category of potential contact with epichlorohydrin by the number of years worked in that category, and then adding the findings for all categories. Information on

potential confounders was obtained from interviews, using a structured questionnaire, with study subjects or their next of kin, and from plant medical records. Subjects were also classified according to high-risk employment before and after working at the plant. Odds ratios (ORs) were calculated with and without adjustment for cigarette smoking (using detailed information on individuals) and employment in outside industries, but only smoking was found to be a confounder in certain analyses. When smoking was not found to be a confounder in the analyses, unadjusted odds ratios were reported.

Statistically significant (or borderline significant) elevated risks for lung cancer were found among workers in the anthraquinone and epichlorohydrin production area (OR, 2.4; 95% confidence interval [CI]: 1.1–5.2; 21 exposed cases, 24 exposed controls) and, within this area, for anthraquinone production (OR, 12; 95%CI: 1.4–99; six exposed cases, one exposed control), and anthraquinone dye standardization (OR, 3.3; 95%CI: 1.0–11; eight exposed cases, six exposed controls). The odds ratio among workers in the anthraquinone intermediate dye production process was 1.8 (95%CI: 0.6–5.1; eight exposed cases, 10 exposed controls). [The Working Group noted that none of the reported odds ratios was adjusted for smoking because the authors ruled it out as a confounder in their analyses.]

The smoking-adjusted odds ratios among workers with 10 or more years since first employment in the anthraquinone and epichlorohydrin production area was 4.6 (95%CI: 0.9–23). An excess of lung cancer was also found for employees in the epichlorohydrin production process who had worked in the anthraquinone production area (three exposed cases, no exposed controls). For all workers (in the entire plant and not just the anthraquinone production area), the odds ratio for potential exposure to epichlorohydrin was 1.7 (95%CI: 0.7–4.1; 12 exposed cases, 18 exposed controls). The risk was concentrated among individuals with low cumulative or short duration

of potential exposure to epichlorohydrin. [The Working Group noted that the increased risk for workers in the anthraquinone dye area was probably independent of the increased risk associated with exposure to epichlorohydrin because the later analysis included only three of the 21 cases observed among anthraquinone production workers.] Elevated odds ratios were also found for some other production areas or processes, but were not statistically significant.

(d) *Nested case–control study of tumours of the central nervous system*

The relationship between central nervous system tumours and exposure to epichlorohydrin was evaluated in greater detail in a nested case–control study ([Barbone *et al.*, 1994](#)). [The Working Group noted that some of the workers exposed to epichlorohydrin were also exposed to anthraquinone.] Cases included 11 (eight deceased and three living) white men who developed tumours of the central nervous system (seven astrocytomas and glioblastomas, two meningiomas and two other benign tumours) before 1988. For each case, four controls ($n = 44$) were matched on year of birth and employment status at the date of diagnosis (for living cases), and were not known to have died before the date of death or diagnosis of the case. Exposure was assessed as described above for lung cancer. Odds ratios were calculated with and without adjustment for cigarette smoking, outside employment, head radiation, head trauma, history of epilepsy and use of antiepileptic drugs. The author stated that none of these were found to be confounders, and thus unadjusted odds ratios were provided (see [Table 2.1](#)).

Statistically significant risks for central nervous system tumours were found among workers in the anthraquinone dye area; the associated odds ratios and the number of exposed cases with duties involving anthraquinone intermediate dyes and their production within this area were identical (OR, ∞ ; 95%CI: 1.7– ∞ ;

three exposed cases). The only other statistically significant odds ratio was for workers involved in the epoxy resin line in the P&A area; elevated but statistically non-significant risks were observed for maintenance and production activities in the azo dye production area. [Results for epichlorohydrin are presented here because some of the workers exposed to epichlorohydrin were also exposed to anthraquinone.] Detailed analyses of routine exposure to epichlorohydrin found a statistically significant odds ratio for routine potential exposure (OR, 4.2; 95%CI: 0.7–26; four exposed cases) and acute exposure (OR, ∞; 95%CI: 1.5–∞; three exposed cases), and positive associations (not statistically significant) with ‘cumulative potential exposure’ ($P_{\text{trend}} = 0.11$), and ‘duration of routine potential exposure’ ($P_{\text{trend}} = 0.11$). Potential exposure to epichlorohydrin primarily occurred in the epoxy plastic and additives division of the P&A production area. [The Working Group noted that three of the four epichlorohydrin-exposed cases worked in either the anthraquinone intermediate dye or azo dye areas.]

(e) *Cohort study: 1996 follow-up*

The cohort was later expanded and updated to include all (3266) workers (men and women) employed for at least 6 months from 1 January 1952 until 1 January 1996 who were followed until 1 January 1996 (Sathiakumar & Delzell, 2000). The average length of follow-up was 27 years. Local rates were used to calculate SMRs. In addition, Poisson regression analysis was used to estimate the lung cancer risk for subjects in a particular area using subjects who had never worked in the area as the comparison group and adjusting for potential confounding by age, calendar period and employment in other high-risk areas.

Mortality from all causes was significantly decreased (SMR, 0.90; 95%CI: 0.83–0.97; 728 observed deaths). Mortality in the entire cohort was elevated, but not statistically significantly, for several cancers including lymphosarcoma

and cancer of the colon, lung, liver, genital tissue, bladder and the central nervous system. A statistically significantly increased risk of mortality from lung cancer was found among workers in the anthraquinone production (South dyes) area (SMR, 1.68; 95%CI: 1.15–2.37; relative risk for ever versus never exposure, 1.7; 95%CI: 1.1–2.6; 32 exposed cases for both analyses). Both external (SMR) and internal (Poisson regression) analyses by time since first employment and duration of exposure among hourly paid white men showed similar results, with slightly higher risks among longer-term workers than shorter-term workers for those with less than 20 years since first employment; this effect was not observed among workers with more than 20 years since first employment. Mortality was highest among workers with 20 or more years since first employment and duration of employment less than 5 years (see Table 2.1). [The lack of a clear exposure–response relationship could be because length of employment is a poor surrogate for exposure to a carcinogenic substance.]

3. Cancer in Experimental Animals

Carcinogenicity studies of oral administration of anthraquinone to mice and rats have been conducted by the National Toxicology Program (NTP, 2005), the results of which are summarized in Table 3.1.

3.1 Oral administration

3.1.1 Mouse

Groups of 50 male and 50 female B6C3F₁ mice were fed diets containing 0, 833, 2500 or 7500 ppm anthraquinone (equivalent to average daily doses of approximately 90, 265 or 825 and 80, 235 or 745 mg/kg body weight (bw) for males and females, respectively) for 105 weeks. The incidence of hepatocellular adenoma, carcinoma,

Table 3.1 Carcinogenicity studies of oral administration of anthraquinone in the diet to rats and mice

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F ₁ (M) 2 yr	Oral (feed) 0, 833, 2 500 or 7 500 ppm 50 animals/group	Liver (hepatocellular adenoma): 21/50, 32/50, 38/50, 41/49	<i>P</i> = 0.011 (833 ppm) <i>P</i> < 0.001 (2500 ppm) <i>P</i> < 0.001 (7500 ppm) <i>P</i> < 0.001 (trend)	Historical incidence for 2-yr feed studies with untreated control groups (mean ± standard deviation): 440/850 (51.8 ± 8.3%); range, 40–68%
		Liver (hepatocellular carcinoma): 8/50, 13/50, 17/50, 21/49	<i>P</i> = 0.026 (2500 ppm) <i>P</i> < 0.001 (7500 ppm) <i>P</i> < 0.001 (trend)	
		Liver (hepatocellular adenoma or carcinoma): 25/50, 34/50, 41/50, 46/49	<i>P</i> = 0.043 (883 ppm) <i>P</i> < 0.001 (2500 ppm) <i>P</i> < 0.001 (7500 ppm) <i>P</i> < 0.001 (trend)	
		Liver (hepatoblastoma): 1/50, 6/50, 11/50, 37/49	<i>P</i> = 0.053 (833 ppm) <i>P</i> = 0.002 (2500 ppm) <i>P</i> < 0.001 (7500 ppm) <i>P</i> < 0.001 (trend)	
		Liver (hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma): 26/50, 35/50, 43/50, 48/49	<i>P</i> = 0.045 (833 ppm) <i>P</i> < 0.001 (2500 ppm) <i>P</i> < 0.001 (7500 ppm) <i>P</i> < 0.001 (trend)	

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments	
Mouse, B6C3F ₁ (F) 2 yr	Oral (feed) 0, 833, 2 500 or 7 500 ppm 50 animals/group	Liver (hepatocellular adenoma): 6/49, 28/50, 27/50, 40/49	<i>P</i> < 0.001 (833 ppm) <i>P</i> < 0.001 (2500 ppm) <i>P</i> < 0.001 (7500 ppm) <i>P</i> < 0.001 (trend)	Historical incidence for 2-yr feed studies with untreated control groups (mean ± standard deviation): 273/852 (32.0 ± 9.6%); range, 18–56%	
		Liver (hepatocellular carcinoma): 2/49, 3/50, 8/50, 8/49	<i>P</i> = 0.051 (2500 ppm) <i>P</i> = 0.048 (7500 ppm) <i>P</i> = 0.031 (trend)		
		Liver (hepatocellular adenoma or carcinoma): 6/49, 30/50, 30/50, 41/49	<i>P</i> < 0.001 (833 ppm) <i>P</i> < 0.001 (2500 ppm) <i>P</i> < 0.001 (7500 ppm) <i>P</i> < 0.001 (trend)		
		Liver (hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma [including multiple]) (overall rate): 6/49, 30/50, 30/50, 41/49	<i>P</i> < 0.001 (833 ppm) <i>P</i> < 0.001 (2500 ppm) <i>P</i> < 0.001 (7500 ppm) <i>P</i> < 0.001 (trend)		
		Thyroid gland (follicular-cell carcinoma): 0/45, 0/48, 0/48, 2/48	<i>P</i> = 0.042 (trend)		Historical incidence for 2-yr feed studies with untreated control groups (mean ± standard deviation): 2/847 (0.2 ± 0.7%); range, 0–2%
		Thyroid gland (follicular-cell adenoma or carcinoma): 1/45, 1/48, 2/48, 4/48	NS (2500 ppm) NS (7500 ppm) <i>P</i> = 0.078 (trend)		Historical incidence for 2-yr feed studies with untreated control groups (mean ± standard deviation): 15/847 (1.8 ± 1.7%); range, 0–6%

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 /N (M) 2 yr	Oral (feed) 0, 469, 938, 1 875 or 3 750 ppm 50–60 animals/group	Kidney (renal tubule adenoma): 1/50, 3/50, 9/50, 5/50, 3/50	$P = 0.010$ (938 ppm) $P = 0.474$ (trend)	Historical incidence for 2-yr feed studies with untreated control groups (mean \pm standard deviation): 7/902 (0.8 \pm 1.2%); range, 0–4% Historical incidence for 2-yr feed studies with untreated control groups (mean \pm standard deviation): 2/891 (0.2 \pm 0.7%); range, 0–2%
		Urinary bladder (transitional epithelial papilloma): 0/50, 1/50, 3/50, 7/50, 3/49	$P = 0.011$ (1875 ppm) $P = 0.053$ (trend)	
Rat, F344/N (F) 2 yr	Oral (feed) 0, 469, 938, 1 875 or 3 750 ppm 50–60 animals/group	Kidney (renal tubule adenoma): 0/50, 4/50, 9/50, 7/50, 12/49	$P = 0.002$ (938 ppm) $P = 0.011$ (1875 ppm) $P = 0.001$ (3750 ppm) $P = 0.001$ (trend)	Historical incidence for 2-yr feed studies with untreated control groups (mean \pm standard deviation): 1/901 (0.1 \pm 0.5%); range, 0–2%
		Kidney (renal tubule adenoma or carcinoma): 0/50, 6/50, 9/50, 8/50, 14/49	$P = 0.020$ (469 ppm) $P = 0.002$ (938 ppm) $P = 0.006$ (1875 ppm) $P < 0.001$ (3750 ppm) $P < 0.001$ (trend)	
		Urinary bladder (transitional epithelial papilloma or carcinoma): 0/49, 0/49, 0/49, 1/50, 2/49	$P = 0.037$ (3750 ppm) $P = 0.037$ (trend)	Historical incidence for 2-yr feed studies with untreated control groups (mean \pm standard deviation): 2/891 (0.2 \pm 0.7%); range, 0–2%
		Liver (hepatocellular adenoma): 0/50, 2/50, 6/50, 4/50, 3/49	$P < 0.05$ (938 ppm)	Historical incidence for 2-yr feed studies with untreated control groups (mean \pm standard deviation): 4/901 (0.4 \pm 1.1%); range, 0–4%

F, female; M, male; NS, not significant; yr, year or years

From [NTP \(2005\)](#)

and adenoma or carcinoma (combined) was increased with a positive trend in both males and females, and was increased in all groups exposed to 2500 ppm or more. The incidence of hepatoblastoma was statistically significant increased in males exposed to 2500 or 7500 ppm, and one hepatoblastoma occurred in a 7500-ppm female. Thyroid follicular-cell adenomas were present in all groups of females; moreover, two 7500-ppm females developed follicular-cell carcinomas. The incidence of follicular-cell carcinoma and adenoma or carcinoma (combined) in 7500-ppm females exceeded the historical control ranges ([NTP, 2005](#)).

3.1.2 Rat

Groups of 50 male and 50 female F344/N rats were fed diets containing 469, 938 or 1875 ppm anthraquinone for 105 weeks. Further groups of 60 males and 60 females received 0 or 3750 ppm anthraquinone for the same period. These dietary concentrations resulted in average daily doses of approximately 20, 45, 90 and 180 and 25, 50, 100 and 200 mg/kg bw anthraquinone for males and females in the 469-, 938-, 1875- and 3750-ppm groups, respectively. At 2 years, animals in the highest-dose group weighed less than those in the control group. Positive trends in the incidence of renal tubule adenoma and of renal tubule adenoma or carcinoma (combined) in females were observed, and the incidence of adenoma or carcinoma (combined) in all groups of exposed females was significantly increased. Renal tubule carcinomas developed in two 469-ppm, one 1875-ppm and two 3750-ppm females. The incidence of renal tubule adenoma in the 938-ppm males was significantly greater than that in the controls, and that in all exposed groups of males exceeded the historical control range; no renal tubule carcinomas were observed in male rats. Papillomas of the transitional epithelium of the kidney were observed in two males exposed to 938 ppm and one male exposed to 3750 ppm; none occurred in

females. At least one male in each exposed group had a transitional epithelial papilloma of the urinary bladder; the incidence in the 1875-ppm group was significantly greater than that in the controls, and that in groups exposed to 938 ppm or more exceeded the historical control range. A positive trend in the incidence of papilloma or carcinoma (combined) was found in females. The incidence of hepatocellular adenoma in the 938-ppm females was significantly greater than that in controls, and that in females exposed to 938 ppm or more exceeded the historical control range ([NTP, 2005](#)).

[The Working Group noted that tumours of the kidney and urinary bladder, and hepatoblastomas are rare spontaneous neoplasms in experimental animals.]

4. Other Relevant Data

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 male F344 rats, [¹⁴C]anthraquinone was well absorbed (> 99.8% of the administered dose) from the gastrointestinal tract following oral doses ranging from 0.35 to 350 mg/kg bw. Following absorption, anthraquinone was distributed to various tissues, with highest concentrations in the adipose tissue. It was not bioaccumulated in any particular tissue and, by 96 hours after administration, more than 95% of the dose had been metabolized and eliminated in the bile, faeces and urine. 2-Hydroxyanthraquinone was the major metabolite detected in the urine of rats exposed to anthraquinone intravenously or orally ([NTP, 2005](#)). In earlier studies,

1-hydroxy- and 2-hydroxyanthraquinone were identified in the urine of rats that received daily oral doses of anthraquinone (Sato *et al.*, 1956), and 1-hydroxyanthraquinone induced tumours of the liver, stomach and large intestine in rats (Mori *et al.*, 1990). The presence of the sulfate and glucuronide conjugates — 2-hydroxy-9,10-anthraquinone, 8,10-dihydroxyanthracene and 2,9,10-trihydroxyanthracene — in rat urine samples after 4 days of dietary administration of 9,10-anthraquinone has been reported (Sims, 1964).

Single-dose toxicokinetic studies have been conducted in F344/N rats and B6C3F₁ mice (NTP, 2005). Toxicokinetic parameters were derived but no modelling of plasma concentration-time profiles to a best fit curve was conducted. Intravenous administration showed biphasic curves in both male and female rats that are best described as a two-compartment open model, and the half-life for plasma anthraquinone was 10–12 hours. Oral administration by gavage also gave results that best fit a two-compartment model. Maximum concentration values were proportional to dose and were observed at 8–18 hours. The half-life could not be estimated reliably at higher doses. The areas under the curve were also proportional to dose. In mice, similar two-compartment behaviour was estimated with a half-life in plasma of 4 hours following intravenous administration. Following oral administration to mice, the half life was 4–6 hours and the areas under the curve were proportional with dose.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

The genetic toxicology of anthraquinone has been reviewed (Butterworth *et al.*, 2001, 2004; NTP, 2005). The data on the genotoxicity of anthraquinone derived from different bacterial systems are conflicting and inconclusive with regard to its in-vitro mutagenic potential. In particular, positive and negative results have been reported for anthraquinone in *Salmonella* mutation assays. Early studies reported that neither anthraquinone nor its metabolites were genotoxic in *Salmonella* bacterial mutagenicity assays (Brown & Brown, 1976; Gibson *et al.*, 1978; Salamone *et al.*, 1979). In later studies, anthraquinone was found to be mutagenic in the *Salmonella* mutagenicity assay in the absence of metabolic activation (Lieberman *et al.*, 1982; Zeiger *et al.*, 1988). Moreover, none of the negative bacterial studies reported purity (NTP, 2005). [The contradictory results for anthraquinone may be attributable to variable amounts of contaminants resulting from the different production methods or testing methods.] It has been suggested that the carcinogenicity of anthraquinone might be due solely to the presence of 9-nitroanthracene, which was present as a contaminant at a level of approximately 0.1% in the anthraquinone sample (Butterworth *et al.*, 2001, 2004), and was found in the NTP report to be a weak mutagen (NTP, 2005). It was also noted that the mutagens claimed to be other impurities in the test material were point-of-contact carcinogens and that forestomach tumours had not been reported in the 2-year bioassay. The amount of 9-nitroanthracene in the anthraquinone given in the 2-year bioassay was reported to be 0.09% w/w, and the two other contaminants (anthrone and phenanthrene) were present at levels of 0.05% and 0.008% and were also negative or mainly negative in mutagenicity assays (NTP, 2005). These three compounds were the major contaminants identified and it was concluded that they were non-mutagenic or weakly mutagenic (NTP, 2005).

Two rat metabolites of anthraquinone — 1-hydroxy- and 2-hydroxyanthraquinone — were examined for mutagenicity in the *Salmonella* assay ([Tikkanen et al., 1983](#); [Butterworth et al., 2004](#)). Anthrone and 2-hydroxyanthraquinone were reported to be weak mutagens in *S. typhimurium* ([NTP, 2005](#)). Anthrone was also reported to give negative results in several other studies. 2-Hydroxyanthraquinone was negative in TA98 but gave positive results in TA100 in the presence of metabolic activation ([Tikkanen et al., 1983](#); [Butterworth et al., 2004](#)). 1-Hydroxyanthraquinone was negative in the absence of and positive in the presence of metabolic activation in TA1537 ([Butterworth et al., 2004](#)). In another study, 1-hydroxyanthraquinone was negative in TA98 and TA100, whereas 2-hydroxyanthraquinone was positive in TA98 with and without metabolic activation and negative in TA100 ([NTP, 2005](#)).

[NTP \(2005\)](#) indicated that 2-hydroxyanthraquinone is a more potent mutagen in *S. typhimurium* TA98 than 9-nitroanthracene. Based on the amount of 2-hydroxyanthraquinone eliminated in the rat urine and the maximal amount of 9-nitroanthracene to which rats might have been exposed as a consequence of its presence as a contaminant (0.1%) in the anthraquinone sample, it was concluded that the level of 2-hydroxyanthraquinone present in exposed rats was at least 5.8-fold that of 9-nitroanthracene. Because anthraquinone is metabolized to at least one mutagenic metabolite with greater mutagenic potency than 9-nitroanthracene, [NTP \(2005\)](#) concluded that the carcinogenic activity of anthraquinone may occur via a mutagenic mechanism regardless of the presence of the contaminant.

[NTP \(2005\)](#) reported the in-vivo induction of strand breaks in the liver and kidney cells of CD-1 mice treated intraperitoneally with 250 mg/kg bw anthraquinone ([Cesarone et al., 1982](#)) and dose-related increases in micronuclei in Syrian hamster embryo cells treated

with 3.13–25 µg anthraquinone (99% pure)/ml ([Gibson et al., 1997](#)).

In the rodent hepatocyte DNA repair assay, 1-hydroxyanthraquinone gave positive results ([Mori, 1989](#)).

In the [NTP \(2005\)](#) study, male and female mice fed anthraquinone (99.8% pure) in the diet at concentrations of 135–2350 mg/kg bw per day (1875–30 000 ppm) for 14 weeks showed significant increases in the frequency of micronucleated normochromatic erythrocytes in the peripheral blood compared with controls. In an acute bone-marrow micronucleus test performed with male mice given 500–2000 mg/kg bw anthraquinone by intraperitoneal injection, micronucleated red blood cells were slightly decreased in a dose-related manner. [Butterworth et al. \(2001\)](#) reported no significant increase in micronuclei in bone-marrow polychromatic erythrocytes of mice administered anthraquinone by gavage at concentrations of 1250, 2500 and 5000 mg/kg bw.

In the Syrian hamster embryo cell transformation assay, anthraquinone gave negative results ([Kerckaert et al., 1996](#)). Anthraquinone did not induce an increased incidence of mutations in the presence or absence of metabolic activation at the *Tk^{+/+}* locus in L5178Y mouse lymphoma cells ([Butterworth et al., 2001](#)). No significant increase in cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in Chinese hamster ovary cells incubated with anthraquinone (up to 50.0 µg/mL) ([Butterworth et al., 2001](#)).

Anthraquinone was not found to be mutagenic in h1A1v2 human B-lymphoblastoid cells that constitutively express cytochrome P450 (CYP)1A1 ([Durant et al., 1996](#)).

4.3 Mechanistic considerations

In subchronic studies, histological lesions were observed in the kidney, liver, spleen, bone marrow and thyroid glands of male and female

rats and the urinary bladder of female rats. Variably sized eosinophilic hyaline droplets occurred within the renal tubules in treated males and females. α_2 -Globulin was increased to the same degree in all treated male rats. The incidence of hyperplasia of the transitional epithelium of the renal pelvis was increased in all exposure groups in 2-year feed studies. In the 14-week study, the liver weights of all exposed male and female mice were significantly greater than those of the controls. Anthraquinone-treated mice showed centrilobular hypertrophy of hepatocytes with enlarged nuclei (NTP, 2005). Centrilobular hypertrophy of liver cells was also pronounced in 2-year studies of exposure to anthraquinone, but information regarding subcellular structural changes was lacking.

A 32-day feeding study of anthraquinone in F344/N rats (NTP, 2005) investigated CYP activity in the liver, 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 2'-deoxyguanosine concentrations in the liver and kidney and cell proliferation in the liver, kidney and urinary bladder. At day 8, CYP1A1 and CYP2B1 were increased in male and female liver and were treatment-related and treatment- and dose-related, respectively. At the same time-point, 8-OHdG and 2'-deoxyguanosine were both slightly decreased in male and female kidney but variable results were found in the liver. Cell proliferation in males and females was increased in the bladder but not in the kidney. At the end of the 32-day study, males and females both had increased levels of hyaline droplets. However, the nephropathy reported in males was not consistent with chronic progressive nephropathy.

The induction of certain drug-metabolizing enzymes in the liver and small intestine of rats was investigated following intragastric administration of 100 mg/kg anthraquinone for 3 days (Longo *et al.*, 2000). In the liver, anthraquinone induced both CYP1A2 and CYP2B, but not CYP1A1. The mechanism by which hepatic enzymes are induced by anthraquinone and

some related compounds is not known, but it did not induce CYP1A2 or CYP2B in the small intestine mucosa of rats (Longo *et al.*, 2000).

5. Summary of Data Reported

5.1 Exposure data

Anthraquinone is widely used as an intermediate for the manufacture of dyes for fibres and textiles. It is also used as a bird repellent and as an additive in the pulp and paper industry. Workers may be exposed by inhalation of dust or by dermal contact. Anthraquinone may be formed from direct combustion processes in motor vehicle engines or as a result of degradation of polycyclic aromatic hydrocarbons by atmospheric oxidation. It is ubiquitous in the environment, where it is released via wastewater streams during its production and use, and has been detected in air, water (including surface, ground- and drinking-water), soil, plants, fish and animal tissues.

5.2 Human carcinogenicity data

The Working Group identified a series of publications on dye and resin workers in a single facility in the USA who were potentially exposed to anthraquinone during its production or its use to produce anthraquinone intermediates. These publications reported on findings from the initial cohort, nested case-control analyses of lung cancer and central nervous system tumours, and updated findings on an expanded cohort. An excess risk of mortality from lung cancer was found among workers employed in the anthraquinone dye production area in both nested case-control and cohort analyses. Workers in this production area were potentially exposed to anthraquinone, anthraquinone dye intermediates, anthracene, vanadium pentoxide

and epichlorohydrin. Within the anthraquinone dye production area, risk for lung cancer was increased 12-fold for workers producing anthraquinone itself, but this was based on only a few exposed cases. The increased risk did not appear to be due to cigarette smoking, or exposure to asbestos or epichlorohydrin. An excess incidence of central nervous system tumours was also found among workers employed in the anthraquinone dye production area, but this was based on only three exposed cases who may also have been exposed to epichlorohydrin, which was also associated with an increased risk of these tumours. The major limitations of these studies were that: (1) risk estimates were calculated for men employed in anthraquinone and anthraquinone dye production, but exposure to anthraquinone per se was not evaluated; (2) the statistical power to detect effects for specific cancers was limited because of small numbers of exposed cases; and (3) the ability to evaluate potential confounding from other occupational exposures was also limited.

5.3 Animal carcinogenicity data

In a 2-year study, anthraquinone caused an increased incidence of benign or malignant neoplasms in mice and rats. In mice, treatment-related hepatoblastomas occurred in males, and hepatocellular adenomas and carcinomas in males and females. Treatment-related tumour formation in male rats included renal tubule adenoma of the kidney and transitional epithelial papillomas of the urinary bladder. Treatment-related tumours in female rats included renal tubule adenomas and carcinomas combined, transitional epithelial-cell papillomas and carcinomas of the urinary bladder combined, and hepatocellular adenomas.

Tumours of the kidney and urinary bladder, and hepatoblastomas are rare spontaneous neoplasms in experimental animals.

5.4 Other relevant data

While no data were available on the metabolism of anthraquinone in humans, many studies have investigated its metabolism in rodents. Anthraquinone is almost completely absorbed after oral administration and is distributed systemically. There is no evidence of its bioaccumulation. 1- and 2-Hydroxyanthraquinones are the metabolites formed in rodents that are relevant to the mechanistic considerations of the parent compound.

Anthraquinone itself gave conflicting results in bacterial mutagenesis and other genotoxicity studies. 2-Hydroxyanthraquinone, the major urinary metabolite of anthraquinone, is mutagenic. Furthermore, 1-hydroxyanthraquinone has been shown to be carcinogenic in rats. Other mechanisms by which anthraquinone causes cancer are not well established. Increased cell proliferation and cytotoxicity have been observed in the urinary tract and kidneys in treated male and female rats. Although accumulation of hyaline droplets consistent with 2u-globulin nephropathy has been observed in the kidney of treated male rats, a similar phenotype, albeit weaker in severity, has also been observed in females.

There is moderate evidence that genotoxicity may play a role in the mechanism of action for anthraquinone-induced cancer. The relevance of the tumour response in experimental animals to humans cannot be excluded.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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1-AMINO-2,4-DIBROMOANTHRAQUINONE

1. Exposure Data

1.1 Chemical and physical data

From [NTP \(1996, 2002\)](#) and [HSDB \(2010\)](#)

1.1.1 Nomenclature

Chem. Abstr. Services Reg. No.: 81-49-2

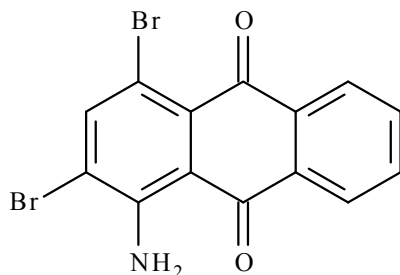
Chem. Abstr. Name: 1-Amino-2,4-dibromo-9,10-anthracenedione

Synonyms: ADBAQ; 2-amino-4-chloro-5-nitrophenol; 1-amino-2,4-dibromoanthra-9,10-quinone; 1-amino-2,4-dibromo-9,10-anthraquinone; 9,10-anthracenedione, 1-amino-2,4-dibromo-; anthraquinone, 1-amino-2,4-dibromo-; dibromoaminoanthraquinone; 2,4-dibromo-1-anthraquinonylamine

RTECS No.: CB5500000

EINECS No.: 201-354-0

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{14}H_7Br_2NO_2$

Relative molecular mass: 381.04

1.1.3 Chemical and physical properties of the pure substance

Description: Odourless reddish brown to orange powder

Melting-point: 226 °C

Vapour pressure: 1.4×10^{-9} mm Hg at 25°C (estimated)

Solubility: 0.015 mg/L in water at 25 °C, < 1 mg/mL in acetone at 23 °C, 1–10 mg/mL in dimethyl sulfoxide at 23 °C and << 1mg/mL in toluene at 23 °C

Flash-point: > 200 °C

Octanol/water partition coefficient: $\log K_{ow}$, 5.31 (estimated)

Henry's law constant: 1.78×10^{-13} atm.m³/mol at 25 °C (estimated)

1.1.4 Technical products and impurities

No data were available to the Working Group.

1.1.5 Analysis

1-Amino-2,4-dibromoanthraquinone has been analysed in various samples by gas chromatography/flame ionization detection and gas chromatography/mass spectrometry ([Games & Hites, 1977](#)).

1.2 Production and use

1.2.1 Production

1-Amino-2,4-dibromoanthraquinone is prepared from 1-aminoanthraquinone by bromination in dilute mineral acids ([HSDB, 2010](#)).

According to the non-confidential information on production volumes submitted to the United States Environmental Protection Agency by companies for chemicals under the 1986–2002 inventory update rule, production in the United States of America ranged between 10 and 500 thousand pounds in 1986 ([HSDB, 2010](#)).

1.2.2 Use

1-Amino-2,4-dibromoanthraquinone is an anthraquinone vat dye that is used as a dye or dye intermediate in the textile industry. Vat dyes are a class of water-insoluble dyes that can easily be reduced to a water-soluble and usually colourless leuco form that can readily impregnate fibres and textiles — typically cotton, wool and cellulose acetate. Their principal properties are brightness and good fastness ([NTP, 1996, 2002](#)). No data were available to the Working Group regarding specific uses of 1-amino-2,4-dibromoanthraquinone.

1.3 Occurrence

1.3.1 Natural occurrence

1-Amino-2,4-dibromoanthraquinone is not known to occur in nature.

1.3.2 Occupational exposure

Occupational exposure by inhalation of dust or by dermal contact to 1-amino-2,4-dibromoanthraquinone can occur during its production and its use as a chemical intermediate in the manufacture of dyes. No specific data were available to the Working Group on occupational exposure to 1-amino-2,4-dibromoanthraquinone.

1.3.3 Environmental occurrence

During its production and use, 1-amino-2,4-dibromoanthraquinone may be released to the environment (e.g., ambient air, water and soil) *via* wastewater streams. 1-Amino-2,4-dibromoanthraquinone was found at concentrations of 92–170 ppb in several samples of raw wastewater from a dye manufacturing plant, but was not detected in the final effluent ([Games & Hites, 1977](#)).

[HSDB \(2010\)](#) reviewed information on and or calculated parameters related to the environmental fate of 1-amino-2,4-dibromoanthraquinone in ambient air, water and soil. It is expected to exist only in the particle phase, but may be susceptible to photolysis by sunlight and particulates in the atmosphere may be removed by wet or dry deposition.

In aquatic environments, 1-amino-2,4-dibromoanthraquinone is expected to adsorb onto suspended solids and sediments and to be immobile. Although it is not clear how 1-amino-2,4-dibromoanthraquinone is removed from the soil or water, its volatilization from water, moist soil surfaces and dry soils and hydrolysis in aquatic environments are unlikely. No data were available to the Working Group on the biodegradation of 1-amino-2,4-dibromoanthraquinone, but its potential for bioconcentration in aquatic environments is high, and its estimated bioconcentration factor in fish is 380 (reviewed by [HSDB, 2010](#)).

1.4 Regulations and guidelines

No data were available to the Working Group.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

Carcinogenicity studies of oral administration of 1-amino-2,4-dibromoanthraquinone to mice and rats in the diet have been conducted ([NTP, 1996](#)), the results of which are summarized in [Table 3.1](#) and [Table 3.2](#).

3.1 Oral administration

3.1.1 Mouse

See [Table 3.1](#)

Groups of 60 male and 60 female B6C3F₁ mice were fed 0, 10 000 or 20 000 ppm 1-amino-2,4-dibromoanthraquinone in the diet for 104 weeks. The average daily consumption was approximately 1690 or 3470 and 1950 or 4350 mg/kg bw 1-amino-2,4-dibromoanthraquinone for males and females, respectively. Ten animals from each group were evaluated histopathologically at 15 months. The incidence of hepatocellular adenoma, and adenoma or carcinoma (combined) was increased in the high-dose groups of males and females at the 15-month interim evaluation. At 2 years, statistically significant increases in the incidence of hepatocellular adenoma and carcinoma in males and females and in that of hepatoblastoma in high-dose males were observed. Squamous-cell papilloma of the forestomach occurred in 10 000-ppm females and 20 000-ppm males and females at the 15-month interim evaluation, and the incidence of squamous-cell papilloma and carcinoma was statistically significantly increased in all treated groups at 2 years. Alveolar/bronchiolar adenomas developed in all treated groups at 15 months, and the incidence of alveolar/bronchiolar adenoma was statistically significantly increased in all exposed groups at 2 years ([NTP, 1996](#)).

3.1.2 Rat

(a) Continuous exposure

See [Table 3.1](#)

Groups of 70 male and 70 female F344/N rats were fed 0, 5000 or 10 000 ppm 1-amino-2,4-dibromoanthraquinone in the diet for 103 weeks. Further groups of 50 males and 50 females were fed 2000 ppm 1-amino-2,4-dibromoanthraquinone for 104 weeks. These dietary concentrations were approximately equal to daily doses of 90, 240 or 490 and 110, 285 or 600 mg/kg body weight (bw) 1-amino-2,4-dibromoanthraquinone for males and females in the 2000-, 5000- and 10 000-groups, respectively. Ten animals from each group were evaluated histopathologically at 9 months. Additional groups of 10 animals from the 0- and 10 000-ppm groups were also evaluated histopathologically at 15 months. At the 15-month interim evaluation, hepatocellular adenoma or carcinoma (combined) occurred in 10/10 males and 9/10 females in the 10 000-ppm group. By the end of the 2-year study, the incidence of hepatocellular adenoma or hepatocellular carcinoma, or hepatocellular cholangiocarcinoma was increased in males and females in the 5000- and 10 000-ppm groups. In the 2000 ppm groups, that of hepatocellular adenoma or carcinoma (combined) was also increased in males and females. Adenomatous polyps (adenoma) of the large intestine (rectum) were found in 6/10 males and 2/10 females in the 10 000 ppm groups at the 15-month interim evaluation, and the incidence of adenomatous polyp (adenoma) or carcinoma of the large intestine (colon and rectum) was significantly increased in all treated groups after 2 years. In the kidney, the incidence of renal tubule adenoma or carcinoma (combined) was significantly increased in all treated groups after 2 years. The incidence of transitional-cell papilloma and carcinoma of the urinary bladder was increased at 2 years in males in the 10 000-ppm group, and in females in the 5000-ppm and 10 000-ppm groups ([NTP, 1996](#)).

Table 3.1 Carcinogenicity studies of oral administration of 1-amino-2,4-dibromoanthraquinone in the diet to rats and mice: effects after the 15-month interim evaluation and 2 years

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumours at the end of 2-year exposure (103–104 weeks)	Significance (logistic regression test)	Tumours at interim evaluations (significance by the Fisher exact test)	Comments
Mouse, B6C3F ₁ (M) 2 yr	0, 10 000, or 20 000 ppm [approximately 0, 1690 or 3470 mg/kg bw/d] 60 animals/group	Liver (hepatocellular adenoma): 10/50, 38/51, 39/50	<i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (20 000 ppm) <i>P</i> < 0.001 (trend)	Groups at 15-mo evaluation (0, 10 000, 20 000 ppm): Liver (hepatocellular adenoma): 0/10, 2/9, 4/10 ^b	Historical incidence for 2-yr feed studies with untreated control groups (mean ± SD): 531/1466 (36.2 ± 14.1%); range, 10–68%
		Liver (hepatocellular carcinoma): 9/50, 18/51, 21/50	<i>P</i> = 0.017 (10 000 ppm) <i>P</i> = 0.003 (20 000 ppm) <i>P</i> = 0.002 (trend)		
		Liver (hepatoblastoma): 0/50, 3/51, 5/50	<i>P</i> < 0.05 (20 000 ppm) (Fisher exact test)	Groups at 15-mo evaluation (0, 10 000, 20 000 ppm): Liver (hepatocellular adenoma or carcinoma): 0/10, 3/9, 4/10 ^b	
		Liver (hepatocellular adenoma or carcinoma): 18/50, 43/51, 42/50	<i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (20 000 ppm) <i>P</i> < 0.001 (trend)		
		Forestomach (squamous-cell papilloma): 0/50, 13/51, 16/50	<i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (20 000 ppm) <i>P</i> < 0.001 (trend)		
		Forestomach (squamous-cell carcinoma): 0/50, 12/51, 13/50	<i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (20 000 ppm) <i>P</i> < 0.001 (trend)	Historical incidence for 2-yr feed studies with untreated control groups (mean ± SD): 22/1474 (1.5 ± 2.0%); range, 0–6%	
		Forestomach (squamous-cell papilloma or carcinoma): 0/50, 19/51, 27/50	<i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (20 000 ppm) <i>P</i> < 0.001 (trend)		
Lung (alveolar/bronchiolar adenoma): 7/50, 26/51, 24/50	<i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (20 000 ppm) <i>P</i> < 0.001 (trend)	Groups at 15-mo evaluation (0, 10 000, 20 000 ppm): Lung (alveolar/bronchiolar adenoma): 0/10, 3/9, 5/10 ^b			

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumours at the end of 2-year exposure (103–104 weeks)	Significance (logistic regression test)	Tumours at interim evaluations (significance by the Fisher exact test)	Comments
Mouse, B6C3F ₁ (M) (contd)		Lung (alveolar/bronchiolar carcinoma): 3/50 (6%), 4/51 (8%), 1/50 (2%)			
		Lung (alveolar/bronchiolar adenoma or carcinoma): 10/50, 28/51, 25/50	<i>P</i> < 0.001 (10 000 ppm) <i>P</i> = 0.002 (20 000 ppm) <i>P</i> < 0.001 (trend)		Historical incidence for 2-yr feed studies with untreated control groups (mean ± SD): 265/1469 (18.0 ± 7.6%); range, 4–32%
Mouse, B6C3F ₁ (F) 2 yr	0, 10 000 or 20 000 ppm [approximately 0, 1950 or 4350 mg/kg bw/d] 60 animals/group	Liver (hepatocellular adenoma): 6/50, 45/50, 49/50	<i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (20 000 ppm) <i>P</i> < 0.001 (trend)	Groups at 15-mo evaluation (0, 10 000, 20 000 ppm): Liver (hepatocellular adenoma): 0/10, 2/10, 7/10 ^a	
		Liver (hepatocellular carcinoma): 0/50, 23/50, 27/50	<i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (20 000 ppm) <i>P</i> < 0.001 (trend)		
		Liver (hepatocellular adenoma or carcinoma): 6/50, 46/50, 50/50	<i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (20 000 ppm) <i>P</i> < 0.001 (trend)	Groups at 15-mo evaluation (0, 10 000, 20 000 ppm): Liver (hepatocellular adenoma or carcinoma): 0/10, 2/10, 8/10 ^a	Historical incidence for 2-yr feed studies with untreated control groups (mean ± SD): 247/1462 (16.9 ± 10.7%); range, 3–42%
		Forestomach (squamous-cell papilloma): 2/50, 16/50, 27/50	<i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (20 000 ppm) <i>P</i> < 0.001 (trend)	Groups at 15-mo evaluation (0, 10 000, 20 000 ppm): Forestomach (squamous-cell papilloma): 0/10, 4/10 ^b , 2/10	
		Forestomach (squamous-cell carcinoma): 0/50, 12/50, 11/50	<i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (20 000 ppm) <i>P</i> = 0.002 (trend)		
		Forestomach (squamous-cell papilloma or carcinoma): 2/50, 25/50, 34/50	<i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (20 000 ppm) <i>P</i> < 0.001 (trend)		Historical incidence for 2-yr feed studies with untreated control groups (mean ± SD): 33/1470 (2.2 ± 3.1%); range, 0–14%

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumours at the end of 2-year exposure (103–104 weeks)	Significance (logistic regression test)	Tumours at interim evaluations (significance by the Fisher exact test)	Comments
Mouse, B6C3F ₁ (F) (contd)		Lung (alveolar/bronchiolar adenoma): 4/50, 17/50, 13/49	<i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (20 000 ppm) <i>P</i> < 0.001 (trend)	Groups at 15-mo evaluation (0, 10 000, 20 000 ppm): Lung (alveolar/bronchiolar adenoma): 0/10, 3/10, 2/10	Historical incidence for 2-yr feed studies with untreated control groups (mean ± SD): 110/1469 (7.5 ± 5.0%); range, 2–26%
		Lung (alveolar/bronchiolar adenoma or carcinoma): 4/50, 17/50, 15/49	<i>P</i> = 0.005 (10 000 ppm) <i>P</i> = 0.001 (20 000 ppm) <i>P</i> = 0.006 (trend)		
		Large intestine, colon (adenomatous polyp (adenoma)): 0/50, 1/40, 1/59, 3/50	NS (2000 ppm) NS (5000 ppm) <i>P</i> = 0.081 (10 000 ppm) <i>P</i> = 0.027 (trend)	Groups at 15-mo evaluation (0 or 10 000 ppm): Large intestine, rectum (adenomatous polyp (adenoma)): 0/10, 6/10 ^a	
Rat, F344/N (M) 2 yr	0, 2000, 5000 or 10 000 ppm [approximately 0, 90, 240 or 490 mg/kg bw/d] 50–70 animals/group	Liver (hepatocellular adenoma): 1/50, 2/40, 40/59, 34/50	<i>P</i> < 0.001 (2000 ppm) <i>P</i> < 0.001 (5000 ppm) <i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (trend)	Groups at 15-mo evaluation (0 or 10 000 ppm): Liver (hepatocellular adenoma): 0/10, 2/10	
		Liver (hepatocellular carcinoma): 1/50, 12/40, 55/59, 46/50	<i>P</i> < 0.001 (2000 ppm) <i>P</i> < 0.001 (5000 ppm) <i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (trend)	Groups at 15-mo evaluation (0 or 10 000 ppm): Liver (hepatocellular carcinoma): 0/10, 7/10 ^a	
		Liver (hepatocholangiocarcinoma): 0/5, 0/40, 6/59, 2/50	<i>P</i> < 0.05 (5000 ppm)		
		Liver (hepatocellular adenoma or carcinoma): 2/50, 25/40, 57/59, 47/50	<i>P</i> < 0.001 (2000 ppm) <i>P</i> < 0.001 (5000 ppm) <i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (trend)	Groups at 15-mo evaluation (0 or 10 000 ppm): Liver (hepatocellular adenoma or carcinoma): 0/10, 10/10 ^a	Historical incidence for 2-yr feed studies with untreated control groups (mean ± SD): 450/1350 (3.3 ± 3.6%); range, 0–10%

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumours at the end of 2-year exposure (103–104 weeks)	Significance (logistic regression test)	Tumours at interim evaluations (significance by the Fisher exact test)	Comments
Rat, F344/N (M) (contd)		Large intestine, colon (adenomatous polyp (adenoma)): 0/50, 1/40, 1/59, 3/50	NS (2000 ppm) NS (5000 ppm) $P = 0.081$ (10 000 ppm) $P = 0.027$ (trend)	Groups at 15-mo evaluation (0 or 10 000 ppm): Large intestine, rectum (adenomatous polyp (adenoma)): 0/10, 6/10 ^a	Historical incidence for 2-yr feed studies with untreated control groups (mean \pm SD): 1/1353 (0.1 \pm 0.4%); range, 0–2% (includes all carcinomas of the large intestine)
		Large intestine, colon (carcinoma): 0/50, 0/40, 1/59, 4/50	NS (2000 ppm) NS (5000 ppm) $P = 0.046$ (10 000 ppm) $P = 0.003$ (trend)		
		Large intestine, rectum (adenomatous polyp (adenoma)): 0/50, 13/40, 51/59, 40/50	$P < 0.001$ (2000 ppm) $P < 0.001$ (5000 ppm) $P < 0.001$ (10 000 ppm) $P < 0.001$ (trend)		
		Large intestine, rectum (carcinoma): 0/50, 1/40, 10/59, 15/50	$P = 0.48$ (2000 ppm) $P < 0.003$ (5000 ppm) $P < 0.001$ (10 000 ppm) $P < 0.001$ (trend)		
		Large intestine, all sites (adenomatous polyp (adenoma)): 0/50, 13/40, 51/59, 40/50	$P < 0.001$ (2000 ppm) $P < 0.001$ (5000 ppm) $P < 0.001$ (10 000 ppm) $P < 0.001$ (trend)		
		Large intestine, all sites (carcinoma): 0/50, 1/40, 11/59, 17/50	$P < 0.444$ (2000 ppm) $P < 0.001$ (5000 ppm) $P < 0.001$ (10 000 ppm) $P < 0.001$ (trend)		
		Kidney (renal tubule adenoma): 2/50, 10/40, 11/59, 14/50	$P < 0.007$ (2000 ppm) $P < 0.014$ (5000 ppm) $P < 0.001$ (10 000 ppm) $P < 0.001$ (trend)	Groups at 15-mo evaluation (0 or 10 000 ppm): Kidney (renal tumour adenoma): 0/10, 2/10	
		Kidney (renal tubule carcinoma): 0/50, 0/40, 2/59, 1/50	NS		

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumours at the end of 2-year exposure (103–104 weeks)	Significance (logistic regression test)	Tumours at interim evaluations (significance by the Fisher exact test)	Comments
Rat, F344/N (M) (contd)		Kidney (renal tubule adenoma or carcinoma): 2/50, 10/40, 13/59, 15/50	<i>P</i> = 0.007 (2000 ppm) <i>P</i> < 0.005 (5000 ppm) <i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (trend)		Historical incidence for 2-yr feed studies with untreated control groups (mean ± SD): 15/1350 (1.1 ± 1.7%); range, 0–6%
		Urinary bladder (transitional-cell papilloma): 0/50, 1/38, 2/58, 8/50	<i>P</i> = 0.459 (2000 ppm) <i>P</i> = 0.192 (5000 ppm) <i>P</i> < 0.004 (10 000 ppm) <i>P</i> < 0.001 (trend)		
		Urinary bladder (transitional-cell carcinoma): 0/50, 0/38, 1/58, 4/50	NS (2000 ppm) <i>P</i> = 0.491 (5000 ppm) <i>P</i> < 0.022 (10 000 ppm) <i>P</i> < 0.001 (trend)		
		Urinary bladder (transitional cell papilloma or carcinoma): 0/50, 1/38, 3/58, 12/50	<i>P</i> = 0.459 (2000 ppm) <i>P</i> < 0.096 (5000 ppm) <i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (trend)		Historical incidence for 2-yr feed studies with untreated control groups (mean ± SD): 3/1329 (0.2 ± 0.6%); range, 0–2%
Rat, F344/N (F) 2 yr	0, 2000, 5000 or 10 000 ppm [approximately 0, 110, 285 or 600 mg/kg bw/d] 50–70 animals/group	Liver (hepatocellular adenoma): 0/50, 28/40, 47/60, 29/48	<i>P</i> < 0.001 (2000 ppm) <i>P</i> < 0.001 (5000 ppm) <i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (trend)	Groups at 15-mo evaluation (0 or 10 000 ppm): Liver (hepatocellular adenoma): 0/10, 5/10 ^b	
		Liver (hepatocellular carcinoma): 0/50, 12/40, 57/60, 45/48	<i>P</i> < 0.001 (2000 ppm) <i>P</i> < 0.001 (5000 ppm) <i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (trend)	Groups at 15-mo evaluation (0 or 10 000 ppm): Liver (hepatocellular carcinoma): 0/10, 6/10 ^a	
		Liver (hepatocholangiocarcinoma): 0/50, 0/40, 11/60 ^a , 13/48 ^a	[Only Fisher exact test given for this tumour in the report]		
		Liver (hepatocellular adenoma or carcinoma): 0/50, 33/40, 59/60, 45/48	<i>P</i> < 0.001 (2000 ppm) <i>P</i> < 0.001 (5000 ppm) <i>P</i> < 0.001 (10 000 ppm) <i>P</i> < 0.001 (trend)	Groups at 15-mo evaluation (0 or 10 000 ppm): Liver (hepatocellular adenoma or carcinoma): 0/10, 9/10 ^a	Historical incidence for 2-yr feed studies with untreated control groups (mean ± SD): 9/1351 (0.7 ± 1.5%); range, 0–6%

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumours at the end of 2-year exposure (103–104 weeks)	Significance (logistic regression test)	Tumours at interim evaluations (significance by the Fisher exact test)	Comments
Rat, F344/N (F) (contd)		Large intestine, colon (adenomatous polyp [adenoma]): 0/50, 1/40, 2/60, 2/49 (carcinoma): 0/50, 1/40, 2/60, 1/49	NS NS	Groups at 15-mo evaluation (0 or 10 000 ppm): Large intestine, rectum (adenomatous polyp (adenoma)): 0/10, 2/10	Historical incidence for 2-yr feed studies with untreated control groups: 0/1351 (includes all carcinomas of the large intestine)
		Large intestine, rectum (adenomatous polyp [adenoma]): 0/50, 27/40, 53/60, 43/49	$P < 0.001$ (2000 ppm) $P < 0.001$ (5000 ppm) $P < 0.001$ (10 000 ppm) $P < 0.001$ (trend)		
		Large intestine, rectum (carcinoma): 0/50, 1/40, 19/60, 7/49	$P = 0.466$ (2000 ppm) $P < 0.001$ (5000 ppm) $P < 0.001$ (10 000 ppm) $P < 0.001$ (trend)		
		Large intestine, all sites (adenomatous polyp [adenoma]): 0/50, 28/40, 53/60, 43/49	$P < 0.001$ (2000 ppm) $P < 0.001$ (5000 ppm) $P < 0.001$ (10 000 ppm) $P < 0.001$ (trend)		
		Large intestine, all sites (carcinoma): 0/50, 2/40, 21/60, 8/49	$P = 0.201$ (2000 ppm) $P < 0.001$ (5000 ppm) $P < 0.001$ (10 000 ppm) $P < 0.001$ (trend)		Historical incidence for 2-yr feed studies with untreated control groups (mean \pm SD): 1/1348 (0.1 \pm 0.4%); range, 0–2%
		Kidney (renal tubule adenoma): 0/50, 3/40, 16/60, 16/48	$P = 0.049$ (2000 ppm) $P < 0.001$ (5000 ppm) $P < 0.001$ (10 000 ppm) $P < 0.001$ (trend)		
		Kidney (renal tubule carcinoma): 0/50, 0/40, 0/60, 2/48	NS		
		Kidney (renal tubule adenoma or carcinoma): 0/50, 3/40, 16/60, 16/48	$P = 0.049$ (2000 ppm) $P < 0.001$ (5000 ppm) $P < 0.001$ (10 000 ppm) $P < 0.001$ (trend)		

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumours at the end of 2-year exposure (103–104 weeks)	Significance (logistic regression test)	Tumours at interim evaluations (significance by the Fisher exact test)	Comments
Rat, F344/N (F) (contd)		Urinary bladder (transitional-cell papilloma): 0/50, 2/40, 7/60, 9/46	$P = 0.201$ (2000 ppm) $P = 0.012$ (5000 ppm) $P = 0.003$ (10 000 ppm) $P < 0.001$ (trend)	Groups at 15-mo evaluation (0 or 10 000 ppm): Urinary bladder (transitional-cell papilloma): 0/10, 1/10	
		Urinary bladder (transitional-cell carcinoma): 0/50, 0/40, 8/60, 16/46	NS (2000 ppm) $P = 0.008$ (5000 ppm) $P < 0.001$ (10 000 ppm) $P < 0.001$ (trend)	Groups at 15-mo evaluation (0 or 10 000 ppm): Urinary bladder (transitional-cell carcinoma): 0/10, 2/10	
		Urinary bladder (transitional-cell papilloma or carcinoma, overall rate): 0/50, 2/40, 17/60, 26/46	$P = 0.201$ (2000 ppm) $P < 0.001$ (5000 ppm) $P < 0.001$ (10 000 ppm) $P < 0.001$ (trend)	Groups at 15-mo evaluation (0 or 10 000 ppm): Urinary bladder (squamous-cell carcinoma): 0/10, 2/10	Historical incidence for 2-yr feed studies with untreated control groups (mean \pm SD): 3/1334 (0.2 \pm 0.6%); range, 0–2%

^a $P < 0.01$ ^b $P < 0.05$

bw, body weight; d, day or days; F, female; M, male; mo, month or months; NS, not significant; SD, standard deviation; yr, year or years

From [NTP \(1996\)](#)

Table 3.2 Carcinogenicity studies of oral administration of 1-amino-2,4-dibromoanthraquinone in the diet to rats: 9-month stop-exposure group and evaluations at 9 or 15 months

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumours (significance by the Fisher exact test)	Comments
Group 1: Rat, F344/N (M) 9 mo	0, 20 000 ppm 10 animals/group	Liver (hepatocellular adenoma or carcinoma): 0/10, 2/10	
Group 2: Rat, F344/N (M) 9-mo stop exposure then no treatment until 15 mo	0, 20 000 ppm 10 animals/group	Liver (hepatocellular adenoma or carcinoma): 0/10, 9/10 ^a Large intestine (adenomatous polyp [adenoma]): 0/10, 3/10 Kidney (renal tubule adenoma): 0/10, 3/10	
Group 3: Rat, F344/N (M) 15 mo	0, 20 000 ppm 20 animals/group	Liver (hepatocellular adenoma or carcinoma): 0/10, 20/20 ^a Large intestine (adenomatous polyp [adenoma]): 0/10, 7/20 ^b Urinary bladder (transitional-cell papilloma): 0/10, 3/19; (transitional-cell carcinoma): 0/10, 1/19; (squamous-cell carcinoma): 0/10, 1/19 Kidney (renal tubule adenoma): 0/10, 1/20	Controls were the same as those for Group 1
Group 1: Rat, F344/N (F) 9 mo	0, 20 000 ppm 10 animals/group	Liver (hepatocellular adenoma or carcinoma): 0/10, 2/10	
Group 2: Rat, F344/N (F) 9-mo stop exposure then no treatment until 15 mo	0, 20 000 ppm 10 animals/group	Liver (hepatocellular adenoma or carcinoma): 0/10, 8/10 ^a Large intestine (adenomatous polyp [adenoma]): 0/10, 5/10 ^b Kidney (renal tubule adenoma): 0/10, 3/10	
Group 3: Rat, F344/N (F) 15 mo	0, 20 000 ppm 20 animals/group	Liver (hepatocellular adenoma or carcinoma): 0/10, 16/18 ^a Large intestine (adenomatous polyp [adenoma]): 0/10, 3/17 Kidney (renal tubule adenoma): 0/10, 2/17 Urinary bladder (transitional-cell papilloma): 0/10, 1/18; (transitional-cell carcinoma): 0/10, 1/18; (squamous-cell papilloma): 0/10, 1/18; (squamous-cell carcinoma): 0/10, 4/18	Controls were the same as those for Group 1

^a $P < 0.01$ ^b $P < 0.05$

F, female; M, male; mo, month or months

From [NTP \(1996\)](#)

(b) *Stop exposure*

See [Table 3.2](#)

Groups of 40 male and 40 female F344/N rats were fed 20 000 ppm 1-amino-2,4-dibromoanthraquinone in the diet for 9 or 15 months. After 9 months of exposure, 10 males and 10 females were evaluated histopathologically (9-month interim evaluation groups). At the same time, 10 males and 10 females were fed control diet until the end of the 15-month evaluation (9-month stop exposure), and 20 males and 20 females continued to receive 20 000 ppm 1-amino-2,4-dibromoanthraquinone for the 15-month period (15-month exposure groups). The approximate daily consumption of 1-amino-2,4-dibromoanthraquinone was 1335 and 1790 mg/kg bw for males and females in the 9-month stop exposure groups and 1115 and 1435 mg/kg bw for males and females in the 15-month exposure groups, respectively. After 9 months of exposure, hepatocellular adenoma or carcinoma (combined) occurred in treated males and females. In males, rare neoplasms at other sites included one adenomatous polyp (adenoma) in the large intestine and one transitional-cell papilloma in the urinary bladder. In the 9-month stop exposure and 15-month exposure groups, hepatocellular adenoma or carcinoma (combined) occurred in most treated males and females. Adenomatous polyp (adenoma) of the rectum occurred in 3/10 males and 5/10 females in the 9-month stop exposure group, and in 7/20 males and 3/17 females in the 15-month exposure group. Renal tubule adenoma occurred in 3/10 males and 3/10 females in the 9-month stop exposure group and 1/20 males and 2/17 females in the 15-month exposure group. Transitional-cell papilloma (3/19 males, 1/18 females) or carcinoma (1/19 males, 1/18 females) of the urinary bladder developed in the 15-month exposure group. In addition, squamous-cell carcinoma of the urinary bladder occurred in 1/10 males and 4/10 females in the 15-month exposure group ([NTP, 1996](#)).

[The Working Group noted that tumours of the kidney, urinary bladder and large intestine in male and female rats, and forestomach tumours and hepatoblastomas in experimental animals are rare spontaneous neoplasms.]

4. Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Human exposure to 1-amino-2,4-dibromoanthraquinone occurs primarily through dermal contact. Specific data on possible oral exposure to 1-amino-2,4-dibromoanthraquinone in humans were not available to the Working Group. Inhalation exposure to 1-amino-2,4-dibromoanthraquinone is considered to be highly unlikely because of its very low vapour pressure, but dye-contaminated dust particles could be inhaled ([NTP, 1996](#)).

4.1.2 Experimental systems

In rats, a single oral dose of 2, 23, 118, 814 or 1473 mg/kg bw [¹⁴C]-labelled 1-amino-2,4-dibromoanthraquinone was readily absorbed from the gastrointestinal tract and distributed to most soft tissues ([NTP, 1996](#)). The percentage of the oral dose that was absorbed was inversely proportional to the dose level administered: 90% of the lowest dose (2 mg/kg) but only 2% of the high dose (814 mg/kg) was absorbed ([NTP, 1996](#)). A single intravenous dose of [¹⁴C]-labelled 1-amino-2,4-dibromoanthraquinone administered to rats was rapidly and widely distributed to all tissues, and the highest concentrations of radioactivity were found in the lungs, kidneys, small intestine, liver, adipose tissue and adrenal glands, but no quantitative data on tissue distribution of radioactivity were reported ([NTP, 1996](#)). The

majority of 1-amino-2,4-dibromoanthraquinone was metabolized within 2 h after administration because only a small amount (less than 3%) of ^{14}C attributed to the parent compound was recovered from either the blood or urine. Adipose tissue contained primarily unmetabolized 1-amino-2,4-dibromoanthraquinone after 24 h, but other tissues, such as the liver, muscle and skin, contained mostly 1-amino-2,4-dibromoanthraquinone metabolites. However, 1-amino-2,4-dibromoanthraquinone metabolites, excreted primarily in the faeces and urine, have not been identified or characterized (NTP, 1996).

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

Limited data are available on the genetic and related effects of 1-amino-2,4-dibromoanthraquinone in experimental systems, because their evaluation has been hindered by the insolubility of this compound in water (NTP, 2005). No data on the mammalian genotoxicity of 1-amino-2,4-dibromoanthraquinone *in vivo* were available to the Working Group.

(a) *Salmonella reverse mutation assay*

1-Amino-2,4-dibromoanthraquinone (100–10 000 $\mu\text{g}/\text{plate}$) was tested for its induction of gene mutations in several strains of *S. typhimurium* using a preincubation protocol in the presence and absence of metabolic activation (NTP, 1996). *Salmonella* strains included those that revert by base-pair substitutions (TA100 and TA1535) and those that revert by frameshift mutations (TA98 and TA1537). 1-Amino-2,4-dibromoanthraquinone was mutagenic in the absence of microsomal metabolic activation in

strains TA98 and TA1537, and, in the presence of metabolic activation, reverse mutation was not induced in strain TA98, whereas strain TA1537 yielded an equivocal response (Haworth *et al.*, 1983; NTP, 1996). It was weakly mutagenic in strain TA100 (in the presence and absence of metabolic activation) and non-mutagenic in strain TA1535 (in the presence and absence of metabolic activation). The variability of the test results may be due to the tendency of 1-amino-2,4-dibromoanthraquinone to precipitate at concentrations of 100 $\mu\text{g}/\text{plate}$ and above (NTP, 1996).

(b) *Chromosomal aberrations and sister chromatid exchange*

1-Amino-2,4-dibromoanthraquinone was tested in cultured Chinese hamster ovary cells for its induction of chromosomal aberrations in the presence and absence of metabolic activation (Loveday *et al.*, 1990; NTP, 1996). Two separate trials conducted without metabolic activation (Loveday *et al.*, 1990) yielded equivocal results. In one trial, exposure to 1-amino-2,4-dibromoanthraquinone at 3.02 or 10.10 $\mu\text{g}/\text{mL}$ significantly increased the percentage of chromosomal aberrations ($P < 0.05$), but, in the second trial, there was no significant increase. However, in the presence of metabolic activation, 1-amino-2,4-dibromoanthraquinone failed to increase the incidence of chromosomal aberrations (Loveday *et al.*, 1990; NTP, 1996).

1-Amino-2,4-dibromoanthraquinone induced sister chromatid exchange in Chinese hamster ovary cells in a dose-dependent manner in the presence or absence of metabolic activation. In the absence of metabolic activation, it induced a $\geq 20\%$ increase in sister chromatid exchange compared with controls at a concentration of 10 $\mu\text{g}/\text{mL}$, but not at lower concentrations (Loveday *et al.*, 1990; NTP, 1996).

(c) *Mouse lymphoma L5178Y/Tk^{+/-} cells*

1-Amino-2,4-dibromoanthraquinone did not induce mutations at the thymidine kinase (*Tk^{+/-}*) locus in mouse lymphoma cells in the absence or presence of metabolic activation at a concentration of 25 µg/mL. Micronucleus formation was not assessed because of the limited solubility of 1-amino-2,4-dibromoanthraquinone in culture medium ([Harrington-Brock et al., 1991](#)).

(d) *Toxicity in Daphnia magna*

In the *Daphnia magna* immobilization assay, 1-amino-2,4-dibromoanthraquinone was shown to be highly toxic under visible light and simulated solar radiation ([Wang et al., 2009](#)). Increased levels of reactive oxygen species *in vivo* were detected by fluorescence of 2',7'-dichlorofluorescein in the presence of both simulated solar radiation and 1-amino-2,4-dibromoanthraquinone, suggesting that toxicity to *D. magna* is due to the induction of oxidative stress and subsequent damage to biological macromolecules such as proteins, DNA, carbohydrates and polyunsaturated fatty acids. However, the specific nature of the macromolecular alterations has not been reported. The photo-induced oxidative damage to *D. magna* was reduced by antioxidants, including vitamin C, vitamin E and β-carotene ([Wang et al., 2009](#)). These observations demonstrated that photosensitization reactions that resulted in the generation of superoxide anion and singlet oxygen species contributed to the photo-induced toxicity of 1-amino-2,4-dibromoanthraquinone and imply an ecological risk of dyes and their intermediates under natural sunlight ([Wang et al., 2009](#)).

(e) *Photo-excitation-related DNA damage in vitro*

Some anthraquinones undergo photo-modification or photosensitization reactions in aqueous or organic solutions ([Brinson et al., 2005](#)). Anthraquinones can serve as an electron

acceptor chromophore to initiate DNA oxidation on photoexcitation with ultraviolet light ([Abou-Elkhair et al., 2009](#)). Photoexcitation of anthraquinone dyes in association with DNA *in vitro* has been shown to cause DNA damage, mainly at guanine residues ([Abou-Elkhair et al., 2009](#)). It is also known that thymine residues form oxidation products in preference to adenine, suggesting a significantly higher reactivity of the thymine radical cation compared with that of the adenine radical cation ([Abou-Elkhair et al., 2009](#)). Specific data on guanine or adenine oxidation in DNA with 1-amino-2,4-dibromoanthraquinone were not available to the Working Group.

(f) *Alterations in oncogenes and suppressor genes in tumours*

[Hayashi et al. \(2001\)](#) examined 1-amino-2,4-dibromoanthraquinone-induced tumours in B6C3F₁ mice for mutations in the *H-ras* or *K-ras* genes. The tumours examined included squamous-cell papillomas and carcinomas of the forestomach, and alveolar/bronchiolar adenomas and carcinomas of the lung. Point mutations in the *ras* proto-oncogene were analysed in DNA isolated from paraffin-embedded mouse forestomach and lung tumours induced by diets containing 10 000 or 20 000 ppm 1-amino-2,4-dibromoanthraquinone for 2 years. The predominant types of mutation observed at a higher frequency were A to T transversions and A to G transitions in codon 61 of *K-ras* (lung tumours) or *H-ras* (forestomach tumours), suggesting that 1-amino-2,4-dibromoanthraquinone or its metabolites target adenine bases in the *ras* proto-oncogene ([Hayashi et al., 2001](#)). No A to T transversions at the second base of CAA (codon 61) were detected in tumours from control animals. These mutations may play a role in multi-organ carcinogenesis.

4.3 Other mechanistic considerations

Despite the widespread use and potential for significant human exposure, available data on the biological effects of 1-amino-2,4-dibromoanthraquinone are limited.

4.3.1 Liver toxicity

[Fleischman et al. \(1986\)](#) studied toxicity in male and female F344/N rats and B6C3F₁ mice fed a diet containing 0, 0.25, 0.50, 1.00, 2.50 and 5.00% 1-amino-2,4-dibromoanthraquinone for 13 weeks. Lethargy and emaciation were noted in both sexes of rats at the 2.50% and 5.00% dose levels. The liver was the main target organ in both species, and the liver/bw ratio increased at all doses. 1-Amino-2,4-dibromoanthraquinone-treated rats developed chronic toxic hepatitis with hepatocytomegaly, centrilobular vacuolar degeneration and necrosis. Serum markers of liver injury were elevated in male and female rats at doses as low as 0.5% (w/w) and were consistently elevated at doses of 2.5% and 5.0%, and regenerative nodules were observed in the liver of rats fed 5.00%. The kidneys exhibited hyaline droplet degeneration of the proximal tubules. Uterine atrophy was noted in female rats at dose levels of 1.00% and higher. In this 13-week study, 1-amino-2,4-dibromoanthraquinone was considered to be markedly toxic in rats and minimally toxic in mice.

4.3.2 Altered hepatocellular foci

[Lilja et al. \(1985\)](#) fed female Fischer rats 1% or 2% 1-amino-2,4-dibromoanthraquinone in the diet for up to 15 months and noted an increase in γ -glutamyltransferase-positive hepatocellular foci at 9 and 15 months in the 2% group, and at 15 months in the 1% group. [The Working Group noted that γ -glutamyltransferase is a marker for preneoplastic foci induced by genotoxic chemicals, but is generally not expressed in tissues

treated with chemicals that induce tumours by a receptor-mediated mechanism.]

1-Amino-2,4-dibromoanthraquinone was tested in a neonatal rat liver focus model for its potential both as an initiator and promoter ([Maronpot et al., 1989](#)). In the test for initiation potential, newborn (< 24 hour old) rats were injected intraperitoneally with 0.07 mg/g bw 1-amino-2,4-dibromoanthraquinone and, at 21 days, were weaned and fed a diet containing phenobarbital (500 ppm) as a promoting agent for either 75 or 300 days. Based on altered hepatocyte foci and liver tumour incidence, no initiating activity of 1-amino-2,4-dibromoanthraquinone was found. To test for liver tumour promoting activity, neonatal rats were initiated with a subcarcinogenic dose of the initiator *N*-nitrosodiethylamine (4 μ g/g bw) and then fed 1-amino-2,4-dibromoanthraquinone at concentrations of 5000 and 10 000 ppm in the diet for 75–300 days, at which time they were killed. In low-dose females, 1-amino-2,4-dibromoanthraquinone caused a significant ($P < 0.05$) reduction in the number of altered hepatocellular foci at 75 days but a significant ($P < 0.01$) increase at 300 days. In low-dose and high-dose males, no significant changes in the number of altered foci were noted at 75 and 300 days. However, in male rats, the mean focus volume was significantly increased in the low-dose group at 75 days and in the high-dose group at 300 days. No significant difference in the incidence of grossly visible liver tumours was noted. The authors attributed the equivocal trends to the unpalatability of the feed containing 1-amino-2,4-dibromoanthraquinone.

5. Summary of Data Reported

5.1 Exposure data

1-Amino-2,4-dibromoanthraquinone is widely used as an intermediate for the manufacture of dyes for fibres and textiles. Workers may be exposed by inhalation of dust or by dermal contact. 1-Amino-2,4-dibromoanthraquinone may be released into the environment via wastewater streams during its production and use.

5.2 Human carcinogenicity data

No human cancer studies were identified that evaluated exposure to 1-amino-2,4-dibromoanthraquinone.

5.3 Animal carcinogenicity data

In one feeding study, administration of 1-amino-2,4-dibromoanthraquinone for 2 years caused an increased incidence of lung adenoma, and benign and malignant neoplasms of the liver (including hepatoblastomas) and of the forestomach in male and female mice. In male and female rats, it caused an increased incidence of benign and malignant neoplasms of the liver (including cholangiocarcinomas), large intestine, kidney and urinary bladder. In the same study, groups of rats were killed at 15 months (including rats exposed for 9 months followed by 6 months without treatment or rats exposed continuously for 15 months). At this time-point, tumours of the large intestine, kidney and urinary bladder and benign and malignant liver tumours were observed in treated male and female rats. There was consistency in the tumour sites observed between the 15-month and 2-year experiments.

Tumours of the kidney, urinary bladder, and large intestine in male and female rats, and tumours of the forestomach and hepatoblastomas in experimental animals are rare spontaneous neoplasms.

5.4 Other relevant data

Although 1-amino-2,4-dibromoanthraquinone is metabolically degraded *in vivo* relatively rapidly, no data on the structure of its metabolites were available.

Few studies on the genetic and biological effects of 1-amino-2,4-dibromoanthraquinone have been published, partly due to its poor insolubility in water. 1-Amino-2,4-dibromoanthraquinone was mutagenic in the absence of microsomal metabolic activation in bacteria, but did not induce mutations in rodent cells when tested at lower concentrations. It induced chromosomal aberrations and sister chromatid exchange in mammalian cells *in vitro* but the results were inconsistent. The compound was cytotoxic to the liver *in vivo* in a 13-week feeding study in rats at doses comparable with those tested in the 2-year carcinogenesis bioassay. Analyses of forestomach and lung tumours that developed in 1-amino-2,4-dibromoanthraquinone-treated mice revealed point mutations in the *ras* proto-oncogene. The predominant types of mutation were A to T transversions and A to G transitions, suggesting that 1-amino-2,4-dibromoanthraquinone or its metabolites target adenine bases in the *ras* proto-oncogene.

Based on the available data, there is moderate evidence of a genotoxic mechanism in the carcinogenicity of 1-amino-2,4-dibromoanthraquinone in rats and mice. The relevance of the tumour response in experimental animals to humans cannot be excluded.

6. Evaluation

6.1 Cancer in humans

There is inadequate evidence in humans for the carcinogenicity of 1-amino-2,4-dibromoanthraquinone.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1-amino-2,4-dibromoanthraquinone.

6.3 Overall evaluation

1-Amino-2,4-dibromoanthraquinone is *possibly carcinogenic to humans (Group 2B)*.

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2-NITROTOLUENE

2-Nitrotoluene was considered by a previous IARC Working Group in 1995 ([IARC, 1996](#)). Since that time new data have become available, which have been incorporated in this *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.: 88-72-2

Chem. Abstr. Name:

1-Methyl-2-nitrobenzene

IUPAC Systematic Name:

1-methyl-2-nitrobenzene

Synonyms: 2-Methylnitrobenzene;

2-methyl-1-nitrobenzene; *ortho*-methyl-

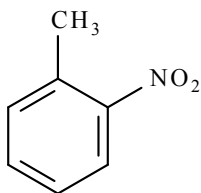
nitrobenzene; *ortho*-mononitrotoluene;

ortho-nitrotoluene; 2-nitrotoluol;

ortho-nitrotoluol

EINECS No.: 201-853-3

1.1.2 Structural and molecular formulae and relative molecular mass



$C_7H_7NO_2$

Relative molecular mass: 137.14

1.1.3 Chemical and physical properties of the pure substance

Description: Yellowish liquid at ambient temperatures ([O'Neil et al., 2006](#))

Boiling-point: 220.4 °C ([O'Neil et al., 2006](#))

Melting-point: -9.3 °C ([O'Neil et al., 2006](#))

Density: 1.162 at 15 °C ([O'Neil et al., 2006](#))

Spectroscopy data: Infrared (prism [4692], grating [437]), ultraviolet (UV) [1292], nuclear magnetic resonance (proton [676], C-13 [857]) and mass spectral data have been reported ([Sadler Research Laboratories, 1980](#))

Solubility: Slightly soluble in water (652 mg/L at 30 °C); soluble in benzene, diethyl ether, ethanol and petroleum ether ([O'Neil et al., 2006](#))

Volatility: Vapour pressure, 0.188 mm Hg at 20 °C; relative vapour density (air = 1), 4.72 ([IARC, 1996](#); [HSDB, 2004](#))

Stability: Combustible when exposed to heat or open flame; potentially reacts explosively with alkali ([Sax & Lewis, 1989](#))

Octanol/water partition coefficient (P): log P, 2.30 ([O'Neil et al., 2006](#))

Conversion factor: $mg/m^3 = 5.6 \times ppm$ (calculated from: $mg/m^3 = (\text{relative molecular mass}/24.45) \times ppm$, assuming

a temperature of 25 °C and pressure of 101 kPa.

1.1.4 Technical products and impurities

2-Nitrotoluene is available commercially at a purity of $\geq 99.5\%$, and containing 3-nitrotoluene (0.2%) and 4-nitrotoluene (0.01%) as typical impurities ([European Commission, 2008](#)).

1.1.5 Analysis

Levels of 2-nitrotoluene can be determined in workplace air by drawing the air sample through a solid sorbent tube containing silica gel, desorbing with methanol and analysing with gas chromatography and flame ionization detection. The range of detection for this method is 1.97–9.86 mg/m³ for a 30-L air sample ([NIOSH, 1998](#)).

2-Nitrotoluene can be measured in surface or groundwater by pre-concentration through salting-out extraction with acetonitrile and sodium chloride and analysis with high performance liquid chromatography with dual wavelength UV detection ([US EPA, 2006](#)). It can also be determined in water and wastewater by homogeneous liquid–liquid extraction or dispersive liquid–liquid micro-extraction and analysis with gas chromatography with flame ionization detection ([Ebrahimzadeh et al., 2007, 2009](#)).

1.2 Production and use

1.2.1 Production

Nitrotoluenes are produced by the nitration of toluene with an aqueous acidic mixture of sulfuric acid and nitric acid at an initial temperature of 25 °C that is slowly raised to 35–40 °C. The resulting product contains 55–60% 2-nitrotoluene, 3–4% *meta*-nitrotoluene and 35–40% *para*-nitrotoluene. The isomers can be separated by a combination of fractional distillation and crystallization ([Dugal, 2005](#)).

In 1993, the production of mononitrotoluenes in the United States of America was 26 000 tonnes, of which approximately 16 120 tonnes were the *ortho* isomer, 780 tonnes were the *meta* isomer and 9100 tonnes were the *para* isomer ([Dugal, 2005](#)). In 1984, the yearly production capacity for mononitrotoluenes (all isomers) in the western world was approximately 200 000 tonnes ([Booth, 1991](#)). 2-Nitrotoluene is listed as a high production volume chemical, and, according to data submitted by companies under the Inventory Update Rule, production of 2-nitrotoluene in the USA was between 10 million and 50 million pounds [4.5 million and 22.7 million tonnes] for every 4-year reporting period between 1986 and 2002 ([US EPA, 2004](#)).

2-Nitrotoluene is produced by 10 companies in the USA, five companies in the People's Republic of China, three companies in the United Kingdom, two companies each in Canada and China, Hong Kong Special Administrative Region, and one company each in the Czech Republic, Germany, India, Italy, Japan and Switzerland ([Chemical Sources International, 2010](#)). Other sources indicated that 2-nitrotoluene was produced by one company in the USA ([HSDB, 2004](#)), four companies in Germany and one company each in Belgium, Italy, Spain and the United Kingdom ([IUCLID, 2000](#)).

1.2.2 Use

2-Nitrotoluene is primarily used in the production of derivatives, including *ortho*-toluidine, 2-amino-4-chlorotoluene, 2-amino-6-chlorotoluene and *ortho*-toluidine-4-sulfonic acid, which are intermediates in the production of various azo dyes ([IARC, 1996](#)). It is also used in the manufacture of (or manufacture of intermediates for) other dyes, such as magenta, sulfur dyes, rubber chemicals, agricultural chemicals and explosives ([HSDB, 2004](#)).

Table 1.1 Estimated occupational exposure to 2-nitrotoluene

Route	Work activity	Exposure	Comment
Inhalation	All	2.85–5.61 mg/m ³	1 h
		0.35–0.7 mg/m ³	8-h TWA
Dermal	Sampling	0–21 mg/d	Exposed area of 210 cm ²
	Filling and emptying tanks	420 mg/d	Exposed area of 420 cm ²
	Maintenance	0–840 mg/d	840 cm ²
	All	420 mg/d	Reasonable worst case

d, day or days; h, hour or hours; TWA, time-weighted average

From [European Commission \(2008\)](#)

1.3 Occurrence

1.3.1 Natural occurrence

ortho-Nitrotoluene is not known to occur as a natural product.

1.3.2 Occupational exposure

Occupational exposure to 2-nitrotoluene may occur during its production and use. The major routes of exposure are inhalation and dermal contact ([European Commission, 2008](#)). It is estimated that approximately 150 workers are potentially exposed to 2-nitrotoluene in the European Union (EU; [European Commission, 2008](#)).

According to information on manufacturing firms in the EU, 2-nitrotoluene is typically produced and used in a closed system and is usually processed within the manufacturing plants ([European Commission, 2008](#)). Exposure to 2-nitrotoluene may occur during sampling, loading and unloading tanks and drums and maintenance. Occupational monitoring data for exposure to 2-nitrotoluene are available from the major producers for the period 1996–2000. 2-Nitrotoluene was measured in personal air samplers for a full shift. For manufacturing workers, mean concentrations were 0.057 mg/m³ for distillation activities in 1991–2000 and 0.075 mg/m³ for nitration activities in 1996–99. Workers involved in the processing of 2-nitrotoluene in 1995–2000 were exposed to

mean levels of 0.050–0.062 mg/m³, and those involved in filling drums or tanks in 1997–99 were exposed to mean levels of 0.030–0.052 mg/m³. Mean short-term occupational exposure measurements ranged from 0.109 to 0.250 mg/m³ for processing activities. Levels of occupational exposure to 2-nitrotoluene ranged from 0.01 to 0.0545 mg/m³ (19 samples) at a company where it was used to produce dinitrotoluenes. The EU noted that detailed information on work activities, sampling and analytical methods were not reported, but estimated that the highest value for a shift (0.280 mg/m³) could be regarded as a reasonable worst-case exposure level. Based on these data and information on sampling and maintenance activities ([Table 1.1](#)), inhalation exposure (8-hour time-weighted average) was estimated to range from 0.35 to 0.7 mg/m³ for all activities. Dermal exposure was estimated at 420 mg per day; the highest exposures were deemed to occur during maintenance activities ([Table 1.1](#)).

2-Nitrotoluene was detected in the ambient air of a chemical manufacturing plant in New Jersey (USA) at a concentration of 47 ng/m³, and at concentrations up to 2 mg/m³ in the nitrotoluene production area of a chemical plant that produced pharmaceuticals and explosives ([IARC, 1996](#)). Annual average predicted levels in the air obtained from data on emissions reported by industry at three sites in Italy were below 0.01 mg/m³ ([European Commission, 2008](#)). The

Table 1.2 Estimates of releases of 2-nitrotoluene from production and processing

Location	Air (kg per day)	Wastewater (kg per day)
Local		
Site A	0.07 ^a	0.57 ^a
Site B	0.125 ^b	0.015
Site C	1.64 ^b	1.08
Regional ^c	1.35	0.978
Continental ^d	1.05	0.758

^a Calculated from data in kilograms per year, assuming 365 days of production and processing

^b No data received; estimate based on general default emission factors

^c Model used emission and production data from Site C, the largest (worst case) source

^d Total amount used and produced in the European Union minus the regional data

From [European Commission \(2008\)](#)

mean 8-hour time-weighted average at a Chinese plant that manufactured di- and trinitrotoluenes was 0.75 mg/m³ (range, undetected–4.29 mg/m³) ([Jones et al., 2005a](#)). [The Working Group noted the lack of data on worldwide occupational exposure.]

1.3.3 Environmental occurrence

(a) Releases

2-Nitrotoluene can be released into the environment (primarily into air and water) during its manufacture and use. It may also be formed from the degradation of di- or trinitrotoluenes and can be released into the environment from di- or trinitrotoluenes-manufacturing plants or industries that use these chemicals, such as munitions-production facilities.

Data on releases into the air and water from 2-nitrotoluene production and processing were obtained from three industrial sites by the European Union, and were used to estimate regional and continental releases ([Table 1.2](#)). Releases into the air at the three sites ranged from 0.07 to 1.64 kg per day, and those into water ranged from 0.015 to 1.08 kg per day. The data submitted by industry showed that no sewage sludge from industrial sewage treatment plants was spread at two sites, and that the sludge was sent off-site for composting before being spread

at the third site. Modelling using worst-case values predicted emissions to the soil of 1.35 kg per day at the regional scale and 1.05 kg per day at the continental scale.

(b) Ambient air

[HSDB \(2004\)](#) and the [European Commission \(2008\)](#) reviewed information and calculated parameters related to the environmental fate of nitrotoluene in ambient air, water and soil. 2-Nitrotoluene released into the air is expected to exist entirely in the vapour phase and is degraded in the atmosphere by a reaction with photochemically produced hydroxyl radicals, with an estimated atmospheric half-life of 23 days ([European Commission, 2008](#)) or 42 days ([HSDB, 2004](#)). It may also be degraded by photolysis; the major products formed are 2-methyl-6-nitrophenol and 2-methyl-4-nitrophenol ([European Commission, 2008](#)).

Limited environmental monitoring data are available. 2-Nitrotoluene was detected in the ambient air in Japan at a concentration of 44 µg/m³ ([European Commission, 2008](#)). In the USA, it was detected at 0.03 and 0.9 ng/m³ in two ambient air samples collected in Boise, ID, in the winter of 1986–87 and at 0.047 µg/m³ in ambient air at a manufacturing plant in New Jersey ([Pellizzari, 1978](#); [NTP, 2008](#)). [The Working

Table 1.3 Environmental occurrence of 2-nitrotoluene in effluents, wastewater or surface water near industrial sites

Location	Source	Sample	Concentration (µg/L)	Reference
Effluents and wastewater				
India	Nitrotoluene-manufacturing plant	Wastewater – acid Wastewater – alkaline	87 000–102 000 53 000–80 000	Swaminathan et al. (1987)
Radford, VA, USA	Trinitrotoluene-manufacturing plant	Effluent	320–16 000	Nay (1972) , Howard et al. (1976)
USA	Production and purification of 2,4,6-trinitrotoluene	Wastewater	20–140	Spanggord et al. (1982a)
USA	Dinitrotoluenes manufacturing plant	Raw effluent	7800	Webb et al. (1973) , Howard et al. (1976)
USA	Trinitrotoluene plant	Raw effluent	150	Webb et al. (1973) , Howard et al. (1976)
NR	Paper mill	Waste-treatment lagoon	Detected	Webb et al. (1973) , Howard et al. (1976)
Teheran, Islamic Republic of Iran	Research facility	Wastewater	110	Ebrahimzadeh et al. (2007, 2009)
Ground- or surface water near or at munitions or military sites				
Germany	Near former munitions-manufacturing plant	<i>Hischagen/Waldhof – surface water</i> 2 brooks River Losse (adjacent)	0.4; 7.4 1.2	Feltes et al. (1990)
		<i>Clausthal-Zellerfeld – surface water</i> 2 ponds River Oder (adjacent)	0.4; 22 < 0.01	

Table 1.3 (continued)

Location	Source	Sample	Concentration (µg/L)	Reference
USA	Munitions-manufacturing plants	<i>Groundwater</i>		ATSDR (2007)
		Texas	4600	
		Illinois	21 000	
		<i>Tennessee</i>	140 000	
		Tennessee (same plant)		
		Well-water	42 600	Best et al. (2001)
		Groundwater	2900	NTP (2008)
		<i>Wisconsin (former plant)</i>		ATSDR (2007)
		Off-site well	0.095	
		Ground-water (4/17 samples)	0.16–17 ^b	
	<i>Missouri – surface water</i>	0.12 (max)		
USA	Former munitions plant/current nuclear weapons assembly/disassembly plant 1999–2005	Texas – groundwater ^a		Pantex (2005)
		Ogallala aquifer	ND–2.9	
		Perched aquifer	ND–5	
Massachusetts, USA	Military training facility	Groundwater	25	ATSDR (2007)

^a Not detected every year, and not detected in 2005, last year of monitoring

^b *ortho*-Nitrotoluene and *para*-nitrotoluene combined

ND, not detected; NR, not reported

Table 1.4 Environmental occurrence of 2-nitrotoluene in water or sediments

Country	Year	Location	Concentration ($\mu\text{g/L}$)	Reference	
Surface water					
Netherlands	1972	<i>Waal River</i> Brakel	3.1–16	European Commission (2008)	
		<i>Rhine River</i>			
	1978	Maassluis	1–10		
	1978	Lobith	3–10		
	1979		1–3		
	1981–84		max., 0.8–3.0		
Germany	1981	Gorinchem	3 (max)	European Commission (2008)	
		<i>Rhine River</i>			
	1987	Wiesbaden	< 0.02–0.35		
	1987	Köln	0.05–0.37		
	1987	Düsseldorf	0.08–0.46		
	1989	Lippe	≤ 2.45		
		<i>River Elbe</i>			
	1997	Schmika	0.15–0.20		
	NR	<i>River Elbe</i>			Feldes et al. (1990)
		Brunsbüttel	0.05		
	Brokdorf	0.08			
		Lauenburg	0.4		
Europe	1992–93	River Elbe	40 (mean)	Götz et al. (1998)	
Europe	1999	River Danube	< 0.02 (90%)	European Commission (2008)	
	1999	River Rhine	0.5 (90%)		
	1999	River Elbe	0.06 (max)		
China	NR	Yellow River	72–483	He et al. (2006)	
	NR	Soughua River	150–8600	Lang et al. (1993)	
	2006	Daliao River watershed ^a (surface water and sediment)	ND–3708	Men et al. (2010)	

Table 1.4 (continued)

Country	Year	Location	Concentration (µg/L)	Reference
Groundwater				
France	1987	Degrémont	90–165	Duguet <i>et al.</i> (1988)
Netherlands	1974	River Waal	4.5 (average) ^b	Meijers & van der Leer (1976)
	1974	River Maas	18.1 (max) ^b 0.3 (max) ^b	
Germany	NR	River Rhine	10	
Sediment				
Japan	1976		3.4–140	European Commission (2008)
Drinking-water				
Germany	NR	NR	Detected	Zoeteman (1980)

^a Sampling from surface water (aqueous and particles) and sediment from 28 sites (main river and branch streams) in the watershed including the Hun River, the Taizi River and the Daliao River; some of the branches were contaminated by wastewater from various industries, including oil refining iron ore and cement and chemical engineering.

^b Includes 2- and 4-nitrotoluene

ND, not detected; NR, not reported

Table 1.5 Estimated human daily intake of 2-nitrotoluene

Source	Regional intake (mg/kg bw per day)
Air	7390×10^{-12}
Drinking-water	$96\,700 \times 10^{-12}$
Fish	$100\,000 \times 10^{-12}$
Leaf crops	3720×10^{-12}
Root crops	8820×10^{-12}
Meat	4.44×10^{-12}
Milk	13.1×10^{-12}
Total	$217\,000 \times 10^{-12}$

From [European Commission \(2008\)](#)

Group noted the large differences in concentration data, which reflect the nature and location of the sampling.]

(c) Water

2-Nitrotoluene released into water is not expected to adsorb (or only slightly) to suspended solids and sediment. Because 2-nitrotoluene absorbs UV light strongly, it is susceptible to photochemical degradation. Volatilization is expected to play a minor role in its environmental fate in water and sediments. Chemical hydrolysis is not expected to be important in the removal of 2-nitrotoluene from aquatic environments, and its bioaccumulation is low. It may be removed from aquatic environments by biodegradation, but not under conditions in which acclimation of the same population of bacteria or other microorganisms is unlikely to occur, such as surface or running waters ([HSDB, 2004](#)).

Environmental monitoring data on 2-nitrotoluene in effluents, wastewater or surface water near industrial sites are provided in [Table 1.3](#) and those in water or sediments are given in [Table 1.4](#). 2-Nitrotoluene was found at highest concentrations (up to 102 000 µg/L) in the wastewater of a manufacturing plant in India ([Swaminathan et al., 1987](#)).

2-Nitrotoluene is a break-down product of di- and trinitrotoluenes. It was detected in

the wastewater or effluent (concentrations up to 16 000 µg/L) from di- and trinitrotoluenes-manufacturing plants, in surface water near former munitions-manufacturing plants in Germany (concentrations up to 22 µg/L), in ground- (concentrations up to 140 000 µg/L) and surface water (concentrations up to 0.12 µg/L) at several munitions-manufacturing plants in the USA, in groundwater at a military training facility (maximum concentration, 25 µg/L), and in wastewater from a paper mill (40 µg/L) and from a research facility (181 µg/L) ([Table 1.3](#)).

Numerous studies have detected 2-nitrotoluene in rivers, namely in the River Waal, the River Rhine, the River Maas and the River Elbe ([Table 1.4](#)). Most, but not all, of the reported values were below 1 µg/L; one study reported a mean concentration of 40 µg/L in samples from the River Elbe taken in Germany ([Götz et al., 1998](#)). Much higher levels (ranging up to 8600 µg/L) have been reported in rivers in China ([Men et al., 2010](#)). 2-Nitrotoluene was also detected in groundwater samples taken in France ([Duguet et al., 1988](#)) and in drinking-water in Germany ([Kool et al., 1982](#)).

(d) Soil

In soil, 2-nitrotoluene is predicted to have moderate mobility based on an estimated soil absorption coefficient of 420. Volatilization of 2-nitrotoluene is expected from moist soil (based on a Henry' Law constant of 1.25×10^{-5} atm.m³/mol) but not from dry soil (based on its vapour pressure of 0.188 mm Hg) ([HSDB, 2004](#)). However, it is unlikely to be removed from the soil by oxidation, chemical hydrolysis or biodegradation. The [European Commission \(2008\)](#) estimated that the half-lives for biodegradation were 300 days in soil and 3014 days in sediment.

2-Nitrotoluene is suspected to be a pollutant at munitions sites, but no data are available. It was found at a concentration of 1.4 ppm in soil contaminated with trinitrotoluene at a historical military testing site ([Radtke et al., 2002](#)).

Table 1.6 Occupational exposure limits and guidelines for 2-nitrotoluene

Country	Year	Concentration (mg/m ³)	Interpretation
Argentina ^a	2007	11 (sk)	TWA
Australia	2008	11 (sk)	TWA
Austria	2006	0.5	TRK
Belgium	2002	11 (sk)	TWA
Bulgaria ^a	2007	11 (sk)	TWA
Colombia ^a	2007	11 (sk)	TWA
Denmark	2002	12 (sk)	TWA
Germany	NR	5 (sk) 10	MAK STEL
Jordan ^a	2007	11 (sk)	TWA
Mexico	2004	30 60	TWA STEL
New Zealand	2002	11 (sk)	TWA
Norway	1999	5.5	TWA
Republic of Korea	2006	11 (sk)	TWA
Singapore ^a	2007	11 (sk)	TWA
Sweden	2005	6 11 (sk)	TWA STEL
United Kingdom	2000	30 (sk) 60	TWA STEL
USA			
ACGIH (TLV)	2007	11 (sk) 60 (sk)	TWA STEL
OSHA (PEL)	1994	30 (sk)	TWA
NIOSH (REL)	1992	11 (sk)	TWA
Viet Nam ^a	2007	11 (sk)	TWA

^a These countries follow the recommendations of the ACGIH threshold limit values.

ACGIH, American Conference of Governmental Industrial Hygienists; MAK, Maximale Arbeitsplatz-Konzentration; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; REL, recommended exposure limit; sk, absorption through the skin may be a significant source of exposure; STEL, short-term exposure limit; TLV, threshold limit value; TRK, Technische Richtkonzentrationen; TWA, time-weighted average

From [IUCLID \(2000\)](#); [RTECS \(2009\)](#); [ACGIH \(2010\)](#)

1.3.4 Estimated human intake of ortho-nitrotoluene

The [European Commission \(2008\)](#) developed a model to predict indirect exposure to 2-nitrotoluene from the environment using data collected from three industrial sites in Italy (see Section 1.3.2). The estimated daily dose for the region was 2.17×10^{-7} mg/kg body weight (bw); the estimated intakes from various other sources are provided in [Table 1.5](#).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for 2-nitrotoluene in several countries are presented in [Table 1.6](#).

Table 2.1 Cohort study of dyestuff workers

Study location and period	Study population	Follow-up period	Exposure assessment	Organ site	Exposure categories	No. of cases/deaths	Relative risk (95% CI)	Comments
Northern Italy 1922–70	906 male dyestuff workers	1946–76	Work histories accessed from personal and factory records; workers assigned to 10 categories related to the type of dyes that they produced	All cancers Bladder cancer	Total cohort	96	2.65 [2.15–3.24]	National rates; workers in the total cohort exposed to benzidine and α - and β -naphthylamine; 2-nitrotoluene was used to manufacture fuchsin and safranin T.
					Total cohort	36	29.27 [20.50–40.52]	
					Fuchsin and safranin T	5	62.5 [20.29–145.85]	

CI, confidence interval
From [Rubino et al. \(1982\)](#)

2. Cancer in Humans

No studies of human cancer were identified that evaluated specific exposure to 2-nitrotoluene. Occupational exposure to 2-nitrotoluene may occur during the manufacture of magenta. Although the Working Group was aware of studies that reported exposure to dinitrotoluene and trinitrotoluene, they did not specifically mention exposure to 2-nitrotoluene and were therefore not reviewed.

2.1 Cohort studies

2.1.1 Background

Magenta production is classified by IARC as *carcinogenic to humans (Group 1)*, based on *sufficient evidence* that the production of magenta causes bladder cancer in humans. IARC also concluded that there was *inadequate evidence* in humans for the carcinogenicity of magenta itself ([IARC, 2010](#)). The IARC evaluation was based

on a review of one case report ([Rehn, 1895](#)), two cohort studies ([Case & Pearson, 1954](#); [Rubino et al., 1982](#)) of magenta-manufacturing workers (not exposed to 1- or 2-naphthylamine or benzidine) and one case–control study of occupational exposure to magenta ([Vineis & Magnani, 1985](#)). One of the cohort studies discussed the process used to manufacture New Magenta (New Fuchsin, a component of magenta) at a dyestuff factory in northern Italy ([Rubino et al. 1982](#); [IARC, 2010](#)). In this factory, fuchsin was manufactured by two different processes, both of which involved exposure to 2-nitrotoluene. In the first process, 2-nitrotoluene was produced as an intermediate (during the conversion of toluene to *ortho*-toluidine); in the second process, it was used as a raw ingredient ([Rubino et al., 1982](#)). The other studies of magenta production did not describe the manufacturing process, and are not reviewed here because it is not known whether the subjects were exposed to 2-nitrotoluene (for a review of these studies, see [IARC, 2010](#)).

2.1.2 Dyestuff manufacturing workers

See [Table 2.1](#)

[Rubino et al. \(1982\)](#) conducted a retrospective cohort study of dyestuff workers in a plant that manufactured aromatic amines and used them in azo dyes. The factory began its operations in 1922, and the manufacture of β -naphthylamine was discontinued in 1960. The cohort comprised 906 men who had worked at the plant for at least 1 month between 1922 and 1970. Vital status (95.8% complete) was accessed using personal records and from municipal registries, and cause of death was obtained from death certificates. Deaths were observed from 1946 to 1976, and expected numbers of deaths were calculated using national rates for 1951–76. Information on exposure was obtained from personal and factory records, and workers were classified into 10 different dye-manufacturing categories, including the manufacture of α - and β -naphthylamine and benzidine, which are known bladder carcinogens. Workers involved in the manufacture of these dyes were excluded from the other dye categories and workers involved in the other manufacturing categories did not change jobs. Of the 868 men with available personal records, 53 were involved in the manufacture of fuchsin and safranine T, and were potentially exposed to 2-nitrotoluene, and other known or suspected carcinogens such as *ortho*-toluidine and 4,4'-methylenebis(2-methylaniline).

Among the total cohort, a statistically significant excess of mortality from all cancers was found (ratio of observed versus expected [standardized mortality ratio (SMR)], 2.65 [95% confidence interval (CI): 2.15–3.24]; 96 observed deaths) due primarily to cancers of the bladder (SMR, 29.27 [95%CI: 20.50–40.52]; 36 exposed deaths), but also cancers of the lung (SMR, 1.78 [95%CI: 0.97–2.98]; five exposed deaths), larynx (SMR, 3.55 [95%CI: 1.15–8.28]; five exposed deaths) and oesophagus (SMR, 4.72 [95%CI: 1.53–11.01]; 10 exposed deaths).

Among fuchsin- and safranine T-manufacturing workers potentially exposed to 2-nitrotoluene, a large excess of mortality from bladder cancer was found (SMR, 62.5 [95%CI: 20.29–145.85]; five exposed cases). Findings for other cancer sites (such as the lung, larynx and oesophagus) were not reported. [It is not possible to evaluate whether 2-nitrotoluene played a causal role in bladder cancer in this study because the workers were also exposed to *ortho*-toluidine, which causes bladder cancer in humans and is classified by IARC as *carcinogenic to humans* (Group 1, [IARC, 2010](#)).]

3. Cancer in Experimental Animals

Carcinogenicity studies of oral administration of 2-nitrotoluene in the diet to mice and rats have been conducted ([NTP, 2002](#)), the results of which are summarized in [Table 3.1](#) (see also [Dunnick et al., 2003](#)).

3.1 Oral administration

3.1.1 Mouse

Groups of 60 male and 60 female B6C3F₁ mice were fed diets containing 0 (controls), 1250, 2500 or 5000 ppm 2-nitrotoluene (equivalent to average daily doses of approximately 0, 165, 360 or 700 and 150, 320 or 710 mg/kg bw in males and females, respectively) for 105 weeks. All males in the 2500- and 5000-ppm groups died before the end of the study. Treated males and females had an increased incidence of haemangiosarcoma located in the skeletal muscle, subcutaneous tissue or mesentery and carcinoma of the large intestine (caecum). In treated female mice, the incidence of hepatocellular adenoma and carcinoma was increased ([NTP, 2002](#); [Dunnick et al., 2003](#)).

Table 3.1 Carcinogenicity studies of 2-nitrotoluene by oral administration in the diet to mice (2-year study) and rats (2-year study and 3-month stop-exposure study)

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumours	Significance (poly-3-test)
Mouse, B6C3F ₁ (M) 2 yr	0, 1250, 2500 or 5000 ppm 60 animals/group	Circulatory system (haemangiosarcoma): 4/60, 17/60, 55/60, 60/60	<i>P</i> < 0.001 (1250 ppm) <i>P</i> < 0.001 (2500 ppm) <i>P</i> < 0.001 (5000 ppm) <i>P</i> < 0.001 (trend)
		Large intestine (caecum, carcinoma): 0/60, 12/60, 9/60, 0/60	<i>P</i> < 0.001 (1250 ppm) <i>P</i> < 0.001 (2500 ppm) <i>P</i> < 0.001 (trend)
Mouse, B6C3F ₁ (F) 2 yr	0, 1250, 2500 or 5000 ppm 60 animals/group	Circulatory system (haemangiosarcoma): 0/60, 2/60, 3/60, 50/60	<i>P</i> < 0.001 (5000 ppm) <i>P</i> < 0.001 (trend)
		Large intestine (caecum, carcinoma): 0/60, 1/60, 4/60, 3/60	<i>P</i> = 0.024 (trend)
		Liver (hepatocellular adenoma): 7/60, 5/59, 19/59, 29/60	<i>P</i> = 0.006 (2500 ppm) <i>P</i> < 0.001 (5000 ppm) <i>P</i> < 0.001 (trend)
		Liver (hepatocellular carcinoma): 2/60, 4/59, 6/59, 16/60	<i>P</i> < 0.001 (2500 ppm) <i>P</i> < 0.001 (5000 ppm) <i>P</i> = 0.024 (trend)
		Liver (hepatocellular adenoma or carcinoma): 9/60, 9/59, 24/59, 39/60	<i>P</i> < 0.001 (2500 ppm) <i>P</i> < 0.001 (5000 ppm) <i>P</i> < 0.001 (trend)

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumours	Significance (poly-3-test)	
Rat, F344 (M) 2 yr	0, 625, 1250 or 2000 ppm; 2000 or 5000 ppm 3 mo stop- exposure 60 animals/group	Mesothelium (malignant mesothelioma): 2/60, 20/60, 29/60, 44/60, 44/60, 54/60	<i>P</i> < 0.001 (625 ppm)	
			<i>P</i> < 0.001 (1250 ppm)	
			<i>P</i> < 0.001 (2000 ppm)	
			<i>P</i> < 0.001 (trend)	
			<i>P</i> < 0.001 (2000 ppm, stop-exposure)	
			<i>P</i> < 0.001 (5000 ppm, stop-exposure)	
			<i>P</i> < 0.001 (trend, stop-exposure)	
			Skin (subcutaneous lipoma): 0/60, 4/60, 13/60, 13/60, 10/60, 12/60	<i>P</i> = 0.041 (625 ppm)
				<i>P</i> < 0.001 (1250 ppm)
				<i>P</i> < 0.001 (2000 ppm)
		<i>P</i> < 0.001 (trend)		
		<i>P</i> < 0.01 (2000 ppm, stop-exposure)		
		Skin (fibroma): 5/60, 46/60, 52/60, 59/60, 45/60, 52/60	<i>P</i> < 0.001 (625 ppm)	
			<i>P</i> < 0.001 (1250 ppm)	
			<i>P</i> < 0.001 (2000 ppm)	
			<i>P</i> < 0.001 (trend)	
			<i>P</i> < 0.001 (2000 ppm, stop-exposure)	
		Skin (fibrosarcoma): 0/60, 7/60, 17/60, 20/60, 8/60, 12/60	<i>P</i> < 0.001 (625 ppm)	
			<i>P</i> < 0.001 (1250 ppm)	
			<i>P</i> < 0.001 (2000 ppm)	
<i>P</i> < 0.001 (trend)				
<i>P</i> < 0.001 (2000 ppm, stop-exposure)				
Skin (fibroma or fibrosarcoma): 5/60, 47/60, 55/60, 59/60, 47/60, 53/60	<i>P</i> < 0.001 (625 ppm)			
	<i>P</i> < 0.001 (1250 ppm)			
	<i>P</i> < 0.001 (2000 ppm)			
	<i>P</i> < 0.001 (trend)			
	<i>P</i> < 0.001 (2000 ppm, stop-exposure)			
			<i>P</i> < 0.001 (5000 ppm, stop-exposure)	
			<i>P</i> < 0.001 (trend, stop-exposure)	

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumours	Significance (poly-3-test)
Rat, F344 (M) (contd)		Mammary gland (fibroadenoma): 0/60, 7/60, 10/60, 2/60, 13/60, 20/60	$P = 0.004$ (625 ppm) $P < 0.001$ (1250 ppm) $P < 0.001$ (trend) $P < 0.001$ (2000 ppm, stop-exposure) $P < 0.001$ (5000 ppm, stop-exposure) $P < 0.001$ (trend, stop-exposure)
		Liver (hepatocellular adenoma): 2/60, 3/60, 3/60, 7/60, 3/60, 4/60	$P = 0.006$ (2000 ppm) $P = 0.007$ (trend)
		Liver (cholangiocarcinoma): 0/60, 0/60, 0/60, 0/60, 0/60, 3/60	$P = 0.034$ (5000 ppm, stop-exposure) $P = 0.025$ (trend, stop-exposure)
		Liver (hepatocellular adenoma or carcinoma): 3/60, 3/60, 3/60, 8/60, 3/60, 6/60	$P = 0.007$ (2000 ppm) $P = 0.009$ (trend) $P = 0.029$ (5000 ppm, stop-exposure) $P = 0.03$ (trend, stop-exposure)
		Lung (alveolar/bronchiolar adenoma): 1/60, 5/60, 1/60, 2/60, 3/60, 8/60	$P < 0.001$ (5000 ppm, stop-exposure) $P < 0.001$ (trend, stop-exposure)
		Lung (alveolar/bronchiolar adenoma or carcinoma) 2/60, 5/60, 1/60, 2/60, 3/60, 11/60	$P < 0.001$ (5000 ppm, stop-exposure) $P < 0.001$ (trend, stop-exposure)
		Circulatory system (haemangioma or haemangiosarcoma): 1/60, 3/60, 1/60, 2/60, 0/60, 4/60	$P = 0.037$ (5000 ppm, stop-exposure) $P = 0.041$ (trend, stop-exposure)
Rat, F344 (F) 2 yr	0, 625, 1250 or 2000 ppm 60 animals/group	Skin (fibroma): 3/60, 3/60, 18/60, 20/60	$P < 0.001$ (1250 ppm) $P < 0.001$ (2000 ppm) $P < 0.001$ (trend)
		Skin (fibroma or fibrosarcoma): 3/60, 3/60, 21/60, 22/60	$P < 0.001$ (1250 ppm) $P < 0.001$ (2000 ppm) $P < 0.001$ (trend)
		Mammary gland (fibroadenoma): 23/60, 47/60, 52/60, 56/60	$P < 0.001$ (625 ppm) $P < 0.001$ (1250 ppm) $P < 0.001$ (2000 ppm) $P < 0.001$ (trend)
		Liver (hepatocellular adenoma): 1/60, 0/59, 1/60, 6/60	$P = 0.048$ (2000 ppm) $P = 0.005$ (trend)

F, female; M, male; mo, month or months; yr, year or years

From [NTP \(2002\)](#), [Dunnick et al. \(2003\)](#)

3.1.2 Rat

2-Nitrotoluene caused early tumour formation in a 13-week study in male F344/N rats fed 0, 625, 1250, 2500, 5000 or 10 000 ppm in the diet; mesothelioma of the tunica vaginalis (3/10) was observed in the 5000-ppm group ([Dunnick, 1993](#); [Dunnick et al., 1994](#)).

In a subsequent 26-week study in male F344/N rats fed 0 or 5000 ppm 2-nitrotoluene in the diet, mesothelioma of the tunica vaginalis (5/20 at 5000 ppm) and cholangiocarcinoma (2/20 at 5000 ppm) occurred after 13 weeks of treatment followed by a 13-week recovery period. In the 26-week exposure arm of this study, mesothelioma of the tunica vaginalis (7/20 at 5000 ppm) and cholangiocarcinoma (1/20 at 5000 ppm) also developed ([NTP, 2000](#)).

A 105-week feeding study was conducted in male and female F344/N rats. A stop-exposure study (exposure for 13 weeks then no further treatment until the end of the 105 weeks) was also conducted in male F344/N rats. In the main study, groups of 60 males and 60 females were fed diets containing 0 (controls), 625, 1250 or 2000 ppm 2-nitrotoluene (equivalent to average daily doses of approximately 0, 25, 50 or 90 and 0, 30, 60 or 100 mg/kg bw in males and females, respectively) for 105 weeks. In the 13-week stop-exposure study, groups of 60 male rats were fed diets containing 2000 or 5000 ppm 2-nitrotoluene (equivalent to average daily doses of approximately 125 or 315 mg/kg bw) for 13 weeks and were maintained on control diet for the remainder of the 105-week study period. The control group of 60 male rats that received untreated feed in the main study served as the control group for the stop-exposure study. All males in the 2000-ppm group in the main study, all males in the 5000-ppm stop-exposure group and all but three males in the 1250-ppm group in the main study died before the end of the 105 weeks. In the main study, 2-nitrotoluene increased the incidence of mesothelioma, skin lipoma, hepatocellular

adenoma or carcinoma (combined), cholangiocarcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) in males, and that of mammary gland fibroadenoma, skin fibroma and/or fibrosarcoma and hepatocellular adenoma in males and females. At the end of 2 years, the incidence of mesothelioma, skin lipoma, fibroma and fibrosarcoma, hepatocellular adenoma or carcinoma (combined), cholangiocarcinoma, alveolar/bronchiolar adenoma or carcinoma (combined), haemangioma or haemangiosarcoma (combined), and mammary gland fibroadenoma was also increased in male rats in the stop-exposure study ([NTP, 2002](#); [Dunnick et al., 2003](#)).

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

(a) Absorption, distribution and excretion

[Ahlborg et al. \(1985\)](#) studied workers in a chemical factory that produced pharmaceuticals and explosives, including primarily trinitrotoluene but also small amounts of 2-nitrotoluene as a contaminant, and found that only those workers exposed to trinitrotoluene had urine that was mutagenic in *Salmonella typhimurium* TA98 in the absence of metabolic activation; the addition of a metabolic activation system increased the mutagenic potency of the urine, but not significantly. Only trinitrotoluene, and none of the other nitrotoluenes, induced the observed mutagenic activity. A follow-up study showed the presence of diazo-positive metabolites in the urine, but the assay method could not ascribe these to any specific compound ([Ahlborg et al., 1988](#)).

(b) Metabolism

[Jones et al. \(2005a\)](#) analysed urinary metabolites in workers who manufactured dinitrotoluenes and trinitrotoluene in Liaoning, Liaoning Province, China. Nitrobenzoic acids were found in 96% and 73% of the urine samples from workers exposed to 2-nitrotoluene, and 4-nitrotoluene respectively, and air concentrations of neither dinitrotoluenes nor 2-nitrotoluene correlated with the concentrations of nitrobenzoic acids. The air concentrations of 2-nitrotoluene and 4-nitrotoluene were $759 \pm 836 \mu\text{g}/\text{m}^3$ and $685 \pm 500 \mu\text{g}/\text{m}^3$, respectively, and the concentrations ($\mu\text{mol}/\text{L}$) of the urinary metabolites were 4.25 ± 5.76 2-nitrobenzoic acid, 0.33 ± 0.65 2-nitrobenzyl alcohol, 0.12 ± 0.40 4-nitrobenzoic acid and 0.01 ± 0.03 4-nitrobenzyl alcohol ([Jones et al., 2005a](#); [Sabbioni et al., 2006](#)). Thus, nitrobenzoic acids are the primary urinary metabolites in humans exposed to 2-nitrotoluene and 4-nitrotoluene.

*4.1.2 Experimental systems**(a) Absorption, distribution, and excretion*

[Chism et al. \(1984\)](#) collected excreta 72 hours after administration of a single oral dose (200 mg/kg bw) of radiolabeled 2-nitrotoluene to male F344 rats. The radiolabel was excreted rapidly (86% within 24 hours). The major route of excretion was urinary; 70–85% of the dose was excreted in the urine in 72 hours. Within that period, 5–13% and 0–0.1% of the dose were excreted in the faeces and in exhaled air, respectively. 2-Nitrobenzoic acid was the major metabolite excreted in the urine 72 hours after administration (29% of the dose); other metabolites identified included 2-nitrobenzyl glucuronide (14% of the dose) and S-(2-nitrobenzyl)-N-acetylcysteine (12% of the dose).

(b) Metabolism

[deBethizy & Rickert \(1984\)](#) incubated 2-nitrotoluene with isolated male F344 rat hepatocytes for up to 1 hour and identified the following metabolites: 2-nitrobenzyl alcohol (52%), 2-nitrobenzyl glucuronide (28%), an unidentified metabolite (20%) and 2-nitrobenzoic acid (3%). [Chism & Rickert \(1985\)](#) administered 200 mg/kg bw 2-nitrotoluene to male and female F344 rats; 12 hours later, males had excreted 28.6% and females had excreted 9.6% of the dose in the bile. Of the material excreted in the bile, 77% in males (22% of the dose) and 86% in females (8.3% of the dose) was 2-nitrobenzyl glucuronide. Inhibition of enterohepatic circulation by bile-duct cannulation resulted in a decrease in liver macromolecular covalent binding of 98 and 85%, respectively, in males and females, showing that enterohepatic circulation is necessary for the hepatic macromolecular covalent binding of 2-nitrotoluene in both sexes of F344 rats.

In rodents, 2-nitrotoluene undergoes oxidation and conjugation in the liver to form *ortho*-nitrobenzyl alcohol and nitrobenzyl glucuronide, which are then excreted into the intestine via the bile and converted to aminobenzyl alcohol by intestinal microflora through reduction of the nitro group. The aminobenzyl alcohol is then re-absorbed and metabolized in the liver, resulting in an electrophilic compound that can bind to hepatic DNA — possibly a nitrenium ion ([Chism & Rickert, 1985](#)) specific to 2-methylaniline. Hepatic DNA adducts and haemoglobin adducts, together with hepatic DNA damage (assessed by the unscheduled DNA synthesis assay), have been found in 2-nitrotoluene-treated rats (see Section 4.2.2a; [Doolittle et al., 1983](#); [Jones & Sabbioni, 2003](#)).

4.2 Genetic and related effects

4.2.1 Humans

(a) Haemoglobin adducts

[Jones et al. \(2005b\)](#) found a wide variety of haemoglobin adducts among workers in a factory that produced di- and trinitrotoluenes, one of which was a 2-methylaniline–haemoglobin adduct, which derives specifically and only from 2-nitrotoluene.

(b) Mutations

There were no reports of 2-nitrotoluene-induced mutations in humans. However, workers exposed to nitrotoluenes (including 2-nitrotoluene) in a factory in China had highly mutagenic urine, with up to 141.6 revertants/mL-equivalent for enzymatically hydrolysed urine (with β -glucuronidase and arylsulfatase) for the 75th percentiles in *S. typhimurium* strain YG1041 in the absence of metabolic activation ([Sabbioni et al., 2006](#)). [The Working Group noted that this value is higher than that generally found in the urine of unexposed subjects (< 10 revertants/mL-equivalent)].

(c) Chromosomal effects

[Sabbioni et al. \(2006\)](#) found an increased frequency of chromosomal aberrations (including gaps) in the circulating blood lymphocytes of factory workers exposed to a variety of nitrotoluenes; the concentration of 2-nitrotoluene in air was $759 \pm 836 \mu\text{g}/\text{m}^3$. Exposure to 2-nitrotoluene resulted in the excretion of 2-nitrotoluene metabolites in the urine (see Section 4.1.1b).

4.2.2 Experimental systems

(a) DNA and haemoglobin adducts

A single oral dose of radiolabelled 2-nitrotoluene given to male F344 rats resulted in the covalent binding of radioactivity to liver DNA, which was maximal 12 hours after administration. Prior

administration of inhibitors of sulfotransferase (SULT) markedly reduced the level of bound material, indicating that 2-nitrotoluene requires SULT for its conversion to a form that binds to liver DNA *in vivo* ([Rickert et al., 1984](#)). After incubation of radiolabelled 2-aminobenzyl alcohol with calf-thymus DNA, male F344 rat hepatic cytosol and 3'-phosphoadenosine 5'-phosphosulfate, the radiolabel bound covalently to DNA; this binding was prevented by the addition of a SULT inhibitor, 2,6-dichloro-4-nitrophenol. The authors suggested that 2-aminobenzyl sulfate was probably the metabolite that bound to DNA ([Chism & Rickert, 1989](#)).

Although a single oral administration of 2-nitrotoluene by gavage to female Wistar rats did not produce any detectable DNA adducts in the liver, it did produce hydrolysable haemoglobin adducts ([Jones & Sabbioni, 2003](#)). When male WELS-Fohm rats were given 2-nitrotoluene on 5 days a week for 12 weeks, haemoglobin adducts were formed, which were identified as 2-methylaniline after mild base treatment. Hepatic DNA adducts of methylaniline at guanosine and adenosine were also formed, and there was a strong linear relationship between the frequencies of both haemoglobin and DNA adducts and dose, suggesting that haemoglobin adducts could be a useful surrogate marker for hepatic DNA adducts ([Jones et al., 2003](#)).

(b) DNA damage

2-Nitrotoluene administered by gavage to male F344 rats induced unscheduled DNA synthesis, which is a measure of DNA damage. However, unscheduled DNA synthesis was not observed in female rats or germ-free male rats ([Doolittle et al., 1983](#)). This study highlighted the obligatory role of intestinal bacteria in the metabolic activation of 2-nitrotoluene, most probably via nitroreductase activity provided by the gut flora, as well as sex differences in the ability of 2-nitrotoluene to cause DNA damage.

In standard cell-culture methods that include serum, 2-nitrotoluene did not induce unscheduled DNA synthesis in the spermatocytes of rats exposed *in vivo* ([Working & Butterworth, 1984](#)). Similarly, it did not induce unscheduled DNA synthesis *in vitro* in rat hepatocytes or hepatocytes from six human subjects ([Butterworth et al., 1989](#)). However, in defined serum-free media, 2-nitrotoluene did induce unscheduled DNA synthesis in primary rat hepatocytes ([Parton et al., 1995](#)). The positive results in this study probably reflected the ability of serum-free medium to retain the integrity of the cell membrane and cell-surface receptors more efficiently than serum-containing medium.

2-Nitrotoluene did not induce DNA damage in the *rec* assay (deficiency in recombination) in *Bacillus subtilis* ([Shimizu & Yano, 1986](#)).

(c) Mutations

Numerous studies have confirmed that 2-nitrotoluene is not mutagenic in a variety of *Salmonella* strains in the presence or absence of metabolic activation ([Chiu et al., 1978](#); [Tokiwa et al., 1981](#); [Spanggord et al., 1982b](#); [Haworth et al., 1983](#); [Shimizu & Yano, 1986](#)).

(d) Chromosomal effects

In Chinese hamster ovary cells, 2-nitrotoluene induced sister chromatid exchange in the presence of metabolic activation, but gave an equivocal response in its absence, and did not induce chromosomal aberrations in the presence or absence of metabolic activation ([Galloway et al., 1987](#)). It did not induce chromosomal aberrations but did induce polyploidy in Chinese hamster lung cells ([Ishidate et al., 1988](#)). 2-Nitrotoluene did not induce micronuclei in the bone marrow of male mice or rats injected intraperitoneally, but gave equivocal results for the induction of micronuclei in the peripheral blood of male mice. No micronuclei were seen in the peripheral blood erythrocytes of female

mice administered 2-nitrotoluene in the diet for 13 weeks ([NTP, 2002](#)).

(e) Alterations in oncogenes and suppressor genes in tumours

Several studies have investigated mutations and altered gene expression in various types of tumour induced by 2-nitrotoluene in the National Toxicology Program ([NTP, 2002](#)) rodent bioassay. The predominant tumours in mice were haemangiosarcomas. [Hong et al. \(2003\)](#) found that 13/15 (87%) of the haemangiosarcomas of the skeletal muscle, subcutaneous tissue or mesentery had mutations in at least one of the following genes: *ras*, *Tp53* or β -catenin (*Catnb*). In particular, 11/15 (73%) had missense mutations in *Tp53* exons 5–8 and 7/15 (47%) had deletions at exon 2 splice-sites or smaller deletions in the *Catnb* gene. In contrast, only 1/15 (7%) of the tumours had mutations in the *K-ras* gene. Spontaneous haemangiosarcomas lacked both p53 and β -catenin protein (encoded by *Catnb*) expression and *ras* mutations. The mutations in *Tp53* and *Catnb* may have resulted from the genotoxic effects of 2-nitrotoluene and played a role in the pathogenesis of the haemangiosarcomas in 2-nitrotoluene-treated mice.

Tumours of the large intestine (caecum) were the other type of tumour induced by 2-nitrotoluene in mice; this was especially unusual, because it had not been observed among either controls or treated mice in more than 500 carcinogenicity studies conducted by the NTP ([Sills et al., 2004](#)). These gland-forming tumours were positive for cytokeratin 20 and negative for cytokeratin 7. Accumulation of β -catenin protein was found in 8/10 (80%) of the caecal carcinomas, and increased cyclin D1 and p53 protein expression was detected in 8/11 (73%); there was no difference in the expression of adenomatous polyposis coli protein between caecal tumours and normal colon tissue. All tumours had mutations in exon 2 in the *Catnb* gene (which corresponds to exon 3 in the human gene), 9/11 (82%) had mutations in

exon 7 of *Tp53* and 9/11 (82%) had specific G→T transversions at codons 10 or 12 in *K-ras*. These mutations and alterations in proteins activate signal-transduction pathways (*K-ras* and *Catnb*), disrupt the cell cycle and by-pass G₁-arrest (*Tp53*, cyclin D1). These changes are common features of human colon cancer and probably contribute to the pathogenesis of the large intestinal cancers in mice treated with 2-nitrotoluene.

(f) *Changes in gene expression*

Starting at 6 weeks of age, mice were fed 2-nitrotoluene for 2 weeks; livers were then collected and microarray analysis was performed among 20 842 genes. Several cancer-related genes, including the fragile histidine triad gene, the WW domain-containing oxidoreductase gene and the epidermal growth factor receptor gene, were downregulated, whereas P21, Cyclin G1, the nicotinamide adenine dinucleotide phosphate:quinone oxidoreductase gene and the regulator of G-protein signalling 16 gene were upregulated. The early changes in gene expression observed in this study involved apoptosis- and cell cycle-related genes ([Iida et al., 2005](#)).

[Kim et al. \(2005\)](#) evaluated the ability of 2-nitrotoluene, and various other agents, to alter gene expression in mouse lymphoma L5178Y cells, which do not have a functional *Tp53* gene and are more sensitive to DNA damage. Differences were found between genotoxic and non-genotoxic (such as 2-nitrotoluene) agents for genes involved in cell-cycle control, stress response and immune response; however, no gene cluster with altered expression was found that was specific for the four carcinogenic agents studied.

Microarray analysis of peritoneal mesotheliomas induced by 2-nitrotoluene in male rats found alterations in the expression of the insulin-like growth factor 1 gene, the *p38* mitogen-activated protein-kinase gene, *Wnt/Catnb* and the integrin signalling pathways gene; these changes

are also observed in human mesotheliomas ([Kim et al., 2006](#)).

4.3 Other mechanistic considerations

4.3.1 *Effects on cell physiology*

[Sabbioni et al. \(2006\)](#) evaluated a wide variety of biomarkers among nitrotoluene workers in a factory in China, and found that haemoglobin adduct levels were higher in workers who were positive for glucose and/or protein in their urine, and for urobilinogen. The levels of bilirubin and urea in serum were significantly lower in workers with high levels of haemoglobin adducts, and their white blood cell count was lower. [The positive results for protein and glucose in the urine could be caused by tubular or glomerular damage induced by nitrotoluenes.] The nephrotoxicity of nitrotoluenes has been documented in miners exposed to dinitrotoluene ([Brüning et al., 1999, 2002](#)) and in rodents exposed to 2-nitrotoluene ([NTP, 1992](#)).

[Sabbioni et al. \(2006\)](#) also found that increased exposure to nitrotoluenes among these workers resulted in increased levels of alkaline phosphatase and decreased levels of alanine aminotransferase. Albumin and total protein decreased, but the concentration of haemoglobin and red blood cell count increased with increased exposure to nitrotoluenes. [The high levels of alkaline phosphatase and the low level of serum proteins could indicate hepatotoxicity, which, together with liver cancer, were noted in the [NTP \(2002\)](#) rodent study of 2-nitrotoluene.]

4.3.2 *Structure-activity relationships*

Many studies have investigated the metabolism, DNA binding, DNA damage, and carcinogenicity associated with various nitrotoluenes. For example, 2- but not 3- or 4-nitrotoluene induces unscheduled DNA synthesis ([Doolittle et al., 1983](#)), and 2-nitrotoluene binds covalently

to liver DNA *in vivo* ([Jones et al., 2003](#)). In addition, 2-nitrotoluene, similarly to 2,6-dinitrotoluene, requires SULT for its conversion to a form that binds covalently to liver DNA ([Rickert et al., 1984](#)).

4.4 Susceptibility

4.4.1 Genetic polymorphisms and enzyme induction

[Sabbioni et al. \(2006\)](#) evaluated various genotypes among a group of workers exposed to nitrotoluenes (including 2-nitrotoluene) and found that the glutathione S-transferase M1 (*GSTM1*)-positive genotype was significantly more common in controls than in the exposed group [an example of the ‘healthy-worker effect’]. This probably reflected an elevated susceptibility of the *GSTM1*-null genotype to the adverse effects of exposure to dinitrotoluene, such as nausea (odds ratio, 8.8; 95%CI: 2.4–32.2). The levels of methylaniline–haemoglobin adducts, which are derived only from 2-nitrotoluene, were elevated among workers with the *GSTM1*-positive genotype, as well as among those with the *N*-acetyltransferase 1 (*NAT1*) rapid genotype. When the concentration of 2-nitrotoluene in the air was also considered, the correlation between the *NAT2* slow genotype and methylaniline–haemoglobin adducts was quite high ($r = 0.97$; $P < 0.01$). There was a lesser association ($P < 0.1$) between the levels of methylaniline–haemoglobin adducts and the *SULT1A1* Arg/Arg genotype and the *SULT1A2* Asn/Asn genotype. The results suggest that *N*-hydroxyarylamine sulfonation may be more important than *N*-sulfonation for the formation of haemoglobin adducts following exposure to nitrotoluene. The high-activity genotype, which induces high levels of the sulfuric acid ester, may cause most of the metabolite to solubilize back to the *N*-hydroxy derivative, thus giving an additional opportunity for the formation of the haemoglobin adducts.

4.5 Mechanisms of carcinogenesis

A study in rats found a high correlation between levels of 2-nitrotoluene-induced hepatic DNA adducts and haemoglobin adducts, indicating that haemoglobin adducts are a reasonable surrogate for hepatic DNA adducts in rats ([Jones et al., 2003](#)) and, by extension, possibly in humans. Thus, the high levels of haemoglobin adducts measured in Chinese factory workers ([Sabbioni et al., 2006](#); see Section 4.4.1), including methylaniline–haemoglobin adducts, which derive directly from 2-nitrotoluene, may indicate the presence of nitrotoluene-derived hepatic DNA adducts, although these were not measured directly. The association with SULT enzymes, based on the genotyping data among the factory workers and nitrotoluene–haemoglobin adducts, also supports the possible presence of nitrotoluene–DNA adducts — the formation of which in rats requires SULT activity.

Nearly all of the Chinese factory workers exposed to a mixture of nitrotoluenes had nitrobenzoic acids in their urine, and 2-nitrobenzoic acid, which is derived directly from 2-nitrotoluene, is mutagenic in the absence of metabolic activation in *Salmonella* ([Sundvall et al., 1984](#)). A wide variety of other nitrobenzoic acids, nitrobenzyl alcohols and nitrosulfonic acids are also mutagenic ([Grummt et al., 2006](#)). The factory workers also had highly mutagenic urine, indicative of systemic exposure to genotoxins, which was probably due to benzyl alcohol metabolites of nitrotoluenes, especially those of the dinitrotoluenes, because of the high correlation between dinitrotoluene-derived haemoglobin adducts and urinary mutagenicity ([Sabbioni et al., 2006](#)). Thus, there is strong evidence that a genotoxic mechanism underlies the induction of tumours by 2-nitrotoluene in rodents and possibly humans.

5. Summary of Data Reported

5.1 Exposure data

2-Nitrotoluene is isolated from mixtures of nitrotoluene stereoisomers and is produced by nitration of toluene with an aqueous sulfuric/nitric acid mixture. Occupational exposure to 2-nitrotoluene can occur during its production or use in the production of azo dyes, such as *ortho*-toluidine, magenta and sulfur dyes, as well as rubber, agricultural chemicals and explosives.

2-Nitrotoluene can be released into the environment (primarily in air and water) during its manufacture and use. It has been detected in wastewater near 2-nitrotoluene-manufacturing plants and in ambient air and surface water. It may also be formed from the degradation of di- or trinitrotoluenes and has been detected in wastewater, effluents, groundwater, surface water and soil near facilities that produced or used these chemicals, namely munitions-manufacturing plants.

5.2 Human carcinogenicity data

No studies of human cancer were identified that evaluated specific exposure to 2-nitrotoluene; however, one cohort study of dyestuff workers stated that exposure to 2-nitrotoluene occurred (from its use as a raw ingredient or formation as an intermediate) during the manufacturing process of New Fuchsin (a component of magenta). Magenta production, but not magenta, is classified by IARC as *carcinogenic to humans (Group 1)*. In this study, a very large excess of mortality from bladder cancer was found among fuchsin- and safranine T-manufacturing workers, who were potentially exposed to 2-nitrotoluene. These workers were also exposed to *ortho*-toluidine, which causes bladder cancer and is classified by IARC as *carcinogenic to humans (Group 1)*; thus, it is not possible to

evaluate whether 2-nitrotoluene played a causal role in the incidence of bladder cancer.

5.3 Animal carcinogenicity data

In a 2-year study, male and female mice fed 2-nitrotoluene in the diet had an increased incidence of haemangiosarcoma and carcinoma of the large intestine (caecum). The incidence of hepatocellular adenoma and carcinoma was also increased in females.

2-Nitrotoluene caused mesothelioma of the tunica vaginalis and cholangiocarcinomas after 13 or 26 weeks of oral administration in the diet to male rats. In a 2-year study in male and female rats, administration of 2-nitrotoluene in the diet increased the incidence of lipoma, fibroma and fibrosarcoma of the skin, mesothelioma, mammary gland fibroadenoma, hepatocellular adenoma or carcinoma (combined), cholangiocarcinoma and alveolar bronchiolar adenoma or carcinoma (combined) in male rats. Treatment-related tumours in female rats included skin fibroma, and skin fibroma or fibrosarcoma (combined), mammary gland fibroadenoma and hepatocellular adenoma. In a 2-year stop-exposure study in which male rats were exposed to 2-nitrotoluene for 13 weeks and then maintained on untreated diet until the end of the experiment, an increase in the incidence of mesothelioma, skin lipoma, fibroma and fibrosarcoma, cholangiocarcinoma, hepatocellular adenoma or carcinoma (combined), alveolar/bronchiolar adenoma or carcinoma (combined), and haemangioma or haemangiosarcoma (combined) was also observed. Thus, even these short exposures to 2-nitrotoluene induced cancer.

There was consistency in the tumour response across the 13-week, 26-week, 2-year and stop-exposure studies.

5.4 Other relevant data

In rodents, 2-nitrotoluene undergoes oxidation and conjugation in the liver to form 2-nitrobenzyl alcohol glucuronide, which is then excreted into the intestine via the bile and converted to aminobenzyl alcohol by intestinal microflora through reduction of the nitro group. The aminobenzyl alcohol is then re-absorbed and metabolized in the liver, resulting in an electrophilic compound that can bind to liver DNA and form DNA adducts. A similar pathway probably operates in humans, because 2-nitrotoluene is primarily metabolized to nitrobenzoic acids, which have been found as urinary metabolites in both humans and rodents.

Urine from workers exposed to a mixture of nitrotoluenes containing 2-nitrotoluene was mutagenic in bacteria. 2-Nitrobenzoic acid, which is derived directly from 2-nitrotoluene, is mutagenic in bacteria in the absence of metabolic activation. Many other nitrobenzoic acids, nitrobenzylalcohols and nitrosulfonic acids are also mutagenic.

Exposure of rats to 2-nitrotoluene induced a specific protein adduct, 2-methylaniline–haemoglobin, and liver DNA adducts of 2-methylaniline at guanosine and adenosine, which were found to be well correlated. This observation suggests that the haemoglobin adducts may serve as a reasonable biomarker of a systemic genotoxic effect.

In workers exposed to a mixture of nitrotoluenes containing 2-nitrotoluene, elevated levels of chromosomal aberrations were found in circulating blood lymphocytes, as were 2-methylaniline–haemoglobin adducts. Because this protein biomarker is specific to exposure to 2-nitrotoluene and was found to be correlated with the formation of DNA adducts in rat liver, and because the urine of exposed workers is mutagenic, there is strong evidence to suggest that genotoxic metabolites of 2-nitrotoluene are formed in exposed humans. Furthermore, the levels of haemoglobin adducts are also influenced by genotype, including those

of glutathione S-transferase, *N*-acetyltransferase (rapid and slow) and sulfotransferase 1A1 genes.

Although exposure of humans to a mixture of nitrotoluenes containing 2-nitrotoluene results in genotoxic effects (i.e. mutagenic urine), 2-nitrotoluene is generally not mutagenic in standard in-vitro mutagenicity assays in the presence or absence of metabolic activation, presumably because the complex metabolism required for its activation is not present in such assays.

Analysis of mutations and changes in gene expression in 2-nitrotoluene-induced haemangiosarcomas in mice found that mutations in *Tp53* and β -catenin probably resulted from the genotoxic effects of 2-nitrotoluene and played a role in the formation of the tumours. Similarly, mutations in β -catenin, *Tp53* and *K-ras* were found in 2-nitrotoluene-induced tumours of the large intestine in mice, and these are common features of human colon cancer. In 2-nitrotoluene-induced mesotheliomas in rats, alterations in expression were found in various genes such as insulin-like growth factor, p38 mitogen-activated protein-kinase, *Wnt*/ β -catenin and integrin-signalling pathways. Alterations in the expression of these genes are also found in human mesotheliomas.

Overall, there is strong evidence that a genotoxic mechanism underlies the induction of tumours in 2-nitrotoluene-exposed rodents. The evidence for the relevance of this mechanism to humans is strong.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 2-nitrotoluene.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2-nitrotoluene.

6.3 Overall evaluation

2-Nitrotoluene is *probably carcinogenic to humans (Group 2A)*.

6.4 Rationale

In making the overall evaluation, the Working Group took into consideration that there is strong evidence that a mutational mechanism underlies the induction of tumours in both rodents and humans exposed to 2-nitrotoluene.

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DIETHANOLAMINE

Diethanolamine was considered by a previous IARC Working Group in 2000 ([IARC, 2000](#)). Since that time new data have become available, which have been incorporated to this *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.: 111-42-2

Deleted Chem. Abstr. Serv. Reg. No.:
8033-73-6

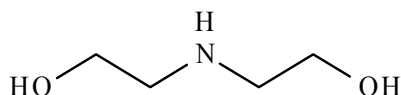
Chem. Abstr. Name: 2,2'-Iminobis[ethanol]

IUPAC Systematic Name:

2-(2-Hydroxyethylamino)ethanol

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-hydroxyethylamino)ethanol; iminodiethanol; *N,N'*-iminodiethanol; 2,2'-iminodi-1-ethanol; diethylolamine.

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

Description: Deliquescent prisms; viscous liquid with a mild odour of ammonia ([O'Neil et al., 2006](#))

Boiling-point: 268.8 °C ([O'Neil et al., 2006](#))

Melting-point: 28 °C ([O'Neil et al., 2006](#))

Density: 1.0940 at 25 °C ([O'Neil et al., 2006](#))

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, 2000](#))

Solubility: Miscible with water, methanol, acetone, ethanol, chloroform and glycerine; soluble at 25 °C in benzene (4.2%), ether (0.8%), carbon tetrachloride (< 0.1%) and *n*-heptane (< 0.1%); slightly soluble to insoluble in petroleum ether ([O'Neil et al., 2006](#))

Vapour pressure: 0.00037 hPa at 25 °C ([IUCLID, 2000](#))

Stability and reactivity: Stable at usual use temperatures; incompatible with some metals, halogenated organics, nitrites,

strong acids and strong oxidizers ([Dow Chemical Company, 1999](#))

Octanol/water partition coefficient (*P*): log *P*, -1.43 ([Sangster, 2006](#))

Conversion factor: $\text{mg/m}^3 = 4.30 \times \text{ppm}$ (calculated from: $\text{mg/m}^3 = (\text{relative molecular mass}/24.45) \text{ppm}$, assuming a temperature of 25 °C and a pressure of 101 kPa)

1.1.4 Technical products and impurities

Diethanolamine is commercially available with the following specifications: purity, 99% min.; monoethanolamine, 0.5% max.; triethanolamine 0.5% max.; and water content, 0.15% max. ([Huntsman Corporation, 2008](#)). A lower grade of diethanolamine is commercially available with the following specifications: purity, 55% min.; monoethanolamine, 5% max.; triethanolamine 40% max.; and water content, 1% max. ([Elarum, 2010](#)). In Europe, diethanolamine is typically marketed with the following specifications: purity, > 99%; triethanolamine, 1% max.; monoethanolamine, 0.5% max.; and water content, 0.2% max. ([OECD, 2007](#)).

Diethanolamine is also available as a blend of 83–87% diethanolamine and 13–17% deionized water with monoethanolamine and triethanolamine present as impurities at a maximum concentration of 1% ([Huntsman Corporation, 2007](#)).

1.1.5 Analysis

Diethanolamine can be determined in workplace air by drawing the air sample through aqueous hexanesulfonic acid and analysing with ion chromatography. The range for this method is 0.30–19.5 mg for a 100-L air sample ([NIOSH, 2003](#)).

Diethanolamine can be determined in air by drawing the air sample through sampling tubes containing XAD-2 resin coated with 10% 1-naphthylisothiocyanate. Samples are analysed

by desorbing the adsorbent with dimethylformamide and quantitating the amine derivative by high performance liquid chromatography using ultraviolet detection ([OSHA, 2010](#)).

Exposure to diethanolamine from metal working fluids has been determined by high performance liquid chromatography/mass spectrometry analysis of aqueous hand-washing solutions and personal air samples collected on acid-treated glass fibre filters ([Henriks-Eckerman et al., 2007](#)).

Levels of diethanolamine in shampoo products can be determined by liquid chromatography/thermal energy analysis after conversion to *N*-nitrosodiethanolamine with acetic acid and sodium nitrite ([Chou, 2005](#)).

1.2 Production and use

1.2.1 Production

Diethanolamine is produced by reacting ethylene oxide with ammonia. In most production facilities, ethylene oxide and ammonia are reacted in a batch process that yields a crude mixture of ethanolamine, diethanolamine and triethanolamine. The mixture is then distilled to separate and purify the individual compounds ([Edens & Lochary, 2004](#)).

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 ([IARC, 2000](#)).

It has been estimated that 45 900 and 75 400 tonnes of diethanolamine were produced in the USA in 1972 and 1983, respectively ([HSDB, 2010](#)). Estimated annual production of diethanolamine in the USA over three decades is presented in [Table 1.1](#).

Worldwide production of ethanolamines in 1985 was approximately (thousand tonnes per

Table 1.1 Estimated annual production of diethanolamine in the USA (thousand tonnes)

Year	1960	1965	1970	1975	1980	1985	1989	1995
Production	24	35	42	39	56	76	92	149

From [Edens & Lochary \(2004\)](#)

year): USA, 220; western Europe, 145; south-eastern Asia, 40; South America, 18; and eastern Europe, 4. Of the world production of ethanolamines in 1985, approximately 50% was monoethanolamine, 30–35% diethanolamine and 15–20% triethanolamine ([Hammer *et al.*, 1987](#)).

The annual world capacity for the ethanolamines in 2005 was estimated at 1 510 000 tonnes, subdivided into 400 000 tonnes for Europe (eight production sites), 780 000 tonnes for North and South America (seven production sites), 30 000 tonnes for the Middle East (one production site) and 300 000 tonnes for the Asia/Pacific region (11 production sites). No data on individual capacities for diethanolamine were available ([OECD, 2007](#)).

Information available in 2010 indicated that diethanolamine was manufactured by 29 companies in the USA, seven companies in Mexico, three companies each in the People's Republic of China and the United Kingdom, two companies each in Canada, Germany, China (Hong Kong SAR) and India, and one company each in Belgium, Slovak Republic and Switzerland ([Chemical Sources International, 2010](#)). Other sources indicated that diethanolamine was produced by five companies in the USA (HSDB, 2010), five companies in Germany, three companies in the United Kingdom, three companies in the Netherlands and one company each in Austria, Belgium, Denmark and Sweden ([IUCLID, 2000](#)).

1.2.2 Use

Diethanolamine is widely used in the preparation of diethanolamides and diethanolamine salts of long-chain fatty acids that are formulated

into soaps and surfactants used in liquid laundry and dishwashing detergents, cosmetics, shampoos and hair conditioners. Diethanolamine is also used in the production of lubricants in the textile industry, in industrial gas purification to remove acid gases and as an emulsifier and dispersing agent in preparations of agricultural chemicals. Diethanolamine is used in metalworking fluids for cutting, stamping and die-casting operations as a corrosion inhibitor. In the production of detergents, cleaners, fabric solvents and metalworking fluids, diethanolamine is used for acid neutralization and soil deposition. Aqueous diethanolamine solutions are used as solvents for numerous drugs that are administered intravenously. Shampoos and hair dyes may contain free diethanolamine as a component and/or as a contaminant of fatty acid alkanolamides, generally in the range of 0.2–10% ([Bailey, 2007](#)). Diethanolamine is used with sulfolane in the sulfinol process to absorb carbon dioxide and hydrogen sulfide gases ([NTP, 1999a](#); [Edens & Lochary, 2004](#); [OECD, 2007, 2008](#)).

[Table 1.2](#) presents estimates of uses in major applications in the USA ([Knaak *et al.*, 1997](#)).

The database for substances in preparations in Nordic countries lists a wide variety of uses of diethanolamine registered in Denmark, Norway, Sweden and Finland. In 2004, 520 preparations containing diethanolamine, accounting for a total volume of 19 865.8 tonnes, were registered in Denmark. In Norway, Sweden, and Finland, 103 (856.8 tonnes), 307 (459.0 tonnes), and 75 (132.7 tonnes) products were registered, respectively. Use categories included intermediates, cleaning/washing agents, paints, lacquers and varnishes, surface treatments, cutting fluids, pH-regulation agents, impregnation materials, surface-active agents, corrosion inhibitors, process regulators, colouring agents, reprographic agents, lubricants and additives. Its use in consumer preparations was indicated for products registered in Norway and Sweden ([SPIN, 2006](#); [OECD, 2008](#)).

Table 1.2 Major uses of diethanolamine in the USA

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. \(1997\)](#)

1.3 Occurrence and exposure

1.3.1 Natural occurrence

Diethanolamine is not known to occur as a natural product.

1.3.2 Occupational exposure

Diethanolamine is present in water-based machining and grinding fluids (soluble oils, semi-synthetic and synthetic metalworking fluids) and has been detected in workplace air in the metal manufacturing industry. It was detected in bulk metalworking fluids at levels ranging from 4 to 5% ([Kenyon et al., 1993](#)). Recent exposure to diethanolamine can be inferred from studies showed dermal sensitivity among workers exposed to metalworking fluids ([Geier et al., 2004a, b](#)). Moreover, the presence of *N*-nitrosodiethanolamine in bulk fluids and in the urine of exposed workers may provide indirect evidence for the exposure to diethanolamine from these fluids ([Ducos & Gaudin, 2003](#)).

According to the 1981–83 National Occupational Exposure Survey ([NIOSH, 1999](#)), 800 000 workers (many of whom were metalworkers) in the USA were potentially exposed to diethanolamine.

The median air concentration of diethanolamine in nine machine shops in Finland was found to be 64 µg/m³ ([Henriks-Eckerman et al., 2007](#)).

The presence of diethanolamine has also been reported in wetting fluids used in road paving. A level of 0.05 mg/m³ 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 limit of detection (0.02 mg/m³) ([Levin et al., 1994](#)). In a study in Germany (1992–94), diethanolamine was detected in samples of metalworking fluids at a range of 0–44% (*n* = 69). The proportion of samples in which diethanolamine was present steadily declined from 90 to 60% over the study period ([Pfeiffer et al., 1996](#)).

In 1996, 51 samples of cooling lubricant concentrates from the German market were analysed. Of these, six (12%) showed diethanolamine concentrations of more than 0.2%, with a maximum concentration reaching 0.85%. The occurrence of diethanolamine levels above 0.2% in these concentrates declined from 80% (1991–92), to 53% (1993), 25% (1994), 21% (1995), and 12% (1996). The reduction was due to a change in the composition of the coolant fluids that followed regulatory requirements in Germany (see Section 1.4). The detected residues above 0.2% were not due to the direct addition of diethanolamine as an ingredient, but to contamination by other components in the coolant fluids ([Kaup et al., 1997](#)).

At a site in Germany, diethanolamine is produced in one production plant and is processed further within eight other operations and

plants. Between January 2001 and December 2006, data on 53 workplace exposures covering all operations were collected by means of personal air sampling. The reported data are 8-hour time-weighted average (TWA) values for shifts. In the production plant, the highest value recorded was 0.026 mg/m³; at the filling stations, the maximum value recorded was 0.062 mg/m³; and the overall range of the measurements (53) was < 0.019–0.062 mg/m³ (OECD, 2008).

1.3.3 Environmental occurrence

Production of diethanolamine and its wide use in industrial and consumer products may result in its release into 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 (EPA) Toxics Release Inventory, air emissions of diethanolamine from 358 industrial facilities in 1994 were approximately 149 200 kg in the USA (US EPA, 1996). According to the National Pollutant Release Inventory (NPRI) of Canada, on-site releases of diethanolamine into the air from 74 facilities amounted to about 40 000 kg/year (Environment Canada, 1995).

(b) Water

Surface water discharges of diethanolamine from 358 industrial facilities in 1994 in the USA amounted to 100 350 kg, as reported in the Toxics Release Inventory (US EPA, 1996). On-site releases of diethanolamine (and its salts) to water from 74 facilities in Canada amounted to about 26 000 kg/year, 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).

Diethanolamine was not detected in a study carried out in 1978 in any of the 21 samples taken from surface water in Japan (Japanese Department of Environmental Health, 1985). Diethanolamine was detected in German surface waters of the Rivers Elbe at 0.34–0.58 µg/L, Mulde at 2.54–4.6 µg/L, Neibe at 0.72–1.8 µg/L and Rhine at 0.30–0.59 µg/L (Pietsch *et al.*, 2001; OECD, 2008).

(c) Soil

Releases of diethanolamine to the land and underground from 358 industrial facilities in the USA in 1994 (as reported to the Toxics Release Inventory) amounted to 77 050 kg and 36 850 kg, respectively (US EPA, 1996). Canadian on-site releases of diethanolamine (and its salts) to land and underground amounted to about 118 000 kg and 497 000 kg/year, respectively, as reported to the NPRI (Environment Canada, 1995).

1.3.4 Occurrence in personal care products

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 19%. Diethanolamine also occurs as a contaminant in triethanolamine products (see Table 1.3).

Potential exposure to diethanolamine in personal care products arises from the use of alkanolamides of diethanolamine, which are condensation products of diethanolamine and fatty acids (e.g. cocamide diethanolamine, a reaction product of diethanolamine and coconut oil-derived fatty acids). Cocamide diethanolamine, lauramide diethanolamine, linoleamide diethanolamine and oleamide diethanolamine are fatty acid diethanolamides that may contain 4–33% diethanolamine, and are present in cosmetics at concentrations of < 0.1–50% (Dea, 1986).

Table 1.3 Diethanolamine content of several condensates

Product	Diethanolamine content	Reference
Coconut oil acid diethanolamine condensate	18.2%	NTP (2001)
Lauric acid diethanolamine condensate	0.83%	NTP (1999a)
Oleic acid diethanolamine condensate	0.19%	NTP (1999c)
Triethanolamine	0.49%	NTP (1999d)

Twenty shampoo products were analysed and 19 were found to contain diethanolamine at levels ranging from 140 to 15 200 ppm ([Chou, 2005](#)). In a substudy to assess skin absorption, a commercially available body lotion was found to contain 1.8 mg/g diethanolamine ([Craciunescu et al., 2009](#)). In a study of skin penetration, two representative shampoo formulations containing coconut diethanolamide at a concentration of 4% were found to contain 0.98% diethanolamine; two shampoos and a bubble bath containing 4.75% lauramide diethanolamine contained 0.25% diethanolamine; a leave-on emulsion containing 2% triethanolamine contained 0.008% diethanolamine; and an oxidative hair dye containing 4.7% lauramide diethanolamine contained 0.25% diethanolamine, while two other hair dye products containing 1.4% lauramide diethanolamine contained 0.075% diethanolamine ([Brain et al., 2005](#)). In a study of the penetration of cosmetic products through intact human skin, a shampoo containing cocamide diethanolamine was found to include 0.092% free diethanolamine, and a second shampoo containing lauramide diethanolamine included 0.28% free diethanolamine ([Kraeling et al., 2004](#)).

1.3.5 Detection in body fluids and daily exposure estimates

After about 3 or 4 weeks of using a body lotion containing 1.8 mg/g diethanolamine, plasma concentrations of the compound in three volunteer subjects ranged from 3 to 7 nmol/mL

([Craciunescu et al., 2009](#)) [data were read from a graph].

[Craciunescu et al. \(2006\)](#) provided exposure estimates of 8–200 mg/kg per day from daily use of shampoo. An alternative calculation using a lower diethanolamine content in shampoo and lower skin penetration rates suggested that the exposure to diethanolamine for a 60-kg adult would be in the range of 0.2–2 µg/kg per day ([Bailey, 2007](#)). [The Working Group noted the large discrepancy in the estimated values between the two studies.]

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for diethanolamine are presented in [Table 1.4](#).

The Food and Drug Administration (FDA) permits the use of diethanolamine as a component of adhesives in food packaging, as an indirect food additive, as a component of uncoated or coated food contact surfaces 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 USA ([FDA, 2010](#)).

A technical standard in Germany limits the level of diethanolamine in water-mixable cooling lubricants to 0.2% ([Kaup et al., 1997](#)).

Table 1.4 Occupational exposure limits and guidelines for diethanolamine

Country	Year	Concentration (mg/m ³)	Interpretation
Argentina ^a	2007	11	TWA
Australia	2008	13	TWA
Belgium	2002	2 (sk)	TWA
Bulgaria ^a	2007	11	TWA
Columbia ^a	2007	11	TWA
Denmark	2002	2	TWA
France	2006	15	VME
Jordan ^a	2007	11	TWA
Netherlands	2003	2	TWA
New Zealand	2002	13 (sk)	TWA
Norway	1999	15	TWA
Republic of Korea	2006	15	TWA
Russian Federation	2003	5 (sk)	STEL
Singapore ^a	2007	11	TWA
Sweden	2005	15	TWA
		30 (sk)	STEL
Switzerland	2006	1	MAK-week
		1	KZG-week
USA			
ACGIH ^a	2007	2	TWA
NIOSH	1995	15	TWA
Viet Nam ^a	2007	11	TWA

^a These countries follow the recommendations of the ACGIH threshold limit values.

ACGIH, American Conference of Governmental and Industrial Hygienists; KZG, Kurz Zeit Gedächtnis; MAK, Maximale Arbeitsplatz-Konzentration; NIOSH, National Institute of Occupational Safety and Health; sk, skin notation; STEL, short-term exposure limit; TWA, time-weighted average; VME, Valeur Moyenne d'Exposition

From [RTECS \(2009\)](#) and [ACGIH \(2010\)](#)

2. Cancer in Humans

The Working Group was not aware of any study that specifically examined the risk of cancer among persons exposed to diethanolamine. While diethanolamine is found in personal care products, no epidemiological studies evaluating human cancer in association with diethanolamine were identified. However, ethanolamines have been used as additives in metalworking fluids since the 1950s and are present in wetting fluids used in asphalt paving. Exposures to these agents occur as complex mixtures and there is a large database of studies on workers exposed in these occupational settings. In light of the complex mixtures, and concomitant

occupational exposures, any observed elevations in risk 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. The data on metalworking fluids are reviewed below, although a formal evaluation by the Working Group is not provided.

There are four major types of metalworking fluid: straight (generally mineral oils), soluble and semi-synthetic (straight oils diluted with water and additives) and synthetic (water and additives with no oil). [Exposure assessments for soluble and synthetic are often combined for analysis.] Ethanolamines — either diethanolamine or triethanolamine — are common additives to

soluble, semi-synthetic and synthetic metalworking fluids (see Section 1). Diethanolamine may also be present as an unintended impurity of intended triethanolamine or fatty acid diethanolamide additives. 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, potential exposure to *N*-nitrosodiethanolamine occurred in all of the studies considered. The use of diethanolamine and nitrites together as additives to metalworking fluids can lead to the formation of *N*-nitrosodiethanolamine. Therefore, workers in any study that noted exposure to *N*-nitrosodiethanolamine would also have been exposed to the diethanolamine from which the nitroso derivative was formed. In this review, only studies that included workers exposed to water-based (soluble, synthetic and semi-synthetic) metalworking fluids were included.

[IARC \(2000\)](#) previously reviewed several studies that examined the risk of cancer among workers potentially exposed to diethanolamine and *N*-nitrosodiethanolamine through metalworking fluids. Virtually all the cohorts described included workers with exposure to soluble, semi-synthetic or synthetic fluids. Only studies with potential exposure to ethanolamines (no studies indicated diethanolamine alone) were considered by the Working Group. The previous *IARC Monograph* ([IARC, 2000](#)) concluded that small excesses were observed for cancers at various sites, in particular the stomach, oesophagus and larynx. In those studies, only associations with the use of soluble oils or synthetic fluids were presented and no results were given specifically in relation to exposure to diethanolamine.

Studies reviewed previously included two proportionate mortality studies, two cohort studies and two nested case-control studies. The proportionate mortality studies included a study of workers employed at a bearing-manufacturing plant ([Park et al., 1988](#)) and a study

of workers employed at two large automotive engine manufacturing plants ([Park & Mirer, 1996](#)) in the USA. The cohort studies analysed the mortality of Swedish men employed in the grinding or turning departments of a company producing bearing rings ([Järvholm et al., 1986](#); [Järvholm & Lavenius, 1987](#)), and that of a large cohort of 46 384 workers employed in three facilities manufacturing automotive parts in the USA ([Eisen et al., 1992](#)).

The later cohort ([Eisen et al., 1992](#)) represents the most extensive database and the findings were reported in a series of publications. Exposure was assessed for all three types of metalworking fluid (straight, soluble and synthetic) ([Eisen et al., 1992](#); [Tolbert et al., 1992](#)). Findings for the follow-up from 1940 to 1984 were reported by [Eisen et al. \(1992\)](#). [Tolbert et al. \(1992\)](#) reported the results of a cohort study of 33 619 persons who had worked for at least 3 years before 1985 in two of the three facilities studied by [Eisen et al. \(1992\)](#). Case-control studies nested among the members of the cohort studied by [Eisen et al. \(1992\)](#) were reported in [Eisen et al. \(1994\)](#) for laryngeal cancer and [Sullivan et al. \(1998\)](#) for oesophageal cancer.

The present Working Group examined results of studies of workers exposed to water-based metalworking fluids published since the previous review. These included an update of the major cohort of automobile workers in the USA ([Eisen et al., 1992](#)) and a series of publications related to that cohort, an independent cohort study of workers in an engine plant ([Kazerouni et al., 2000](#)) and a population-based case-control study of urinary bladder cancer ([Colt et al., 2011](#)).

[Eisen et al. \(2001\)](#) extended the follow-up of the [Eisen et al. \(1992\)](#) cohort for an additional 10 years for 46 399 automobile manufacturing workers with potential exposure to metalworking fluids. In external analyses, significant excesses in risks were found for leukaemia and cancers of the liver, lung and stomach among white workers and pancreatic cancer in black

workers. In some circumstances, the risks were higher in the extended follow-up period than in the entire period of observation. In internal analyses using the unexposed workers as a reference group, workers with the highest cumulative exposure ($\text{mg}/\text{m}^3\text{-years}$) to grinding with soluble metalworking fluids (modelled as a categorical variable) had a significant increased risk for skin cancer and indications for an increased risk of cancer of the larynx, with some evidence of a trend ($P = 0.065$). When exposure to different types of metalworking fluids was modelled as a continuous variable, significant increases were observed for an exposure–response to synthetic metalworking fluids and liver cancer and exposure to soluble metalworking fluids and cancers of the skin and prostate. Excess risks of borderline significance were observed for other sites.

A series of analyses and subanalyses stemmed from the [Eisen et al. \(2001\)](#) cohort ([Zeka et al., 2004](#); [Agalliu et al., 2005a, b](#); [Bardin et al., 2005](#); [Thompson et al., 2005](#); [Malloy et al., 2007](#); [Friesen et al., 2009](#); [Mehta et al., 2010](#); [Costello et al., 2011](#)). Each study assessed cumulative exposure to the three types of metalworking fluid (straight, soluble and synthetic) using a common quantitative exposure matrix. These studies used a variety of analytical methods (such as analysis of relevant biological time windows of exposure and latency) to evaluate risks at specific cancer sites. [An advantage of many of these studies is that they examined cancer incidence rather than mortality.] A few studies have also calculated risk estimates for exposure to specific components of metalworking fluids (i.e. ethanolamines and nitrosamines).

Exposure to soluble metalworking fluids was associated with an elevated risk for cancers of the breast ([Thompson et al., 2005](#)), prostate ([Agalliu et al., 2005a, b](#)) and skin (melanoma) ([Costello et al., 2011](#)) but not with cancers of the urinary bladder ([Friesen et al., 2009](#)), lung ([Friesen et al., 2009](#)), larynx, oesophagus, stomach ([Zeka et al.,](#)

[2010](#)), rectum ([Malloy et al., 2007](#)) or hepatobiliary tract ([Bardin et al., 2005](#)).

None of the studies found a statistically significant association with exposure to synthetic metalworking fluids. A marginally significant inverse relationship for lung cancer was observed for exposure to synthetic fluids ([Mehta et al., 2010](#)). Significant elevated risks for several cancer sites were associated with exposure to straight metalworking fluids. [The Working Group noted the complexity of the exposure assessment because workers were often exposed to multiple types of metalworking fluid, the types of fluids had changed over time (the use of water-based fluids has increased) and workers often changed jobs.]

[Kazerouni et al. \(2000\)](#) updated the mortality experience of 11 838 workers ([Decoufle, 1976, 1978](#)) exposed to a variety of metalworking fluids, including cutting-oil mist. [The Working Group inferred that the description of metalworking fluid defined by the authors included straight, soluble, synthetic and semi-synthetic types.] Exposures were qualitatively categorized as ‘heavy, moderate or low’ based on job history. Among workers exposed to oil mist, significantly (or of borderline significance) elevated standardized mortality ratios were observed for cancers of the liver and biliary tract, testis and lung and Hodgkin disease; the magnitude of effect was higher among heavily exposed workers. Among workers with heavy exposure to oil mists, mortality from lung cancer was higher for those with ≥ 5 years of exposure compared with those with < 5 years of exposure [although the confidence intervals overlapped].

[Colt et al. \(2011\)](#) conducted a large, population-based case–control study in the USA of 1158 incident urinary bladder cancer cases and 1402 population controls. Men reporting use of metalworking fluids at a personal interview had a significantly elevated risk for urinary bladder cancer after adjusting for demographic factors, tobacco smoking and employment in

other high-risk occupations. [The Working Group noted that the type of metalworking fluid was not specified and exposure was based on self-reporting.]

[The Working Group noted that the mixed and varied exposures may explain the variability of the results of the different studies and also make the attribution of excesses of cancer observed to any single agent very difficult. It is probable that most of the cohorts studied included workers exposed to water-reduced metalworking fluids, who were probably exposed to diethanolamine by skin penetration and inhalation. Because the exposures reviewed here occur were to complex mixtures, the metalworking fluid environment might be evaluated better as an exposure circumstance.]

3. Cancer in Experimental Animals

3.1 Skin application

3.1.1 Mouse

Groups of 50 male and 50 female B6C3F₁ mice, 6 weeks of age, received dermal applications of 0, 40, 80 or 160 mg/kg body weight (bw) diethanolamine (purity, > 99%) in 95% ethanol on 5 days per week for 103 weeks. Survival of treated males was similar to that of vehicle controls, but that of treated females was significantly 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 incidence of hepatocellular adenoma, hepatocellular carcinoma and of hepatocellular

adenoma and carcinoma (combined) in all dosed groups was significantly greater than that 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 incidence of hepatoblastoma in the mid- and high-dose groups was significantly increased compared with vehicle controls (0/50, 2/50, 8/50 ($P = 0.004$, pairwise comparison) and 5/50 ($P = 0.028$, pairwise comparison) in the control, low-, mid- and high-dose groups, respectively). In female mice, the incidence of hepatocellular adenoma and carcinoma was significantly higher than that in the vehicle controls (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). The incidence of renal tubule adenoma in males showed an 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 single sectioning and extended-step sectioning were combined, the incidence was: 1/50, 6/50, 8/50 and 7/50 ($P = 0.046$, poly-3 trend test) for the control, low-, mid- and high-dose groups, respectively ([NTP, 1999b](#); [Table 3.1](#)).

[The Working Group noted that tumours of the kidney and hepatoblastomas are rare spontaneous neoplasms in experimental animals.]

3.1.2 Rat

Groups of 50 male and 50 female F344/N rats, 6 weeks of age, received dermal applications of diethanolamine (purity, > 99%) in 95% ethanol on 5 days per week for 103 weeks. Males received 0, 16, 32 or 64 and females received 0, 8, 16 or 32 mg/kg bw. Survival rates for treated males and females were similar to those of corresponding

Table 3.1 Carcinogenicity studies of exposure to diethanolamine in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mouse, B6C3F ₁ (M, F) 105 wk NTP (1999b)	Dermal application 0, 40, 80 or 160 mg/kg bw (M, F), 5x/wk, 103 wk 50/group rats	Liver (hepatocellular adenoma): M-31/50 (62%), 42/50 (84%), 49/50 (98%), 45/50 (90%) F-32/50 (64%), 50/50 (100%), 48/50 (96%), 48/50 (96%)	$P > 0.001$ (trend in M, F) (poly-3 test)	> 99% pure
		Liver (hepatocellular carcinoma): M-12/50 (24%), 17/50 (34%), 33/50 (66%), 34/50 (68%) F-5/50 (10%), 19/50 (38%), 38/50 (76%), 42/50 (84%)	$P > 0.001$ (trend in M, F) (poly-3 test)	
		Liver (hepatoblastoma): M-0/50, 2/50 (4%), 8/50 (16%), 5/50 (10%)	$P = 0.004$ (mid-dose M) $P = 0.028$ (high-dose M)	
		Kidney (renal tubule adenoma, standard evaluation): M-1/50 (2%), 4/50 (8%), 6/50 (12%), 6/50 (12%)	$P = 0.049$ (trend in M) (poly-3 test)	
		Kidney (renal tubule adenoma, standard + extended evaluations): M-1/50 (2%), 6/50 (12%), 8/50 (16%), 7/50 (14%)	$P = 0.046$ (trend in M) (poly-3 test)	

bw, body weight; F, female; M, male; wk, week or weeks

vehicle-control groups. The mean body weight of the high-dose males was lower than that of the vehicle controls from week 8 and that of the high-dose females was lower than that of the vehicle controls from week 97. There was no increase in tumour incidence in treated groups compared with the vehicle controls ([NTP, 1999b](#)).

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, received dermal applications of diethanolamine in 95% ethanol. The diethanolamine used was from the same chemical batch as that used in the study in B6C3F₁ mice ([NTP, 1999b](#)). The diethanolamine was administered in 200- μ L volumes, five times a week for 20 weeks. The concurrent negative-control groups were treated with 200 μ L 95% ethanol. The positive-control group was treated with 1.25 μ g 12-*O*-tetradecanoylphorbol 13-acetate (approximately 99% pure) twice a week for 20 weeks. The doses of diethanolamine selected were based on the maximum tolerated dose used earlier ([NTP, 1999b](#)) and were 5, 10 or 20 mg/mouse per application (higher than the maximum tolerated dose). 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 3 weeks. Animals that did not survive to 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, 18/20 of which developed multiple papillomas, 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 (by dermal application

studies) in B6C3F₁ mice and F344/N rats of fatty acid-diethanolamine (coconut oil acid, lauric acid and oleic acid diethanolamine) condensates conducted by the National Toxicology Program ([NTP, 1999c](#), [d](#), [2001](#)). The same three condensates were also tested in the transgenic Tg.AC and *Tp53*^{+/-} mouse models ([Spalding et al., 2000](#)). The Working Group concluded that these studies could not be used in an 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 that these studies were not designed as, and did not represent, conventional or adequate carcinogenesis bioassays of diethanolamine.]

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

Studies of the penetration of [¹⁴C]diethanolamine from cosmetic formulations (shampoos hair dyes and body lotions) through human skin samples indicated that approximately 0.1% of the applied dose of shampoo and hair dye formulations was absorbed into the receptor fluid after 5–30 minutes; in a 72-hour repeated-dose study with a body lotion formulation, nearly 30% of applied diethanolamine accumulated in the skin and approximately 1% was absorbed into the receptor fluid ([Kraeling et al., 2004](#)). A subsequent study found similar levels of permeation of diethanolamine from cosmetic formulations through human skin ([Brain et al., 2005](#)). [These studies did not address the permeability of diethanolamine through the skin of young

children, the effects of elevated temperatures associated with bathing or showering, or the effect of abrasions that alter skin integrity on the dermal absorption of diethanolamine.]

Diethanolamine was absorbed by human liver slices and incorporated into phospholipids ([Mathews et al., 1995](#)).

4.1.2 Experimental systems

The absorption and distribution of dermally applied [¹⁴C]diethanolamine in 95% ethanol was characterized in F344 rats and B6C3F₁ mice ([Mathews et al., 1997](#); [IARC, 2000](#)). In both species, the percentage absorbed 48 hours after a single administration increased with increasing dose (rats, 2.1–27.5 mg/kg bw; mice, 8–81 mg/kg bw). At comparable dose levels, the percentage absorbed in mice (30–40%) was about 2.5 times higher than that in rats (10–20%). In contrast to carcinogenicity studies, the studies of disposition of diethanolamine prevented oral exposure through grooming activities by gluing a wire mesh over the area of skin application. Tissue/blood ratios of [¹⁴C] were substantially greater than 1 for all tissues examined (adipose, brain, heart, kidney, liver, lung, muscle, skin and spleen), with the greatest accumulation (tissue/blood > 100) in the liver and kidney of rats and mice. [The 2.5-fold increase in absorption of diethanolamine in mice compared with rats does not appear to account for the differential liver tumour response in these species: 100% incidence in mice exposed to 40 mg/kg or higher and the lack of a liver tumour response in rats exposed to up to 64 mg/kg bw.]

Diethanolamine was well absorbed in rats after oral exposure. Forty-eight hours after a single oral dose of 7 mg/kg bw [¹⁴C]diethanolamine, 22% of the recovered dose was excreted in the urine and 60% of the dose remained in the tissues ([Mathews et al., 1997](#)). The tissue distribution of [¹⁴C] was similar after oral, intravenous and dermal administration. After daily

oral administration of 7 mg/kg bw [¹⁴C]diethanolamine to rats, diethanolamine equivalents accumulated in the tissues at high concentrations during 4–8 weeks of repeated treatment, and reached a level of 0.3 mg/g tissue in the liver. In a comparative study of the penetration of [¹⁴C]diethanolamine through excised skin samples obtained from multiple species, the permeability rate constant for an aqueous solution of diethanolamine (37% w/w) through mouse skin was approximately 10 times higher than that through rat skin and about 20 times higher than that through human skin ([Sun et al., 1996](#)).

After a single intravenous or oral administration of diethanolamine, rats predominantly excreted the parent compound in the urine ([Mathews et al., 1997](#)); after repeated oral administration, the parent compound was still the major product excreted in the urine but *N*-methylated metabolites were also detected. The parent compound also accounted for the majority of radioactivity extracted from the liver and brain of rats administered [¹⁴C]diethanolamine; two minor metabolites identified in tissues were *N*-methyldiethanolamine and *N,N*-dimethyldiethanolamine ([Mathews et al., 1995](#)).

Diethanolamine is known to be incorporated into membrane phospholipids ([Artom et al., 1949](#); [Barbee & Hartung, 1979](#); [IARC, 2000](#)). It can be *O*-phosphorylated and *N*-methylated to metabolites that are incorporated into polar head groups as aberrant membrane phospholipids (phosphoglyceride and sphingomyelin analogues) via the ethanolamine metabolic pathway ([Mathews et al., 1995](#)). Following repeated exposure of rats to diethanolamine, the extent of methylation and accumulation of aberrant sphingomyelinoid lipids in tissues increased. *N*-Nitrosodiethanolamine, a hepatocarcinogen, was not detected in the urine, blood or gastric contents of B6C3F₁ mice that were administered 160 mg/kg per day diethanolamine by dermal application or oral gavage with or without 140

ppm (≈ 40 mg/kg per day) sodium nitrite in their drinking-water for 2 weeks ([Stott et al., 2000](#)). Thus, liver tumour induction in mice is unlikely to be due to nitrosation of diethanolamine to this mutagenic nitrosamine.

4.2 Toxic effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

Toxicology studies of diethanolamine were conducted in male and female F344 rats and B6C3F₁ mice for 13 weeks by administration in the drinking-water (160–5000 ppm, \approx equivalent to daily doses of 15–440 mg/kg bw for rats; and 630–10 000 ppm, \approx equivalent to daily doses of 100–1700 mg/kg bw for mice) and by topical application (five times a week at daily doses of 32–500 mg/kg bw in rats and 80–1250 mg/kg bw in mice) ([Melnick et al., 1994a, b](#)). In both species, diethanolamine induced dose-dependent toxic effects at multiple organ sites. In rats, induced toxic responses were observed in the bone marrow (poorly regenerative microcytic anaemia), kidney (increased weight, tubular necrosis, decreased renal function, and tubular mineralization), brain and spinal cord (demyelination), testis (seminiferous tubule degeneration) and skin (ulceration, inflammation, hyperkeratosis and acanthosis at site of application). In mice, diethanolamine induced toxicity in the liver (hepatocellular cytological alterations and single-cell necrosis), kidney (nephropathy and tubular epithelial necrosis in males), heart (cardiac myocyte degeneration) and skin (ulceration, inflammation, hyperkeratosis and acanthosis at site of application). Minimal to mild cytological alterations in the liver of mice comprised multiple hepatocyte changes, including enlarged cells that were frequently multinucleated,

increased nuclear pleomorphism, and increased eosinophilia; these changes were observed in all treated groups of male and female mice, except for females that received topical applications of 80 mg/kg bw. No liver lesions were observed in rats exposed to diethanolamine by either route. The mechanism of diethanolamine toxicity at multiple organ sites in rats and mice is unknown, but may be related to its high tissue accumulation and effects on phospholipid metabolism resulting in alterations in membrane structure and function ([Melnick et al., 1994a, b](#)).

4.3 Genetic and related effects

4.3.1 Experimental systems

The genetic toxicology of diethanolamine has been reviewed ([IARC, 2000](#)). Diethanolamine was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, TA1538, or TA98, in *Escherichia coli* WP2 uvrA, or in L5178Y mouse lymphoma cells incubated in the presence or absence of metabolic activation systems. It did not induce sister chromatid exchange or chromosomal aberrations in cultured Chinese hamster ovary cells or mitotic gene conversion in *Saccharomyces cerevisiae*. Diethanolamine did not increase the frequency of micronuclei in peripheral erythrocytes of B6C3F₁ mice exposed to 80–1250 mg/kg by dermal application for 13 weeks ([NTP, 1999b](#)).

Diethanolamine (10–500 μ g/mL, 0.1–5.0 mM) induced morphological transformation in Syrian hamster embryo (SHE) cells cultured for 7 days in media containing 28 μ M [3 μ g/mL] choline; however, this effect was prevented by supplementation of the medium with excess choline (30 mM [3.125 mg/mL]) ([Lehman-McKeeman & Gamsky, 2000](#)). Diethanolamine also inhibited choline uptake and decreased phosphatidylcholine synthesis by these cells. The latter changes were also prevented by supplementation of the medium with 30 mM choline.

Based on these findings, the authors suggested that diethanolamine-induced morphological transformation of SHE cells was due to inhibition of choline uptake. [Because the high level of choline supplementation may have blocked diethanolamine uptake by the cells and any subsequent cellular effects of this agent, a study of morphological transformation in SHE cells by choline deficiency would help to determine whether reduced choline uptake alone was sufficient to induce cell transformation.]

Primary cultures of hepatocytes isolated from B6C3F₁ mice were grown in the presence of diethanolamine (4.5 mM [473 mg/mL]) or in choline-deficient medium (0.86 μM, 0.09 mg/L) for 48 hours and evaluated for DNA methylation status in GC-rich regions ([Bachman et al., 2006](#)). Both diethanolamine and choline-deficient treatments resulted in 54 regions of altered methylation, of which 43 and 49 regions were hypomethylations, respectively, and only one hypermethylation with each treatment. Based on the size of polymerase chain reaction products of methylation-sensitive restriction digests, the authors concluded that 39 of the 54 regions of altered methylation (72%) were similar after both diethanolamine and choline-deficient treatments. Thus, the authors suggested that, by inhibiting choline uptake into cells, diethanolamine may decrease the supply of S-adenosyl methionine (SAM), the main methyl donor for many methylation reactions, leading to hypomethylations in promoter regions of genes and consequent alterations in gene expression.

Four-day-old *Drosophila melanogaster* females were given 4% sucrose solutions containing 5, 10, 20, 40 or 80% diethanolamine for 24 hours; after a 2-hour recovery period, they were mated with 7-day-old males ([Muñoz & Barnett, 2003](#)). The frequencies of meiotic non-disjunction in *Drosophila* oocytes were analysed in the progeny from three successive 24-hour broods. Diethanolamine induced similar increases in the frequencies of female

non-disjunction (chromosome malsegregation) at all concentrations. The authors suggested that the induction of aneuploidy may be a genotoxic effect of diethanolamine.

DNA was isolated from sections of liver tumours obtained in the 2-year dermal application study of diethanolamine in B6C3F₁ mice ([NTP, 1999b](#)) and analysed for genetic alterations in β-catenin *Catnb* and H-*ras* genes ([Hayashi et al., 2003](#)). *Catnb* encodes β-catenin protein, which is involved in cell–cell adhesion and Wnt signal transduction. Genetic alterations in exon 2 of the latter gene included deletion mutations and point mutations that occurred at much higher frequencies in liver neoplasms from diethanolamine-exposed mice compared with controls. The frequency of *Catnb* mutations was 100% in diethanolamine-induced hepatoblastomas, 32% in hepatocellular neoplasms from mice exposed to diethanolamine, and 10% in control hepatocellular neoplasms. These findings indicate the occurrence of in-vivo mutagenesis in diethanolamine-induced tumours. Hepatocellular neoplasms obtained from mice exposed to diethanolamine lacked H-*ras* codon 61 mutations; this is in sharp contrast to spontaneous liver tumours in this strain of mice, in which the frequency of H-*ras* mutations is slightly higher than 50%.

4.4 Mechanistic data

4.4.1 Hepatic choline deficiency

Diethanolamine induces hepatic choline deficiency in mice ([Lehman-McKeeman et al., 2002](#)), probably due to the inhibition of choline uptake ([Lehman-McKeeman & Gamsky, 2000](#)). Application of diethanolamine in 95% ethanol to the skin of B6C3F₁ mice at doses of 0, 20, 40, 80 or 160 mg/kg bw on 5 days a week for 4 weeks caused decreases in liver concentrations of choline, phosphocholine, phosphatidylcholine, glycerophosphocholine and SAM, while levels of S-adenosyl homocysteine were increased.

Phosphocholine, which is the intracellular storage form of choline, was most sensitive to treatment with diethanolamine. These metabolic changes were qualitatively similar to those caused by feeding diets deficient in choline to mice ([Lehman-McKeeman et al., 2002](#)) or rats ([Pomfret et al., 1990](#)) for 2 weeks. Based on these changes and earlier studies that observed the induction of liver neoplasms in rats and mice fed choline-deficient diets ([Newberne et al., 1982](#)), the authors suggested that diethanolamine-induced hepatocarcinogenicity is due to the induction of choline deficiency and the consequent reduction in hepatic levels of SAM (a methyl donor for methyltransferases); the latter change can lead to DNA hypomethylation and altered gene expression. However, in the study by [Lehman-McKeeman et al. \(2002\)](#), there was no effect on hepatic levels of SAM in mice treated with 40 mg/kg bw, a dose that produced a significant increase in the incidence of hepatocellular neoplasms in male and female mice in a carcinogenicity study ([NTP, 1999b](#)). Liver choline levels have not been measured in mouse kidney or in rat liver.

4.4.2 Cell proliferation

Dermal exposure of B6C3F₁ mice to diethanolamine in 95% ethanol at daily doses of 0, 20, 40, 80 or 160 mg/kg bw was reported to increase hepatocyte proliferation (assessed by the 7-day 5-bromo-2'-deoxyuridine (BrdU) labelling index) over 13 weeks of treatment without affecting the number of apoptotic cells ([Mellert et al., 2004](#)), but there was no clear dose-response in the liver. Because diethanolamine is known to cause syncytia formation (polyploidization; enlarged, multinucleated hepatocytes) in mice ([Melnick et al., 1994a](#)), an increase in the hepatocyte labelling index, which is a measure of DNA synthesis, may not reflect a true increase in hepatocyte number.

The BrdU labelling index was increased in primary cultures of mouse or rat hepatocytes incubated with 5–750 µg/mL diethanolamine for 24 hours; supplementation of the culture medium with choline reduced the level of diethanolamine-induced DNA synthesis, and reducing the concentration of choline in the culture medium (in the absence of diethanolamine) also caused increases in DNA synthesis. In contrast, DNA synthesis was not increased in cryopreserved human hepatocytes incubated with diethanolamine or in culture medium depleted of choline ([Kamendulis & Klaunig, 2005](#)).

Interestingly, the effects of reduced choline uptake in mouse neural precursor cells appear to be converse to those reported in mouse hepatocytes. In neural precursor cells, treatment with 3 mM [312 µg/mL] diethanolamine reduced choline uptake and decreased intracellular levels of phosphocholine; however, these changes resulted in a reduced BrdU labelling index and increased DNA fragmentation (apoptosis) ([Niculescu et al., 2007](#)). The effects of diethanolamine on DNA synthesis and apoptosis were prevented by choline supplementation of the growth medium. Based on these findings, the authors concluded that prenatal exposure to diethanolamine may adversely affect brain development.

4.5 Mechanisms of carcinogenesis

Induction of choline deficiency has been proposed as the means by which diethanolamine induces liver neoplasms in mice ([IARC, 2000](#); [Lehman-McKeeman et al., 2002](#); [Kamendulis & Klaunig, 2005](#); [Leung et al., 2005](#)). Choline is an essential nutrient that can be acquired from the diet or by de-novo synthesis via the sequential methylation of phosphatidylethanolamine, catalysed by phosphatidylethanolamine *N*-methyltransferase using SAM as the methyl donor ([Zeisel, 2008](#)). This pathway is activated when cellular levels of choline are low, resulting

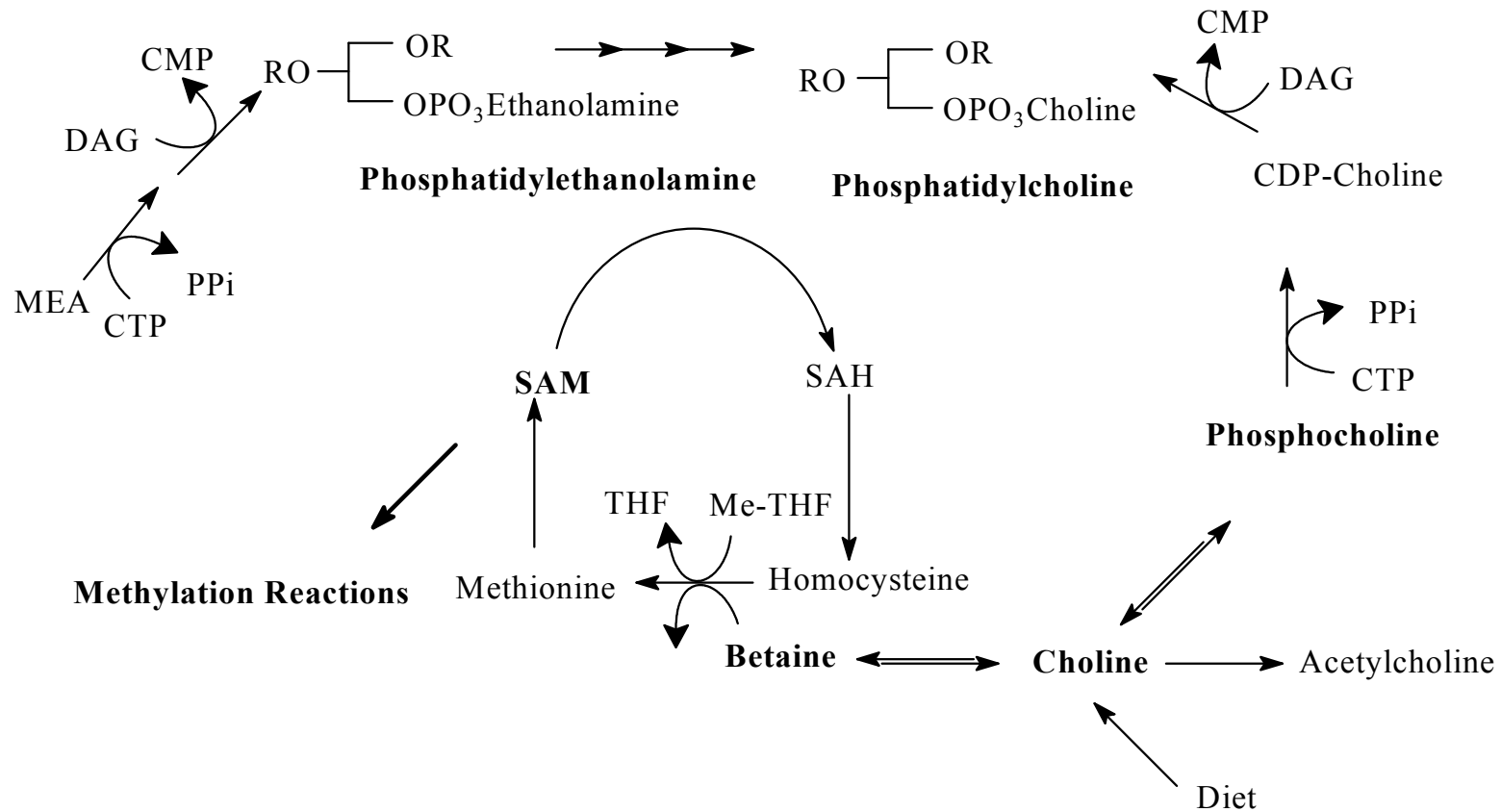
in greater utilization of SAM. Pathways of choline formation and utilization are shown in Fig. 4.1. Choline is involved in numerous physiological functions; the major fate of choline is conversion to phosphatidylcholine after its phosphorylation to phosphocholine. Acetylation of choline produces the neurotransmitter acetylcholine. Dietary choline is also the major source of methyl groups via its oxidation to betaine and subsequent conversion to SAM. Depletion of intracellular choline leads to a reduced availability of SAM and can result in hypomethylation of DNA, altered expression of genes that regulate growth and possible tumour development.

The basis for the proposed mechanism of diethanolamine-induced choline deficiency in the induction of liver tumours includes the following: (a) dermal exposure of mice to maximum tolerated doses of diethanolamine resulted in reductions in levels of choline, choline metabolites and SAM in the liver ([Lehman-McKeeman *et al.*, 2002](#)); (b) the induction of morphological transformation in SHE cells by diethanolamine was prevented by the addition of excess choline ([Lehman-McKeeman & Gamsky, 2000](#)); (c) the inhibition of choline uptake by diethanolamine in SHE and Chinese hamster ovary cells was prevented by the addition of excess choline to the culture medium ([Lehman-McKeeman & Gamsky, 1999](#); [Lehman-McKeeman & Gamsky, 2000](#)); (d) DNA methylation status was similarly altered (mainly hypomethylations) in isolated mouse hepatocytes grown in the presence of diethanolamine or in choline-deficient medium ([Bachman *et al.*, 2006](#)); (e) increases in DNA synthesis in primary cultures of mouse or rat hepatocytes incubated with diethanolamine were prevented by the addition of excess choline ([Kamendulis & Klaunig, 2005](#)); (f) *N*-nitrosodiethanolamine was not detected in mice that were administered diethanolamine by dermal application with or without sodium nitrite in their drinking-water ([Stott *et al.*, 2000](#)).

Several issues raise uncertainties about the reliability of the proposed choline deficiency mechanism: (a) no effect on hepatic levels of SAM was observed in mice administered a dose of diethanolamine (40 mg/kg bw) that produced a significant increase in hepatocellular neoplasms ([Lehman-McKeeman & Gamsky, 2000](#)); (b) studies of induced choline deficiency have not been evaluated in mouse kidney, the second site of tumour induction by diethanolamine in mice; (c) although rats are highly sensitive to choline deficiency, the 2-year carcinogenicity study of diethanolamine found no evidence of a liver tumour response in this species ([NTP, 1999b](#)). The lack of published studies on the effects of maximum tolerated doses on liver levels of choline and choline metabolites in rats creates a critical gap in the data on the proposed mechanism of choline-deficiency in the induction of liver tumours by this chemical; (d) the hallmark of dietary choline deficiency is a fatty liver; however, a fatty liver was not diagnosed in rats or mice exposed to diethanolamine; (e) the detection of mutations in the β -catenin gene in liver tumours from diethanolamine-exposed mice indicates that in-vivo mutagenesis may be involved ([Hayashi *et al.*, 2003](#)). No studies have been reported on the mutational profile in liver tumours induced in mice fed a choline-deficient diet.

Several factors relate to the applicability of the proposed mechanism of diethanolamine-induced choline deficiency in mice for the evaluation of carcinogenic risks to humans. Similar to studies in rats and mice, diethanolamine was absorbed by human liver slices and incorporated into phospholipids. The finding that healthy adults deprived of dietary choline develop signs of organ dysfunction (e.g. fatty liver), which was reversed by consumption of a high-choline diet, contributed to the recognition of choline as an essential nutrient; the current recommended adequate intake of choline is 425 mg/day for women and 550 mg/day for men ([Zeisel & da](#)

Fig. 4.1 Interrelationship between the intracellular pathways of choline and methionine



CDP-choline, cytidyl diphosphate-choline; CMP, cytidyl monophosphate; CTP, cytidyl triphosphate; DAG, diacylglycerol; MEA, monoethanolamine; PC, phosphatidylcholine; PPi, pyrophosphate; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; THF, tetrahydrofolate
 Reprinted from [Leung et al. \(2005\)](#) with permission from Elsevier.

[Costa, 2009](#)). Variations in dietary requirements for choline have been attributed in part to polymorphisms in genes involved in choline metabolism. The rodent choline-deficient model has been widely used to understand the progression of non-alcoholic steatohepatitis ([Larter & Yeh, 2008](#)), a risk factor for liver cancer in humans ([Severi et al., 2010](#)). Thus, the choline-deficient mechanism is relevant to humans; however, the relationship between exposure to diethanolamine, a reduction in liver choline levels and the development of liver cancer in humans is not known.

[Sufficient data that would allow reliable comparisons of the sensitivity of humans and rodents to diethanolamine-induced choline deficiency are not available. Data are also lacking on tissue levels of diethanolamine in rodents and humans following repeated exposures to this chemical and the effects on intracellular concentrations of choline and choline metabolites. Tissue levels of diethanolamine are dependent on the frequency of exposure and factors that affect dermal absorption, e.g. skin temperature. The level of dietary intake of choline may also affect sensitivity to agents such as diethanolamine that competitively inhibit its intracellular uptake. In this regard, it is worth noting that the ingestion of choline from diets fed to rodents in the carcinogenicity study ([NTP, 1999b](#)) or to mice in the study that demonstrated the induction of hepatic choline deficiency ([Lehman-McKeeman et al., 2002](#)) (0.05–0.2%, equivalent to 300–320 mg/kg bw) provided approximately 35–40 times higher intakes of choline on a per kilogram basis than the current adequate intake amount recommended for humans.]

The induction of liver tumours in mice by diethanolamine was suggested to be a consequence of choline deficiency. This mechanism is applicable to human health, especially for subgroups that are highly susceptible to dietary choline deficiency. Other possible mechanisms include diacylglycerol accumulation and

activation of protein kinase C ([Leung et al., 2005](#)), incorporation of diethanolamine into membrane phospholipids and generation of lipid second messengers, e.g. aberrant ceramides ([Mathews et al., 1995](#); [NTP, 1999b](#)), and induction of aneuploidy ([Muñoz & Barnett, 2003](#)).

5. Summary of Data Reported

5.1 Exposure data

Diethanolamine has been produced since the 1930s by the reaction of ethylene oxide with ammonia. Occupational exposure can occur through its production and use in metalworking fluids and coolant fluids, textile processing, and industrial gas purification. Exposure of the general population to diethanolamine occurs through dermal contact with cosmetics, soaps and detergents where it is present as a contaminant in fatty acid-diethanolamide surfactants.

5.2 Human carcinogenicity data

Numerous studies have investigated exposure to metalworking fluids and the risk of cancer in workers who were probably exposed to diethanolamine and other agents. Excess risks of cancer were observed among workers exposed to metalworking fluids that probably contained diethanolamine. However, these studies cannot distinguish the carcinogenic effect of diethanolamine alone from that of the complex mixture. No studies were identified that evaluated human cancer associated with the use of personal care products that contain diethanolamine.

5.3 Animal carcinogenicity data

In male and female mice, dermal application of diethanolamine increased the incidence of hepatocellular carcinoma and hepatocellular adenoma in males and females, and of hepatoblastoma in males. The incidence of renal tubule adenoma was also increased in males. Dermal application of diethanolamine did not induce tumours in rats.

Tumours of the kidney and hepatoblastomas are rare spontaneous neoplasms in experimental animals.

5.4 Other relevant data

Diethanolamine is absorbed only weakly through human skin. No data were available on the absorption of diethanolamine after other routes of exposure in humans.

A genotoxic mechanism is supported by the induction of aneuploidy in *Drosophila* and the elevated frequency of mutations in β -catenin *Catnb* genes in liver tumours induced by diethanolamine. However, diethanolamine was not genotoxic in most in-vitro systems and did not increase the frequency of micronuclei in exposed mice.

A mechanism for liver tumour induction of diethanolamine in mice that involves the inhibition of choline uptake in the liver has been proposed based on the reduced levels of choline metabolites observed in the liver of mice exposed to diethanolamine, the fact that supplementation of incubation medium with choline prevented diethanolamine-induced morphological transformation in Syrian hamster embryo cells and prevented diethanolamine-induced increases in DNA synthesis in rat hepatocytes, and the similarity in DNA hypomethylation in mouse hepatocytes grown in either the presence of diethanolamine or choline-deficient medium. This hypothesis is challenged by the finding that reductions in hepatic levels of S-adenosyl

methionine do not occur at all doses of diethanolamine that induced liver tumours in mice, and the fact that the hallmark liver phenotype for choline deficiency, i.e. fatty liver, was not observed in rats or mice exposed to diethanolamine.

There is weak evidence that a genotoxic mechanism is involved in the induction of liver tumours by diethanolamine. There is moderate experimental support for choline deficiency as a mechanism for diethanolamine-induced liver cancer in rodents. The human relevance of this mechanism to humans cannot be excluded, especially for subgroups that are highly susceptible to dietary choline deficiency. No mechanistic data are available on the induction of kidney tumours by diethanolamine.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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COCONUT OIL DIETHANOLAMINE CONDENSATE

1. Exposure Data

1.1 Chemical and physical data

Coconut oil diethanolamine condensate is a mixture of diethanolamides of the fatty acids that constitute coconut oil, which is composed of approximately 48.2% lauric acid (12:0), 18% myristic acid (14:0), 8.5% palmitic acid (16:0), 8% caprylic acid (8:0), 7% capric acid (10:0), 6% oleic acid (18:1, n-9), 2.3% stearic acid (18:0) and 2% linoleic acid (18:2, n-6) ([NTP, 2001](#)).

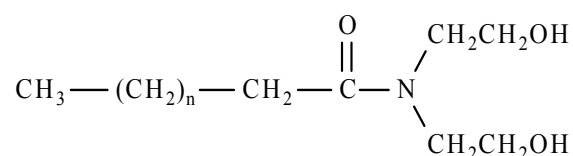
1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 68603-42-9
Deleted Chem. Abstr. Serv. Reg. Nos: 8036-48-4; 8040-31-1; 8040-33-3; 12751-06-3; 53028-62-9; 56448-72-7; 56832-66-7; 63091-31-6; 66984-58-5; 67785-10-8; 67785-14-2; 71343-51-6; 71343-71-0; 83652-14-6; 87714-18-9; 90651-47-1; 118104-13-5; 153189-69-6; 186615-78-1 (ChemID plus)

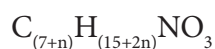
Chem. Abstr. Name: Amides, coco, N,N-bis(hydroxyethyl)

Synonyms: N,N-Bis(hydroxyethyl)coco amides; N,N-bis(hydroxyethyl)coco fatty acid amides; cocamide DEA; cocamide diethanolamine; coco diethanolamides; coco diethanolamine; coco fatty acid diethanolamides; coconut DEA; coconut diethanolamides; coconut oil diethanolamides; coconut oil diethanolamine

1.1.2 Structural and molecular formulae and relative molecular mass



$n = 5, 7, 9, 11, 13, 15$



Relative molecular mass: range, 231–371

1.1.3 Chemical and physical properties of the pure substance

Description: Clear, amber-coloured liquid with a faint coconut odour ([CTFA, 1986](#))

Boiling-point: 169–275 °C

Melting-point: 23–35 °C ([CTFA, 1986](#))

Density: 0.99 g/cm³ at 20 °C ([IUCLID, 2000](#))

Solubility: Miscible with water at 20 °C; produces an alkali in aqueous solution ([CTFA, 1986](#))

Octanol/water partition coefficient (P): log P, 3.52 ([IUCLID, 2000](#))

1.1.4 Technical products and impurities

Coconut oil diethanolamine condensate is available in several grades, which differ on the basis of the molar ratio of coconut oil

methyl esters and diethanolamine used during their manufacture; the purest product is obtained with a molar ratio of 1:1. Free diethanolamine is present in the final product at concentrations ranging from 4 to 8.5% (CTFA, 1986).

In one lot of commercial coconut oil diethanolamine condensate to be used in animal toxicology studies, impurities identified by high-performance liquid chromatography analysis with ultraviolet detection included diethanolamine (18.2%), alkanolamides of unsaturated acids, amine salts of the acids and *N*-nitrosodiethanolamine (219 ppb) (NTP, 2001).

Trade names: Trade names for coconut oil diethanolamine condensate include: Agent 565-14RC; Alkamide 2104; Alkamide CDE; Alkamide CDO; Alkamide DC 212S; Amicol CDE 1; Amicol CDE 2; Amicol CDE-G; Amidet B 112; Amidet SB 13; Aminol HCA; Aminol KDE; Amisol CD; Amisol CD-E; Arcalon 12; CDE 100; Calamide C; Calimide C; Carsamide CA; Cedemide CX; Cedemide DX; Clindrol 200CGN; Clindrol 206CGN; Clindrol Superamide 100CG; COA; Cocamide DEA; Cocamide diethanolamine; Coco diethanolamides; Coco diethanolamine; Coco fatty acid diethanolamides; Coconut DEA; Coconut diethanolamides; Coconut oil diethanolamides; Colamid C; Comperlan COD; Comperlan KD; Comperlan LS; Comperlan PD; Conco Emulsifier K; Crillon CDY; Cyclomide CD; Diterol 87; Elromid KD 80; Empilan 2502; EMP 6501; Empilan CDE; Empilan CDE/FF; Ethylan A 15; Ethylan LD; Eur-Amid; Foamid C; Foamole 2AC; Homelead CD; Incromide CA; Lauridit KDG; Marlamid D 1218; Marpon MM; Mazamide 80; Monamid 150AD; Monamid 150DR; Monamid 705; Monamid ADD; Monolube 29-78; *N,N*-Bis(hydroxyethyl) coco amides; *N,N*-Bis(hydroxyethyl) coco amides; Naxonol CO; Naxonol PN 66; Ninol 11CM; Ninol 1281; Ninol 2012 Extra; Ninol 2012E; Ninol 40CET; Ninol 40CO; Ninol 49CE; Ninol P 621; Nissan Stafoam DF; Oramix DL 200; P and G Amide 72; Profan 128 Extra; Profan

EX 24; Profan Extra 24; Purton CFD; Rewomid DC 212S; Rewomid DL 240240; Rolamid CD; Schercomid CDA; Schercomid SCO; Stafoam DF; Stafoam DF 4; Stafoam DFC; Standamid KD; Standamid KDO; Standamid SD; Standamid SD-K; Steinamid DC 212S; Steinamid DC 212SE; Tohol N 220XM; Varamide A 10; Varamide A 2; Varamide MA 1; Vicamid 528; Witcamide 5133; Witcamide 82.

1.1.5 Analysis

Analytical methods to determine the diethanolamide composition of coconut oil diethanolamine condensate have been reported using gas chromatography (O'Connell, 1977) and high-performance liquid chromatography (Nakae & Kunihiro, 1978).

1.2 Production and use

1.2.1 Production

Coconut oil diethanolamine condensate is produced by a condensation reaction at a 1:1 or 1:2 molar ratio of the appropriate fatty acids (methyl cocoate, coconut oil, whole coconut acids or stripped coconut fatty acids) to diethanolamine at temperatures of up to 170 °C and in the presence of an alkaline catalyst. The 1:2 mixture of fatty acid (or methyl fatty acid) to diethanolamine results in a lower-quality diethanolamide with residues of ethylene glycol and free diethanolamine. The 1:1 mixture produces a higher-quality diethanolamide with much less free amine, which is consequently used at lower concentrations than the 1:2 diethanolamide (CTFA, 1986).

It has been estimated that 10 300 and 8650 tonnes of coconut oil diethanolamine condensate were produced in the United States of America in 1977 and 1985, respectively (HSDB, 2010).

Information available in 2010 indicated that coconut oil diethanolamine condensate

was produced by 10 companies in Mexico, three companies in the USA, two companies each in France and China, Hong Kong Special Administrative Region, and one company each in India and Pakistan ([Chemical Sources International, 2010](#)). Other sources indicated that it was produced by 15 companies in the USA ([HSDB, 2010](#)), four companies in the United Kingdom, three companies each in Germany and Spain, two companies each in Italy and Sweden, and one company each in Belgium, France, and the Netherlands ([IUCLID, 2000](#)).

1.2.2 Use

Fatty acid diethanolamides, including coconut oil diethanolamine condensate, are widely used in cosmetics. In 1985, coconut oil diethanolamine condensate was reported to be present in nearly 600 cosmetic formulations of bath oil, shampoo, conditioner, lipstick and hair dye. The concentration of diethanolamide in these preparations ranged from 1 to 25%. Non-cosmetic applications include use as a surfactant in soap bars, light-duty detergents and dishwashing detergents and as a delinting agent for cottonseed ([CTFA, 1986](#)).

Coconut oil diethanolamine condensate is used as a corrosion inhibitor in water-based soluble, semi-synthetic and synthetic metal-working fluids and in polishing agents ([Byers, 2006](#)). It is also used widely as an antistatic agent in plastics, e.g. in polyethylene film for food packaging and rigid poly(vinyl) chloride. It has been employed in combination with metallic salts as an antistatic for polystyrene and in impact-resistant rubber polystyrene blends ([HSDB, 2010](#)).

1.3 Occurrence

1.3.1 Natural occurrence

Coconut oil diethanolamide is not known to occur in nature.

1.3.2 Occupational exposure

Occupational exposure to coconut oil diethanolamide in various materials has been inferred from reports of dermatitis, verified by a patch test, among workers; materials identified were barrier creams, hand-washing liquids and metalworking fluids that contained coconut oil diethanolamine ([Grattan *et al.*, 1989](#); [Pinola *et al.*, 1993](#)), hydraulic mining oil ([Hindson & Lawlor, 1983](#)), and materials used in a printing facility ([Nurse, 1980](#)).

1.3.3 Personal care and cleaning products

Exposure to coconut oil acid diethanolamine condensate may occur by skin contact with cosmetic formulations of bath oil, shampoo, conditioner, lipstick, hair dye, soap bars, light-duty detergents and dishwashing detergents ([NTP, 2001](#)).

The composition of 2354 registered washing and cleaning agents in the Danish Product Register Data Base in 1992 was reviewed. Of these, 12/70 automotive cleaners, 3/250 detergents for washing textiles, 36/118 dishwashing fluids, 11/94 floor polishes, 83/507 general cleaners, 127/200 shampoos, 9/115 high-pressure cleaning agents and 75/224 skin cleaners contained coconut diethanolamide ([Flyvholm, 1993](#)).

1.3.4 Environmental occurrence

A total of 35 samples of coastal water and 39 samples of harbour sediment collected from several hot spots on the Spanish coast in 1999–2000 were analysed. Coconut diethanolamide was detected in seawater collected from Spanish harbours at Barcelona (< 0.05–4.2 µg/L), Tarragona (< 0.05–24 µg/L) and Almeria (< 0.05 µg/L), and in sediment collected from several locations on the southern and eastern coasts. It was detected in bottom sediment taken from harbours in Barcelona (90–830 µg/kg), Tarragona (30–150 µg/kg), Almeria (275–720 µg/kg),

Almerimar (250–750 µg/kg), Aguadulce (85–820 µg/kg), Cadiz (230 µg/kg), Sancti Petri (380 µg/kg), Sotogrande (120 µg/kg), Duquesa (310 µg/kg), Estepona (220–440 µg/kg), Banus (120–140 µg/kg) and Marbella (110–190 µg/kg). Coastal sediment collected in San Fernando adjacent to an untreated urban wastewater discharge point contained 2710 µg/kg coconut diethanolamide ([Petrović *et al.*, 2002](#)).

Sludge from five sewage-treatment plants in Spain, Portugal and Germany was found to contain C9 coconut diethanolamide (not detected–0.2 mg/kg dry weight), C11 coconut diethanolamide (0.3–6.2 mg/kg dry weight), C13 coconut diethanolamide (0.2–10.5 mg/kg dry weight), C15 coconut diethanolamide (not detected–7 mg/kg dry weight) and C17 coconut diethanolamide (not detected–5.5 mg/kg dry weight) ([Petrović & Barceló, 2000](#)).

1.4 Regulations and guidelines

No occupational exposure limits or recommended guidelines for maximum safe levels in drinking-water have been established for coconut oil diethanolamine condensate.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Skin application

3.1.1 Mouse

Groups of 50 male and 50 female B6C3F₁ mice, 6 weeks of age, received dermal applications of 0, 100 or 200 mg/kg body weight (bw) coconut oil diethanolamine condensate (purity,

> 99%) in 95% ethanol (0, 50 or 100 mg/mL ethanol) on 5 days a week for 2 years. Survival of treated males and females was similar to that of the vehicle-control group. The mean body weights of treated males were similar to those of the controls throughout the study; those of the 100- and 200-mg/kg females were lower than those of the controls from weeks 93 and 77, respectively. In male mice, the incidence of hepatocellular adenoma was significantly greater in both treated groups than in vehicle controls (22/50 (44%), 35/50 (70%) and 45/50 (90%); $P = 0.002$ and $P < 0.001$, respectively, poly-3 trend test, for control, low-dose and high-dose groups, respectively), and that of hepatocellular carcinoma was significantly greater in the high-dose group than in vehicle controls (12/50 (24%), 12/50 (24%) and 20/50 (40%); $P = 0.041$ poly-3 trend test). In addition, the incidence of hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma combined in the low- and high-dose groups was significantly increased compared with that in the vehicle-control group (29/50 (56%), 39/50 (78%), $P = 0.011$ poly-3 trend test), and 49/50 (98%, $P < 0.001$ poly-3 trend test) in the control, low- and high-dose groups, respectively). The incidence of hepatoblastomas in high-dose males was significantly increased compared with controls ($P = 0.03$). In females, the incidence of hepatocellular neoplasms was significantly higher in treated groups than in vehicle controls (hepatocellular adenoma: 32/50 (64%), 44/50 (88%, $P = 0.006$ poly-3 trend test), and 43/50 (86%, $P = 0.005$ poly-3 trend test); hepatocellular carcinoma: 3/50 (6%), 21/50 (42%) and 32/50 (64%, $P < 0.001$ poly-3 trend test) in the control, low- and high-dose groups, respectively). In addition, the incidence of hepatocellular adenoma and hepatocellular carcinoma combined in the low- and high-dose groups was significantly increased in comparison with the vehicle-control group (33/50 (66%), 46/50 (92%) and 48/50 (96%, $P < 0.001$ poly-3 trend test)). The incidence of renal tubular adenoma (1/50 (2%),

1/50 (2%), 7/50 14%) and renal tubular adenoma or carcinoma combined (1/50 (2%), 1/50 (2%), 9/50 (18%)) was significantly increased in high-dose males ($P < 0.05$ poly-3 trend test). One high-dose female had a renal tubular adenoma ([NTP, 2001](#)).

[The Working Group noted that tumours of the kidney and hepatoblastoma are rare spontaneous neoplasms in experimental animals.]

3.1.2 Rat

Groups of 50 male and 50 female F344/N rats, 6 weeks of age, received dermal applications of 0, 50 or 100 mg/kg bw coconut oil acid diethanolamine condensate (purity, > 99%) in 95% ethanol on 5 days a week for 2 years. Survival rates for treated males and females were similar to those of corresponding vehicle controls, as were mean body weights. No increase in the incidence of tumours was observed in treated groups compared with the vehicle controls ([NTP, 2001](#)).

4. Other Relevant Data

In 2-year studies of dermal application of coconut oil diethanolamine condensate in B6C3F₁ mice (0, 100 or 200 mg/kg bw) and F344/N rats (0, 50 or 100 mg/kg bw), neoplasms were induced in male (liver and kidney) and female (liver) mice, while there was no evidence of neoplastic effects in either sex of rats ([NTP, 2001](#)). This pattern of tumour response is the same as that of diethanolamine applied to the skin of mice and rats ([NTP, 1999](#)). The content of unreacted diethanolamine in the coconut oil diethanolamine condensate was estimated to be approximately 18.2%, and the increased incidence of neoplasms in mice was associated with the level of free diethanolamine that was present in the solutions of diethanolamine condensate tested ([NTP, 1999, 2001](#)). Sufficient free diethanolamine was present in the coconut

oil diethanolamine condensate to account for the tumour responses observed in mice. If the tumour responses were due to diethanolamine, then the results of the dermal carcinogenicity study of coconut oil diethanolamine condensate confirm the findings of the study on diethanolamine, and Section 4 of the *Monograph* on diethanolamine (in this volume) would pertain to this *Monograph*.

4.1 Absorption, distribution, metabolism, excretion

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

Although no studies have been conducted specifically on coconut oil diethanolamine condensate, a mixture of diethanolamides and the fatty acids found in coconut oil, a study on the disposition of lauric acid (the major fatty acid present in coconut oil) diethanolamine condensate (1:1) in rats after intravenous, oral and dermal administration and in mice after intravenous and dermal administration has been reported ([Mathews et al., 1996](#)). Lauramide diethanolamine was absorbed by rats and mice after oral or dermal administration, and was rapidly cleared from all tissues except adipose. Unlike diethanolamine, lauramide diethanolamine did not accumulate in the tissues of rats after repeated dermal applications. The major route of excretion of the administered dose was as polar metabolites in the urine (80–90%) of both species. No unchanged diethanolamine, diethanolamine-derived metabolites or parent compound were detected in the urine. Lauramide diethanolamine was readily metabolized *in vivo* and *in vitro*; metabolites identified in the plasma and urine of treated rats, and in media from human and rat liver slices incubated with this

compound reflected ω - and ω -1 to 4 hydroxylation followed by β -oxidation to chain-shortened carboxylic acids. Thus, fatty acid diethanolamine condensates are readily metabolized by hydroxylation of the fatty acid moiety, but are resistant to hydrolysis of the amide linkage. Therefore, metabolism of lauramide diethanolamine does not release free diethanolamine in rats or mice.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

Coconut oil diethanolamine condensate was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100 or TA1535, or in L5178Y mouse lymphoma cells incubated in the presence or absence of metabolic activation systems (see Tables E1 and E2 in [NTP, 2001](#)). It did not induce sister chromatid exchange or chromosomal aberrations in cultured Chinese hamster ovary cells in the presence or absence of metabolic activation systems (see Tables E3 and E4 in [NTP, 2001](#)). Coconut oil diethanolamine condensate increased the frequencies of micronucleated erythrocytes in the peripheral blood of male and female B6C3F₁ mice that received dermal applications for 14 weeks (see Table E5 in [NTP, 2001](#)).

4.3 Mechanisms of carcinogenesis

The tumour response in mice exposed to coconut oil diethanolamine condensate appears to be due to the presence of free diethanolamine in the solution tested. This suggestion is based on the association of the mouse liver tumour responses with exposures to diethanolamine in studies of diethanolamine condensates with varying levels of diethanolamine contamination. Because the

frequency of micronucleated erythrocytes was not increased in mice exposed to diethanolamine ([NTP, 1999](#)), free diethanolamine in the solution tested does not account for the observed increase in frequency of micronucleated erythrocytes in mice exposed to coconut oil diethanolamine condensate ([NTP, 2001](#)), which may act via a genotoxic mechanism.

5. Summary of Data Reported

5.1 Exposure data

Coconut oil diethanolamine condensate is a mixture of amides produced by the condensation of coconut oil fatty acids with diethanolamine. Exposure of the general population occurs through dermal contact due to its wide use as a surfactant in cosmetics, soaps and detergents. Occupational exposure may occur by inhalation and skin absorption from some metalworking fluids. Coconut oil diethanolamine condensate may contain diethanolamine as a contaminant.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

In a study of dermal application in mice, coconut oil diethanolamine condensate increased the incidence of hepatocellular carcinoma and hepatocellular adenoma in males and females, and of hepatoblastoma in males. The incidence of renal tubule adenoma and carcinoma combined was also increased in males. In a study of dermal application in rats, no increase in tumour incidence was observed.

Tumours of the kidney and hepatoblastoma are rare spontaneous neoplasms in experimental animals.

5.4 Other relevant data

No data on the absorption, distribution, metabolism or excretion of coconut oil diethanolamine condensate were available to the Working Group.

The amide linkage between diethanolamine and the fatty acid moiety is resistant to metabolic hydrolysis.

The carcinogenic effects of the coconut oil diethanolamine condensate used in the cancer bioassay may be due to the levels of diethanolamine (18.2%) in the solutions tested. Mechanistic data are very weak to evaluate the carcinogenic potential of coconut oil diethanolamine condensate *per se*.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of coconut oil diethanolamine condensate.

6.3 Overall evaluation

Coconut oil diethanolamine condensate is *possibly carcinogenic to humans (Group 2B)*.

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DI(2-ETHYLHEXYL) PHTHALATE

This substance was considered by previous Working Groups, in October 1981 ([IARC, 1982](#)), March 1987 ([IARC, 1987](#)) and February 2000 ([IARC, 2000](#)). 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

From [ATSDR \(2002\)](#), [HSDB \(2010\)](#) and [Lide \(2010\)](#).

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; 109630-52-6;
126639-29-0; 137718-37-7; 205180-59-2;
275818-89-8; 607374-50-5

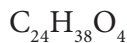
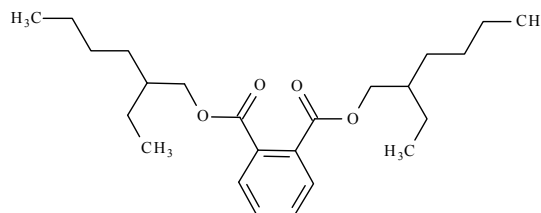
Chem. Abstr. Name: Di(2-ethylhexyl)
phthalate

IUPAC Systematic Name: Bis(2-ethylhexyl)
phthalate

Synonyms: 1,2-Benzenedicarboxylic acid,
bis(2-ethylhexyl) ester; 1,2-benzenedicar-
boxylic acid, 1,2-bis(2-ethylhexyl) ester;
bis(2-ethylhexyl) benzene-1,2-dicarboxy-
late; bis(2-ethylhexyl) 1,2-benzenedicar-
boxylate; bis(2-ethylhexyl) *o*-phthalate;
DEHP; diethylhexyl phthalate; di-2-
ethylhexyl phthalate; dioctyl phtha-
late; di-sec-octyl phthalate; ethylhexyl
phthalate; 2-ethylhexyl phthalate; octyl

phthalate; phthalic acid, bis(2-ethylhexyl)
ester; phthalic acid di(2-ethylhexyl) ester;
phthalic acid dioctyl ester

1.1.2 Structural and molecular formulae and relative molecular mass



Relative molecular mass: 390.56

1.1.3 Chemical and physical properties of the pure substance

Description: Colourless liquid with almost
no odour

Boiling-point: 384 °C

Melting-point: -55 °C

Density: 0.981 g/cm³ at 25 °C

Solubility: Sparingly soluble in water
(0.27 mg/L at 25 °C); slightly soluble in
carbon tetrachloride; soluble in blood and

fluids containing lipoproteins; miscible with mineral oil and hexane

Volatility: Vapour pressure, 1.42×10^{-7} mm Hg at 25 °C

Octanol/water partition coefficient: $\log K_{ow}$, 7.6

Conversion factor in air: 1 ppm = 15.94 mg/m³

1.1.4 Technical products and impurities

Technical products are generally reported to be of high purity (> 99.7%). The impurities found are mainly other phthalates. Some technical formulations may contain bisphenol A (CAS No. 80-05-7) as an additive in the range 0.025–0.5% ([European Commission, 2008](#)).

Trade names for di(2-ethylhexyl) phthalate (DEHP) include: Bisoflex 81; Bisoflex DOP; Compound 889; Corflex 400; DEHP; Diacizer DOP; Dioctyl phthalate; DOF; DOF (Russian plasticizer); DOP; ESBO-D 82; Ergoplast FDO; Ergoplast FDO-S; Etalon; Etalon (plasticizer); Eviplast 80; Eviplast 81; Fleximel; Flexol DOD; Flexol DOP; Garbeflex DOP-D 40; Good-rite GP 264; Hatco DOP; Jayflex DOP; Kodaflex DEHP; Kodaflex DOP; Monocizer DOP; NSC 17069; Octoil; Octyl phthalate; Palatinol AH; Palatinol AH-L; Palatinol DOP; Pittsburgh PX 138; Plasthall DOP; Reomol D 79P; Sansocizer DOP; Sansocizer R 8000; Sconamoll DOP; Sicol 150; Staflex DOP; Truflex DOP; Vestinol AH; Vynecizer 80; Vynecizer 80K; Witcizer 312 ([SciFinder, 2010](#)).

1.1.5 Analysis

Detection and quantification of very low levels of DEHP 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 ([ATSDR, 2002](#)).

Selected methods for the analysis of DEHP in various matrices are presented in [Table 1.1](#).

1.2 Production and use

1.2.1 Production

DEHP 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 ([ATSDR, 2002](#)). It was first produced in commercial quantities in Japan around 1933 and in the United States of America in 1939 ([IARC, 2000](#)).

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

The global production of DEHP in 1994 was estimated to be between 1 and 4 million tonnes. The production volume of DEHP in western Europe was 595 000 tonnes/year in 1997 but had decreased to 221 000 in 2004. Some 800 plants in the European Union (EU) use DEHP or preparations that contain DEHP ([European Commission, 2008](#)). The European Commission reported that 1 million tonnes of DEHP were used in Europe in 2000 ([IUCLID DataBase, 2000](#)).

DEHP was first used commercially in the USA in 1949. During the period 1950–54, its production in the USA was 106 thousand tonnes, and, by 1965–69, had risen to 655 thousand tonnes ([Peakall, 1975](#)). From 1982 to 1986, production of DEHP in the USA increased from 114 to 134 thousand tonnes, but, in 1994, was 117 500 tonnes ([Anon., 1996](#)). Production in Japan in 1995 was 298 000 tonnes and that in Taiwan, China, in 1995 was 207 000 tonnes, down from 241 000 tonnes in 1994 ([Anon., 1996](#)). It was estimated that 109 thousand tonnes of dioctyl

Table 1.1 Selected methods for the analysis of di(2-ethylhexyl) phthalate

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Collect on cellulose ester membrane filter; desorb with carbon disulfide	GC/FID	10 µg/sample	NIOSH (2003) [Method 5020]
Drinking-water and source water	Extract in liquid–solid extractor; elute with dichloromethane; concentrate by evaporation	GC/MS	0.5 µg/L	EPA (1995a) [Method 525.2]
Drinking-water	Extract in liquid–liquid extractor; isolate; dry; concentrate	GC/PID	2.25 µg/L	EPA (1995b) [Method 506]
Wastewater, municipal and industrial	Extract with dichloromethane; dry; exchange to hexane and concentrate	GC/ECD	2.0 µg/L	EPA (1999a) [Method 606]
	Extract with dichloromethane; dry; concentrate	GC/MS	2.5 µg/L	EPA (1999b) [Method 625]
	Add isotope-labelled analogue; extract with dichloromethane; dry over sodium sulfate; concentrate	GC/MS	10 µg/L	EPA (1999c) [Method 1625]
Groundwater, leachate, soil, sludge and 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)	EPA (1996) [Method 8061A]
Serum	Precipitate proteins; add internal standard; extract with <i>n</i> -heptane	GC/MS	10 µg/L	Buchta <i>et al.</i> (2003)

GC, gas chromatography; ECD, electron capture detection; FID, flame ionization detection; MS, mass spectrometry; PID, photoionization detection

phthalates were produced in the USA in 1999 ([ATSDR, 2002](#)).

Information available in 2010 indicated that DEHP was produced by 23 companies in the USA, 19 companies in Mexico, nine companies in China, four companies in the United Kingdom, three companies in Germany, two companies each in China (Hong Kong Special Administrative Region), India and Japan, and one company each in Belgium, Bulgaria, Canada, the Czech Republic, France, the former state union of Serbia and Montenegro, South Africa and Switzerland ([Chemical Sources International, 2010](#)). A European source indicated that DEHP was produced by five companies each in Germany and Italy, four companies each in the Netherlands and the United Kingdom, three companies each in Austria and France, two companies in Belgium and one company each in Finland, Spain and Sweden ([IUCLID DataBase, 2000](#)).

1.2.2 Use

As a plasticizer, the primary function of DEHP is to soften otherwise rigid plastics and polymers. Plastics may contain from 1 to 40% DEHP by weight. An estimated 90% of DEHP is used as a plasticizer for polyvinyl chloride (PVC) polymers ([Toxics Use Reduction Institute, 2005](#)). In the EU, 95% of DEHP is used as a plasticizer in polymer products ([European Commission, 2008](#)).

The uses of DEHP fall into two major categories: polymer uses (e.g. consumer products such as footwear, shower curtains and toys, medical devices and commercial/industrial uses) and non-polymer uses (e.g. dielectric fluids, paints, adhesives and inks). Non-polymer uses represent less than 5% of the total DEHP used in the USA. Approximately 45% of total consumption of DEHP in the USA is for plasticizing various industrial and commercial products. Industrial and commercial uses of DEHP include resilient flooring, wall covering, roofing, aluminium

foil coating/laminating, paper coating, extrudable moulds and profiles, electronic component parts, and wire and cable coating and jacketing. Medical devices comprise approximately 25% of total manufacturing use of DEHP in the USA. Medical devices that contain DEHP include PVC sheet materials such as intravenous bags, and tubing used in a variety of medical applications ([Toxics Use Reduction Institute, 2005](#)).

1.3 Occurrence

Concern regarding exposure to DEHP rose to prominence when [Jaeger & Rubin \(1970\)](#) reported its presence in human blood that had been stored in PVC bags. The same authors later reported the presence of DEHP in tissue samples of the lung, liver and spleen from patients who had received blood transfusions ([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 DEHP 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 DEHP-containing plastics has resulted in its becoming a ubiquitous environmental contaminant ([Huber et al., 1996](#)). Many data sources show that nearly all populations absorb DEHP and excrete its metabolites in their urine in measurable amounts. Within these general population studies, concentrations in body fluids (and presumably exposures) vary substantially, and the 95th percentile exposure is 10-fold or higher than the median ([CDC, 2009](#)).

1.3.1 Natural occurrence

DEHP is not known to occur naturally.

1.3.2 Occupational exposure

According to the 1981–83 National Occupational Exposure Survey, 341 800 workers in the USA were potentially exposed to DEHP. Occupational exposure to DEHP may occur during its manufacture and its use, mostly as a plasticizer of PVC (compounding, calendaring and coating operations). Printing and painting occupations also account for a large number of workers being potentially exposed (NOES, 1999). Occupational exposure to DEHP occurs by inhalation, essentially in the form of an aerosol (mist), because of its very low vapour pressure (Fishbein, 1992). Indeed, DEHP aerosols are used to test the efficacy of high-efficiency particulate air filters during their manufacture (Roberts, 1983).

Few data are available on levels of occupational exposure to DEHP (Table 1.2). Huber *et al.* (1996) observed that concentrations in air reported in older studies were well above (up to 60 mg/m³) those determined later; these older studies, however, reported concentrations of total phthalates.

Urinary levels of DEHP, its metabolites and total phthalates have been shown in a few studies to be higher in DEHP-exposed workers than in unexposed workers and in post-shift samples than in pre-shift samples (Liss *et al.*, 1985; Nielsen *et al.*, 1985; Dirven *et al.*, 1993).

Exposure to DEHP may occur concurrently with that to other compounds, 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).

A biological monitoring survey of workers exposed to DEHP was conducted in a factory using PVC plastisols. Three urinary metabolites of DEHP — mono(2-ethylhexyl) phthalate

(MEHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP) and 2-ethylhexanoic acid (2-EHA) — were quantified in five workers using a plastisol (containing 33% DEHP) and in five unexposed workers (controls) during 5 days with pre- and post-shift sampling. In the first stage, plastisols are prepared in a closed workshop where PVC resins are blended with DEHP and other ingredients in containers, then routed to the neighbouring workshop where glass bottles are automatically dipped into vats filled with plastisols. The bottles are then dried after passage in an oven at 160 °C. Around 100 tonnes of DEHP were consumed by the factory per year. Every day, two of the investigated workers, who wore gloves and protective clothes, were involved in the preparation of plastisols. The other participating workers oversaw the automatic chain and fed plastisols into where flasks were dipped. Median concentrations of pre- and post-shift urinary samples in the exposed workers ($n = 62$) were 12.6 and 28.7 µg/L for MEHP, 38.6 and 84.4 µg/L for MECPP and 20.4 and 70.6 µg/L for 2-EHA, respectively. In the controls ($n = 29$), the corresponding values were 4.8 and 4.7 µg/L for MEHP, 15.1 and 12.4 µg/L for MECPP and 21.8 and 20.5 µg/L for 2-EHA, respectively. There was a significant increase (Mann–Whitney U-test, $P < 0.05$) in post-shift excretion by exposed workers versus unexposed controls and in post-shift versus pre-shift concentrations only in the exposed workers. No air samples were reported (Gaudin *et al.*, 2010).

In 2003–05, 156 workers were recruited from eight sectors in which materials containing diethyl phthalate (DEP), dibutyl phthalate (DBP) and/or DEHP were used during part of their regular job duties. Companies included one company from each of seven manufacturing sectors: phthalate manufacture, PVC film, PVC compounding, vehicle filters, rubber hoses, rubber gaskets and rubber boots; and 13 companies from nail-only salons. For MEHP, geometric mean concentrations of mid-shift to end-shift

Table 1.2 Workplace air levels of di(2-ethylhexyl) phthalate (DEHP)

Production	Country	Air concentration (mg/m ³)		Sampling	No. of samples	Reference
		Mean	Range			
DEHP-manufacturing plant	USA			Personal, whole-shift		Liss & Hartle (1983)
Chemical operators, technicians and maintenance workers			ND–4.11		50 ^a	
Polyvinyl chloride (PVC)-processing industry	Sweden			Personal, 2 h		Nielsen et al. (1985)
Thick film department: calender operators/machine attendants		0.4 ^b	0.1–0.8		16	
PVC-processing plants	Finland			Area, 1.5–3 h		Vainiotalo & Pfäffli (1990)
Extrusion		0.05	0.02–0.08		4	
Extrusion		0.3	0.1–0.5		5	
Calendering		0.5	0–1		7	
Hot embossing		0.05	0.03–0.07		5	
Welding		0.3	0.25–0.35		4	
Injection moulding		0.02	0.01–0.03		2	
Compounding		0.02	0.01–0.03		5	
Thermoforming		0.02	0–0.04		2	
High-frequency welding		< 0.02				
PVC-processing plants	Netherlands			Personal, 2 h		Dirven et al. (1993)
<i>Boot factory</i>						
Mixing process		0.26	0.1–1.2		16	
Extruder process		0.12	0.05–0.28		11	
<i>Cable factory</i>						
Mixing process		0.18	0.01–0.81		8	
Extruder process		0.24	0.01–1.27		13	
Various plants	USA			Personal, 4–5 h		Roberts (1983)
Two aerosol filter testing facilities			0.01–0.14			
PVC sheet-processing plant			0.06–0.29		3	

^a Only six measurements were above the detection limit.

^b Presented as total phthalates, but DEHP was the main plasticizer.

ND, not detected

Table 1.3 Estimated daily intake of di(2-ethylhexyl) phthalate by the population of Canada

Substrate/medium	Estimated intake for various age ranges (ng/kg body weight 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

^a Assumed to weigh 7 kg, breathe 2 m³ air, drink 0.75 L water and ingest 35 mg soil

^b Assumed to weigh 13 kg, breathe 5 m³ air, drink 0.8 L water and ingest 50 mg soil

^c Assumed to weigh 27 kg, breathe 12 m³ air, drink 0.9 L water and ingest 35 mg soil

^d Assumed to weigh 57 kg, breathe 21 m³ air, drink 1.3 L water and ingest 20 mg soil

^e Assumed to weigh 70 kg, breathe 23 m³ air, drink 1.5 L water and ingest 20 mg soil

Calculated by the Working Group based on the assumptions by [Meek & Chan \(1994\)](#)

samples (in µg/L) were significantly increased in PVC compounding (from 13.0 to 24.0), rubber hose manufacture (from 6.08 to 8.70) and rubber boot manufacture (from 4.98 to 9.21). Increases were also observed in PVC film manufacture, rubber gasket manufacture and nail salons, but these did not achieve statistical significance ([Hines et al., 2009](#)).

Using the same data set, phthalate metabolite concentrations measured in the workers' end-shift urine samples were used in a simple pharmacokinetic model to estimate daily phthalate intake. DEHP intake estimates based on three of its metabolites combined were 0.6–850 µg/kg per day; the two highest geometric mean intakes occurred in PVC film manufacture (17 µg/kg per day) and PVC compounding (12 µg/kg per day) ([Hines et al., 2011](#)).

1.3.3 Environmental occurrence

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

DEHP is considered a priority and/or hazardous pollutant in Canada ([Meek & Chan, 1994](#); [Meek et al., 1994](#); [Environment Canada, 1995](#)), the Netherlands ([Wams, 1987](#)) and the USA ([Kelly et al., 1994](#)), 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, precipitations, 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](#); [ATSDR, 2002](#); [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.

Human daily intakes of DEHP from various exposure pathways have been estimated (see [Table 1.3](#)).

(a) Biota

DEHP was measured in water, sediment and in several species of fish at six sites from the Ogun river catchments, Ketu, Lagos (Nigeria). The concentration of DEHP in water ranged from 255 to 390 µg/L at the six sites, and that in sediment ranged from 20 to 820 µg/kg. The concentration of DEHP in fish species ranged from 40 to 150 µg/kg in *Tilapia* sp., from 40 to 110 µg/kg

in *Chrysichthys* sp. and from 30 to 300 µg/kg in *Synodontis* sp. The concentration of phthalates in fish did not differ between the species and was not correlated with water or sediment concentration ([Adeniyi et al., 2011](#)).

Phthalate compounds in sediments and fishes were investigated in 17 rivers in Taiwan, China, to determine the relationships between levels of phthalates in sediment and aquatic factors, and biota–sediment accumulation factor for phthalates. The highest concentrations of DEHP in fish samples were found in *Liza subviridis* (253.9 mg/kg dry weight) and *Oreochromis niloticus niloticus* (129.5 mg/kg dry weight). The biota–sediment accumulation factors of DEHP in *L. subviridis* (13.8–40.9) and *O. niloticus niloticus* (2.4–28.5) were higher than those in other fish species, indicating that the living habits of fish and physical–chemical properties of phthalates, such as their octanol/water partition coefficient, may influence the bioavailability of phthalates in fish. Mean concentrations (range) of DEHP during the low-flow season and high-flow season were 4.1 (<0.05–46.5) and 1.2 (<0.05–13.1) mg/kg body weight (bw), respectively. Concentrations of DEHP in sediments were significantly affected by temperature, suspended solids, ammonia–nitrogen and chemical oxygen demand ([Huang et al., 2008](#)).

Levels of DEHP were measured in blood samples obtained from 33 breeding monkeys and 10 wild monkeys in Japan. In breeding monkeys, the total average amount of DEHP was 0.1939 ± 0.1050 µg/mL, and was slightly higher in male monkeys (0.2348 ± 0.0955 µg/mL) than in female monkeys (0.1642 ± 0.1057 µg/mL). In wild monkeys, the total average amount of DEHP was 0.4674 ± 0.1346 µg/mL, and was slightly higher in males (0.4703 ± 0.1436 µg/mL) than in females (0.4443 µg/mL) ([Asaoka et al., 2000](#)).

(b) Air

The Environmental Protection Agency (EPA) Toxic Release Inventory reported that 86 tonnes of DEHP were released into the environment in the USA in 2008, of which 72 tonnes were released into the air from 215 facilities that manufactured and used this chemical, combined with 1588.6 tonnes that were transferred offsite (see [Table 1.4](#)). In 1997, air emissions of DEHP from 312 industrial facilities in the USA amounted to 110.5 tonnes ([National Library of Medicine, 2011](#)). In Canada, 27 tonnes of DEHP were released into the air in 1995, according to the Canadian National Pollutant Release Inventory ([Environment Canada, 1995](#)). DEHP concentrations of up to 300 ng/m³ have been found in urban and polluted air, and levels between 0.5 and 5 ng/m³ have been reported in the air of oceanic areas ([WHO, 1992](#)).

DEHP released into the air can be carried for long distances in the troposphere and 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](#)).

DEHP in air has been monitored in the North Atlantic, the Gulf of Mexico and on Enewetak Atoll in the North Pacific at levels that ranged from not detectable to 4.1 ng/m³ ([Giam et al., 1978, 1980](#); [Atlas & Giam, 1981](#)). Concentrations of DEHP in the atmosphere of the northwestern Gulf of Mexico averaged 1.16 ng/m³ for 10 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](#)).

Similar levels of DEHP in air (between 0.5 and 5 ng/m³; mean, 2 ng/m³) have been found in the Great Lakes ecosystem (Canada and the USA). The concentration of DEHP in precipitation ranged from 4 to 10 ng/L (mean, 6 ng/L). Atmospheric fluxes to the Great Lakes are a

Table 1.4 Environmental Protection Agency Toxic Release Inventory, 2008, results for 215 facilities in the USA

On-site environmental release (form R)	Pounds	Tonnes
Total air release	159 506	72
Total water release	4 163	1.9
Total underground injection release	0	0
Total land release	25 830	11.7
Total disposal (environmental release)	189 499	86
Off-site waste transfer (form R)	Pounds	Tonnes
Total publicly owned treatment works transfer	4 222	1.9
Total other off-site locations transfer	3 502 285	1588.6
Total off-site waste transfer	3 506 507	1590.5
Total environmental release and off-site waste transfer	3 696 006	1676.5

From National Library of Medicine (2011)

combination of dry and wet removal processes. The total deposition of DEHP 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, DEHP was measured at 14 monitoring stations (53 samples), and the mean air concentration was 2.0 ng/m³ (range, 0.3–77 ng/m³), with an average fallout of 23.8 µg/m² per month (range, 6.0–195.5 µg/m² per month). The total annual fallout of DEHP in Sweden was estimated to be 130 tonnes ([Thurén & Larsson, 1990](#)).

During 1995, four sets of samples of the breathable fraction of atmospheric particulates, including phthalates, from two monitoring stations were measured in the air of the Rieti urban area in Italy. The concentrations of DEHP ranged from 20.5 to 31 ng/m³ (normalized) and from 34.8 to 503.5 ng/m³ (normalized) at the two monitoring stations, respectively ([Guidotti et al., 1998](#)).

The concentration of DEHP in the air at Lyngby, Denmark, was calculated to be 22 ng/m³ based on the analysis of snow samples ([Løkke & Rasmussen, 1983](#)), and levels of 26–132 ng/m³ 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, USA, in 1978 ranged from 10 to 17 ng/m³ ([Bove et al., 1978](#)).

There is a paucity of data concerning concentrations of DEHP in indoor air, although its volatilization from plastic products has been noted ([Wams, 1987](#)). DEHP has been shown to account for 69 and 52% of the total amount of phthalates adsorbed to sedimented dust and particulate matter, respectively, in several Oslo (Norway) dwellings. It was found at levels of 11–210 µg/100 mg sedimented dust in 38 dwellings and at levels of 24–94 µg/100 mg suspended particulate matter (mean ± standard deviation [SD], 60 ± 30) in six dwellings. It was suggested that suspended particulate exposure to DEHP is one- to threefold higher than the estimated vapour-phase exposure ([Øie et al., 1997](#)).

Levels of phthalates were measured in 48-hour personal air samples collected from parallel cohorts of pregnant women in New York City, USA ($n = 30$), and in Krakow, Poland ($n = 30$). Spot urine samples were collected during the same 48-hour period from the New York women ($n = 25$). DEHP was present in 100% of the air and urine samples. The air concentrations of DEHP (shown in [Table 1.5](#)) were higher in Krakow

Table 1.5 Airborne exposure of pregnant women to di(2-ethylhexyl) phthalate ($\mu\text{g}/\text{m}^3$)

Area	No.	Median	Mean \pm SD	Range
New York City, USA	30	0.22	0.22 \pm 0.10	0.05–0.41
Krakow, Poland	30	0.37	0.43 \pm 0.24	0.08–1.1

SD, standard deviation
From [Adibi et al. \(2003\)](#)

(median, $0.37 \mu\text{g}/\text{m}^3$) than in New York (median, $0.22 \mu\text{g}/\text{m}^3$). The urinary concentrations of the metabolite MEHP among the New York women were ($\mu\text{g}/\text{g}$ creatinine): median, 4.60; mean, 40.5 ± 98.4 ; range, 1.80–449. MEHP levels were not correlated with measured personal levels of DEHP ([Adibi et al., 2003](#)).

The same investigators measured airborne DEHP and DEHP metabolite concentrations in spot urine samples collected from 246 pregnant Dominican and African-American women in New York City, USA. Other phthalates were also measured, and 48-hour personal air samples ($n = 96$ women) and repeated indoor air samples ($n = 32$ homes) were also collected. DEHP was detected in 100% of personal air samples (mean, $0.18 \mu\text{g}/\text{m}^3$; range, 0.16 – $0.21 \mu\text{g}/\text{m}^3$). Indoor air sample results were: mean, $0.09 \mu\text{g}/\text{m}^3$; range, 0.08 – $0.10 \mu\text{g}/\text{m}^3$. Urine samples for DEHP metabolites showed an approximately 50-fold increased range from the lowest 5th percentile to the upper 95th percentile. For MEHP (the least concentrated of the metabolites), the results were: mean, 4.8 ng/mL; range, 4.0–5.8 ng/mL. The urinary levels of MEHP were not correlated with measured airborne concentrations ([Adibi et al., 2008](#)).

(c) Water and sediments

In general, concentrations of DEHP in fresh-water are in the range of < 0.1 – $10 \mu\text{g}/\text{L}$, although occasionally much higher values have been observed ($\sim 100 \mu\text{g}/\text{L}$) when water basins are surrounded by large numbers of industrial plants ([WHO, 1992](#)).

Surface water discharges of DEHP from 195 industrial facilities in 2010 in the USA amounted to 555 kg, as reported in the EPA Toxic Release Inventory ([National Library of Medicine, 2011](#)). DEHP has been detected in 24% of 901 surface water supplies at a median concentration of $10 \mu\text{g}/\text{L}$ and in 40% of 367 sediment samples at a median concentration of $1000 \mu\text{g}/\text{kg}$ in samples recorded in the STORET database in the USA ([Staples et al., 1985](#)). DEHP concentrations in water from Galveston Bay, Texas, ranged from < 2 to $12\,000 \text{ ng}/\text{L}$ (average, $600 \text{ ng}/\text{L}$) ([Murray et al., 1981](#)), somewhat higher than those found earlier for the Mississippi Delta (23 – $225 \text{ ng}/\text{L}$; average, $70 \text{ ng}/\text{L}$) and the Gulf of Mexico coast (6 – $316 \text{ ng}/\text{L}$; average, $130 \text{ ng}/\text{L}$) ([Giam et al., 1978](#)). Levels of DEHP up to $720 \text{ ng}/\text{L}$ were found in two sampling stations of the Mississippi River in the summer of 1984 ([DeLeon et al., 1986](#)).

Levels of dissolved DEHP in samples from the River Mersey estuary, Liverpool, United Kingdom, ranged from 0.125 to $0.693 \mu\text{g}/\text{L}$ ([Preston & Al-Omran, 1989](#)). Levels of up to $1.9 \mu\text{g}/\text{L}$ DEHP were found in rivers of the greater Manchester area, United Kingdom ([Fatoki & Vernon, 1990](#)), and at unspecified levels as contaminants in all the samplings of the Elbe River and its tributaries in Germany during the period 1992–94 ([Franke et al., 1995](#)). Levels of DEHP in two rivers in southern Sweden were 0.32 – $3.10 \mu\text{g}/\text{L}$ and 0.39 – $1.98 \mu\text{g}/\text{L}$. The highest value was in samples taken near an industrial effluent discharge ([Thurén, 1986](#)). In a 12-day survey in the Netherlands, DEHP levels ranging from 0.2 to $0.6 \mu\text{g}/\text{L}$ were found in the River Rhine near Lobith and levels ranging from < 0.1

to 0.3 ng/L were found in Lake Yssel ([Ritsema et al., 1989](#)).

Levels of DEHP 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](#)).

DEHP has been reported in the leachate from municipal and industrial landfills at levels ranging from < 0.01 to 200 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](#)).

Because DEHP is lipophilic, it tends to be adsorbed onto sediment, which serves as a sink ([WHO, 1992](#)). It has been measured in rivers and lake sediments in Europe ([Schwartz et al., 1979](#); [Giam & Atlas, 1980](#); [Preston & Al-Omran, 1989](#); [Ritsema et al., 1989](#)) and in river and bay sediments in the USA ([Peterson & Freeman, 1982](#); [Ray et al., 1983](#); [Hollyfield & Sharma, 1995](#)), at concentrations that ranged from 0.021 to 70 mg/kg. Near direct discharge points from industry in Sweden and Malaysia, concentrations of DEHP 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, China ([Jeng, 1986](#)).

In experimental studies of a marine environment of Narragansett Bay, RI, USA, biodegradation by the surface microlayer biota was shown to account for at least 30% of the removal of DEHP ([Davey et al., 1990](#)).

Water solubility is a major factor that limits the degradation of phthalate esters under methanogenic conditions. In a study of the degradation of DEHP and its intermediate hydrolysis products, 2-ethylhexanol (2-EH) and MEHP in a methanogenic phthalic acid ester-degrading

enrichment culture at 37 °C, the culture readily degraded 2-EH via 2-EHA to methane; MEHP was degraded to stoichiometric amounts of methane with phthalic acid as a transient intermediate; while DEHP remained unaffected throughout the 330-day experimental period ([Ejlertsson & Svensson, 1996](#)).

In a study of treatment efficiency, the fate of six phthalates was investigated throughout all the processes in the wastewater-treatment plant of Marne Aval (France). The plant treats wastewater from a highly populated area and was used as a pilot station for the development of nitrification processes. At each step of treatment, DEHP was always the major compound (9–44 µg/L) present in wastewater. In sludge, the prevailing compound was also DEHP (72 µg/g). For the studied period, the removal efficiency of DEHP from wastewater was about 78%. Downstream of the treatment plant discharge, DEHP concentrations remained below the European norm for surface water (1.3 µg/L) ([Dargnat et al., 2009](#)).

In a study of a large number of organic pollutants, water samples were collected at 15 sites from five main stream sections of the Yangtze River in Jiangsu Province (China). In three of the main sections, DEHP concentrations were [mean (range) µg/L]: 0.836 (0.469–1.33); 0.771 (0.352–1.07); and 1.123 (0.582–2.05). In the remaining two sections, DEHP was below the limit of quantitation ([He et al., 2011](#)).

Phthalate ester plasticizers were determined in rivers and dams of the Venda region, South Africa. Generally, the highest concentrations of phthalates were found as DBP and DEHP. DEHP levels at nine sites ranged from 0.3 to 2.18 mg/L ([Fatoki et al., 2010](#)).

A selection of 30 primarily estrogenic organic wastewater contaminants was measured in several influent/effluent wastewater samples from four municipal wastewater treatment plants and effluents from one bleached kraft pulp mill in Canada. DEHP was detected at 6–7 µg/L in municipal effluents ([Fernandez et al., 2007](#)).

A series of xenoestrogens, including DEHP, was measured in various matrices collected in Germany: 116 surface water samples, 35 sediments from rivers, lakes and channels, 39 sewage effluents, and 38 sewage sludges. DEHP was the dominant phthalate, concentrations of which ranged from 0.33 to 97.8 µg/L (surface water), 1.74 to 182 µg/L (sewage effluents), 27.9 to 154 mg/kg dry weight (sewage sludge) and 0.21 to 8.44 mg/kg (sediment) ([Fromme et al., 2002](#)).

Water and soil samples were taken from different agricultural areas in Almeria (Spain). The level of DEHP was below the limit of quantification (0.05 mg/kg) in soil samples whereas it was detected in water samples at concentrations ranging from 0.19 to 0.88 µg/L ([Garrido Frenich et al., 2009](#)). A study was designed to provide information on both the occurrence and concentration build-up of phthalate plasticizers along a heavily urbanized transect of the Seine River in Paris, France. For surface waters, eight or nine sampling sessions were performed at six Seine River locations and at one Marne River site. Surface water samples were manually sampled from July 2006 to November 2007, with a sampling frequency of about 2 months. For settleable particles, sediment traps were set during a 4-week period at four sampling sites. Of the four frequently observed pollutants, DEHP was predominant and exhibited the highest concentrations, the median of which was 1.00 µg/L and the maximum was 14.63 µg/L ([Gaspero et al., 2009](#)).

Surface sediment samples from five tidal marshes along the coast of California, USA, were analysed for organic pollutants. In sediments from Stege Marsh, all phthalate compounds measured in the study were found at levels higher than other contaminants. Creek bank sediment at one station exhibited a very high concentration of DEHP (32 000 ng/g) compared with other stations. Excluding this station, DEHP was most abundant with concentration levels of 235–3000 ng/g (median, 1630 ng/g) in Stege

Marsh sediments. DEHP concentrations found in sediments from most stations fell within the range found in other contaminated areas but were much higher than ambient San Francisco Bay sediment levels (200 ng/g) ([Hwang et al., 2006](#)).

(d) Soil

The principal source of DEHP release to land is disposal of industrial and municipal waste to landfills ([Swedish Environmental Protection Agency, 1996](#); [Bauer & Herrmann, 1997](#); [ATSDR, 2002](#)). Releases of DEHP to land from 298 industrial facilities in the USA in 1997 amounted to 32 tonnes (National Library of Medicine, 2011). According to the Canadian National Pollutant Release Inventory, 33 tonnes of DEHP were released from Canadian facilities onto the land ([Environment Canada, 1995](#)).

Five soils and leachate-sprayed soils from the Susquehanna River basin in the states of Pennsylvania and New York (USA) had levels of DEHP 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 DEHP in soil collected in the vicinity of a DEHP manufacturing plant amounted to up to 0.5 mg/kg ([Persson et al., 1978](#)).

DEHP has been identified in at least 737 of the 1613 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List. However, the number of sites evaluated for DEHP is not known ([ATSDR, 2002](#)).

One hundred and thirty-nine samples from 20 wastewater-treatment plants, seven sludge-treatment (thermal drying) and three composting sludge plants in Catalonia (Spain) were taken between 2001 and 2003. Of these, 92.8% had DEHP concentrations that ranged from 1.9 to 100 mg/kg dry matter. Ten samples had concentrations from above 100 to 3513.8 mg/kg dry matter ([Abad et al., 2005](#)).

Soil samples collected randomly within the Muledane open dump, Thohoyandou, Limpopo province, South Africa, had mean values for DEHP of 0.03 ± 0.01 mg/kg (Adeniyi *et al.*, 2008).

Contamination by phthalates in 23 arable soils throughout China was investigated to evaluate the present pollution situation. Among the phthalates, DEHP was dominant and was detected in all 23 soils at concentrations that ranged from 0.2 to 5.98 mg/kg. A distinct feature of phthalate pollution in China was that the average concentration in northern China was higher than that in southern China. A close relationship was observed between the concentration of phthalates in soils and the consumption of agricultural film which suggests that the application of agriculture film might be a significant source of phthalate pollution in arable soils in China (Hu *et al.*, 2003).

(e) Food

The most common route of human exposure to DEHP is through food contamination. The average daily exposure from food in the USA was estimated in 1992 to be about 0.3 mg/day per individual, with a maximum exposure of 2 mg/day (WHO, 1992).

DEHP 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; Gilbert, 1994). It can originate from PVC wrapping materials, manufacturing processes or from the animals that produced the milk or meat (Giam & Wong, 1987; Gilbert, 1994; Sharman *et al.*, 1994). The highest levels of DEHP have been measured in milk products, meat and fish as well as in other products that have a high fat content. The use of DEHP in food contact applications is reported to be decreasing (Page & Lacroix, 1995).

DEHP 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 levels of phthalates (expressed as DEHP equivalents) in the raw milk of between 0.12 and 0.28 mg/kg. After processing, DEHP was concentrated in cream at levels of up to 1.93 mg/kg, whereas low-fat milk contained < 0.01–0.07 mg/kg. In the United Kingdom, pooled milk samples from doorstep delivery in different regions of the country contained < 0.01–0.09 mg/kg DEHP. Concentrations of DEHP in 10 samples of retail cream and 10 samples of butter obtained in the United Kingdom ranged from 0.2 to 2.7 mg/kg and 2.5 to 7.4 mg/kg, respectively. Thirteen retail milk and cream products from Spain had levels of DEHP ranging from < 0.01 to 0.55 mg/kg (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 DEHP from plasticized tubing used in commercial milking equipment. Concentrations for each individual cow averaged 0.03 mg/kg and rose to 0.05 mg/kg in the central collecting tank. In control milk samples obtained by hand-milking, the concentration of DEHP was below 0.005 mg/kg. In Norway and the United Kingdom, DEHP in milk tubing has been replaced by other types of plasticizer, such as di(2-ethylhexyl) adipate and diisodecyl phthalate (Castle *et al.*, 1990). An investigation of residues of DEHP in retail whole milk samples from 14 Danish dairies in August 1989 (about 6 months after the use of DEHP-plasticized milk tubing was banned in Denmark) revealed a mean concentration below 50 µg/L (Petersen, 1991).

Retail samples of Canadian butter and margarine wrapped in aluminium foil–paper laminate were found to contain DEHP at levels of up to 11.9 mg/kg. Ten samples of butter (454 g each) had levels of DEHP 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 DEHP in the total wrapper and the corresponding food. When DEHP was not present in the wrapper, a background level of DEHP of about 3–7 mg/kg was found in butter while, when it was present, an average level of 9.4 mg/kg was found (Page & Lacroix, 1992).

DEHP was found in both the packaging and in several contacted foods sampled in a 1985–89 survey as part of the Canadian Health Protection Branch Total Diet Program. Average levels of 65 µg/kg DEHP in beverages and 290 µg/kg in foods were associated with the use of DEHP-plasticized cap or lid seals in a variety of glass-packaged foods. DEHP was found in 14 types of cheese at levels of up to 5.5 mg/kg [average, 2.4 mg/kg] and, on a butter-fat basis, these levels averaged about 8 mg/kg DEHP. Levels in factory-packaged fish were 0.2 mg/kg in halibut and 2.1 mg/kg in pollack and those in two smoked salmon samples were 0.3 and 3.9 mg/kg. DEHP 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 DEHP 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 those in total diet cereal products ranged from 0.02 to 1.5 mg/kg. A low incidence and low levels of DEHP were found in total diet samples of fruit and vegetables (mostly not detected to 0.07 mg/kg) (Page & Lacroix, 1995). DEHP was detected in 80, 71, 94 and 52% of Italian plastic-packaged salted meat, jam, baby food and milk samples, respectively, and in all the cheese and vegetable soup samples. The mean DEHP 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 DEHP, with hardly

any differences between the food items (Gruber *et al.*, 1998). DEHP was found in commercial fish (pooled samples of 10 individuals each; mg/kg wet weight): 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).

DEHP was found in 12 glass-bottled drinking-water samples (sealed with caps with a plastic internal gasket) at levels ranging from 2.4 to 17.7 µg/L (mean, 6.0 µg/L). It was also found in 12 polyethylene terephthalate-bottled drinking-water samples (sealed with caps with a plastic internal gasket) at levels ranging from 2.7 to 31.8 µg/L (mean, 10.5 µg/L) (Vitali *et al.*, 1993).

A study designed to quantify dietary intake of phthalates and of diethylhexyl adipate, using duplicate diet samples, and to compare these data with those calculated based on urinary levels of primary and secondary phthalate metabolites, was conducted in Germany. Twenty-seven female and 23 male healthy subjects aged 14–60 years collected daily duplicate diet samples over 7 consecutive days. The median (95th percentile) daily intake of DEHP via food was 2.4 (4.0) µg/kg bw. Its metabolite, MEHP was detectable only at minor concentrations in the food samples, thus conversion of DEHP to MEHP and dietary intake of MEHP were negligible. When comparing dietary DEHP intake from the previous day with intake of DEHP calculated from concentrations of urinary DEHP metabolites, significant correlations were observed for most of the metabolites. The investigators concluded that food was the major source of intake of DEHP (Fromme *et al.*, 2007a).

DEHP was analysed in 29 total diet samples, in 11 samples of baby food and in 11 samples of infant formulae from Denmark. Twenty-nine 'copies' of a 24-hour daily diet were prepared and collected by randomly selected adults. A selection of 11 different types of ready-to-use baby food in glass jars were sampled in retail shops and analysed before their last day of use. Different types of fruit, cereal, rice mixed with fruit or

meat mixed with vegetables were represented. The 11 types of infant formulae were bought in retail shops. In the total diet samples, DEHP was present at levels above the limit of quantitation in 11/29 samples; in a further six samples the level was above the limit of detection but below the limit of quantification. In baby food and infant formulae, two of 11 samples were above the limit of quantification for each category. Maximum and minimum mean concentrations in the total diet samples were 0.11 and 0.18 mg/kg DEHP, respectively ([Petersen & Breindahl 2000](#)).

In a study to estimate the influence of air concentrations of DEHP on its levels in food, concentrations in the atmosphere and in four vegetable crops cultivated on land surrounding a plastics production factory in China were determined. The DEHP concentrations (means) in air at sites 0.2, 0.4, 0.8 and 1.6 km from the production building were 9.4–12.8, 5.8–9.6, 1.6–5.0 and 0.04–0.27 $\mu\text{g}/\text{m}^3$ dry weight, respectively, and were highest downwind and lowest upwind from the factory. Similarly, vegetables accumulated the highest DEHP contents downwind and the lowest quantities upwind from the plant. The highest accumulated DEHP contents of field mustard, bok choy, eggplant and cowpea were 52.0 ± 3.1 , 43.1 ± 2.2 , 36.2 ± 2.8 and 19.4 ± 0.47 mg/kg dry weight, respectively ([Du et al., 2010](#)).

In a study designed to evaluate the migration of six phthalic acid esters in orange juice packaged in PVC bottles, the concentration of DEHP in the orange juice was lower than the limit of detection (1.11 ng/mL) during the first 2 months of storage after production, but was detectable after 3 months. The concentration increased with duration of storage and reached 662 ng/mL after 12 months, at which time the expiration date came into force ([Guo et al., 2010](#)).

Packaging materials intended for direct food contact were acquired from the Brazilian retail market and analysed for their plasticizer content. DEHP was identified in films and closure seals

at concentrations ranging from 15 to 44% (w/w) ([Freire et al., 2006](#)).

A total of 98 samples of cap-sealing resins for bottled foods from in and around Yokohama City (Japan) were purchased in 1993, 1995 and 1997–99. DEHP was detected in seven of 16 samples purchased in 1993 or 1995, at concentrations ranging from 8.4 to 48.2%; the seven positive samples were all from imported bottled foods. For 1997–99, among the 61 samples of imported bottled foods, 11 contained DEHP. DEHP was also detected in seven of the 21 samples of domestic (Japanese) bottled foods at concentrations of 19.6–31.2% ([Hirayama et al., 2001](#)).

(f) *Exposure from medical devices*

DEHP at concentrations of up to 40% by weight is generally used as a plasticizer in PVC materials that 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) ([Toxics Use Reduction Institute, 2005](#)). It is known to leach from PVC blood packs into whole blood, platelet concentrates and plasma during storage; DEHP increases in concentration with storage time and is converted by a plasma enzyme to the metabolite, MEHP ([Rock et al., 1978](#)). DEHP 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](#)). Patients receiving blood products or undergoing treatments requiring extracorporeal blood circulation may be exposed by leaching of DEHP from PVC bags and tubing ([Wams, 1987](#); [WHO, 1992](#)).

DEHP was detected in whole blood at levels ranging from 16.8 to 52.6 mg/L and in packed cells at levels ranging from 32.6 to 55.5 mg/L in PVC blood bags stored at 5 °C. These levels increased with duration of storage. The average

content was 6.7 ± 4.6 mg/L in cryoprecipitate and 7.4 ± 2.8 mg/L in fresh frozen plasma. Both values were independent of the storage period (Sasakawa & Mitomi, 1978).

The accumulation of DEHP in platelet-poor plasma stored for seven and 14 days in PVC bags sterilized by steam, ethylene oxide or irradiation revealed 7-day storage levels of DEHP 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 DEHP in six patients varied between 3.4 and 11.1 mg/L, while those of MEHP ranged from 2.4 to 15.1 mg/L. In newborn infants subjected to a single exchange transfusion, concentrations of DEHP in plasma from the blood taken from the transfusion set varied between 36.8 and 84.9 mg/L, while those of MEHP ranged between 3.0 and 15.6 mg/mL (Sjöberg *et al.*, 1985a). The concentrations in blood of both DEHP and MEHP were similar in PVC bags stored for 4 days or less (Rock *et al.*, 1986).

The degree of exposure to DEHP was assessed in 11 patients undergoing haemodialysis treatment for renal failure and showed that, on average, an estimated 105 mg DEHP was extracted from the dialyser during a single 4-hour dialysis session, with a range of 23.8–360 mg. Time-averaged circulating concentrations of MEHP during the session (1.33 ± 0.58 mg/L) were similar to those of DEHP (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 DEHP over the course of a year, with a range of 3.7–56 g (Pollack *et al.*, 1985). DEHP 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 pre-dialysis patients, DEHP was not detected (< 0.1 mg/L); in no case was the

hydrolysis product MEHP detected (< 0.4 mg/L) (Nässberger *et al.*, 1987). A comparative evaluation of haemodialysis tubing plasticized with DEHP and that plasticized with tri-2-ethylhexyl trimellitate was made in 11 patients (10 men, one woman) with chronic renal failure undergoing haemodialysis for a period of 6 months. During treatment with tubing containing DEHP, the plasma level of DEHP 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 DEHP and its derivatives MEHP, 2-EH and phthalic acid in seven elderly patients undergoing regular CAPD were compared with those in six aged-matched healthy controls during a 4-hour dwell period. Serum concentrations of DEHP 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 MEHP in the fluid of CAPD bags before use was four times higher than that of the parent compound DEHP. During the first 4 hours of dwell time, the concentrations of MEHP and 2-EH 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 DEHP 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 not statistically significantly (0.135 mg/L; range, 0.073–0.659 mg/L; $P = 0.062$) (Mettang *et al.*, 1996a).

Levels of DEHP ranging from < 1 to 4100 mg/L have been reported in the condensate from water traps of six respirators. Estimation of the inhalation exposure to DEHP of five artificially ventilated pre-term infants over a 24-hour period yielded values ranging between 1 µg/h and 4200 µg/h. DEHP (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](#))

Plateletpheresis concentrates were transfused to thrombocytopenic adult haematological patients (seven men, five women) in Austria. Serum DEHP levels were assessed before and after transfusion and after storage for 5 days. The median DEHP concentration in plateletpheresis concentrates was 10.9 mg/L (range, 5.7–23.7), representing a median total dose of 3.5 mg DEHP (range, 1.6–8.8 mg) to the patient. Median serum DEHP levels increased from 192 ng/mL (range, 10–532 ng/mL) at baseline to 478 ng/mL (range, 142–1236 ng/mL) after transfusion. DEHP was also measured on days 1, 3, 4 and 5 in plateletpheresis concentrates that were either stored in donor plasma ($n = 5$) or with addition of T-Sol ($n = 5$). In samples taken shortly after the plateletpheresis procedure, a mean level of 1.88 µg/mL (range, 0.41–3.2 µg/mL) DEHP was found in concentrates suspended in donor plasma compared with 6.59 µg/mL (range, 2.09–10.67 µg/mL) on day 5 ($P = 0.0002$). Similarly, mean DEHP levels increased from 0.75 µg/mL (range, 0.29–1.0 µg/mL) in concentrates suspended in 35% of donor plasma to 1.9 µg/mL (range, 0.5–3.25 µg/mL; $P = 0.0023$) in those suspended in 65% of the T-Sol additive solution ([Buchta et al., 2005](#)).

DEHP and MEHP levels, migration patterns and metabolism in blood products were examined for the detailed assessment of exposure to DEHP. From the Japanese Red Cross Society, 78 blood products (red blood cell concentrate, $n = 18$; irradiated red blood cell concentrate, $n = 18$; whole blood, $n = 18$; blood platelets, $n = 18$; and frozen

plasma, $n = 6$) were sampled in January 2003 for use in this study. The levels of DEHP and MEHP detected and the ratio of MEHP to DEHP ([MEHP concentration per DEHP concentration in samples] $\times 100\%$) were: 6.8–36.5 µg/mL, 0.3–4.3 µg/mL and $6.0 \pm 2.6\%$, respectively, in red blood cell products released from medical blood bags; 7.4–36.1 µg/mL, 0.3–3.3 µg/mL and $5.7 \pm 2.0\%$, respectively, in irradiated red blood cell concentrate products; 15.0–83.2 µg/mL, 0.5–9.7 µg/mL and $7.8 \pm 4.1\%$, respectively, in whole blood products; 1.8–15.0 µg/mL, 0.1–4.6 µg/mL and $15.2 \pm 8.4\%$, respectively, in platelet products; and 11.6–18.5 µg/mL, 1.1–2.5 µg/mL and $10.5 \pm 2.3\%$, respectively, in fresh frozen plasma products. The levels of DEHP and MEHP in all blood products ranged from 1.8 to 83.2 µg/mL and from 0.1 to 9.7 µg/mL, respectively, and increased with increasing storage time. In addition, whole blood products stored in PVC bags had the highest DEHP levels compared with other blood products. These results indicate that the maximum level of human exposure to DEHP released from blood bags would be 0.7 mg/kg bw/time ([Inoue et al., 2005](#)).

Sixteen patients undergoing coronary artery bypass grafting in Japan were randomly divided into two groups of eight. Group A had tubing containing DEHP in the circuit, and group B had no DEHP in the tubing. The plasma level of DEHP at the end of the operation was significantly increased compared with that before anaesthesia in both groups (group A: from 103 ± 60 to 2094 ± 1046 ng/mL; group B: from 135 ± 60 to 472 ± 141 ng/mL), and was significantly higher in group A than in group B ([Takahashi et al., 2008](#)).

DEHP and MEHP were measured in the supernatant of centrifuged samples of 10 whole blood units collected in citrate-phosphate-dextrose buffer from healthy adult blood donors. The level of DEHP significantly increased 12.6-fold from 34.3 ± 20.0 (SD) µM [13.4 ± 7.8 (SD) mg/L] on day 1 to 433.2 ± 131.2 (SD) µM

[169.2 ± 51.2 (SD) mg/L] on day 42, and that of MEHP significantly increased 20.2-fold from 3.7 ± 2.8 (SD) µM [1 ± 0.8 (SD) mg/L] on day 1 to 74.0 ± 19.1 (SD) µM [20.6 ± 5.3 (SD) mg/L] on day 42 ([Rael et al., 2009](#)).

The exposure of six plasma donors, six discontinuous-flow platelet donors and six continuous flow platelet donors to DEHP was measured in Germany, where each donor can perform up to 26 plateletphereses or up to 40 plasmapheresis procedures per year. Exposure was calculated by determining three specific metabolites in urine: mono(2-ethyl-5-hydroxyhexyl) phthalate [MEHHP]; mono(2-ethyl-5-oxohexyl)phthalate [MEOHP] and MEHP. Maximum concentrations in urine samples were found after the continuous-flow plateletpheresis procedure: 826 µg/L (range, 241–1346 µg/L) MEHHP, 774 µg/L (range, 302.3–1153 µg/L) MEOHP and 266 µg/L (range, 69.2–536.5 µg/L) MEHP (means for the six volunteers). The corresponding mean values for discontinuous-flow donors, plasma donors and controls were: 416.3, 416.3 and 148.1 µg/L; 86.3, 91.6 and 41.9 µg/L; and 52.3, 38.2 and 15.5 µg/L, respectively. The excretion of metabolites was significantly ($P < 0.0001$) higher for both plateletpheresis techniques compared with plasmapheresis and controls, and continuous-flow plateletpheresis led to significantly higher ($P < 0.0001$) levels of excretion than discontinuous-flow plateletpheresis. Mean absolute exposures to DEHP were 1.2 mg for discontinuous- and 2.1 mg for continuous-flow plateletpheresis, and those for plasmapheresis (0.37 mg) were in the range of the controls (0.41 mg). Mean doses of DEHP for both plateletpheresis techniques (18.1 and 32.3 µg/kg per day) were close to or exceeded the reference dose of the EPA and the tolerable daily intake value of the EU on the day of the apheresis ([Koch et al., 2005a](#)).

(g) Building materials

[Afshari et al. \(2004\)](#) measured phthalate emissions from PVC flooring, polyolefine flooring, a refrigerator list, two electric cables, PVC skirting and floor wax. Samples were taken in exhaust air from the chambers after 6, 35, 62, 105 and 150 days from the start of the experiment. PVC flooring was tested for an additional 100 days. Polyolefine covered with wax did not emit DEHP. The other materials resulted in a maximum concentration of approximately 1 µg/m³ DEHP. The concentration of DEHP in each chamber increased slowly until a rather stable level was reached after 150 days.

In a field study of floor dust from 15 Danish schools, the mean concentration of DEHP was 3.214 mg/g dust, the 90th percentile value was 6.404 mg/g and the 95th percentile was 7.063 mg/g ([Clausen et al., 2003](#)).

Room air samples from 59 apartments and 74 kindergartens in Berlin (Germany) were tested in 2000 and 2001 for the presence of phthalates. These substances were also measured in household dust from 30 apartments. For air samples in apartments, the levels of DEHP at 59 sites were: 191 (mean), 156 (median), 390 (95%) and 615 ng/m³ (maximum). In kindergartens, the levels in 73 samples were: 599 (mean), 458 (median), 1510 (95%) and 2253 ng/m³ (maximum). With a contribution of approximately 80% of all values, the main phthalate in house dust was DEHP, with median values of 703 mg/kg (range, 231–1763 mg/kg). No statistically significant correlation could be found between air and dust concentration. The estimated daily intake of DEHP for an adult (in µg/kg per day) was 0.05 from indoor air and 0.11 from dust. For a child, the estimated daily intake (in µg/kg per day) was 0.06 for air and 5.97 for dust ([Fromme et al., 2004](#)).

The presence of a target set of phthalates was investigated in the interior of 23 used private cars during the summer and winter in Italy. DEHP

was detected in six of the vehicles at detectable concentrations that ranged from 200 to 1400 ng/m³ (Geiss *et al.*, 2009).

In 2003, in a village in a rural part of southern Germany, internal exposure to DEHP of 36 nursery schoolchildren (aged 2 ± 6 years), 15 parents and four teachers was determined and compared. The DEHP metabolites MEHHP, MEOHP and MEHP were determined in the first morning urine. The sum of the three DEHP metabolites in the urine of children and adults was 90.0 and 59.1 µg/L, respectively (median values; $P = 0.074$). Concentrations of the secondary metabolites MEHHP (median, 49.6 versus 32.1 µg/L; $P = 0.038$) and MEOHP (median, 33.8 versus 19.6 µg/L; $P = 0.015$) were significantly higher in children than in adults, whereas those of MEHP were low in both adults and children (median, 6.6 µg/L versus 9.0 µg/L). Creatinine-adjusted values more accurately reflected the dose taken up (with respect to body weight) in children and adults. Total creatinine-adjusted levels of DEHP metabolites in urine were significantly higher in children than in adults (median values, 98.8 versus 50.9 µg/g creatinine; $P < 0.0001$), as were those of both secondary metabolites, MEHHP (55.8 versus 28.1 µg/g creatinine; $P < 0.0001$) and MEOHP (38.3 versus 17.2 µg/g creatinine; $P < 0.0001$). Creatinine-corrected concentrations for the monoester MEHP in children and adults were very similar (8.7 versus 8.6 µg/g creatinine (Koch *et al.*, 2004a).

Urine samples of the general German population were examined for human-specific metabolites of phthalates. The study subjects were inhabitants of Erlangen, a city in southern Germany, and its vicinity. Eighty-five subjects were sampled (median age, 33 years; range, 7–64 years; 53 women: median age, 29 years; 32 men: median age, 36 years). First morning urine was collected in mid-April 2002, and metabolites of DEHP — MEHP, MEHHP and MEOHP — were measured in urine, from which the daily

intake of DEHP was determined. A median intake of 13.8 µg/kg bw per day and an intake at the 95th percentile of 52.1 µg/kg bw per day were estimated. Twelve per cent of the subjects (10/85 samples) within this group had intake greater than 37 µg/kg bw per day, and 31% (26/85 samples) had values higher than the reference dose (20 µg/kg bw per day) of the US Environmental Protection Agency (Koch *et al.*, 2003a).

(h) Sludge

Organic residues from four wastewater-treatment plants in Seville, Spain, were analysed between January and October 2005. All the treatment plants carried out anaerobic biological stabilization of the sludge. DEHP was found in all of the sludge samples analysed at concentrations of 12–345 mg/kg dry mass. Mean concentrations of DEHP in primary, secondary and anaerobically-digested dehydrated sludges and in compost samples were 53, 65, 159 and 75 mg/kg dry mass, respectively (Aparicio *et al.*, 2009).

Concentrations of DEHP and its metabolites were measured in the sludges from several sewage-treatment plants in the Province of Québec, Canada. DEHP was found at concentrations ranging from 15 to 346 mg/kg in primary, secondary, digested, dewatered or dried sludges. Metabolites were detected in almost all sludges, except those that had undergone a drying process at high temperature (Beauchesne *et al.*, 2008).

In Switzerland, sewage sludges from different catchment areas were sampled: four samples containing predominantly domestic sewage were obtained from separate sewer systems (type A); six samples containing a mixture of domestic sewage, stormwater runoff and small amounts of industrial wastewater were obtained from combined sewer systems (type B); and two samples containing a mixture of domestic sewage, stormwater runoff and higher amounts of industrial wastewater were obtained from combined sewer systems (type C). DEHP was

found in all 12 samples at concentrations ranging from 21 to 114 mg/kg dry mass. There was no apparent difference in concentration between the three types of source ([Berset & Etter-Holzer, 2001](#)).

The presence of nonylphenols, nonylphenol ethoxylates and eight phthalates was analysed in urban stormwater and sediment from three catchment areas in Sweden. Emission loads for these substances were then calculated for a specific urban catchment area. A level of ≤ 48 $\mu\text{g/g}$ DEHP was found in the sediment, and aqueous concentrations of up to 5.0 $\mu\text{g/L}$ DEHP were detected ([Björklund et al., 2009](#)).

A variety of contaminants, including phthalates, was measured in source-separated compost and digestate samples from Switzerland that were selected to cover a wide variety of variables that may influence organic pollutant concentrations, including treatment processes (composting/digestion), origin (urban/rural) and composition of input material (green waste with/without crude organic kitchen waste), and season of collection. The median concentration of DEHP was 280 $\mu\text{g/kg}$ dry weight ($n = 6$). The highest value (1990 $\mu\text{g/kg}$ dry weight) was detected in an urban digestate sample containing organic kitchen waste, as well as fruit and vegetable residues from grocery stores ([Brändli et al., 2007](#)).

In a study that measured the occurrence of 43 semi-volatile organic compounds in sewage sludges collected from 11 wastewater-treatment plants in mainland and Hong Kong Special Administrative Region, China, DEHP was detected in the sludge of nine of the 11 sites. The mean DEHP concentration was 21 mg/kg dry weight, with detectable values ranging from 4.4 to 108 mg/kg dry weight ([Cai et al., 2007](#)).

Seventeen sewage sludges were studied for organic pollutants that the EU has proposed be controlled for land application. Samples were collected in 2002–03 from different wastewater-treatment plants of the Valencian Community (Spain) by third parties (inspectors of the regional

government) at the points they considered to be representative during 1 day of sampling. Results by type of treatment were: anaerobic (10 facilities), range 40–325 mg/kg dry weight; aerobic (three facilities), 2–80 mg/kg; and without digestion (four facilities), 50–350 mg/kg ([Gomez-Rico et al., 2007](#)).

(i) Total exposure of the general population

The Centers for Disease Control and Prevention ([CDC, 2009](#)) in the USA analysed urine samples from a representative population sample of 2605 persons for DEHP metabolites, as part of the National Health and Nutrition Examination Survey for 2003–4. The metabolites measured were MEHP, MEHHP, MEOHP and MCEPP. Results were presented as geometric means and selected percentile (in $\mu\text{g/g}$ creatinine) by age group, sex and ethnic group for each metabolite. Results for MEHP are presented in [Table 1.6](#). For the total population, the geometric mean was 2.20 $\mu\text{g/g}$ (95% confidence interval [CI], 2.01–2.41 $\mu\text{g/g}$), with a 95th percentile value of 25.4 $\mu\text{g/g}$ (95%CI: 16.7–34.7 $\mu\text{g/g}$). For each subgroup, the ratio of the 95th percentile to the median value was 10-fold or greater. Urinary concentrations in the youngest age group (6–11 years) were somewhat higher than those of adults: 3.00 $\mu\text{g/g}$ (95%CI: 2.30–3.93 $\mu\text{g/g}$) versus 2.14 $\mu\text{g/g}$ (95%CI: 1.98–2.31 $\mu\text{g/g}$). Comparisons between sex and ethnicity are also presented in [Table 1.6](#). Geometric mean and percentile values for the four metabolites are compared in [Table 1.7](#). Compared with the geometric mean value of 2.20 $\mu\text{g/g}$ (95%CI: 2.01–2.41 $\mu\text{g/g}$) for MEHP, the concentrations of the other metabolites were substantially higher: MEHHP, 20.4 $\mu\text{g/g}$ (95%CI: 18.7–22.3 $\mu\text{g/g}$); MEOHP, 13.6 $\mu\text{g/g}$ (95%CI: 12.4–14.8 $\mu\text{g/g}$); and MCEPP, 32.6 $\mu\text{g/g}$ (95%CI: 29.6–36.0 $\mu\text{g/g}$). Similar 10-fold or greater ratios of the 95th percentile to the median values, and similar relative values for children compared with adults were observed for each of the metabolites.

Table 1.6 Urinary mono(2-ethylhexyl) phthalate (in µg/g of creatinine) for the population in the USA, 2003–4

	Geometric mean (95% CI)	Selected percentiles (95% CI)				No.
		50th	75th	90th	95th	
Total	2.20 (2.01–2.41)	1.89 (1.68–2.19)	4.31 (3.84–4.74)	10.8 (8.72–13.8)	25.4 (16.7–34.7)	2605
Age group						
6–11 yr	3.00 (2.30–3.93)	2.80 (1.93–4.09)	5.86 (4.69–7.70)	14.3 (8.54–24.4)	28.7 (14.1–45.3)	342
12–19 yr	2.07 (1.74–2.48)	1.88 (1.60–2.23)	4.25 (3.19–5.62)	11.6 (6.83–23.2)	24.8 (11.6–37.9)	729
> 20	2.14 (1.98–2.31)	1.84 (1.63–2.08)	4.14 (3.78–4.40)	10.5 (8.38–12.9)	25.6 (15.9–36.3)	1534
Sex						
Male	2.01 (1.82–2.21)	1.71 (1.46–1.89)	4.14 (3.49–4.81)	10.4 (7.68–16.2)	23.3 (15.1–41.1)	1250
Female	2.40 (2.15–2.69)	2.16 (1.84–2.40)	4.40 (3.97–4.89)	10.9 (8.27–16.0)	27.0 (17.5–34.6)	1355
Ethnic group						
Mexican Americans	2.12 (1.74–2.59)	1.94 (1.50–2.42)	4.06 (3.29–4.93)	9.38 (5.72–15.4)	16.8 (9.86–38.6)	652
Non-Hispanic blacks	2.56 (2.24–2.92)	2.28 (2.02–2.78)	5.17 (4.48–6.83)	13.2 (10.5–16.2)	27.5 (18.4–36.0)	699
Non-Hispanic whites	2.12 (1.91–2.35)	1.82 (1.60–2.13)	4.11 (3.49–4.42)	10.7 (7.42–15.1)	27.0 (15.1–37.4)	1088

CI, confidence interval; yr, years

Adapted from [CDC \(2009\)](#)

Table 1.7 Urinary concentrations of selected di(2-ethylhexyl) phthalate metabolites (in µg/g of creatinine) for the total population in the USA, 2003–4

	Geometric mean (95% CI)	Selected percentiles (95% CI)				No.
		50th	75th	90th	95th	
Mono(2-ethylhexyl) phthalate	2.20 (2.01–2.41)	1.89 (1.68–2.19)	4.31 (3.84–4.74)	10.8 (8.72–13.8)	25.4 (16.7–34.7)	2605
Mono(2-ethyl-5-hydroxyhexyl) phthalate	20.4 (18.7–22.3)	17.7 (16.3–19.6)	35.8 (30.5–43.3)	93.5 (74.0–128)	182 (134–262)	2605
Mono(2-ethyl-5-oxohexyl) phthalate	13.6 (12.4–14.8)	12.1 (11.0–12.9)	24.3 (20.9–27.8)	63.0 (47.8–75.8)	118 (94.1–153)	2605
Mono(2-ethyl-5-carboxypentyl) phthalate	32.6 (29.6–36.0)	27.0 (24.3–30.6)	54.6 (48.0–63.5)	139 (109–186)	251 (192–356)	2605

CI, confidence interval

Adapted from [CDC \(2009\)](#)

In a study to assess the contribution of different food types to phthalate exposure, associations between dietary intake (assessed by a 24-hour dietary recall) for a range of food types (meat, poultry, fish, fruit, vegetables and dairy) and phthalate metabolites measured in the urine were analysed using multiple linear regression modelling. MEHP levels in the urinary samples (in ng/g creatinine) were: median, 1.9; mean, 6.5; geometric mean, 2.2; and range, 0.09–294. Other metabolites showed a similar skewed distribution. The levels of metabolites of DEHP were associated with the consumption of poultry ([Colacino et al., 2010](#)).

Median daily intakes of DEHP among German university students were calculated to be between 2.4 and 4.2 µg/kg bw per day based on DEHP metabolites measured in the urine, and were associated with the industrial production of DEHP in Germany with a correlation coefficient of > 0.9 ([Helm, 2007](#)).

Daily intake of phthalates was estimated from urinary metabolite levels in Japan between late May and early June 2004. Thirty-six volunteers (35 adults and one child; aged 4–70 years), 26 of whom were aged 20–29 years and 25 of whom were men, participated. Most of the participants lived in the Tokyo-Yokohama area. The median concentration of MEHP was 5.0 µg/L (geometric SD, 2.52 µg/L). From this, the investigators estimated a mean DEHP intake of 2.7 (range, 2.0–3.3) µg/kg bw per day ([Itoh et al., 2007](#)).

[Wittassek & Angerer \(2008\)](#) reported that, based on urinary phthalate metabolite concentrations estimated in 102 German subjects aged 6–80 years, the median daily intake of DEHP was 2.7 µg/kg bw per day. They noted that, in general, children had higher exposures than adults ([Wittassek & Angerer 2008](#)).

In a retrospective human biomonitoring study, 24-hour urine samples taken from the German Environmental Specimen Bank for Human Tissues were analysed for a series of phthalate ester metabolites. The samples were

collected from 634 subjects (predominantly students; age range, 20–29 years; 326 women, 308 men) between 1988 and 2003 ($n \geq 60$ per year). Based on urinary metabolite excretion, the daily intakes of the parent phthalates were estimated and the chronological course of phthalate exposure was investigated. Metabolites of all five phthalates were detectable in over 98% of the urine samples. For the DEHP metabolite, MEHP, the median value declined from 9.8 µg/g creatinine in 1988 to 5.5 µg/g creatinine in 2003. The median over the whole period was 7.3 µg/g creatinine for the total cohort, with similar values for men and women. Other metabolites showed similar trends. For the entire study period, the daily intake estimated from the metabolite levels (µg/kg bw per day) was: median, 3.5; upper 95th percentile, 10.1; and range, 0.19–39.8. These levels decreased from 1988 (median, 3.9; upper 95th percentile, 9.9; range, 0.78–39.8) to 2003 (median, 2.4; upper 95th percentile, 5.7; range, 0.82–7.1) ([Wittassek et al., 2007a](#)).

The excretion of DEHP and its metabolites were studied by analysing first morning urine samples from 53 women and 32 men, aged 7–64 years (median, 34.2 years), living in northern Bavaria (Germany) who were not occupationally exposed to phthalates. Concentrations were found to vary considerably between subjects with differences spanning more than three orders of magnitude. Median concentrations of excreted DEHP metabolites were 46.8 µg/L MEHHP (range, 0.5–818 µg/L), 36.5 µg/L MEOHP (range, 0.5–544 µg/L), and 10.3 µg/L MEHP (range, < 0.5 [limit of quantification]–177 µg/L). A strong correlation was found between the excretion of MEHHP and MEOHP (correlation coefficient $r = 0.991$) indicating close metabolic proximity of these two parameters but also the absence of any contaminating interference ([Koch et al., 2003b](#)).

(j) *Exposure of infants and children*

[Zhang et al. \(2009\)](#) assessed maternal–fetal exposure to phthalates to investigate whether in-utero exposure to phthalates is associated with low birth weight (LBW). They examined phthalate concentrations in specimens of maternal blood, cord blood and meconium from a total of 201 newborn–mother pairs (88 LBW cases and 113 controls) residing in Shanghai (China) during 2005–06 in a nested case–control study on pregnancy outcomes. Median DEHP concentrations in maternal blood and cord blood varied from 0.5 to 0.7 mg/L and did not differ between controls and mothers with LBW infants. In contrast, highly significant differences were found between control and LBW mothers for the levels of the metabolite MEHP. Median MEHP levels in maternal blood were: controls, 1.4 mg/L (range, 1.2–2.1 mg/L); and LBW, 2.9 mg/L (range, 1.8–3.5 mg/L). Levels in cord blood were: controls, 1.1 mg/L (range, 0.9–1.7 mg/L); and LBW, 2.5 mg/L (range, 1.6–3.4 mg/L). Levels in neonatal meconium were: controls, 2.9 mg/g (range, 1.8–4.4 mg/g); and LBW, 5.5 mg/g (range, 3.4–9.3 mg/g).

Exposure of children to DEHP by migration from PVC toys and other articles into saliva has been reported. Until the early 1980s, DEHP was the predominant plasticizer used in soft PVC products for children. Since that time, it has been replaced in most countries by other plasticizers, in particular di(isononyl) phthalate ([Steiner et al., 1998](#); [Wilkinson & Lamb, 1999](#)).

The levels of phthalate esters were analysed in a total of 86 human milk samples collected among 21 breast-feeding mothers over a 6-month postpartum time, in the city of Kingston, Ontario, Canada, in 2003–04. DEHP was the predominant ester with an arithmetic mean value of 222 ng/g (range, 156–398 ng/g). Weak correlations between lipid content and levels of phthalate esters were observed. The levels of phthalate esters in human milk fluctuated over

the 6-month period. The mean daily intake of DEHP over the first 6-month period for a 7-kg infant consuming 750 g milk (breast-feeding) was estimated at 167 µg/day ([Zhu et al., 2006](#)).

The presence of several phthalate metabolites was analysed in breast milk from healthy mothers living in southern Italy. Milk samples from 62 healthy mothers (mean age ± SD, 29.42 ± 5.28 years; range, 18–41 years) from the Brindisi and Tricase areas were randomly collected within approximately 7 days postpartum during March 2006 and September 2006. Women enrolled in the study did not undergo surgical interventions and/or ongoing medical treatments, including intravenous infusions or blood transfusions, during the period immediately preceding the sample collection. MEHP was found in all samples: median, 8.4 µg/L (95%CI: 7.6–10.0 µg/L); 95th percentile, 28.5 µg/L; maximum, 109 µg/L. No oxidized metabolites of DEHP were found ([Latini et al., 2009](#)).

In 2001, 42 primiparae from southern Sweden (median age, 29 years; range, 23–39 years) provided breast milk, blood and urine samples 2–3 weeks after delivery. In breast milk, DEHP was detected in 39/42 samples (median, 9.0 ng/mL; range, 0.45–305 ng/mL) and MEHP was detected in 16/42 samples (median, 0.49 ng/mL; maximum, 6.5 ng/mL). In blood, DEHP was detected in 17/36 samples (median, 0.50 ng/mL; maximum, 129 ng/mL) and MEHP was detected in six of 36 samples (median, 0.49 ng/mL). MEHP was detected in all 38 samples of urine (median, 9 ng/mL; range, 2.9–57 ng/mL). No correlations existed between urinary concentrations and those found in milk or blood/serum for individual phthalate metabolites ([Högberg et al., 2008](#)).

The disposition of DEHP and MEHP during a single exchange transfusion was investigated in four newborn infants. The amounts of DEHP and MEHP 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 DEHP

originated from parts of the transfusion set other than the blood bag. Immediately after the transfusions, the plasma levels of DEHP 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 hours ([Sjöberg et al., 1985b](#)).

Measurement of serum levels of DEHP in 16 newborn infants undergoing exchange transfusion indicated an undetectable level (< 1 mg/L) before the exchange but levels ranging from 6.1 to 21.6 mg/L (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 DEHP ranged from 12.3 to 87.8 mg/L and, in six newborn infants receiving a third blood unit, the serum levels ranged from 24.9 to 93.1 mg/L ([Plonait et al., 1993](#)).

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 DEHP after 14 and 24 days of ECMO support were 26.8 and 33.5 mg/L, respectively. DEHP levels of 3.5, 1.0 and 0.4 mg/kg were found in the liver, heart and testicular tissues, respectively, and trace quantities were found in the brain. The rate of DEHP extraction from the model PVC circuits was linear with time (rate, 3.5 and 4.1 mg/L per hour). The exposure to DEHP for a 4-kg infant on ECMO support for 3–10 days was estimated to be 42–140 mg/kg bw ([Shneider et al., 1989](#)).

A more recent study of 18 infants on ECMO life support also reported leaching of DEHP 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 DEHP was 8.3 ± 5.7 mg/L. For a 4-kg infant, the estimated exposure over 3–10 days was 10–35 mg/kg bw. No leaching of DEHP from heparin-coated PVC circuits was detected ([Karle et al., 1997](#)).

Exposure to DEHP in six critically ill premature newborns was assessed by measuring the levels of three DEHP metabolites in 41 urine samples. MEHHP and MEOHP were detected in all 41 samples, and MEHP was detected in 33. For the 33 samples, the geometric mean, 5% percentile and 95% percentile urinary concentrations were: MEOHP, 1617, 243 and 10 413 ng/mL; MEHHP, 2003, 290 and 13 161 ng/mL; and MEHP, 100, 6.22 and 704 ng/mL. Concentrations per gram of creatinine were approximately eightfold higher than results in nanograms per millilitre ([Calafat et al., 2004](#)).

[Green et al. \(2005\)](#) studied 54 neonates admitted to neonatal intensive care units in Boston, MA, USA, for at least 3 days between 1 March and 30 April 2003. The exposures of infants to DEHP were classified based on medical products used: the low-exposure group included infants receiving primarily bottle and/or gavage feedings; the medium-exposure group included infants receiving enteral feedings, intravenous hyperalimentation and/or nasal continuous positive airway pressure; and the high-exposure group included infants receiving umbilical vessel catheterization, endotracheal intubation, intravenous hyperalimentation and in-dwelling gavage tube. Urinary MEHP levels increased monotonically with DEHP exposure. For the low-, medium- and high-exposure groups, median (interquartile range) MEHP levels were 4 (18), 28 (58) and 86 (150) ng/mL, respectively ($P = 0.004$). After adjustment for institution and sex, urinary MEHP levels among infants in the high-exposure group were 5.1 times those among infants in the low-exposure group ($P = 0.03$).

In a follow-up report, the levels of three metabolites of DEHP were measured in the urine of the 54 infants. Urinary concentrations stratified by intensiveness (in ng/mL) were: MEHP – low, 4; medium, 28; high, 86; MEHHP – low, 27; medium, 307; high, 555; and MEOHP – low, 29; medium, 286; high, 598. Urinary concentrations of MEHHP and MEOHP among infants in

the high-intensiveness group were 13–14 times those among infants in the low-intensiveness group ($P \leq 0.007$). A structural equation model confirmed the specific monotonic association between intensiveness of product use and biological measures of DEHP. Inclusion of the oxidative metabolites, MEHHP and MEOHP, strengthened the association between intensiveness of product use and biological indices of DEHP exposure over that observed with MEHP alone ([Weuve et al., 2006](#)).

In Germany, urine samples were taken from 254 children aged 3–14 years during 2001 and 2002, and were analysed for concentrations of the DEHP metabolites, MEHP, MEHHP and MEOHP. In addition, DEHP was analysed in house dust samples collected with vacuum cleaners in the homes of the children. The geometric means of the urinary levels of MEHP, MEHHP and MEOHP (in $\mu\text{g/L}$) were 7.9, 52.1 and 39.9, respectively. MEHHP and MEOHP concentrations were highly correlated ($r = 0.98$). The correlations of MEHHP and MEOHP with MEHP were also high ($r = 0.72$ and 0.70 , respectively). The concentrations of MEHHP and MEOHP were 8.0-fold and 6.2-fold those of MEHP. The ratios of MEHHP to MEOHP and MEOHP to MEHP decreased with increasing age. Boys showed higher urinary concentrations than girls for all three metabolites of DEHP. Children aged 13–14 years had the lowest mean urinary concentrations of the secondary metabolites. The house dust analyses revealed DEHP contamination of all samples with a geometric mean of 508 mg/kg dust. No correlation could be observed between the levels of any of the urinary DEHP metabolites and those of DEHP in house dust ([Becker et al., 2004](#)).

Another study estimated the daily DEHP intake of children who lived in two different urban areas in the city of Berlin and in two rural villages in the northern part of Germany. First morning urine samples for the analysis of the DEHP metabolites MEHHP, MEOHP and

MEHP and individual data (age, body weight, body height) from 239 children (aged 2–14 years; median, 8.5 years) were collected between March 2001 and March 2002. Two calculation models based upon the volume and the creatinine-related urinary metabolite concentrations were applied. Applying the volume-based model, a median daily DEHP intake of 7.8 $\mu\text{g/kg}$ bw per day, a 95th percentile of 25.2 $\mu\text{g/kg}$ bw per day and a maximum of 140 $\mu\text{g/kg}$ bw per day were estimated. Using the creatinine-based model, the median daily intake was estimated at 4.3 $\mu\text{g/kg}$ bw per day with a 95th percentile of 15.2 $\mu\text{g/kg}$ bw per day and a maximum of 409 $\mu\text{g/kg}$ bw per day. In general, exposure to DEHP decreased with increasing age. The median for children aged 2–4 years was 5.7 (creatinine-based) or 10.7 (volume-based) $\mu\text{g/kg}$ bw per day. For children aged 12–14 years, the median was 2.7 (creatinine-based) or 4.8 (volume-based) $\mu\text{g/kg}$ bw per day. Comparing boys and girls in the whole age range, values were slightly higher in boys than in girls ($P = 0.023$ in the creatinine-based model and $P = 0.097$ in the volume-based model). While median values were comparable in both estimation models (4.9 versus 3.9 $\mu\text{g/kg}$ bw per day in the creatinine-based model and 8.4 versus 7.4 $\mu\text{g/kg}$ bw per day in the volume-based model), the 95th percentile was about twice as high for boys as for girls. Differences in gender were particularly pronounced in the youngest children, between 2 and 4 years of age ($P < 0.004$ in both calculation models) ([Wittassek et al., 2007b](#)).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines for DEHP in several countries are presented in [Table 1.8](#).

In 1989, the EPA required that DEHP be subject to registration as a pesticide in the USA under the Federal Insecticide, Fungicide, and Rodenticide Act. This requirement was cancelled in 1998 ([RTECS, 2009](#)).

Table 1.8 Occupational exposure limits and guidelines for di(2-ethylhexyl) phthalate

Country	Year	Concentration (mg/m ³)	Interpretation
Argentina ^a	2007	5	TWA
Australia	2008	5	TWA
		10	STEL
Belgium	2002	5	TWA
		10	STEL
Bulgaria ^a	2007	5	TWA
Colombia ^a	2007	5	TWA
Denmark	2002	3	TWA
Finland	1999	5	TWA
		10 (sk)	STEL
France	2006	5	VME
Germany	2005	10	MAK
Hungary	2000	10	TWA
		40 (sk)	STEL
Japan	2007	5	OEL
Jordan ^a	2007	5	TWA
Korea, Republic of	2006	5	TWA
		10	STEL
Mexico	2004	5	TWA
		10	STEL
New Zealand	2002	5	TWA
		10	STEL
Norway	1999	3	TWA
Philippines	1993	5	TWA
Poland	1999	1	MAK (TWA)
		5	MAK (STEL)
Russian Federation	2003	1	STEL
Singapore ^a	2007	5	TWA
Sweden	2005	3	TWA
		5	STEL
Switzerland	2006	5	MAK-wk
United Kingdom	2005	5	TWA
		10	STEL
USA			
ACGIH (TLV)	2007	5	TWA
OSHA (PEL)	1994	5 (general industry, shipyards, construction, federal contractors)	TWA
NIOSH (REL air)	1992	5	TWA
		10	STEL
MSHA (air)	1971	5	TWA
Viet Nam ^a	2007	5	TWA

a These countries follow the recommendations of the ACGIH threshold limit values.

ACGIH, American Conference of Governmental Industrial Hygienists; MAK, maximale Arbeitsplatz-Konzentration; MSHA, Mine Safety and Health Administration; NIOSH, National Institute of Occupational Safety and Health; OEL, occupational exposure limit; OSHA, Occupational Safety and Health Administration; PEL, permissible exposure limit; REL, recommended exposure limit; sk, absorption through the skin may be a significant source of exposure; STEL, short-term exposure limit; TLV, threshold limit value; TWA, time-weighted average; VME, valeur moyenne d'exposition [average exposure value]; wk, week

From [IUCLID DataBase \(2000\)](#); [RTECS \(2009\)](#); [ACGIH \(2010\)](#)

The World Health Organization established an international drinking-water guideline for DEHP of 8 µg/L in 1993 ([WHO, 2008](#)). The [EPA \(1998\)](#) has set the maximum contaminant level for DEHP in drinking-water at 6 µg/L in the USA.

The [Food and Drug Administration \(1999\)](#) permits the use of DEHP in the USA 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% weight 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 come into contact with food and as a food-packaging plasticizer for foods with a high water content.

The European Union has ruled that DEHP shall not be used in toys and childcare articles at concentrations greater than 0.1% by mass of the plasticised material ([Council of Europe, 2005](#)).

A reference dose of 20 µg/kg bw per day was set by the [EPA \(2007\)](#).

2. Cancer in Humans

The epidemiological studies that evaluated cancer risk and exposure specifically to DEHP are very limited, and include an occupational cohort study of DEHP production workers that was reviewed by a previous IARC Working Group ([IARC, 2000](#)), and a case–control study of breast cancer. However, several studies evaluated cancer risk and exposure to plastics, particularly in occupational settings and the PVC industry. Workers who make PVC plastics or products are potentially exposed to phthalate plasticizers, including DEHP, which is the most common, and these products can contain 1–40% DEHP

by weight. Some studies of workers making PVC plastics are briefly reviewed in this section if they included analyses for exposure to phthalate plasticizers ([Hagmar *et al.*, 1990](#)) or phthalates ([Heineman *et al.*, 1992](#)), or if they specifically mentioned that exposure to DEHP occurred or that DEHP was a suspected etiological agent ([Hagmar *et al.*, 1990](#); [Selenskas *et al.*, 1995](#); [Hardell *et al.*, 1997, 2004](#)). These comprise one cohort study, three case–control studies of testicular cancer, one case–control study of pancreatic cancer and one case–control study of multiple myeloma.

Long-term dialysis patients are liable to experience elevated exposures to DEHP, through frequent and protracted exposure to substances leached from surgical tubing during dialysis; however, the Working Group was not aware of any study of dialysis patients for which study methods were adequate for the evaluation of carcinogenic risks associated with DEHP.

Exposure to DEHP has been associated with some hormone-related outcomes (e.g. endometriosis, thyroid hormone disruption and testicular dysgenesis syndrome), thus studies of breast and testicular cancer are of special interest.

2.1 Studies specific for exposure to DEHP

2.1.1 Occupational exposure

[Thiess *et al.* \(1978\)](#) evaluated the mortality of 221 workers in a DEHP production plant in Germany, who were followed between 1940 and 1976. Most subjects (135/221) were hired after 1965 and the process was completely enclosed in 1966. No information on the level of exposure was provided. Only eight deaths from all causes occurred during the follow-up period; one from pancreatic cancer (0.13 expected) and one from urinary bladder papilloma (0.01 expected) occurred among workers with a long duration of exposure (≥ 20 years). No further report on a

longer follow-up for this cohort was available to the Working Group. [The Working Group noted that the majority of the cohort members were employed after exposure levels had been considerably reduced. The methods for this study were poorly described and power was inadequate to detect a potential excess risk.]

2.1.2 Case-control study

(a) Cancer of the breast

[López-Carrillo et al. \(2010\)](#) conducted a case-control study in northern Mexico to evaluate the association between urinary levels of nine phthalate metabolites and breast cancer (see [Table 2.1](#)). They interviewed 233 women with breast cancer and 221 age-matched controls from 2007 to 2008, and collected sociodemographic and reproductive characteristics and first morning void urine samples before any treatment. Exposure assessment was based on the measurement of biomarkers: no data on personal habits involving exposure to phthalates were available. Phthalate metabolites, detected in at least 82% of all women, were measured in urine samples by isotope dilution/high-performance liquid chromatography coupled to tandem mass spectrometry. After adjusting for risk factors and other phthalates, increased odds ratios for breast cancer were associated with urinary concentrations of four DEHP metabolites: MEHP, MEHHP, MEOHP, and MECPP; however, this increased risk was only statistically significant for MECPP, with a dose-response trend ($P = 0.047$). A non-significant negative association was observed for MEOHP. With regard to other phthalate metabolites, urinary concentrations of DEP and monoethyl phthalate (MEP) metabolites were positively associated with breast cancer (odds ratio of highest versus lowest tertile, 2.20; 95%CI: 1.33–3.63; P for trend < 0.01). In contrast, significant negative associations were found for monoisobutyl phthalate (MiBP), monobenzyl phthalate (MBzP) and mono(3-carboxylpropyl)

phthalate (MCP) metabolites. The odds ratios for the sum of all nine metabolites (including five non-DEHP phthalate metabolites), for the 2nd and 3rd versus the 1st tertile were 0.94 (95%CI: 0.57–1.56) and 1.41 (95%CI: 0.86–2.31; P for trend = 0.114).

[The Working Group considered that this study had an appropriate design, although the timing of exposure assessment was a concern. Biological samples to measure DEHP metabolites were obtained after diagnosis among cases, before any treatment; metabolites were measured in the urine, and it is not known whether disease status could have affected metabolite levels. A limitation of this study was the lack of consistency in effect between the four DEHP metabolites measured and the lack of a dose-response for all metabolites. Further, it is unclear which metabolite is the best biomarker for exposure to DEHP.]

2.2 Occupational exposure to phthalate plasticizers

2.2.1 Cohort study

See [Table 2.2](#)
[Hagmar et al. \(1990\)](#) reported on the mortality of 2031 Swedish workers at a PVC-processing factory that produced flooring, film and pipes from PVC. DEHP was the major plasticizer used in all of these products. A significant excess of total cancer morbidity (standard incidence ratio, 1.28; 95%CI: 1.01–1.61; 75 cases) and respiratory cancer morbidity (SIR, 2.13; 95%CI: 1.27–3.46; 17 cases) was observed among the PVC-processing workers, but no statistically significant association was found with cumulative exposure to plasticizers. [The Working Group noted that only 6% of the cohort was exposed only to plasticizers.] Respiratory cancer risk was increased in individuals who were exposed to both asbestos and plasticizers but not to vinyl chloride (SIR, 10.70; 95%CI: 2.20–31.20; three cases) and in

Table 2.1 Case-control study of cancer and di(2-ethylhexyl) phthalate (DEHP)

Reference, study location, period	Total cases	Total controls	Control source (hospital, population)	Exposure assessment	Organ site	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates	Comments	
López-Carrillo <i>et al.</i> (2010), Northern Mexico, 2007–08	233 cases identified in 25 tertiary hospitals, aged ≥ 18 yr; response rate, 94.8%; histopathologically confirmed [Note: incident status of cases unspecified, but because urine samples were obtained before any treatment, incident cases are assumed]	221	Population-based selected from lists of the Health Department national surveys; matched 1:1 by age (± 5 yr) and residency; response rate, 99.5%	Measurement of four DEHP metabolites in first morning void urine samples: MEHP, MEHHP, MEOHP, MECPP	Breast (unspecified)	MEHP (µg/g creatinine)				Current age, age at menarche, parity, menopausal status, non-DEHP phthalate metabolites	Results are also described for metabolites of MEP, MnBP, MiBP, MBzP, MCPP
						0.22–3.42	81	1.00			
						3.43–7.51	67	1.03 (0.62–1.69)			
						7.52–257.08	83	1.23 (0.75–2.01)			
								<i>P</i> trend = 0.383			
						MEHHP (µg/g creatinine)					
						2.69–35.61	90	1.00			
						35.62–63.38	53	0.77 (0.46–1.28)			
						63.39–1014.60	88	1.37 (0.84–2.24)			
								<i>P</i> trend = 0.106			
						MEOHP (µg/g creatinine)					
						2.10–23.90	108	1.00			
						24.91–43.10	51	0.60 (0.36–1.00)			
43.11–1230.94	72	0.84 (0.52–1.36)									
		<i>P</i> trend = 0.651									
MECPP (µg/g creatinine)											
11.59–57.88	69	1.00									
57.89–97.67	73	1.27 (0.77–2.10)									
97.68–1742.92	89	1.68 (1.01–2.78)									
		<i>P</i> trend = 0.047									

CI, confidence interval; MnBP, mono(*n*-butyl) phthalate; MBzP, monobenzyl phthalate; MCPP, mono(3-carboxypropyl) phthalate; MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono(2-ethylhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MEP, monoethyl phthalate; MiBP, monoisobutyl phthalate; yr, year or years

Table 2.2 Cohort study of workers potentially exposed to di(2-ethylhexyl) phthalate (DEHP), phthalates or polyvinyl chloride (PVC) plastics

Reference, period	Study population	Follow-up period	Exposure assessment	Organ site	Exposure categories	No. of cases/deaths	Relative risk (95% CI)	Comments				
Hagmar et al. (1990) , 1945–80	2031 PVC-processing workers	1945–85	Workers were exposed to DEHP in addition to other phthalates, PVC, and vinyl chloride	All cancer Respiratory cancer	Exposed to DEHP in addition to other phthalates, PVC and vinyl chloride	75	Morbidity 1.28 (1.01–1.61)	Expected number of deaths and cancer morbidity based on national rates; SMRs and 95% CIs calculated using Poisson distribution; exposure–response relationship were based on person–yr; potential confounding by tobacco smoking and exposure to other agents; limited power to detect exposure relationships and risk of cancer at individual sites; no overall risk estimates given for plasticizers.				
						17	2.13 (1.27–3.46)					
			Workers categorized into four levels (not exposed, low, moderate and high) of exposure to plasticizers, vinyl chloride monomer and asbestos by experts	Respiratory cancer	Cumulative exposure to plasticizers (mg–yr)		Mortality					
						1.22 (0.88–1.71)						
						1.53 (0.73–2.80)						
							Morbidity					
						1	0.52 [0.01–2.90]					
						5	3.73 [1.22–8.71]					
						5	2.13 [0.69–4.97]					
							Test for trend <i>P</i> > 0.05					

CI, confidence interval; SMR, standardized mortality ratio; yr, year or years

workers not exposed to any of the three agents. [The limitations of this study were the small number of workers and exposed deaths/cases of site-specific cancers, potential confounding by tobacco smoking or other risk factors and crude exposure estimates.]

2.2.2 Case-control studies

See [Table 2.3](#)

Three population-based case-control studies — two in Sweden and one in Denmark — evaluated the association between occupational exposure to PVC plastics or products and testicular cancer. A small Swedish study found an increased risk for testicular cancer among men exposed to PVC (OR, 6.6; 95%CI: 1.4–32; seven exposed cases and two exposed controls) ([Hardell et al., 1997](#)). [The Working Group noted that the results were only from living subjects, which could introduce bias if the risk factor were associated with poor prognosis.] In a larger Danish study, no increased risk for testicular cancer was observed for men ever exposed to mainly PVC (OR, 0.7; 95%CI: 0.5–1.2) or plastics in general (OR, 1.0; 95%CI: 0.8–1.2) ([Hansen, 1999](#)). [However, this study had limited statistical power because of small numbers of exposed cases; the prevalence of exposure to PVC for 1 year or more among controls was only 0.5%.] In the second Swedish study ([Hardell et al., 2004](#); [Westberg et al., 2005](#)) of 791 men with germ-cell testicular cancer and 791 matched controls, ever exposure to PVC plastics was associated with an increased risk for testicular cancer (OR, 1.35; 95%CI: 1.06–1.71); a non-significant increased risk was reported for exposure to soft (containing plasticizer) plastics (OR, 1.48; 95%CI: 0.94–2.34; 54 cases and 37 controls) but not to rigid plastics (OR, 1.06; 95%CI: 0.55–2.01; 23 cases and 26 controls). The risk was elevated among workers with a 10-year latency (OR, 1.45; 95%CI: 1.06–1.98). However, odds ratios for exposure decreased with increasing exposure for all four measures of

exposure (duration, maximum intensity, median intensity over the subject work history and cumulative median intensity). [The questions on exposure were focused on PVC in general and not on exposure to specific substances, which could decrease the possibility of detecting an effect due to phthalates.]

[Selenskas et al. \(1995\)](#) conducted a nested case-control study of pancreatic cancer among a cohort of workers employed at a plastics manufacturing and research and development plant in New Jersey, USA ([Dell & Teta, 1995](#)). Individuals with potential exposure to phthalates worked in either the vinyl- and polyethylene-processing department, or the fibres and fabrics department. Vinyl processing involved the compounding and calendaring (a fabrication step) of PVC polymers and copolymers that are produced at other locations. Potential exposure to DEHP, specifically mentioned as being used in this plant, occurred in the production of flexible plastics. A significantly increased risk for pancreatic cancer was observed only in the vinyl- and polyethylene-processing workers (relative risk, 7.15; 95%CI: 1.28–40.1; five exposed cases who had worked for more than 16 years). No trend of increasing risk with increasing duration or latency was observed. [However, there were only nine cases and 40 controls in the combined production areas, so that the number of cases and control in each exposure stratum (duration or latency) was small.] Most of the cases (eight of nine) and controls (34/40) in the vinyl- and polyethylene-processing areas worked in the vinyl- and polyethylene-processing subdepartment in that area. Among these workers, an elevated risk for pancreatic cancer was observed among those exposed for more than 18 years (relative risk, 8.98; 95%CI: 0.90–89.8). All of the cases of pancreatic cancer that occurred in the vinyl- and polyethylene-processing department worked in the building where both vinyl and polyethylene were processed and none of the cases occurred among workers in the building where only polyethylene was processed. [The

Table 2.3 Case-control studies of workers potentially exposed to di(2-ethylhexyl) phthalate (DEHP), phthalates or polyvinyl chloride (PVC) plastics

Reference, location	Study population and methods	Exposure	Effects	Comments
Hardell et al. (1997); Ohlson & Hardell (2000) , Sweden	<i>Population-based, testicular cancer</i> <i>Cases:</i> 148 men aged 30–75 yr with testicular cancer reported to the Swedish Cancer Registry between 1989 and 1992 <i>Controls:</i> 315 men selected from the Swedish Population Registry who were born the same yr as the cases; ORs calculated using conditional logistic regression and analyses made with latency times of 1 and 5 yr	Occupational exposure to PVC plastics assessed via a detailed questionnaire; cumulative exposure calculated by multiplying the exposure level by portion of days worked and number of yr of exposure	Odds ratios (95% CI); cases/controls <i>Exposure to PVC plastics</i> All: 6.6 (1.4–32); 7/2 Low: 2.6 (0.3–32); 2/2 High: NR; 5/0	Small number of exposed cases and controls; retroactive exposure assessment; self-administered questionnaire
Hansen (1999) , Denmark	<i>Population-based, testicular cancer</i> <i>Cases:</i> 3745 men aged 16–75 yr with testicular cancer, identified in the Danish Cancer registry between 1970 and 1989, and members of the national pension fund <i>Controls:</i> 7490 men without cancer randomly selected from the national pension fund and matched to cases on age; odds ratios calculated using conditional regression models adjusting for socioeconomic status and analyses made using lag times of 0, 1, 5, 10 and 15 yr	Employment history obtained from the national pension fund records, and socioeconomic status inferred from occupational titles; workers in the cable manufacturing industry assumed to be exposed to PVC (industry is the largest consumer of PVC and phthalate plasticizers in Denmark)	Odds ratios (95% CI); cases/controls <i>Ever exposed (yr) to mainly PVC</i> All: 0.7 (0.5–1.2); 26/71 < 1: 0.9 (0.5–1.6); 28/39 1–2: 1.3 (0.4–4.7); 4/6 > 2: 0.4 (0.1–1.0); 4/26	Limited documentation; study reported as a peer-reviewed letter to the editor; small number of subjects exposed to mainly PVC for more than 1 yr; potential misclassification of exposure; potential confounding

Table 2.3 (continued)

Reference, location	Study population and methods	Exposure	Effects	Comments
Hardell et al. (2004) ; Westberg et al. (2005) Sweden	<i>Population-based, testicular cancer</i> Cases: 791 men with testicular cancer reported to the Swedish Cancer Registry from 1993 to 1997 Controls: 791 matched men selected from the Swedish population registry; odds ratios calculated by conditional logistic regression model for matched studies and analyses made using lag times, of > 1 yr and > 10 yr; cases and controls with an exposure time shorter than 1 yr before diagnosis classified as unexposed; exposure time dichotomized by the median exposure time of controls (8 yr)	Exposure to PVC plastics obtained from detailed questionnaire on entire working history and exposure specifically to PVC; an expert assessment performed using questionnaire data and five semi-quantitative exposure categories developed: unexposed, insignificant, low, medium and high; five different exposure measures then derived based on the qualitative and quantitative categorization: ever/never exposed, duration, maximum intensity, median intensity, cumulative median intensity	Odds ratios (95% CI); cases/controls Hardell et al. (2004) Ever exposed (latency) to PVC > 1 yr: 1.35 (1.06–1.71); 200/59 > 10 yr: 1.45 (1.06–1.98); 123/97 Type of PVC plastic Rigid plastic: 1.06 (0.55–2.01); 23/26 Soft plastic: 1.48 (0.94–2.34); 54/37 Median intensity of exposures with a 10-yr latency Insignificant: 1.75 (0.51–5.98); 8/6 Low: 1.50 (0.89–2.51); 51/39 Medium: 1.52 (0.98–2.35); 57/46 High: 0.67 (0.19–2.36); 7/6 Westberg et al. (2005) Insignificant, low, medium, high Duration: 1.4, 1.6, 1.1, 1.2 Maximum intensity: 2.3, 1.2, 1.4*, 0.9 Median intensity: 2.6*, 1.3, 1.4, 0.9 Cumulative median intensity: 1.6*, 1.5*, 1.1, 1.0	The study population did not overlap with Hardell et al. (1997) ; ever exposure to PVC occurred in 160 subjects as reported by the subjects and 360 subjects as assessed by the expert assessment; 360 subjects were used for the analysis; small number of exposed subjects in highest exposure category; lack of exposure measures for work tasks * 95% CI does not include 1.0

Table 2.3 (continued)

Reference, location	Study population and methods	Exposure	Effects	Comments
Selenskas et al. (1995) , New Jersey, USA	<i>Nested, pancreatic cancer</i> <i>Cohort:</i> 5594 male workers employed at least 7 mo between 1946 and 1967 at a plastics manufacturing plant (Dell & Teta, 1995) <i>Cases:</i> 28 men who died from pancreatic cancer <i>Controls:</i> 140 men randomly selected from the cohort and matched to the case for yr of birth and survival; risk estimates calculated by conditional logistic regression models for matched studies; exposures within 10 yr of case death excluded	Occupational exposure assessed by employment history and department of work; individuals classified into major production and non-production areas	Relative risk (95% CI) for pancreatic cancer <i>Vinyl and polyethylene processing (9 cases/40 controls)</i> Duration of employment (yr) ≤ 1: 0.54 (0.06–4.57) 1–5: 0.84 (0.16–4.30) 5–16: 0.47 (0.06–3.84) > 16: 7.15 (1.28–40.1) No trend observed with time since first employment <i>Other departments</i> At least five cases of pancreatic cancer also observed in resin pulverizing, resins and varnish, and plant service and maintenance, but no associations observed in the duration of employment or latency strata	Individuals with potential exposure to DEHP or phthalates worked in either the vinyl- and polyethylene-processing department or the fibres and fabrics department; small numbers of subjects; limited power to detect effects of duration of employment and latency because only nine cases and 40 vinyl- and polyethylene-processing workers stratified into five duration categories or five latency categories

Table 2.3 (continued)

Reference, location	Study population and methods	Exposure	Effects	Comments
Heineman et al. (1992) , Denmark	<i>Population-based, multiple myeloma</i> Cases: 1098 men over 18 yr, and diagnosed with multiple myeloma from 1970 to 1984 Controls: 4169 men selected at random from the Danish Central Population Registry and matched to the case on age and yr in which the case was diagnosed; maximum likelihood estimates of the odds ratios and 95% CI calculated adjusting for age; risks for specific substances calculated using a referent group not exposed to that substance; effects of multiple exposure calculated using logistic regression that adjusted for exposure to several agents and age	Employment history obtained from the pension fund and job titles on tax records; information used by experts to assess and assign individuals to categories of exposures to specific substances	Odds ratios (95% CI); cases/control <i>Exposure to phthalates (overall)</i> Possible: 1.3 (0.9–2.0); 34/99 Probable: 2.0 (0.9–4.4); 11/21 <i>Duration of exposure, no lagging</i> Possible 1 mo– < 5 yr: 1.1 (0.6–2.3); 12/41 ≥ 5 yr: 1.2 (0.7–2.2); 17/54 Test for trend $P = 0.21$ Probable 1 mo– < 5 yr: 1.9 (0.4–8.9); 3/6 ≥ 5 yr: 2.5 (0.9–7.0); 7/11 Test for trend $P = 0.02$ When exposure lagged for 10 yr, risk increased with duration of possible exposure but not probable exposure; however, test for trend not significant. <i>Logistic regression adjusting for exposure to vinyl chloride, engine exhaust and gasoline</i> No increase in risk observed with increasing duration of possible or probable exposure under both lagging conditions	Specific estimates given for exposure to phthalates; small numbers of exposed cases and controls in each stratum, especially for probable exposure; some attempt to adjust for multiple exposures, especially vinyl chloride

CI, confidence interval; mo, month or months; NR, not reported; yr, year or years

Working Group noted that the limitations of this study include the small numbers of exposed cases, the categorization of exposure that was not specific to DEHP, the lack of quantitative exposure measures and potential confounding from exposure to occupational agents, tobacco smoking or other risk factors.]

The relationship between multiple myeloma and exposure to phthalates (and other occupational agents) was evaluated in a population-based case-control study among Danish men ([Heineman et al., 1992](#)). Exposure to phthalates was associated with elevated but non-significant odds ratios for multiple myeloma, with a higher risk estimate for probable exposure (OR, 2.0; 95%CI: 0.9–4.4; 11 cases and 21 controls) than possible exposure (OR, 1.3; 95%CI: 0.9–2.0; 34 cases and 94 controls). Risk estimates for probable exposure increased with increasing duration of exposure when latency was not considered (OR for probable exposure greater than 5 years, 2.5; 95%CI: 0.9–7.0; *P* for trend = 0.02). When time since first exposure was lagged for 10 years, risks increased with duration of exposure for possible but not probable exposure. Exposure to vinyl chloride was also associated with an increased risk for multiple myeloma, and the risk estimate increased with increasing duration of exposure. Stratified analysis was conducted to separate the effects of exposure to phthalates from exposure to vinyl chloride. Increased risk estimates were observed for: exposure to phthalates but never to vinyl chloride (OR for 5 or more years, 2.0; 95%CI: 0.1–27; one case); exposure to vinyl chloride but never to phthalates (OR for 5 or more years, 2.6; 95%CI: 0.3–19.2; two cases); and exposure to both (OR, 5.2; 95%CI: 1.0–29.5; four cases). However, the numbers of exposed cases in each stratum were small. In logistic regression analyses that controlled for exposure to vinyl chloride, engine exhaust and gasoline, risk estimates for exposure to phthalates no longer increased with duration of exposure. [If duration of exposure were a poor surrogate for cumulative

exposure, this would lead to a misclassification of exposure and thus make it hard to detect an exposure-response relationship.]

3. Cancer in Experimental Animals

3.1 Inhalation

3.1.1 Hamster

Groups of 65 male and 65 female Syrian golden hamsters, 12 weeks of age, were exposed continuously by whole-body inhalation to DEHP vapour at a concentration of $15 \pm 5 \mu\text{g}/\text{m}^3$ until natural death (almost continuously for 23 months up to a total exposure of 7–10 mg/kg bw per hamster). Controls (80 males and 80 females) were untreated. No significant difference in tumour incidence was observed between the controls and the DEHP-exposed group ([Schmezer et al., 1988](#)).

3.2 Oral administration

See [Table 3.1](#).

3.2.1 Mouse

Groups of 50 male and 50 female B6C3F₁ mice, 5 weeks of age, were maintained on a diet containing 0 (controls), 3000 or 6000 ppm DEHP for 103 weeks. A dose-related decrease in mean body weight gain was observed in female mice from week 25 to the end of the study. The incidence of hepatocellular carcinoma was significantly increased in high-dose males with a significant trend. In females, both the low-dose and the high-dose groups showed an increase in the incidence of hepatocellular carcinoma with a significant trend. The incidence of hepatocellular carcinoma or adenoma (combined) was increased in low- and high-dose males and females compared to their respective controls.

Table 3.1 Carcinogenicity studies of exposure to di(2-ethylhexyl) phthalate (DEHP) in the diet in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F ₁ (M) 103 wk Kluwe et al. (1982) ; NTP (1982) ; Kluwe et al. (1985)	0, 3000 or 6000 ppm 50	Liver (hepatocellular carcinoma): 9/50, 14/48, 19/50* Liver (hepatocellular adenoma or carcinoma, combined): 14/50, 25/48*, 29/50**	* <i>P</i> = 0.022 <i>P</i> = 0.018 (trend) * <i>P</i> = 0.013 ** <i>P</i> = 0.002	> 99.5% pure
Mouse, B6C3F ₁ (F) 103 wk Kluwe et al. (1982) ; NTP (1982) ; Kluwe et al. (1985)	0, 3000 or 6000 ppm 50	Liver (hepatocellular carcinoma): 0/50, 7/50*, 17/50** Liver (hepatocellular adenoma or carcinoma, combined): 1/50, 12/50*, 18/50**	* <i>P</i> = 0.006 ** <i>P</i> < 0.001 <i>P</i> < 0.001 (trend) * <i>P</i> = 0.001 ** <i>P</i> < 0.001	> 99.5% pure
Mouse, B6C3F ₁ (M) 104 wk David et al. (1999, 2000a)	0, 100, 500, 1500 or 6000 ppm 70, 60, 65, 65 and 70	Liver (hepatocellular adenoma or carcinoma, combined): 8/70, 14/60, 21/65*, 27/65*, 37/70* Liver (hepatocellular adenoma): 4/70, 10/60, 13/65, 14/65, 19/70 Liver (hepatocellular carcinoma): 4/70, 5/60, 9/65, 14/65, 22/70	* <i>P</i> < 0.05 [<i>P</i> < 0.0005, trend] [<i>P</i> < 0.0005, trend]	> 99.7% pure; 10–15 animals per group were killed during wk 79 and the remaining animals were autopsied at wk 105; survival was reduced in mice receiving 6000 ppm DEHP.
Mouse, B6C3F ₁ (F) 104 wk David et al. (1999, 2000a)	0, 100, 500, 1500 or 6000 ppm 70, 60, 65, 65 and 70	Liver (hepatocellular adenoma or carcinoma, combined): 3/70, 4/60, 7/65, 19/65*, 44/70* Liver (hepatocellular adenoma): 0/70, 2/60, 4/65, 9/65, 34/70 Liver (hepatocellular carcinoma): 3/70, 2/60/ 3/65, 16/65, 16/70	* <i>P</i> < 0.05 [<i>P</i> < 0.0005, trend] [<i>P</i> < 0.0005, trend]	> 99.7% pure; 10–15 animals per group were killed during wk 79 and the remaining animals were autopsied at wk 105; survival was reduced in mice receiving 6000 ppm DEHP
Mouse, Sv/129 wild-type or Sv/129 <i>Ppara</i> -null (M) 22 mo Ito et al. (2007a)	0, 0.01 or 0.05% NR	Liver (tumours): <i>Ppara</i> -null–1/25, 1/25, 8/31 Liver (tumours): wild-type–0/24, 2/23, 2/20	[NS], see comments	Purity NR; according to the authors, the incidence of liver tumours was higher (<i>P</i> < 0.05) in <i>Ppara</i> -null mice exposed to 0.05% DEHP (8/31; six hepatocellular adenomas, one hepatocellular carcinoma and one cholangiocellular carcinoma) than in those exposed to 0% DEHP (1/25; one hepatocellular carcinoma). The Working Group noted the unusual grouping of cholangiocellular and hepatocellular tumours. When comparing hepatocellular tumours only (7/31 versus 1/25), there was no statistical difference [<i>P</i> = 0.052, Fisher's one-tailed test.]

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M) 103 wk Kluwe et al. (1982) ; NTP (1982) ; Kluwe et al. (1985)	0, 6000 or 12000 ppm 50	Liver (hepatocellular adenoma or carcinoma, combined): 3/50, 6/49, 12/49*	* $P < 0.02$	99.5% pure
Rat, F344 (F) 103 wk Kluwe et al. (1982) ; NTP (1982) ; Kluwe et al. (1985)	0, 6000 or 12000 ppm 50	Liver (hepatocellular adenoma): 0/50, 4/49, 5/50* Liver (hepatocellular carcinoma): 0/50, 2/49, 8/50** Liver (hepatocellular adenoma or carcinoma, combined): 0/50, 6/49***, 13/50****	* $P < 0.05$ ** $P < 0.01$ *** $P < 0.02$ **** $P < 0.001$	99.5% pure
Rat, F344 (M) 104 wk David et al. (1999, 2000b)	0, 100, 500, 2500 and 12500 ppm 80, 50, 55, 65 and 80	Liver (hepatocellular adenoma or carcinoma, combined): 5/80, 5/50, 4/55, 11/65*, 34/80* Liver (hepatocellular adenoma): 4/80, 5/50, 3/55, 8/65, 21/80 Liver (hepatocellular carcinoma): 1/80, 0/50, 1/55, 3/65, 24/80 Pancreas (acinar-cell adenoma): 0/60 (control), 5/59* (high dose)	* $P < 0.05$ [$P < 0.0005$, trend] [$P < 0.0005$, trend] * $P < 0.05$	> 99.7% pure
Rat, F344 (F) 104 wk David et al. (1999, 2000b)	0, 100, 500, 2500 and 12500 ppm 80, 50, 55, 65 and 80	Liver (hepatocellular adenoma or carcinoma, combined): 0/80, 4/50*, 1/55, 3/65, 22/80* Liver (hepatocellular adenoma): 0/80, 3/50, 1/55, 2/65, 8/80 Liver (hepatocellular carcinoma): 0/80, 1/50, 0/55, 1/65, 14/80	* $P < 0.05$ [$P < 0.0005$, trend] [$P < 0.0005$, trend]	> 99.7% pure

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley (SD-CD) (M) 159 wk Voss et al. (2005)	0, 600, 1897 and 6000 ppm 390, 180, 100 and 60	Testicle (benign Leydig-cell tumour): 64/390, 34/180, 21/100, 17/60*	* $P < 0.05$ $P < 0.019$ (trend)	99.7% pure
		Liver (hepatocellular adenoma): 13/167, 3/84, 4/53, 6/31	[NS]	
		Liver (hepatocellular carcinoma): 2/167, 3/84, 0/53, 3/31*	*[$P < 0.05$]	
		Liver (hepatocellular adenoma or carcinoma, combined): 15/167, 6/84, 4/53, 9/31*	* $P < 0.005$ $P = 0.001$ (trend)	

F, female; M, male; mo, months; NR, not reported; NS, not significant; wk, week or weeks

Multiple liver tumours occurred more frequently in exposed mice than in controls. ([Kluwe et al., 1982](#); [NTP, 1982](#); [Kluwe et al., 1985](#)).

Five groups of 70, 60, 65, 65 and 70 male and 70, 60, 65, 65 and 70 female B6C3F₁ mice, 6 weeks of age, were fed 0 (controls), 100, 500, 1500 or 6000 ppm DEHP in the diet for up to 104 weeks. Ten to 15 animals per group were killed during week 79. The remaining animals were autopsied at week 105. Survival was reduced in male and female mice receiving 6000 ppm DEHP. Overall weight gain was significantly lower in 6000-ppm males. Significantly higher liver weight was observed in 500-, 1500- and 6000-ppm males and 6000-ppm females. A significantly higher incidence of hepatocellular adenoma or carcinoma (combined) was observed in 500- (males only), 1500- and 6000-ppm groups (incidences at week 79 and 105 were combined). The incidence of hepatocellular adenoma and of hepatocellular carcinoma was increased in a dose-related manner [P for trend < 0.0005] in males and females ([David et al., 1999, 2000a](#)).

3.2.2 Rat

Groups of 50 male and 50 female Fischer 344 rats, 4–5 weeks of age, were maintained on a diet containing 0 (controls), 6000 or 12 000 ppm DEHP for 103 weeks. A dose-related decrease in mean body weight gain was observed throughout the study in males. In females, body weight gain was reduced in the high-dose group only. In males, the incidence of hepatocellular adenoma and of hepatocellular carcinoma did not differ significantly among the groups. However, in males of the 12 000-ppm group, the incidence of hepatocellular adenoma or carcinoma (combined) differed significantly from that in controls. In females, the incidence of hepatocellular adenoma and of hepatocellular carcinoma was significantly higher in the high-dose groups compared with controls. In addition, the incidence of hepatocellular adenoma or carcinoma

(combined) was significantly higher in low-dose and high-dose females ([Kluwe et al., 1982](#); [NTP, 1982](#); [Kluwe et al., 1985](#)).

Two groups of 10 male Fischer 344 rats, 6 weeks of age, were fed a diet containing 0% (controls) or 2% DEHP (purity, 98%) for 95 weeks. Hepatocellular adenoma or carcinoma (combined) developed in six of 10 rats fed DEHP and none were found in eight controls ($P < 0.005$, χ^2 test) ([Rao et al., 1987](#)). [The Working Group noted the small number of animals per group and the limited reporting of the study.]

Groups of 80, 50, 55, 65 and 80 male and 80, 50, 55, 65 and 80 female Fischer 344 rats, 6 weeks of age, were fed a diet containing 0 (controls), 100, 500, 2500 and 12 500 ppm DEHP for 104 weeks. No significant differences in survival were observed among the groups. Body weight and food consumption were significantly lower ($P < 0.05$) in males and females of the 12 500-ppm group. The incidence of hepatocellular carcinoma or hepatocellular adenoma (combined) was significantly higher in 100-ppm females, 2500-ppm males and 12 500-ppm males and females compared with controls. The incidence of hepatocellular adenoma and of hepatocellular carcinoma was increased in a dose-related manner [P for trend < 0.0005] in males and females. In males of the high-dose group (12 500 ppm), but not in females or in other groups of males, the incidence of acinar-cell adenoma of the pancreas was significantly increased compared with controls ([David et al., 1999, 2000b](#)).

Seven hundred and thirty male Sprague-Dawley (SD-CD) rats were divided into four groups. DEHP was mixed with the diet at 0 (controls), 600, 1897 and 6000 ppm. DEHP-treated animals were fed 5 g DEHP-diet/100 g bw daily on 6 days per week for 159 weeks and received DEHP-free diet on the 7th day only after they had consumed the rest of their DEHP-containing diet. On this basis, the DEHP-treated rats were exposed to 30, 95 and 300 mg/kg bw, respectively. Controls were fed an equicaloric

DEHP-free diet. No difference in survival was observed among the groups. In the high-dose group, the incidence of hepatocellular adenoma or carcinoma (combined) and of benign Leydig-cell tumours was significantly increased and showed a dose-related trend. Time to tumour analysis revealed that DEHP-induced Leydig-cell tumours developed earlier in life ([Voss et al., 2005](#)). [The Working Group noted the large variability in the number of animals per group.]

3.3 Intraperitoneal injection

3.3.1 Hamster

Three groups of 25 male and 25 female Syrian golden hamsters, 6 weeks of age, received intraperitoneal injections of 3 g/kg bw DEHP once a week, once every 2 weeks or once every 4 weeks. A group of 25 males and 25 females served as untreated controls. The animals were observed for life or were killed when moribund. No significant difference in tumour incidence was observed between the DEHP-treated groups and the controls ([Schmezer et al., 1988](#)).

3.4 Genetically modified mouse

3.4.1 Oral administration

Groups of 15 male and 15 female CB6F1-rasH2 mice, 6 weeks of age, were fed DEHP (purity, > 99%) in the diet at concentrations of 0 (controls), 1500, 3000 or 6000 ppm, and groups of 15 male and 15 female wild-type mice were fed concentrations of 0 (controls) or 6000 ppm DEHP in the diet, for 26 weeks. DEHP treatment induced hepatocellular adenomas in 1/15, 2/15 and 4/15 ($P < 0.05$, Fisher's exact probability test) male CB6F1-rasH2 mice in the 1500-, 3000- and 6000-ppm groups, respectively, compared with 0/15 controls. No hepatocellular tumours were observed in females or wild-type mice ([Toyosawa et al., 2001](#); [Usui et al., 2001](#)).

Groups of 15 male and 15 female Tg.AC mice were fed a diet containing 0 (controls), 1500, 3000 or 6000 ppm DEHP for 26 weeks. No increase in the incidence of tumours was observed ([Eastin et al., 2001](#)).

Groups of 15 male and 15 female *Xpa*^{-/-} mice were fed a diet containing 0 (controls), 1500, 3000 or 6000 ppm DEHP, and groups of 15 male and 15 female wild-type (C57BL/6) or *Xpa*^{-/-}/*P53*^{+/-} were fed a diet containing 0 (controls) or 6000 ppm DEHP. The observation period for all groups was 39 weeks. No significant difference in tumour incidence was observed between the DEHP-treated groups and their respective controls ([Mortensen et al., 2002](#)).

DEHP-induced tumorigenesis was compared in wild-type and *Ppara*-null Sv/129 mice. Mice of each genotype, 3 weeks of age, were divided into three groups and fed diets containing 0 (controls), 0.01 or 0.05% DEHP [purity unspecified] for 22 months. The liver tumour incidence in the *Ppara*-null mice was 1/25, 1/25 and 8/31 for the 0, 0.01 and 0.05% doses, respectively. In the wild-type mice, the corresponding incidence was 0/24, 2/23 and 2/20 for the 0, 0.01 or 0.05% doses, respectively. According to the authors, the incidence of liver tumours was significantly higher ($P < 0.05$) in *Ppara*-null mice exposed to 0.05% DEHP (8/31; six hepatocellular adenomas, one hepatocellular carcinoma, one cholangiocellular carcinoma) than in control *Ppara*-null mice (1/25; one hepatocellular carcinoma). [The Working Group noted the unusual grouping of cholangiocellular and hepatocellular tumours. When comparing hepatocellular tumours only (7/31 versus 1/25), there was no statistical difference ($P = 0.052$, Fisher's one-tailed test)] ([Ito et al., 2007a](#)).

3.4.2 Skin application

Groups of 15 male and 15 female Tg.AC mice, 8–9 weeks of age, received daily topical applications of 0 (controls), 100, 200 or 400 mg/kg bw

DEHP (in acetone) on 5 days per week for 28 weeks. DEHP did not increase the incidence of tumours at the site of application ([Eastin et al., 2001](#)). [The Working Group noted the limited reporting of the study.]

3.5 Co-exposure with modifying agents

3.5.1 Mouse

(a) Oral administration

Male B6C3F₁ mice, 4 weeks of age, received a single intraperitoneal injection of 80 mg/kg bw *N*-nitrosodiethylamine (NDEA). At 5 weeks of age, the mice were fed a diet containing 0 (controls) or 3000 ppm DEHP (Aldrich Chemical Co. [purity unspecified]) for 1, 7, 28, 84 or 168 days (and killed after 168 days), or for 168 days (and killed after 252 days). When DEHP was fed for 28, 84 or 168 days (killed after 168 days), or 168 days (killed after 252 days), the incidence of hepatocellular adenoma was increased compared with mice receiving NDEA only (6/29, 5/28, 14/30 [$P < 0.01$] and 5/11 [$P < 0.01$], respectively, versus 6/30 controls) ([Ward et al., 1984](#)).

Groups of 10–20 male B6C3F₁ mice, 4 weeks of age, received a single intraperitoneal injection of 80 mg/kg bw NDEA followed 2 weeks later by exposure to 0 (controls), 3000, 6000 or 12 000 ppm DEHP (Aldrich Chemical, Co. [purity unspecified]) in the diet for 18 months. DEHP increased the incidence of hepatocellular carcinoma (3/10, 10/10 [$P < 0.01$], 18/20 [$P < 0.01$] and 11/20, respectively) ([Ward et al., 1986](#)). [The Working Group noted that the mean survival of the animals treated with 12 000 ppm DEHP was much lower (8.7 months) than that in the 3000-ppm (> 18 months) and 6000-ppm (> 17 months) groups.]

(b) Skin application

Two groups of 25 female SENCAR mice, 7 weeks of age, received a single topical application of 20 µg/animal 7,12-dimethylbenz[*a*]anthracene. Starting 1 week later, one group received twice-weekly applications of 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (2 µg/application) for 2 weeks and then multiple twice-weekly applications of 100 mg DEHP (purity, 99%) for 26 weeks; the other group received TPA only and served as the control group. The animals were killed at experimental week 29. DEHP acted as a second-stage promoter in enhancing skin tumorigenesis (total number of papillomas: 161 versus 55 in controls [$P < 0.01$]; 6.44 papilloma/mouse versus 2.2/control mouse) ([Diwan et al., 1985](#)).

3.5.2 Rat

Groups of 10 female CF-344/CrlBR rats, 6–8 weeks of age, received a single intraperitoneal injection of 150 mg/kg bw NDEA. Two weeks later, they were maintained on a basal diet containing 0 or 1.2% DEHP. Five animals from each group were killed at 3 and 6 months. No liver tumours were observed ([Popp et al., 1985](#)).

Groups of 10 female F344/NCr rats, 5 weeks of age, received a single intraperitoneal injection of 282 mg/kg bw NDEA followed 2 weeks later by exposure to 0 (controls), 3000, 6000 or 12 000 ppm DEHP in the diet for 14 weeks. DEHP did not increase the number or size [details not given] of NDEA-induced hepatocellular proliferative lesions, including hyperplastic foci and hepatocellular tumours ([Ward et al., 1986](#)). [The Working Group noted the limited reporting of the results.]

Two groups of 6–12 male F344 rats were fed 200 ppm 2-acetylaminofluorene in the diet for 7 weeks and were subsequently given 0 (controls) or 12 000 ppm DEHP in the diet for 24 weeks. DEHP did not enhance the occurrence of

2-acetylaminofluorene-induced liver neoplasms ([Williams et al., 1987](#)).

Groups of 20 male F344 rats, 5 weeks of age, were fed 0.05% *N*-ethyl-*N*-hydroxyethylnitrosamine in the diet for 2 weeks and were subsequently maintained on a diet containing 0% (controls) or 1.2% DEHP (Wako Pure Chemical Ind. Ltd [purity unspecified]) for 24 weeks. The incidence (13/20 versus 4/20 in controls) and multiplicity (1.10 ± 1.12 versus 0.20 ± 0.41 in controls) of renal tubule adenoma or carcinoma (combined) were significantly ($P < 0.01$) increased compared with controls ([Kurokawa et al., 1988](#)).

Groups of 15 male F344/DuCrj rats, 6 weeks of age, were given drinking-water containing 0.05% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine for 4 weeks and were then maintained on diets containing 0 (controls), 0.3, 0.6 or 1.2% DEHP. The diets contained also 3.0% uracil for 2 weeks. Surviving animals were killed at the end of experimental week 20. DEHP did not increase the incidence of urinary bladder papilloma ([Hagiwara et al., 1990](#)).

3.5.3 Hamster

Two groups of 25 male and 20–25 female Syrian golden hamsters, 6 weeks of age, were exposed to DEHP vapour in the breathing air at a concentration of 0 (controls) or $15 \pm 5 \mu\text{g}/\text{m}^3$ continuously from 12 weeks of age until natural death. The animals were also given an oral dose of 0.3 mg *N*-nitrosodimethylamine (NDMA) in saline once a week for 30 weeks. DEHP did not increase the incidence of NDMA-induced liver tumours ([Schmezer et al., 1988](#)).

A group of 25 male and 25 female Syrian golden hamsters, 6 weeks of age, received intraperitoneal injections of 3 g/kg bw DEHP once every 4 weeks and were also treated once a week with an oral dose of 1.67 mg/kg bw NDMA. A control group of 25 males and 25 females was treated with NDMA, but no DEHP. The animals

were observed for life or were killed when moribund. DEHP did not increase the incidence of NDMA-induced liver tumours ([Schmezer et al., 1988](#)).

4. Other Relevant Data

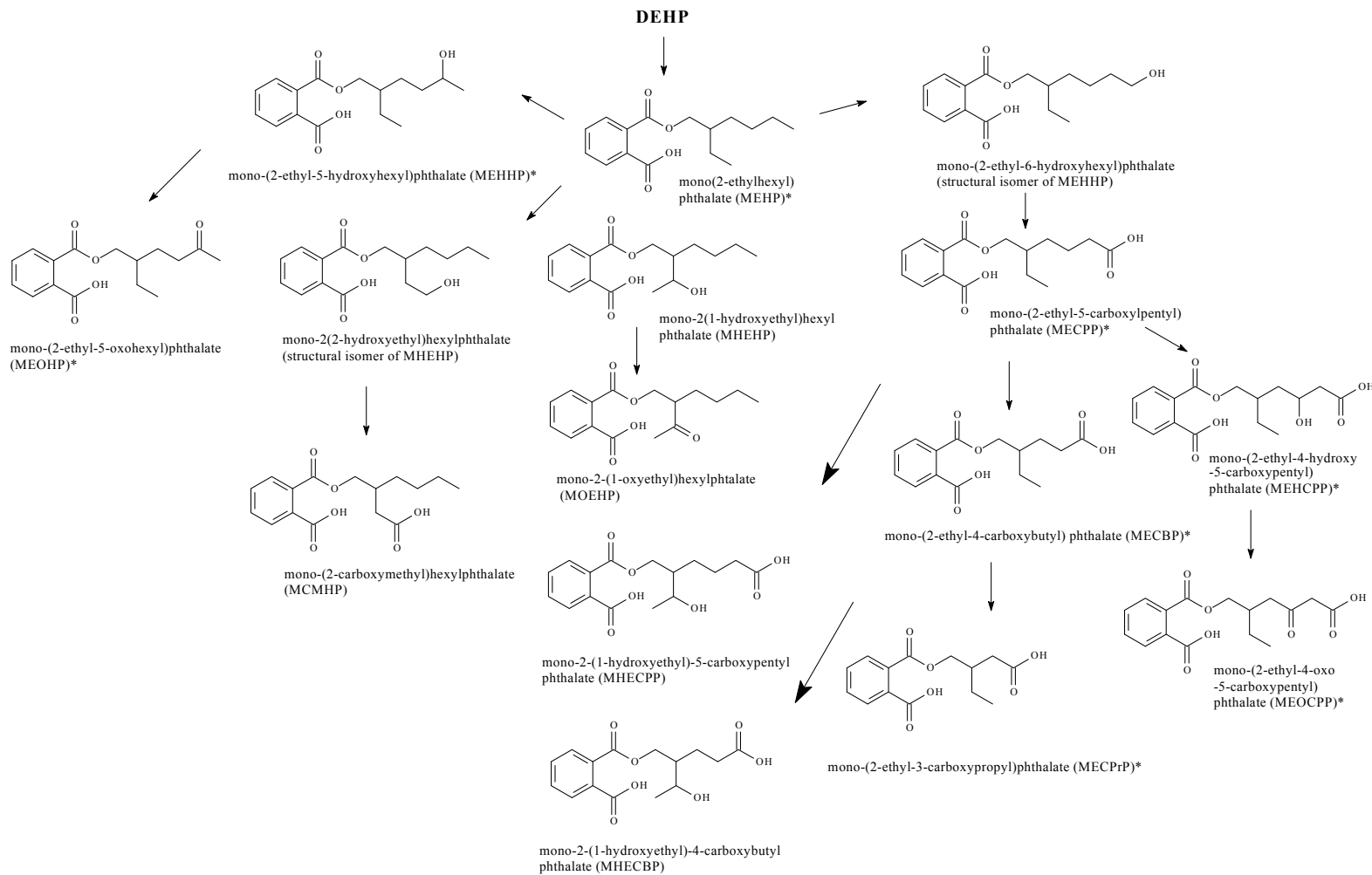
4.1 Absorption, distribution, metabolism and excretion

Human exposure to DEHP can occur via the dermal, inhalation, oral and intravenous routes, and may be high when the compound is released from medical equipment to patients in neonatal intensive care units ([Silva et al., 2006](#)). Since the last review ([IARC, 2000](#)), several studies have been published on absorption, distribution, metabolism and excretion of DEHP in rodents and, more importantly, in humans. The reproductive and developmental toxicity of DEHP has been evaluated twice by an expert panel ([Kavlock et al., 2002, 2006](#)) that provides comments on such studies.

Much attention has been paid to the DEHP metabolite, MEHP. Upon ingestion, DEHP is rapidly metabolized to MEHP by pancreatic lipases in the lumen of the gut in both rodents and humans, before being further converted into oxidative metabolites and glucuronidated for excretion in the urine and faeces. The review by [Silva et al. \(2006\)](#) shows the metabolic schema for humans and rodents (Fig. 4.1).

The approaches for studying the absorption, distribution, metabolism and excretion of DEHP are hampered by its ubiquitous presence in the environment and laboratory equipment, and by its hydrolysis under abiotic conditions to monoesters ([Fromme et al., 2007b](#)). Studies that used radiolabelled DEHP provided the most accurate estimation of its absorption, distribution, metabolism and excretion and metabolites. Two sets of data are most frequently cited and

Fig. 4.1 Suggested mechanisms for di(2-ethylhexyl) phthalate (DEHP) metabolism in humans



(*) Previously identified in rodents
 Reprinted from Toxicology, Vol. no. 219, Silva MJ, Samandar E, Preau JL *et al.*, Urinary oxidative metabolites of di(2-ethylhexyl) phthalate in humans, pp. 22–32. Copyright (2006), with permission from Elsevier.

are most applicable to the characterization of the absorption, distribution, metabolism and excretion of DEHP in humans ([Koch et al., 2004b, 2005b, 2006](#)) and in rodents ([Albro & Thomas, 1973](#); [Albro et al., 1983](#); [Albro, 1986](#); [Albro & Lavenhar, 1989](#)). As discussed below, studies in marmosets have attempted to reflect the human situation and the possible risk of effects of DEHP, but the ability to metabolize and absorb DEHP apparently differs between the two species. Emerging evidence indicates that the effects of DEHP on the metabolism of endogenous and xenobiotic compounds are relevant to its toxicity. The activities of several metabolic enzymes have been shown to be both enhanced and inhibited by exposure to DEHP in experimental animals. As discussed in Section 4.3, DEHP may specifically affect general metabolism through the constitutive androstane receptor (CAR)-2, a nuclear receptor transcription factor. Only one physiologically based pharmacokinetic model is available for predictions on DEHP and MEHP in rats ([Keys et al., 1999](#)). The adequacy of this model is discussed below.

4.1.1 Humans

(a) Absorption, distribution and excretion

Urinary and serum levels of DEHP metabolites were evaluated in a human male volunteer after a single oral dose (48.10 mg; 641 µg/kg bw) of deuterium-labelled DEHP (i.e. the D4-ring-labelled DEHP) to avoid background interference ([Koch et al., 2004b, 2005b](#)). The excretion of two oxidative metabolites, MEHHP and MEOHP, and MEHP was monitored for 44 hours in urine (i.e. 25 samples every 1–4 hours after dosing) and for 8 hours in serum (i.e. five samples before and every 2 hours after dosing). Peak concentrations of the three metabolites were found in serum after 2 hours and of oxidative metabolites in the urine after 4 hours. In serum, all metabolites were unconjugated and the major metabolite was MEHP at a much higher concentration (4.95 mg/L)

than the oxidative metabolites (0.20 mg/L MEHHP and 0.06 mg/L MEOHP). The half-lives for all metabolites in serum were estimated to be shorter than 2 hours. Excretion in the urine followed a multiphase elimination model in which the absorption and distribution phase lasted approximately 4–8 hours. MEHP oxidation products are polar metabolites and consequently showed higher urinary elimination: after 44 hours, 47% of the DEHP dose was excreted in the urine in the form of MEHHP (peak concentration, 10.04 mg/L), MEOHP (6.34 mg/L) and MEHP (3.63 mg/L).

The clearance of DEHP through excretion of its metabolites was studied after three doses of D4-ring-labelled DEHP (low, 4.7 µg/kg bw; medium, 28.7 µg/kg bw; and high, 650 µg/kg bw) were administered to the same volunteer at 1-week intervals ([Koch et al., 2005b](#)). The study identified two new ω-oxidation products, MECPP and mono(2-carboxymethyl)hexyl phthalate (MCMHP). Monitoring was carried out for 44 hours in urine and 8 hours in blood plus in 24-hour urine samples for the medium and low doses. On a molar basis, after 24 hours, 70.5% of the high DEHP dose was excreted in the urine (24.1% as MEHHP, 20.7% as MECPP, 14.6% as MEOHP, 7.3% as MEHP and 3.8% as MCMHP). An additional 3.8% of DEHP was excreted as metabolites on day 2 to give a total of 74.3% DEHP excreted in the urine after 2 days. The elimination half-lives were 5 hours for MEHP, 10 hours for MEHHP and MEOHP, 12–15 hours for MECPP and 24 hours for MCMHP. The proportional metabolite excretion rate relative to DEHP did not vary by dose, and therefore metabolism and excretion were not dose-dependent. Thus, most of the orally administered DEHP was absorbed and then excreted in the urine. Unlabelled metabolites found in the urine of the volunteer in this study indicated that DEHP metabolism was comparable with that found in the general population. [However, the Working Group recognized that higher exposure

levels than the range tested may result in differences in metabolism and excretion as well as other factors related to potentially susceptible subpopulations.]

The above results suggested that the use of secondary DEHP metabolites in urine may give a more accurate estimate of DEHP exposure and dose than MEHP in the blood or urine because of the short half-life of MEHP. However, [Koch et al. \(2005b\)](#) noted that serum MEHP levels in humans were of the same order of magnitude as those in animal studies, despite lower doses in human studies and a dose-normalized area under the curve (AUC) in blood which was 15–100 times higher in the human volunteer than in animals. [Koch et al. \(2005b\)](#) stated that, if it is assumed that MEHP in blood is a surrogate for toxic potential, DEHP would be 15–100 times more toxic in humans than in marmosets or rats.

Previous estimates of human absorption have been reported in two volunteers exposed to non-labelled DEHP ([Schmid & Schlatter, 1985](#)) and extrapolated from studies of absorption in rats ([Rhodes et al., 1986](#)). The data of [Koch et al. \(2004b, 2005b\)](#) showed a much higher fraction of absorption using labelled than non-labelled DEHP. The authors also stressed that, in previous human studies, standard substances were not used to quantify the metabolites and to exclude confounding due to contamination. [Koch et al. \(2004b\)](#) noted that [Peck & Albro \(1982\)](#) found that, 24 hours after intravenous infusion of a platelet concentrate containing DEHP to a cancer patient, almost 60% of the infused dose appeared in the urine as DEHP metabolites.

Several DEHP exposure studies have also noted a greater proportion of oxidative metabolites than MEHP in monitored urine ([Barr et al., 2003](#); [Silva et al., 2006](#); [Weuve et al., 2006](#); [Fromme et al., 2007b](#); [Wittassek et al., 2007a, b](#)). [Silva et al. \(2006\)](#) identified urinary oxidative metabolites of DEHP from individuals with MEHP concentrations about 100 times higher than the median concentrations in the general population of the

USA. Three additional oxidative metabolites were identified: mono(2-ethyl-3-carboxypropyl) phthalate, mono(2-ethyl-4-carboxybutyl) phthalate and mono-2-(1-oxoethyl)hexyl phthalate; the presence of urinary DEHP metabolites containing less than eight carbons in the alkyl chain, that had previously been identified in rodents, were also reported in humans ([Albro et al., 1983](#)), indicating a similar oxidative metabolism of DEHP in humans and rodents. All of these metabolites were identified in human urine based on their chromatographic and mass spectrometric behaviour. Moreover, [Silva et al. \(2006\)](#) noted that all of the above metabolites were identified in three adult volunteers who were not exposed to DEHP via the intravenous route, with MEHHP and MEOHP levels above 500 ng/mL. They concluded that the presence of similar metabolites regardless of dose and route of exposure may indicate a uniform metabolism of DEHP in humans.

Glucuronidation increases urinary clearance of DEHP metabolites, but [Koch et al. \(2004b, 2005b\)](#) noted the absence of glucuronidated MEHP in the blood of the volunteer (results in urine were not reported). [Albro et al. \(1982\)](#) cite urinary data from the two humans given non-radioactive DEHP by [Peck et al. \(1978\)](#) as the basis for species comparisons of the glucuronidation of urinary DEHP metabolites. The levels of free versus conjugated metabolites were reported as 20% versus 80% with no specific information with regard to the form. [Peck & Albro \(1982\)](#) reported the chemical structure of the urinary metabolites in only one of the two patients, that MEHP represented only 11% of the given dose (174.3 mg DEHP), and that approximately 80% of the urinary metabolites were glucuronidated. Therefore, from the available human data, oxidative metabolites and not MEHP appear to be specifically glucuronidated.

In a variability study, MEHP was present in 95% of the samples with secondary metabolites detectable in 100% of the samples ([Fromme et al.,](#)

2007b). The authors reported substantial day-to-day and within-subject variability also after adjustment for creatinine (a reasonable surrogate for bw-adjusted dose). [Hauser et al. \(2004\)](#) found substantial variation in individual day-to-day excretion levels. Such variation in urinary metabolism reflects differences in exposure as well as absorption, distribution, metabolism and excretion between subjects.

[Silva et al. \(2006\)](#) reported that exposure to DEHP in the general population occurs via inhalation rather than dermal absorption and that MEHP may be found in human amniotic fluid ([Silva et al., 2004](#)). Moreover, [Frederiksen et al. \(2007\)](#) cited a body of literature indicating that DEHP is found in human breast milk (unmetabolized or as its primary monoester). [The presence of oxidative metabolites in free (unconjugated) form in breast milk and amniotic fluid may pose additional risks ([Kavlock et al., 2006](#)).]

Exposure studies in humans that measured primary and secondary urinary metabolites suggested age-related differences in their production and/or clearance, with younger children producing higher proportions of MEHHP and MEOHP compared with MEHP ([Kavlock et al., 2006](#); [Wittassek et al., 2007a](#)). Neonates show a further deviation in oxidative DEHP metabolism, with MECPP being the most predominant ([Wittassek & Angerer, 2008](#)). A reduced potential for glucuronidation may lead to slower excretion and higher concentrations of DEHP metabolites in neonates than in older children and adults ([Weuve et al., 2006](#)).

(b) Metabolism

After ingestion, DEHP is rapidly metabolized to MEHP by pancreatic lipases in the lumen of the gut in multiple species ([Albro & Thomas, 1973](#); [Albro & Lavenhar, 1989](#)), and is further converted to oxidative metabolites in both rats and humans ([Silva et al., 2006](#)). The oxidation of MEHP can occur via cytochrome P450 (CYP) 4A, alcohol dehydrogenase (ADH) and aldehyde

dehydrogenase (ALDH), and yields multiple oxidation products of 2-ethylhexanol (2-EH) and of the aliphatic side chain of MEHP ([Fay et al., 1999](#)). Both ω -1 and ω -2 oxidation occur in humans; however, the forms of CYP or other enzymes that are responsible for this oxidative metabolic activity are not known.

[Koch et al. \(2005b\)](#) reported rapid degradation of all DEHP metabolites in blood at room temperature. [Kato et al. \(2004\)](#) reported that human serum lipase activity could almost totally convert DEHP to MEHP. Lipases are also present in breast milk ([Kavlock et al., 2006](#)). [Weuve et al. \(2006\)](#) noted that neonates have elevated gastric lipase activity ([Hamosh, 1990](#)), which may enhance their ability to convert DEHP to MEHP as well as to increase their digestion of milk fats. Because the concentrations of excreted substances are dependent on water intake, toxicokinetics are easier to interpret from measurements in blood than from those in urine. However, measurements of phthalate monoesters are susceptible to contamination in blood from the parent phthalates, which are also hydrolysed to their respective monoesters by serum enzymes ([Kato et al., 2004](#)).

(c) Toxicokinetic models

No pharmacologically based pharmacokinetic model is available in humans to predict the absorption, distribution, metabolism and excretion of DEHP or its metabolites from a given exposure concentration. [Wittassek et al. \(2007a, b\)](#) have extrapolated exposure concentrations from data on urinary excretion in children and adults ([Koch et al., 2005b](#)). The authors did not take into account differences in lipase activity and absorption, distribution, metabolism and excretion between age groups. [The Working Group did not present the results of this extrapolation because the products are estimates of exposure concentration and not outputs of absorption, distribution, metabolism and excretion.]

4.1.2 Experimental systems

(a) Absorption, distribution and excretion

Relatively little information is available regarding the extent to which DEHP is metabolized before or during absorption from the gastrointestinal tract and the available data are limited to rodents. In general, a high level of DEHP hydrolase activity has been found in pancreatic juice, intestinal contents and/or intestinal tissue of many mammalian species ([Albro & Lavenhar, 1989](#)). Most if not all DEHP that reaches the intestines is probably absorbed as hydrolysis products rather than intact diester.

Studies of labelled DEHP in several species have reported that the majority of an oral dose is absorbed and excreted, and that the levels of absorption are different among the species ([Albro *et al.*, 1982](#); [Albro, 1986](#); [Rhodes *et al.*, 1986](#); [Albro & Lavenhar, 1989](#); [Kessler *et al.*, 2004](#)). [Ikeda *et al.* \(1980\)](#) fed 50 mg/kg bw DEHP to rats, dogs and miniature pigs for 21–28 days and then gave ¹⁴C-labelled DEHP on the last day by gavage. By 24 hours, rats were reported to have excreted 84% of the label (urine and faeces), whereas dogs excreted 67% and pigs excreted 37%. [The Working Group noted that the small sample size of these studies limited interpretations regarding interindividual variability and reduced the confidence in comparisons between species.]

[Calafat *et al.* \(2006\)](#) gave unlabelled DEHP to pregnant Sprague-Dawley rats at different doses (11, 33, 100 or 300 mg/kg bw by gavage) and reported urinary concentrations of 1.62, 3.19, 8.11 and 15.9 µg/mL MEHP, respectively. They also provided creatinine-adjusted values.

[Koo & Lee \(2007\)](#) administered a single dose of [¹⁴C]DEHP to assess serum and urinary excretion of DEHP and MEHP in 4-week-old Sprague-Dawley rats. Total radioactivity peaked at 24 hours in the urine and after 24 hours in the serum. Urinary DEHP and MEHP levels peaked at 24 hours only for the highest dose tested. [Results

in the text of the report for urinary concentrations of MEHP at 24 hours differed from those displayed graphically.] Serum concentrations at 24 hours appeared to be proportional to the dose for MEHP (6.22, 36.3 and 169.19 µg/mL following doses of 40, 200 and 1000 mg/kg bw, respectively) but not for DEHP (1.78, 2.38 and 5.3 µg/mL following the same doses, respectively). The peak concentration, the time to reach peak concentration and the AUC were greater for MEHP than for DEHP in both urine and serum but elimination half-lives of DEHP were greater than those of MEHP. [Kessler *et al.* \(2004\)](#) conducted a similar study in Sprague-Dawley rats treated orally with 30–500 mg per day unlabelled DEHP. Normalized AUCs of DEHP were at least one order of magnitude smaller than those of MEHP. Results from the two studies differed in terms of the time at which peak concentrations were achieved in blood or serum samples. Quantitatively, the magnitude of peak concentration achieved was comparable at the highest dose (1000 mg/kg). [Inconsistencies exist between the concentrations of DEHP reported in the blood by [Koo & Lee \(2007\)](#) and [Kessler *et al.* \(2004\)](#).]

In contrast to earlier reports ([Albro *et al.*, 1982](#)), [Calafat *et al.* \(2006\)](#) administered DEHP (0, 11, 33, 100 or 300 mg/kg bw per day) to pregnant Sprague-Dawley rats and reported that MEHP was mostly glucuronidated (87%) in the urine whereas free MEHP predominated in the amniotic fluid (88.2%). They suggested that maternal urinary MEHP levels may be a useful surrogate marker for fetal exposure to DEHP because these values were inter-correlated. It was noted that although glucuronidation increases the hydrophilic properties of the phthalate metabolites, thus enhancing their urinary excretion, it is not known whether their conjugated species are biologically inactive.

Studies of the distribution of labelled DEHP in the tissues of experimental animals have been reported, but in most cases only total radioactivity was monitored with no distinction between

DEHP and its metabolites ([Lindgren et al., 1982](#); [Gaunt & Butterworth, 1982](#); [Rhodes et al., 1986](#); [Isenberg et al., 2000](#); [Ono et al., 2004](#)). Therefore, the tissue distribution of parent DEHP following exposure cannot be ascertained by any route. The liver appears to acquire an initially higher load of DEHP and/or its metabolites ([Albro & Lavenhar 1989](#); [Pugh et al., 2000](#); [Ito et al., 2007a](#)) with second highest specific activity in adipose tissue ([Williams & Blanchfield, 1974](#); [Ikeda et al., 1980](#)). [Ikeda et al. \(1980\)](#) reported radioactivity in the bile of DEHP-exposed dogs and pigs for several days suggesting the possibility of enterohepatic recirculation. Studies of unlabelled DEHP showed no consistent rise in different tissues in animals ([Ljungvall et al., 2004](#); [Rhind et al., 2009](#)).

(b) Metabolism

[Albro \(1986\)](#) reported that pancreatic tissue has the greatest ability to hydrolyse DEHP in CD rats through competition of non-specific lipases that require a phthalate monoester as a substrate. Pancreatic lipase activity per gram of tissue was 400-fold that in intestinal mucosa and 240-fold that in the liver. The only esterase reported to metabolize MEHP was located in liver microsomes and was assumed to be responsible for the formation of urinary phthalic acid found in rats and mice. Liver and kidney oxidation of MEHP appeared to occur via microsomal monooxygenases analogous (or identical) to the CYP-associated fatty acid ω - and (ω -1) hydroxylases ([Albro & Lavenhar, 1989](#)).

[Lake et al. \(1977\)](#) evaluated the rates of intestinal hydrolysis of phthalates, including DEHP, among species and reported them to be: ferrets > rats > humans; baboons have a rate threefold higher than that of ferrets.

[Ito et al. \(2005\)](#) measured the activity of four DEHP-metabolizing enzymes (lipase, uridine 5'-diphospho-glucuronosyl transferase (UGT), ADH and ALDH) in several organs (liver, lungs, kidneys and small intestine) of CD-1 mice, rats

and marmosets. The authors reported that lipase activity was highest in the small intestines of mice, UGT activity was highest in mice, and ADH and ALDH activities were 1.6–3.9 times greater in the livers of marmosets than in those of rats or mice. [Interpretation of the study is limited by the lack of measurement of pancreatic lipase.]

Studies of the effects of age on metabolism are limited and results are contradictory ([Gollamudi et al., 1983](#); [Sjöberg et al., 1985c](#)).

(c) Toxicokinetic models

Currently, only one physiologically based pharmacokinetic model is available for DEHP and MEHP in rats ([Keys et al., 1999](#)). [The adequacy of this model is limited by the data available to the authors at the time it was published and by the difficulties in obtaining important information with which to construct and test the model. However, it also has methodological issues regarding its construction and validation.] Tissue:blood partition coefficients for DEHP were estimated from the *n*-octanol:water partition coefficient, while partition coefficients for MEHP were determined experimentally using a vial equilibration technique. All other parameters were either found in the literature or were estimated. The flow-limited model failed to simulate the available data adequately. Alternative plausible models were explored, including diffusion-limited membrane transport, enterohepatic circulation and MEHP ionization (pH-trapping model), which significantly improved predictions of DEHP and MEHP blood concentrations with respect to the flow-limited model predictions. In the pH-trapping model, non-ionized MEHP is assumed to pass into intracellular compartments where it is mostly ionized and trapped intracellularly until it is de-ionized and released. [No references were given by the authors and there are no known references in the literature stating that MEHP is ionized. The authors appear to base their assumption on structure–activity relationships. It is not readily apparent why MEHP is

assumed to be ionized but DEHP is not (because they do not differ greatly by molecular weight).]

4.2 Genetic and related effects

An examination of the current literature since the publication of the previous *Monograph* ([IARC, 2000](#)) showed that, although in most bacterial systems DEHP gives negative results, a robust response is shown in cell transformation and DNA damage assays.

The information from the original Table 7 of the previous *Monograph* ([IARC, 2000](#)) is provided for reference in [Table 4.1](#) (i.e. genetic and related effects of DEHP, MEHP and other DEHP metabolites), which contains some additional information, as well as some corrections made to the originally reported results and new studies. The genotoxicity of DEHP oxidative metabolites other than MEHP has not been adequately characterized experimentally and no new studies are available.

4.2.1 Humans

(a) DNA adducts

No specific studies were identified regarding DNA adduct formation following exposure to DEHP or its metabolites.

(b) DNA strand breaks

Exposure to DEHP, as detected by its metabolite MEHP, has been associated with increased DNA damage in humans. DEHP is suspected to contribute to the increasing incidence of testicular dysgenesis syndrome. [Hauser et al. \(2007\)](#) assessed human sperm DNA damage following environmental exposure to DEHP using the neutral Comet assay. The urinary levels of phthalate metabolites were similar to those reported among the general population of the USA: the 50th and 95th percentiles were 7.7 ng/mL and 112 ng/mL for MEHP, and 48.5 ng/mL and

601 ng/mL for MEHHP. MEHP concentration was positively associated with a 12% increase in tail DNA relative to the study population median, and with sperm DNA damage after adjusting for MEHP oxidative metabolites. The authors suggested that the negative association of Comet results with the concentrations of oxidative metabolites may serve as a phenotypic marker for DEHP metabolism, indicating lower toxicity if a person is able to metabolize DEHP/MEHP oxidatively to a greater extent. An earlier report ([Duty et al., 2003](#)) with fewer samples and no adjustment for oxidative metabolites did not find a relationship between MEHP and sperm DNA strand breaks.

DEHP has been studied *in vitro* in various human tissues using Comet assays. [Anderson et al. \(1999a\)](#) reported dose-related increased median tail moments (alkaline Comet assay) in both leukocytes at non-toxic DEHP concentrations and in blood lymphocytes. The addition of metabolic activation abolished the effect in the positive leukocyte experiment. The length of exposure was not given. [Biscardi et al. \(2003\)](#) found increased Comet length in human leukocytes exposed for 1 hour to organic extracts of water lyophilisates stored in polyethylene terephthalate bottles. The study was successful in detecting the presence of DEHP, although concentrations were not reported. [Choi et al. \(2010\)](#) reported increased Olive tail moment 24 and 48 hours after exposure to DEHP (0.97 µg/mL) in human hepatocyte HepG2 cells without cytotoxicity. Whether the assay was alkaline or neutral was not clear. [Erkekoğlu et al. \(2010a\)](#) reported increased tail intensity and moment in LNCaP prostate adenocarcinoma cells (24-hour exposure, alkaline Comet assay). The experiments were conducted at half maximal inhibitory concentrations (IC_{50}) that induced cell cytotoxicity (1170 µg/mL). [Park & Choi \(2007\)](#) reported increased tail moment in HeLa cervical epithelial carcinoma cells (24-hour exposure). There was a dose-related increase in tail moments from

Table 4.1 Genetic and related effects of di(2-ethylhexyl) phthalate (DEHP), mono(2-ethylhexyl) phthalate (MEHP) and other DEHP metabolites

Test system	Result ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Bacillus subtilis</i> rec, differential toxicity	–	NT	500 µg/disc	Tomita et al. (1982a)
<i>Salmonella typhimurium</i> , reverse mutation	NT	+	5 mg/plate	Tomita et al. (1982a)
<i>Salmonella typhimurium</i> , forward mutation	–	–	500	Liber (1985)
<i>Salmonella typhimurium</i> TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	9860 µg/plate	Kirby et al. (1983)
<i>Salmonella typhimurium</i> TA100, TA98, reverse mutation	–	–	4000 µg/plate	Robertson et al. (1983)
<i>Salmonella typhimurium</i> TA100, TA98, reverse mutation	–	–	2000 µg/plate	Yoshikawa et al. (1983)
<i>Salmonella typhimurium</i> TA100, TA102, TA98, TA97, reverse mutation	–	–	10 000 µg/plate	Baker & Bonin (1985)
<i>Salmonella typhimurium</i> TA100, TA102, TA98, TA97, reverse mutation	–	–	5000 µg/plate	Matsushima et al. (1985)
<i>Salmonella typhimurium</i> TA100, TA102, TA98, TA97, reverse mutation	–	–	10 000 µg/plate	Nohmi et al. (1985)
<i>Salmonella typhimurium</i> TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	5000 µg/plate	Rexroat & Probst (1985)
<i>Salmonella typhimurium</i> TA100, TA1535, TA98, TA97, reverse mutation	–	–	10 000 µg/plate	Zeiger & Haworth (1985)
<i>Salmonella typhimurium</i> TA100, TA1535, TA1537, TA98, reverse mutation	–	–	10 000 µg/plate	Zeiger et al. (1985)
<i>Salmonella typhimurium</i> TA1537, TA98, TA7001, TA7002, TA7003, TA7004, TA7005, TA7006, reverse mutation	–	–	1000 µg/plate	Gee et al. (1998)
<i>Salmonella typhimurium</i> TA1535/pSK 1002, <i>umu</i> C gene expression system with commercial rat liver metabolic system (<i>n</i> = 3)	–	+	780	Okai & Higashi-Okai (2000)
<i>Salmonella typhimurium</i> TA1535/pSK 1002, <i>umu</i> C gene expression system with fresh Sprague-Dawley rat liver metabolic system (<i>n</i> = 3)	NT	+	780	Okai & Higashi-Okai (2000)
<i>Salmonella typhimurium</i> TA1535/pSK 1002, <i>umu</i> C gene expression system with fresh Sprague-Dawley rat pancreas metabolic system (<i>n</i> = 3)	NT	+	780	Okai & Higashi-Okai (2000)
<i>Salmonella typhimurium</i> TA1535/pSK 1002, <i>umu</i> C gene expression system with fresh Sprague-Dawley rat intestine metabolic system (<i>n</i> = 3)	NT	+	780	Okai & Higashi-Okai (2000)
<i>Salmonella typhimurium</i> TA1535/pSK 1002, <i>umu</i> C gene expression system with fresh Sprague-Dawley rat kidney metabolic system (<i>n</i> = 3)	NT	–	780	Okai & Higashi-Okai (2000)
<i>Salmonella typhimurium</i> TA1535/pSK 1002, <i>umu</i> C gene expression system with fresh Sprague-Dawley rat lung metabolic system (<i>n</i> = 3)	NT	–	780	Okai & Higashi-Okai (2000)
<i>Salmonella typhimurium</i> TA1535/pSK 1002, <i>umu</i> C gene expression system with hog pancreatic lipase (<i>n</i> = 3) + 1 mM cholic acid	NT	+	780	Okai & Higashi-Okai (2000)

Table 4.1 (continued)

Test system	Result ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Salmonella typhimurium</i> TA1535/pSK 1002, <i>umu</i> C gene expression system with hog pancreatic lipase (<i>n</i> = 3) + 5 mM cholic acid	NT	+	780	Okai & Higashi-Okai (2000)
<i>Salmonella typhimurium</i> TA1535/pSK 1002, <i>umu</i> C gene expression system with hog pancreatic lipase (<i>n</i> = 3) + 1 mM deoxycholic acid	NT	+	780	Okai & Higashi-Okai (2000)
<i>Salmonella typhimurium</i> TA1535/pSK 1002, <i>umu</i> C gene expression system with hog pancreatic lipase (<i>n</i> = 3) + 1 mM deoxycholic acid	NT	+	780	Okai & Higashi-Okai (2000)
<i>Escherichia coli</i> WP2 <i>uvrA</i> , reverse mutation	–	–	2000	Yoshikawa et al. (1983)
<i>Saccharomyces cerevisiae</i> , gene conversion	(+)	(+)	5000	Arni (1985)
<i>Saccharomyces cerevisiae</i> , gene conversion	–	–	2000	Brooks et al. (1985)
<i>Saccharomyces cerevisiae</i> , gene conversion	–	–	1000	Inge-Vechtomov et al. (1985)
<i>Saccharomyces cerevisiae</i> D7–144, gene conversion ^c	+	NT	3100	Mehta & von Borstel (1985)
<i>Saccharomyces cerevisiae</i> D7–144, gene conversion ^c	NT	+	1500	Mehta & von Borstel (1985)
<i>Saccharomyces cerevisiae</i> XV185- ¹⁴ C, point mutation ^c	+	NT	12 300	Mehta & von Borstel (1985)
<i>Saccharomyces cerevisiae</i> XV185- ¹⁴ C, point mutation ^c	NT	+	3100	Mehta & von Borstel (1985)
<i>Saccharomyces cerevisiae</i> RM52, point mutation ^c	+	–	12 300	Mehta & von Borstel (1985)
<i>Saccharomyces cerevisiae</i> , gene conversion	–	–	5000	Parry & Eckardt (1985)
<i>Saccharomyces cerevisiae</i> D6, aneuploidy	+	+	5000	Parry & Eckardt (1985)
<i>Saccharomyces cerevisiae</i> D7, mitotic segregation	–	+	5000	Parry & Eckardt (1985)
<i>Saccharomyces cerevisiae</i> , homozygosis	–	–	5000	Arni (1985)
<i>Saccharomyces cerevisiae</i> , homozygosis	–	–	1000	Inge-Vechtomov et al. (1985)
<i>Saccharomyces cerevisiae</i> , reverse mutation	–	–	5000	Arni (1985)
<i>Saccharomyces cerevisiae</i> , reverse mutation	–	–	1000	Inge-Vechtomov et al. (1985)
<i>Saccharomyces cerevisiae</i> , reverse mutation	–	–	5000	Parry & Eckardt (1985)
<i>Saccharomyces cerevisiae</i> , forward mutation	–	–	1000	Inge-Vechtomov et al. (1985)
<i>Saccharomyces pombe</i> , forward mutation	?	–	5900	Loprieno et al. (1985)
<i>Saccharomyces cerevisiae</i> DEL assay and ICR recombination	–	–	200 000	Carls & Schiestl (1994)
<i>Aspergillus nidulans</i> , haploid mutation, non-disjunction and mitotic crossing-over	–	NT	9900	Carere et al. (1985)
<i>Allium cepa</i> (rooting onion), total chromosomal aberration, 96 h immersion (<i>n</i> = 5500 cells) <i>in vivo</i>	–		50	Rank et al. (2002)
<i>Allium cepa</i> (rooting onion), total chromosomal aberration, 96 h immersion (<i>n</i> = 5500 cells) <i>in vivo</i>	+		5	Rank et al. (2002)

Table 4.1 (continued)

Test system	Result ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Tradescantia</i> pollen cells, micronucleus formation, plant mutagenicity test (Trad/MCN test)	+		? ^d	Biscardi et al. (2003)
<i>Drosophila melanogaster</i> , crossing-over/recombination	–		39 000 µg/g food	Würgler et al. (1985)
<i>Drosophila melanogaster</i> , somatic mutation	(+)		6930 µg/cm ² [53300 µg/mL]	Fujikawa et al. (1985)
<i>Drosophila melanogaster</i> , somatic mutation	(+)		780 µg/g food	Vogel (1985)
<i>Drosophila melanogaster</i> , somatic mutation	–		39 000 µg/g food	Würgler et al. (1985)
<i>Drosophila melanogaster</i> , sex-linked recessive lethal mutation	–		20 inj.	Yoon et al. (1985)
<i>Drosophila melanogaster</i> , sex-linked recessive lethal mutation	–		18 600 µg/g food	Zimmering et al. (1989)
<i>Drosophila melanogaster</i> , DNA double strand breakage <i>in vivo</i>	–		7540 µg/g food	Kawai (1998)
<i>Drosophila melanogaster</i> , DNA repair test <i>in vivo</i>	–		7540 µg/g food	Kawai (1998)
<i>Drosophila melanogaster</i> , wing spot test, mutation <i>in vivo</i>	–		7540 µg/g food	Kawai (1998)
<i>Chironomus riparius</i> larvae, mouthpart deformities, <i>in vivo</i> (10 d) (<i>n</i> = 13/dish × 3)	+		1	Park & Kwak (2008)
DNA single-strand breaks, rat hepatocytes <i>in vitro</i>	–	NT	3900	Bradley (1985)
DNA strand breaks, Chinese hamster ovary cells <i>in vitro</i>	–	–	39 000	Douglas et al. (1985)
DNA single-strand breaks, rat or Syrian hamster hepatocytes <i>in vitro</i>	–	NT	9750	Schmezer et al. (1988)
DNA single- and double-strand breaks ^c , Comet assay (alkaline) tail intensity, MA-10 mouse Leydig tumour cell line (24 h) <i>in vitro</i>	+	NT	1170	Erkekoğlu et al. (2010a)
DNA single- and double-strand breaks ^c , Comet assay (alkaline) tail moment, MA-10 mouse Leydig tumour cell line (24 h) <i>in vitro</i>	+	NT	1170	Erkekoğlu et al. (2010a)
Cell viability (24 h), MA-10 mouse Leydig tumour cell line <i>in vitro</i>	+	NT	3.9	Erkekoğlu et al. (2010a)
Reactive oxygen species production (24 h), MA-10 mouse Leydig tumour cell line <i>in vitro</i>	+	NT	1170	Erkekoğlu et al. (2010a)
Unscheduled DNA synthesis, rat primary hepatocytes <i>in vitro</i>	–	NT	3900	Butterworth et al. (1984); Kornbrust et al. (1984); Probst & Hill (1985)
Unscheduled DNA synthesis, rat primary hepatocytes <i>in vitro</i>	–	NT	10 000	Williams et al. (1985)
Unscheduled DNA synthesis, rat primary hepatocytes <i>in vitro</i>	–	NT	1000	Astill et al. (1986)
Unscheduled DNA synthesis, B6C3F ₁ mouse primary hepatocytes <i>in vitro</i>	–	NT	390	Smith-Oliver & Butterworth (1987)
Gene mutation, mouse lymphoma L5178Y cells, <i>Tk</i> ^{+/-} locus <i>in vitro</i>	(+)	–	980	Kirby et al. (1983)
Gene mutation, mouse lymphoma L5178Y cells, <i>Tk</i> ^{+/-} locus <i>in vitro</i>	?	–	2500	Amacher & Turner (1985)

Table 4.1 (continued)

Test system	Result ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Gene mutation, mouse lymphoma L5178Y cells, <i>Tk</i> ^{+/-} locus <i>in vitro</i>	-	-	4900	Myhr et al. (1985)
Gene mutation, mouse lymphoma L5178Y cells, <i>Tk</i> ^{+/-} locus <i>in vitro</i>	(+)	(+)	7.5	Oberly et al. (1985)
Gene mutation, mouse lymphoma L5178Y cells, <i>Tk</i> ^{+/-} locus <i>in vitro</i>	-	-	9800	Styles et al. (1985)
Gene mutation, mouse lymphoma L5178Y cells, <i>Tk</i> ^{+/-} locus <i>in vitro</i>	-	-	250	Astill et al. (1986)
Gene mutation, mouse lymphoma L5178Y cells, ouabain resistance <i>in vitro</i>	-	-	200	Garner & Campbell (1985)
Gene mutation, mouse lymphoma L5178Y cells, ouabain resistance <i>in vitro</i>	-	-	9800	Styles et al. (1985)
Gene mutation, BALB/c-3T3 mouse cells, ouabain resistance <i>in vitro</i>	NT	-	1960	Matthews et al. (1985)
Sister chromatid exchange, Chinese hamster Don cells <i>in vitro</i>	-	NT	3900	Abe & Sasaki (1977)
Sister chromatid exchange, Chinese hamster V79 cells <i>in vitro</i>	+	NT	25	Tomita et al. (1982a)
Sister chromatid exchange, Chinese hamster ovary cells <i>in vitro</i>	-	-	3900	Douglas et al. (1985)
Sister chromatid exchange, Chinese hamster ovary cells <i>in vitro</i>	(+)	-	5000	Gulati et al. (1985)
Sister chromatid exchange, rat liver RL4 cells <i>in vitro</i>	-	NT	1000	Priston & Dean (1985)
Micronucleus formation, Chinese hamster ovary cells <i>in vitro</i>	-	-	3900	Douglas et al. (1985)
Micronucleus formation, rat hepatocytes <i>in vitro</i>	-	NT	3900	Müller-Tegethoff et al. (1995)
Micronucleus formation, Syrian hamster embryo cells <i>in vitro</i>	+	NT	NR	Fritzenschaf et al. (1993)
Chromosomal aberrations, Chinese hamster Don cells <i>in vitro</i>	-	NT	3900	Abe & Sasaki (1977)
Chromosomal aberrations, Chinese hamster lung cells <i>in vitro</i>	-	NT	160	Ishidate & Odashima (1977)
Chromosomal aberrations, Chinese hamster ovary cells <i>in vitro</i>	-	NT	781	Phillips et al. (1982)
Chromosomal aberrations, Chinese hamster liver cells <i>in vitro</i>	-	NT	50	Danford (1985)
Chromosomal aberrations, Chinese hamster ovary cells <i>in vitro</i>	-	-	5000	Gulati et al. (1985)
Chromosomal aberrations, Chinese hamster lung cells <i>in vitro</i>	-	-	4130	Ishidate & Sofuni (1985)
Chromosomal aberrations, rat liver RL4 cells <i>in vitro</i>	-	NT	1000	Priston & Dean (1985)
Chromosomal aberrations, Syrian hamster embryo cells <i>in vitro</i> ^f	-	NT	30 µM [12] 24-h	Tsutsui et al. (1993)
Chromosomal aberrations, Syrian hamster embryo cells <i>in vitro</i> ^f	NT	+	1 µM [0.4] 2-h	Tsutsui et al. (1993)
Aneuploidy, Chinese hamster liver cells <i>in vitro</i>	(+)	NT	50	Danford (1985)
Mitotic aberrations, Chinese hamster primary liver cells <i>in vitro</i>	(+)	NT	50	Parry (1985)
Aneuploidy, rat liver RL4 cells <i>in vitro</i>	-	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, C3H 10T½ mouse cells	-	NT	3.9	Sanchez et al. (1987)

Table 4.1 (continued)

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	+	NT	1 ^g	Barrett & Lamb (1985)
Cell transformation, Syrian hamster embryo cells, clonal assay ^h	+	NT	100	Sanner & Rivedal (1985)
Cell transformation, Syrian hamster embryo cells, clonal assay ^h	+	NT	4	Sanner & Rivedal (1985)
Cell transformation, Syrian hamster embryo cells, clonal assay ^h	+	NT	0.8	Sanner & Rivedal (1985)
Cell transformation, SA7/Syrian hamster embryo cells ⁱ	+	NT	78	Hatch & Anderson (1985)
Cell transformation, SA7/Syrian hamster embryo cells ⁱ	+	NT	507	Hatch & Anderson (1985)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	4 µM [1.5 µg/mL]	Mikalsen <i>et al.</i> (1990)
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	30 µM [12 µg/mL]	Mikalsen & Sanner (1993)
Cell transformation, Syrian hamster embryo cells, clonal assay ^f	+	NT	3 µM [1.2] 48-h	Tsutsui <i>et al.</i> (1993)
Cell transformation, Syrian hamster embryo cells, clonal assay ^f	NT	+	30 µM [12] 2-h	Tsutsui <i>et al.</i> (1993)
Cell transformation, Syrian hamster embryo cells, clonal assay ^f	NT	+	3 µM [1.2] 3-h	Tsutsui <i>et al.</i> (1993)
Cell transformation, Syrian hamster embryo cells, clonal assay (traditional X-radiated feeder layer ^j)	+	NT	5	Pant <i>et al.</i> (2010)
Cell transformation, Syrian hamster embryo cells, clonal assay (conditioned media)	+	NT	2.5	Pant <i>et al.</i> (2010)
Cell transformation, RLV/Fischer rat embryo cells, anchorage independent cell growth ^k	?	NT	NR	Suk & Humphreys (1985)
Cell transformation, RLV/Fischer rat embryo cells, anchorage independent cell growth ^k	+	NT	2000	Suk & Humphreys (1985)
Ornithine decarboxylase superinduction, Syrian hamster embryo cells ^l	-	NT	39	Dhalluin <i>et al.</i> (1998)
DNA strand breaks, Comet assay (alkaline), human leukocytes <i>in vitro</i> (median tail moment)	-, +	NT	390, 3900 ^m	Anderson <i>et al.</i> (1999a)
DNA strand breaks, Comet assay (alkaline), human leukocytes <i>in vitro</i> (median tail moment)	+	-	31 and 156 ^m	Anderson <i>et al.</i> (1999a)
DNA strand breaks, Comet assay (alkaline), human lymphocytes <i>in vitro</i> (median tail moment)	+	-	3.9 and 195 ⁿ	Anderson <i>et al.</i> (1999a)
DNA single and double-strand breaks ^d , Comet assay, human leukocytes <i>in vitro</i> (total comet length; 1 h)	+	NT	NR	Biscardi <i>et al.</i> (2003)
DNA single and double-strand breaks ^d , Comet assay, human leukocytes <i>in vitro</i> (number of cells > 96th distribution; 1 h)	+	NT	NR	Biscardi <i>et al.</i> (2003)
DNA single and double-strand breaks, Comet assay, human hepatocyte HepG2 cell line <i>in vitro</i> (Olive tail moment)	+	NT	0.97 24-h	Choi <i>et al.</i> (2010)

Table 4.1 (continued)

Test system	Result ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA single and double-strand breaks, Comet assay, human hepatocyte HepG2 cell line <i>in vitro</i> (Olive tail moment)	+	NT	0.97 48-h	Choi <i>et al.</i> (2010)
DNA single and double-strand breaks, Comet assay, LNCaP human prostate adenocarcinoma cell line <i>in vitro</i> (tail intensity) ^e	+	NT	1170 24-h	Erkekoğlu <i>et al.</i> (2010b)
DNA single and double-strand breaks, Comet assay, LNCaP human prostate adenocarcinoma cell line <i>in vitro</i> (tail moment) ^e	+	NT	1170 24-h	Erkekoğlu <i>et al.</i> (2010b)
DNA single and double-strand breaks, Comet assay (alkaline), human HeLa cells <i>in vitro</i> (tail moment; <i>n</i> = 3) ^o	+	NT	38 24-h	Park & Choi (2007)
Unscheduled DNA synthesis, human hepatocytes <i>in vitro</i>	-	NT	3900	Butterworth <i>et al.</i> (1984)
Gene mutation, human lymphocytes, <i>TK</i> ^{+/-} and <i>HPRT</i> loci <i>in vitro</i>	-	-	1000	Crespi <i>et al.</i> (1985)
Sister chromatid exchange, human lymphocytes <i>in vitro</i>	-	-	1000	Obe <i>et al.</i> (1985)
Sister chromatid exchange, human lymphocytes (co-culture with rat liver cells) <i>in vitro</i>	-	(+)	39	Lindahl-Kiessling <i>et al.</i> (1989)
Chromosomal aberrations, human lymphocytes <i>in vitro</i>	-	NT	75	Turner <i>et al.</i> (1974)
Chromosomal aberrations, human lymphocytes <i>in vitro</i>	-	NT	60	Stenchever <i>et al.</i> (1976)
Chromosomal aberrations, human lymphocytes <i>in vitro</i>	-	NT	160	Tsuchiya & Hattori (1976)
Aneuploidy, human fetal lung cells <i>in vitro</i>	-	NT	6	Stenchever <i>et al.</i> (1976)
DNA strand breaks, Wistar rat liver <i>in vivo</i>	-		2000 po × 28	Elliott & Elcombe (1987)
DNA oxidative damage, F344 rat liver <i>in vivo</i>	+ ^P		12 000 mg/kg diet, 1 yr	Takagi <i>et al.</i> (1990a)
DNA oxidative damage, F344 rat liver <i>in vivo</i>	+ ^P		12 000 mg/kg diet, 1-2 wk	Takagi <i>et al.</i> (1990b)
DNA single-strand breaks, F344 rat liver <i>in vivo</i>	-		20 000 mg/kg diet, 78 wk	Tamura <i>et al.</i> (1991)
DNA oxidative damage, F344 rat liver <i>in vivo</i>	-		12 000 mg/kg diet, 22 wk	Cattley & Glover (1993)
DNA oxidative damage, 8-OHdG in the liver, male Sprague-Dawley rats <i>in vivo</i>	+		1000 po × 14 d	Seo <i>et al.</i> (2004)
DNA single-strand breaks (quantification of hydroxy DNA ends) in liver, male F344 rats <i>in vivo</i>	-		1.2% diet [12 000 mg/ kg] × 5 mo	Pogribny <i>et al.</i> (2008)
Unscheduled DNA synthesis, F344 rat hepatocytes <i>in vivo</i>	-		500 po, 150 po × 14, or 12 000 mg/kg diet, 30 d + 500 po	Butterworth <i>et al.</i> (1984)

Table 4.1 (continued)

Test system	Result ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Unscheduled DNA synthesis, Sprague-Dawley rat hepatocytes <i>in vivo</i>	–		5000 po	Kornbrust et al. (1984)
Unscheduled DNA synthesis, F344 rat hepatocytes <i>in vivo</i>	–		12 000 mg/kg diet, 28 d	Cattley et al. (1988)
Unscheduled DNA synthesis, B6C3F ₁ mouse hepatocytes <i>in vivo</i>	–		6000 mg/kg diet, 28 d	Smith-Oliver & Butterworth (1987)
Gene mutation, <i>lacI</i> transgenic C57BL/6 mouse liver <i>in vivo</i>	–		6000 mg/kg diet, 120 d	Gunz et al. (1993)
Gene mutation, <i>lacZ</i> gene, <i>lacZ</i> transgenic mouse liver, <i>in vivo</i> (2 wk)	+, +		2333 po × 6	Boerrigter (2004)
Gene mutation, <i>lacZ</i> gene, <i>lacZ</i> transgenic mouse kidney, <i>in vivo</i> (2 wk)	–, –		2333 po × 6	Boerrigter (2004)
Gene mutation, <i>lacZ</i> gene, <i>lacZ</i> transgenic mouse spleen, <i>in vivo</i> (2 wk)	–, –		2333 po × 6	Boerrigter (2004)
Gene mutation, <i>Gpt</i> mutant frequency in liver DNA, <i>Gpt</i> delta transgenic Sprague-Dawley female rats <i>in vivo</i>	–		12 000 ppm [1440 mg/kg/d] × 13 wk (diet)	Kanki et al. (2005)
Micronucleus formation, mice <i>in vivo</i>	–		5000 po	Astill et al. (1986)
Micronucleus formation, B6C3F ₁ mouse erythrocytes <i>in vivo</i>	–		6000 ip × 5	Douglas et al. (1986)
Micronucleus formation, male rat (Sprague-Dawley or F344) hepatocytes, <i>in vivo</i> ^a	?		1000 po	Suzuki et al. (2005)
Micronucleus formation, male rat (Sprague-Dawley or F344) reticulocytes, <i>in vivo</i> ^a			1000 po	Suzuki et al. (2005)
Chromosomal aberrations (aberrant metaphase), Syrian hamster exposed <i>in vivo</i> , embryos cultured <i>in vitro</i> ^f	+		7500 po	Tomita et al. (1982a)
Chromosomal aberrations, F344 rat bone marrow <i>in vivo</i>	–		4900 po × 5	Putman et al. (1983)
Aneuploidy, F344 rat hepatocytes <i>in vivo</i>	–		12 000 diet, 7 d	Hasmall & Roberts (1997)
Proportion in any ploidy class, F344 rat hepatocytes (<i>n</i> = 4) analysed 30 h after first dose	?		950 po × 2	Hasmall & Roberts (2000)
Proliferation in all ploidy classes (greatest in higher classes of octoploid cells), F344 rat hepatocytes (<i>n</i> = 4) analysed 30 h after first dose	+		950 po × 2	Hasmall & Roberts (2000)
Cell transformation, Syrian hamster exposed <i>in vivo</i> , embryos cultured <i>in vitro</i> ^f	+		7500 po	Tomita et al. (1982a)
Sperm morphology, B6C3F ₁ mice <i>in vivo</i>	–		6000 ip × 5	Douglas et al. (1986)
Sperm morphology, Sprague-Dawley rats <i>in vivo</i>	–		5200 ip × 5	Douglas et al. (1986)
Dominant lethal test, ICR Swiss male mice <i>in vivo</i>	+		12.78 mL/kg [12 780 mg/kg] ip	Singh et al. (1974)

Table 4.1 (continued)

Test system	Result ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Dominant lethal test, male mice <i>in vivo</i> (d 1,5,10)	+	NT	1 mL/kg [1000 mg/kg] sc × 3	Autian (1982)
Dominant lethal test, ICR Swiss male mice <i>in vivo</i> (d 1,5,10)	+	NT	1 mL/kg [1000 mg/kg] sc × 3	Agarwal et al. (1985)
Body fluids, Sprague-Dawley rat urine, microbial mutagenicity	-	-	2000 po × 15	DiVincenzo et al. (1985)
Binding (covalent) to F344 rat hepatocyte DNA <i>in vitro</i>	-	NT	390	Gupta et al. (1985)
Binding (covalent) to DNA liver, F344 rat <i>in vivo</i>	-	-	10 000 mg/kg diet, 11 d	Albro et al. (1982)
Binding (covalent) to DNA liver, F344 rat <i>in vivo</i>	-	-	10 000 mg/kg diet, 4 wk	von Däniken et al. (1984)
Binding (covalent) to DNA liver, F344 rat <i>in vivo</i>	-	-	2000 po × 3	Gupta et al. (1985)
Binding (covalent) to DNA liver, F344 rat <i>in vivo</i>	-	-	500 po	Lutz (1986)
Mono(2-ethylhexyl) phthalate (MEHP)				
<i>Bacillus subtilis</i> rec, differential toxicity	+	NT	400 µg/disc [400 µg/mL]	Tomita et al. (1982a)
<i>Salmonella typhimurium</i> TA100, reverse mutation	+	-	1250 µg/plate [350 µg/mL]	Tomita et al. (1982a)
<i>Salmonella typhimurium</i> TA100, reverse mutation	+ (with cytotoxicity)	NT	2500 µg/plate [700 µg/mL]	Tomita et al. (1982a)
<i>Salmonella typhimurium</i> TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	-	-	0.2 µl/plate [100 µg/mL]	Kirby et al. (1983)
<i>Salmonella typhimurium</i> TA100, TA102, TA98, TA97, reverse mutation	-	-	1000 µg/plate [500 µg/mL]	Dirven et al. (1991)
<i>Escherichia coli</i> WP2 B/r	+	NT	700 (without cytotoxicity)	Tomita et al. (1982a)
<i>Saccharomyces cerevisiae</i> strain Bj3505, yeast-based estrogen receptor gene transcription assay (methylated sequences in the ER promoter region)	-	NT	280	Kang & Lee (2005)
Unscheduled DNA synthesis, B6C3F ₁ mouse primary hepatocytes <i>in vitro</i>	-	NT	139	Smith-Oliver & Butterworth (1987)
Gene mutation, mouse lymphoma L5178Y cells, <i>Tk</i> ⁺ locus <i>in vitro</i>	-	-	0.3 µL/mL [300 µg/mL]	Kirby et al. (1983)
Sister chromatid exchange, Chinese hamster V79 cells <i>in vitro</i>	+	NT	25	Tomita et al. (1982a)

Table 4.1 (continued)

Test system	Result ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, Syrian hamster embryo cells <i>in vitro</i>	–	+	10 µM [2.78 µg/mL]	Tsutsui et al. (1993)
Chromosomal aberrations, Syrian hamster embryo cells <i>in vitro</i> ^f	–	NT	83.4 24-h	Tsutsui et al. (1993)
Chromosomal aberrations, Syrian hamster embryo cells <i>in vitro</i> ^f	NT	+	0.84 2-h	Tsutsui et al. (1993)
Cell transformation, Syrian hamster embryo cells ^f	(+)	NT	28 48-h	Tsutsui et al. (1993)
Cell transformation, Syrian hamster embryo cells ^f	NT	+	56 2-h	Tsutsui et al. (1993)
Cell transformation in Syrian hamster embryo cells	–	(+)	100 µM [27.8 µg/mL]	
Cell transformation, Syrian hamster embryo cells, clonal assay	+	NT	23 µM [6.4 µg/mL]	Mikalsen et al. (1990)
Cell transformation, C3H10T½ mouse cells	–	NT	417	Sanchez et al. (1987)
DNA single- and double-strand breaks, Comet assay (alkaline), MA-10 mouse Leydig tumour cell line (24 h) ^e (tail intensity)	+	NT	0.84	Erkekoğlu et al. (2010a)
DNA single- and double-strand breaks, Comet assay (alkaline), MA-10 mouse Leydig tumour cell line (24 h) ^e (tail moment)	+	NT	0.84	Erkekoğlu et al. (2010a)
DNA single- and double-strand breaks, Comet assay (alkaline), MA-10 mouse Leydig tumour cell line (24 h) ^e (cell viability)	–	NT	0.84	Erkekoğlu et al. (2010a)
DNA single- and double-strand breaks, Comet assay (alkaline), MA-10 mouse Leydig tumour cell line (24 h) ^e (reactive oxygen species production)	–	NT	0.84	Erkekoğlu et al. (2010a)
DNA strand breaks, Comet assay, human leukocytes <i>in vitro</i> (median tail moment)	+	NT	28	Anderson et al. (1999a)
DNA single- and double-strand breaks, Comet assay, LNCaP human prostate adenocarcinoma cell line <i>in vitro</i> (24 h) ^e (tail intensity and moment)	+	NT	0.84	Erkekoğlu et al. (2010b)
DNA single- and double-strand breaks, Comet assay, human inferior nasal turbinate epithelial cells <i>in vitro</i> , slow migration of Olive tail moments (% DNA in tail × median migration) > 2	+		1400	Kleinsasser et al. (2004a)
DNA single- and double-strand breaks, Comet assay, human inferior nasal turbinate mucosal cells <i>in vitro</i> , slow migration of Olive tail moments (% DNA in tail × median migration) > 2 and % of DNA in tail	+		28	Kleinsasser et al. (2004b)
DNA single- and double-strand breaks, Comet assay, human peripheral lymphocytes <i>in vitro</i> , slow migration of Olive tail moments (% DNA in tail × median migration) > 2 and % of DNA in tail	+		28	Kleinsasser et al. (2004b)
Unscheduled DNA synthesis, human primary hepatocytes <i>in vitro</i>	–	NT	139	Butterworth et al. (1984)
DNA strand breaks, Wistar rat liver <i>in vivo</i>	–	NT	500	Elliott & Elcombe (1987)
Chromosomal aberrations, Syrian hamster exposed <i>in vivo</i> , embryos cultured <i>in vitro</i>	+		375 × 1 po	Tomita et al. (1982a)

Table 4.1 (continued)

Test system	Result ^a		Dose ^b (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cell transformation, Syrian hamster exposed <i>in vivo</i> embryos cultured <i>in vitro</i>	+		375 × 1 po	Tomita et al. (1982a)
DNA single- and double-strand breaks, Comet assay (neutral), human sperm <i>in vivo</i> ^c (comet extent, % DNA in tail, total distributed moment)	–		IQR increase in urinary concentration (max., 0.4 µg/mL urine)	Duty et al. (2003)
DNA single and double-strand breaks, Comet assay, human sperm <i>in vivo</i> ^c (comet extent, % DNA in tail, total distributed moment)	+		IQR increase in urinary concentration (max., 0.9 µg/mL urine)	Hauser et al. (2007)
Mono(2-ethyl 5-hydroxyhexyl) phthalate				
<i>Salmonella typhimurium</i> TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 µg/plate [500 µg/mL]	Dirven et al. (1991)
Mono(2-ethyl 5-oxohexyl) phthalate				
<i>Salmonella typhimurium</i> TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 µg/plate [500 µg/mL]	Dirven et al. (1991)
Mono(5-carboxyl 2-ethylpentyl) phthalate				
<i>Salmonella typhimurium</i> TA100, TA102, TA98, TA97, reverse mutation	–	–	1000 µg/plate [500 µg/mL]	Dirven et al. (1991)
2-Ethylhexanol				
<i>Bacillus subtilis</i> rec, differential toxicity	–	NT	500	Tomita et al. (1982a)
<i>Salmonella typhimurium</i> TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	1 µL/plate [500 µg/ mL]	Kirby et al. (1983)
Gene mutation, mouse lymphoma L5178 cells, <i>Tk</i> locus <i>in vitro</i>	–	–	0.3 µL/mL [300 µg/ mL]	Kirby et al. (1983)
Phthalic acid				
<i>Bacillus subtilis</i> rec, differential toxicity	–	NT	500 µg/disk	Tomita et al. (1982a)

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

^b LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw per day

^c The authors only recognized a response greater than twofold as positive.

^d The authors examined extracts of lyophilisates of mineral water contaminated with DEHP after storage in plastic (polyethylene terephthalate) bottles but the concentrations of DEHP in the lyophilisates were not given by the authors and a dose–response cannot therefore be assessed.

^e The authors conducted the Comet assay at the half maximal inhibitory concentration only at which cytotoxicity was observed.

^f There was an increase at every dose of DEHP tested (1–300 µM) for aberrant metaphase including chromatid gaps and isochromatid gaps after 2 h of exposure. The authors conducted

Table 4.1 (continued)

a trend test for chromosomal aberrations which was positive for DEHP. In the same experiment, MEHP induced an increase in the percentage of transformed colonies between 2.8 and 28 µg/mL after exposure for 48 hours but the number of colonies scored was half that induced by DEHP and the increases were not statistically significant for MEHP.

⁸ Dose-related increases starting at 1 µg/mL; no statistical analysis made by authors

^h The authors did not conduct statistical analyses of their data but considered a result to be positive if it was greater than 1% transformation. For one experiment, the lowest concentration of DEHP (0.8 µg/mL) gave a positive result of 0.79% which was threefold greater than the concurrent control for that experiment.

ⁱ The authors only recognized a response greater than twofold as positive; in one experiment, there was cytotoxicity as higher levels and the increase at 78 µg/mL gave the same magnitude of response as exposure to 507 µg/mL in another experiment with no cytotoxicity.

^j The authors reported a similar result for 2.5 µg/mL DEHP as that for 5.0 µg/mL using the standard method, but it failed to reach significance.

^k The authors did not conduct statistical analyses of their data and considered a result with a twofold enhancement of survival to be positive and, at two or more concentrations, to be a positive dose–response. The positive control was lost due to contamination; the authors therefore considered one experiment to be null. In a second experiment, DEHP-induced anchorage-independent survival showed a dose–response at 1000 and 2000 µg/mL (61% and 161%). No trend test was made.

^l Positive if followed by 0.16 µM 12-*O*-tetradecanoylphorbol 13-acetate for 5 h

^m Similar responses, one of which was while the other was not statistically significant; no trend test made in the second experiment. In one experiment, a positive response was found at 3900 µg/mL but with significant toxicity and the response was negative at 390 µg/mL.

ⁿ Dose-related increases started at 3.9 µg/mL and were statistically significant at 195 µg/mL, but no trend test was made.

^o The authors did not carry out a trend test but the response was increased in a dose-related manner with statistical significance reached at the half maximal inhibitory concentration (toxicity).

^p No oxidative damage in kidney DNA

^q The authors used 50% and 25% of the half maximal lethal dose. In one laboratory, there was 70% cytotoxicity at 1000 mg/kg and ~60% cytotoxicity in another in the liver (these were the lowest doses tested). In one laboratory, the total number of reticulocytes was reduced. Cytotoxicity rendered the results of this study suspect.

^r Only two doses were tested, with the lowest dose giving a 2.7-fold increase in aberrant metaphase above control levels (3750 mg/kg) but the higher dose (7500 mg/kg) giving a result that reached statistical significance. A similar result was reported for transformation.

^s Sperm DNA damage was associated with monoethylphthalate and MEHP after adjusting for DEHP oxidative metabolites, which may serve as phenotypic markers for DEHP metabolism to 'less toxic' metabolites. Results are given as adjusted regression coefficients (95% confidence interval) for common parameters associated with an interquartile range increase in MEHP and mono(2-ethyl-5-hydroxyhexyl) phthalate when both compounds are included in the same model; 89% of MEHP in the urine was above the level of detection (1.2 ng/mL). The authors hypothesized that % DNA in tail may indicate single-strand breaks compared with comet extent and tail distributed moment (double-strand breaks).

d, day or days; Gpt, guanine phosphoribosyltransferase; h, hour or hours; ip, intraperitoneal; IQR, interquartile ranges; max, maximum; mo, month or months; NR, not reported; 8OH-dG, 8-hydroxydeoxyguanosine; po, oral; sc, subcutaneous; Tk, thymidine kinase; wk, week or weeks; yr, year or years

9.4 µg/mL DEHP (a concentration that induced little cytotoxicity) to 38 µg/mL. The authors did not carry out a trend test and only reported statistical significance at the IC₅₀ concentration (38 µg/mL).

MEHP has also been studied in human tissues *in vitro* using the Comet assay. [Kleinsasser et al. \(2004a\)](#) used three-dimensional mini-organ cultures of inferior nasal turbinate epithelia from 25 donors. Olive tail moment > 2 was increased in the mini-organ cultures and in single-cell epithelial cultures, which were more sensitive to MEHP than the mini-organ cultures at relatively high concentrations (1400 µg/mL).

In a separate study, [Kleinsasser et al. \(2004b\)](#) reported increased Olive tail moments and percentage DNA in tail, in inferior nasal turbinate cultures and in peripheral lymphocytes exposed to MEHP (28 µg/mL). [Anderson et al. \(1999a\)](#) reported increases in median tail moment after exposure to MEHP (28 µg/mL) in human leukocytes.

[Hauser et al. \(2007\)](#) did not report reactive oxygen species or apoptosis in sperm.

Positive results from Comet assays in several human tissues or cell lines were reported at concentrations that did not induce cytotoxicity or apoptosis, resulting in loss of cell viability for DEHP and MEHP. For most of the *in-vitro* studies, positive Comet results were obtained at ~1–10 µg/mL for DEHP and ~30 µg/mL for MEHP. Thus, the parent compound appeared to be more efficient for this end-point.

(c) Mutations

No specific studies were identified regarding the induction of mutations in human tissues after exposure to DEHP or its metabolites.

(d) Chromosomal effects

The results of sister chromatid exchange and chromosomal aberration assays of human lymphocytes treated *in vitro* with DEHP have

been largely negative with the exception of [Lindahl-Kiessling et al. \(1989\)](#) (see [Table 4.1](#)).

(e) Changes in DNA methylation pattern

No DEHP-specific data on DNA methylation in humans were available to the Working Group.

4.2.2 Experimental systems

A large number of *in-vitro* systems have tested the ability of DEHP to induce mutation, transformation and epigenetic changes. Overlaps in context with assays already discussed in Section 4.2.1 are not repeated here. There is an especially robust database for Syrian hamster embryo (SHE) transformation (see [Table 4.1](#)). Overall, tests in *Salmonella* assays have given negative results for DEHP. Below, epigenetic and mutational changes in DEHP-induced tumours are discussed to elucidate the mechanisms of DEHP carcinogenicity.

(a) DNA oxidative damage

No covalent binding following exposure to DEHP *in vivo* has been reported in rat hepatocytes; DNA oxidative damage was previously reported at high concentrations of DEHP ([IARC, 2000](#)).

More recently, [Seo et al. \(2004\)](#) reported that administration of DEHP (14-day exposure) to Sprague-Dawley rats increased levels of 8-hydroxydeoxyguanosine (8-OHdG) (at 1000 mg/kg bw) and malondialdehyde (at 50 mg/kg bw) in liver DNA. Neither end-point was correlated with patterns of enzyme induction associated with peroxisomal proliferation or with the observed decreases in CYP1A1, -1A2, -3A4, UGT or glutathione S-transferase (GST). DEHP (12000 ppm [12000 mg/kg diet] for 22 days) induced expression of DNA repair enzymes (8-oxoguanine glycosylase/lyase, apurinic/apyrimidinic endonuclease, mammalian N-methylpurine-DNA glycosylase and polymerase β) in F334 rats ([Rusyn et al., 2000](#)).

(b) DNA strand breaks

[Pogribny et al. \(2008\)](#) reported no increased DNA strand breaks as measured by the number of 3'-hydroxy DNA ends in F334 rats (1.2% DEHP in the diet for 5 months). Exposure to DEHP (1170 µg/mL) *in vitro* increased tail moment and intensity in the Comet assay in MA-10 mouse Leydig cell tumour lines ([Erkekoğlu et al., 2010b](#)). MA-10 cells showed ~80–60% survival at 3.9–195 µg/mL DEHP, increased tail moment and intensity and decreased cell viability at 0.84 µg/mL MEHP.

(c) Cell transformation

One of the most robust databases for the effects of DEHP, as measured in the SHE cell assay, was the study of neoplastic transformation by chemical carcinogens. [Tsutsui et al. \(1993\)](#) reported that a low concentration of DEHP (1.2 µg/mL) was effective after a longer period of exposure (48 hours) in the absence of exogenous metabolic activity and after shorter time periods in the presence of exogenous activity, with no effect on survival. [Barrett & Lamb \(1985\)](#) and [Sanner & Rivedal \(1985\)](#) also reported a positive response without metabolic activation at low levels of exposure (~1 µg/mL). [Pant et al. \(2010\)](#) demonstrated the feasibility of conducting the SHE cell transformation assay without using an X-ray-irradiated feeder layer and including conditioned media to find a greatly enhanced response at lower exposures to DEHP. [Tomita et al. \(1982a\)](#) exposed Syrian hamsters *in utero* to a single dose of DEHP (7500 mg/kg) and reported a positive transformation assay and chromosomal aberrations in the embryonic cells (see Section 4.2.2(e)).

[Tsutsui et al. \(1993\)](#) reported that MEHP gave negative results in the SHE assay after 48 hours and did not affect survival (up to 28 µg/mL), while it gave positive results for transformation in the presence of exogenous metabolic activation and at a higher concentration (56 µg/mL).

[Mikalsen et al. \(1990\)](#) reported that MEHP was positive at 5.6 µg/mL.

[Park & Kwak \(2008\)](#) exposed *Chironomus riparius* larvae to DEHP *in vivo* and reported increased mouthpart deformities and upregulation of heat shock protein (which interacts with certain cellular proteins including steroid hormone receptors) at a concentration of 1 µg/mL.

(d) Mutations

Most assays using *Salmonella typhimurium* have given negative results for DEHP (see [Table 4.1](#)). [Tomita et al. \(1982a\)](#) were able to induce a positive response with a large dose (5 mg/plate). Using the *umu C* gene expression system in *S. typhimurium*, [Okai & Higashi-Okai \(2000\)](#) reported that exposures to DEHP (780 µg/mL) gave negative results in the absence of activation and weakly positive results in the presence of a commercial metabolic activation mixture. Results were positive in the presence of metabolic activation from rat pancreas; weak but significant activities were reported with metabolic activation from liver and intestine; and no significant activities were observed with metabolic activation from lung and kidney. Significant *umu C* gene expression was obtained with highly purified lipase from porcine pancreas and was enhanced in the presence of bile acids. Metabolic lipase activity in various organs correlated with DEHP genotoxic activity.

[Tomita et al. \(1982a\)](#) reported positive results in *S. typhimurium* exposed to MEHP (700 µg/mL) and concurrent cytotoxicity, in the *Escherichia coli* WP2 *B/r* test system with no cytotoxicity at the same concentration and in the *Bacillus subtilis* differential toxicity test at 400 µg/disc.

[Parry & Eckardt \(1985\)](#) presented positive results for gene conversion and aneuploidy at cytotoxic concentrations of DEHP (5000 µg/mL). Gene conversions, and point and reverse mutations were induced in three strains of *Saccharomyces cerevisiae* D7-144 in the presence of metabolic activation at 1500 µg/mL

DEHP ([Mehta & von Borstel, 1985](#)). Largely negative results have been reported for mutations in *Drosophila melanogaster* exposed to DEHP.

In-vitro results on gene mutation in mouse lymphoma L5178Y cells at the thymidine kinase (*Tk*⁺) locus have been mixed. [Oberly et al. \(1985\)](#) reported weakly positive results at 7.5 µg/mL DEHP, but several other studies reported negative results at much higher concentrations.

The dominant lethal test for mutation was positive in several studies in mice *in vivo* ([Singh et al., 1974](#); [Autian, 1982](#); [Agarwal et al., 1985](#)), with either repeated exposures (three subcutaneous injections of 1 mL/kg bw [1000 mg/kg bw]) or a single dose (injection of 12.78 mL/kg bw [12 780 mg/kg bw]). Dominant lethal mutations in parent germ cells may induce failure of implantation or early death due to unscheduled DNA synthesis, chromosomal breaks or elimination of aberrant chromosomes.

[Boerrigter \(2004\)](#) investigated the response of male and female *lacZ*-plasmid transgenic mice to six doses of 2333 mg/kg bw DEHP, 200 mg/kg bw 4-chloro-6-(2,3-xylidino)-2-pyrimidinylthioacetic acid (WY-14 643) or 90 mg/kg bw clofibrate. Mutant frequencies were significantly elevated (~40%) in the liver but not in kidney or spleen after 21 days of exposure to DEHP and WY-14 643 but not to clofibrate. The pattern of mutation induction matched that of tumour induction in mice for all the three compounds ([Boerrigter, 2004](#)).

[Kanki et al. \(2005\)](#) studied mutagenicity and mutation spectra *in vivo* in an animal model developed for genotoxicity analysis (i.e. guanine phosphoribosyltransferase delta transgenic rats). In the DEHP-treated (12000 ppm [12000 mg/kg diet], ~187 mg/rat per day) rats, about 10 random copies of the transgene lambda EG10 per haploid gene and marked hepatomegaly were reported, although only a few GST-placental form-positive liver cell foci were observed (GST-placental form staining was negative), and no mutagenic activity of DEHP was detected in the liver.

(e) Chromosomal effects

Sister chromatid exchange and micronucleus formation after exposure to DEHP *in vitro* have been investigated in Chinese hamster cells with mostly negative results. [Suzuki et al. \(2005\)](#) reported negative results for micronuclei and micronucleated reticulocytes at cytotoxic concentrations in 4-week-old rats. [Fritzenschaf et al. \(1993\)](#) reported positive micronucleus formation in SHE cells but the exposure level was not given. MEHP (25 µg/mL) increased sister chromatid exchange in Chinese hamster V79 cells ([Tomita et al., 1982a](#)).

Exposure to DEHP *in vitro* did not induce chromosomal aberrations in several assays in Chinese hamster cells. However, chromosomal aberrations were increased in SHE cells in the presence of exogenous metabolic activity after exposure to 0.4 µg/mL DEHP or 0.84 µg/mL MEHP ([Tsutsui et al., 1993](#)). MEHP (375 mg/kg bw) also increased chromosomal aberrations in embryo cells of pregnant Syrian hamsters ([Tomita et al., 1982a](#)). The same cells gave positive results in the SHE transformation assay (see Section 4.2.2(c)).

Results for chromosomal aberration in the *Allium cepa* assay after in-vivo exposure to 5–50 µg/mL DEHP were inconclusive ([Rank et al., 2002](#)).

No alteration of hepatocyte ploidy was reported in hepatocytes of adult F344 rats exposed *in vivo* to DEHP for 7 days ([Hasmall & Roberts, 1997](#)). However, two doses of 950 mg/kg bw DEHP have been reported to increase DNA synthesis after 30 hours of exposure in all ploidy classes and in particular in the octoploid classes (normally representing a small proportion of polyploidy cell in the liver) ([Hasmall & Roberts, 2000](#)).

(f) Changes in DNA methylation pattern

[Pogribny et al. \(2008\)](#) reported no changes in global methylation of total F334 rat liver DNA after exposure to 1.2% DEHP in the diet for 5 months. No statistically significant change in the methylation status of the promoter region of the *GST-pi* gene was reported. Western blot analysis showed an increase in DNA methyltransferase I and *c-myc* expression but not H3K9 or H4K20 histone trimethylation nor changes in the metabolism of methyl donors (*S*-adenosylmethionine and *S*-adenosylhomocysteine content in liver).

[Wu et al. \(2010\)](#) reported a DEHP-induced increase in global DNA methylation status and significantly upregulated RNA expressions of DNA methyltransferases (*Dnmt1*, *Dnmt3a* and *Dnmt3b*) in the testes of male Kunming mouse fetuses after 1 week of maternal exposure to 500 mg/kg bw per day.

4.3 Mechanistic data

4.3.1 Hepatocyte-specific events

(a) Effects of DEHP on peroxisome proliferator-activated receptor (PPAR) activation

Phthalates, including DEHP, are ligands for nuclear receptors PPAR α , - β and - γ ([Issemann & Green, 1990](#); [Dreyer et al., 1992](#); [Göttlicher et al., 1992](#); [Sher et al., 1993](#); [Corton & Lapinskas, 2005](#)).

(i) Humans

No studies could be found which showed evidence that DEHP activates PPAR α in human liver *in vivo*.

In vitro, trans-activation assays were used to assess the activation potential of DEHP, MEHP and 2-EHA for either full-length PPAR subtype ([Maloney & Waxman, 1999](#); [Hurst & Waxman, 2003](#); [Lampen et al., 2003](#); [Lapinskas et al., 2005](#)) or hybrid transcription factors consisting of the PPAR ligand-binding domain

fused with DNA-binding domains of the glucocorticoid receptor ([Lampen et al., 2003](#)) or the transcription factor GAL4 ([Bility et al., 2004](#)). DEHP did not activate human PPAR α or human PPAR γ ([Maloney & Waxman, 1999](#)). However, several studies showed that all three human PPAR subtypes were activated by MEHP ([Maloney & Waxman, 1999](#); [Hurst & Waxman, 2003](#); [Lampen et al., 2003](#); [Bility et al., 2004](#)). In addition, both human PPAR γ isoforms - γ 1 and - γ 2 were activated by MEHP ([Feige et al., 2007](#)). The DEHP metabolite 2-EHA weakly activated human PPAR α but not human PPAR γ ([Maloney & Waxman, 1999](#)). No studies have examined the activation of human PPAR β by DEHP or 2-EHA.

[Rotroff et al. \(2010\)](#) examined the ability of DEHP to activate several response elements in a multiplex human response element transactivation assay in the hepatocellular carcinoma cell line, HepG2. In addition, a modification of the approach was used to generate the human ligand-binding domain of nuclear receptors expressed as a chimera with the yeast GAL4 DNA-binding domain that activated in trans a 5X-UAS_G-TATA promoter linked to a reporter sequence. DEHP was shown to activate PPAR α and PPAR γ significantly (effective concentration for half-maximal response in μ M): PPAR γ _TRANS (46), PPRE_CIS (48), PPAR α _TRANS (50). The PPAR β TRANS assay was negative for DEHP ([Martin et al., 2010](#); <http://actor.epa.gov/actor/faces/ToxCastDB/assay.jsp>).

[Lapinskas et al. \(2005\)](#) determined whether phthalate esters interact directly with human PPAR α or PPAR γ using the scintillation proximity assay. The binding affinity for MEHP to bind to the α and γ subtypes were 15 μ M and 12 μ M, respectively. DEHP was negative in this assay (i.e. binding affinity > 150 μ M). The PPAR β subtype was not examined.

(ii) Experimental systems

The DEHP metabolite MEHP activates mouse PPAR α , PPAR γ and, to a lesser extent, PPAR β in trans-activation assays ([Maloney & Waxman, 1999](#); [Hurst & Waxman, 2003](#); [Lampen *et al.*, 2003](#); [Lapinskas *et al.*, 2005](#); [Feige *et al.*, 2007](#)). In addition, both mouse PPAR γ isoforms — γ 1 and γ 2 — were activated by MEHP ([Feige *et al.*, 2007](#)). 2-EHA weakly activated mouse PPAR α , β and γ ([Lampen *et al.*, 2003](#); [Lapinskas *et al.*, 2005](#)). In one study, 2-EHA failed to activate mouse PPAR γ ([Maloney & Waxman, 1999](#)). DEHP activated PPAR α and PPAR γ , albeit weakly, but not mouse PPAR β ([Lampen *et al.*, 2003](#); [Lapinskas *et al.*, 2005](#)).

(iii) Species differences

DEHP treatment increased the expression of PPAR α in mice and rats but not significantly, while the level of PPAR α appeared to be reduced in marmosets ([Ito *et al.*, 2007b](#)).

[Important species differences in expression and molecular signalling for PPAR α have been reported, and were considered to be of critical importance for the evaluation of human cancer risk from DEHP in the previous IARC evaluation ([IARC, 2000](#)). More recent studies are detailed below to illustrate the state of science in this area.]

Mice and rats express PPAR α at high levels in the liver, whereas human PPAR α is expressed at a lower level in human liver ([Palmer *et al.*, 1998](#)). [Palmer *et al.* \(1998\)](#) used electrophoretic mobility shift assays to determine the level of PPAR α that binds to a peroxisome proliferator response element (PPRE) from the *CYP4A6* gene. In seven lysates in which PPAR α could be detected by the assay, the amounts were about 10 times lower than those detected in the livers of CD-1 or BALB/cByJ mice; for the remainder of the 13 samples, the amount was below the level of detection (more than 20 times lower than in mouse liver). Using the RNase protection assay, a threefold variation in the expression

of full-length PPAR α messenger RNA (mRNA) between human samples was observed. [The Working Group noted that expression of human PPAR α has not been determined in a sufficient number of samples to conclude unequivocally that all populations express less PPAR α than responsive rodents.]

In one study, the expression of human PPAR α protein in one of six humans appeared to approach levels found in mouse livers ([Walgren *et al.*, 2000](#)). [The Working Group noted that, in this study, human PPAR α protein expression was not normalized to housekeeping or loading controls that would help to evaluate whether the proteins in the sample were intact.]

[Ito *et al.* \(2007b\)](#) reported no differences in constitutive PPAR α mRNA expression in CD-1 mice and Sprague-Dawley rats but a lower level of expression in marmosets (*Calithrix jacchus*) (i.e. ~25% of the level in mice).

There is evidence that human and rodent PPAR α differ in their ability to be activated by PPAR α agonists, as would be expected given that the amino acid sequences within the ligand-binding domains differ between species. The mouse and rat PPAR α ligand-binding domains are 94% similar to that of human PPAR α ([Sher *et al.*, 1993](#); [Mukherjee *et al.*, 1994](#); [Tugwood *et al.*, 1996](#)). The activation of the human PPARs by MEHP required higher concentrations for to achieve the same levels as the corresponding mouse receptor ([Maloney & Waxman, 1999](#); [Hurst & Waxman, 2003](#); [Lampen *et al.*, 2003](#); [Bility *et al.*, 2004](#)).

A truncated human PPAR α variant exists that may determine responses between species. This truncated form, identified in several laboratories and called hPPAR α -8/14 ([Tugwood *et al.*, 1996](#)), hPPAR α_{sv} ([Palmer *et al.*, 1998](#)), PPAR α_{tr} ([Gervois *et al.*, 1999](#)) and PPAR α 2 ([Hanselman *et al.*, 2001](#)), lacks exon 6 due to alternative splicing, resulting in a human PPAR α that lacks the hinge region and ligand-binding domain. In transactivation assays *in vitro*, this form acts as a dominant

negative, inhibiting the ability of the wild-type receptor to activate transcription, possibly by titrating out limiting amounts of co-activators such as C/EBP binding protein/p300 ([Gervois et al., 1999](#)). The level of the truncated human PPAR α mRNA ranges from 10 to 50% of full-length human PPAR α mRNA ([Palmer et al., 1998](#); [Gervois et al., 1999](#); [Roberts et al., 2000](#); [Hanselman et al., 2001](#)). In the [Palmer et al. \(1998\)](#) study, the authors used RNase protection assays to determine that the truncated form accounts for 28–42% of the protected fragments in each of 10 samples. In mice, rats and rabbits, this level is below 10%, whereas, in cynomolgus monkeys, the ratios of the truncated to full-length form approach those in humans ([Hanselman et al., 2001](#)). One study concluded that the level of the truncated form does not correlate with responsiveness to PPAR α agonists ([Hanselman et al., 2001](#)); however, this study only measured primary human hepatocyte mRNA levels of the acyl-coenzyme A oxidase gene, an inappropriate biomarker of human PPAR α activity given that this gene does not appear to be regulated by PPAR α in the same way as the rodent gene.

(b) *Effects of DEHP on markers of PPAR activation*

PPAR α activation in the liver has been also characterized indirectly by assessing: (1) increases in the size and/or numbers of peroxisomes in cells; (2) increases in acyl-coenzyme A oxidase expression, protein or activity; (3) increased expression, protein levels or activity of CYP4A protein, a ω -lauric acid hydroxylase; and/or (4) increases in the levels of carnitine acyl-coenzyme A transferase (CAT). [The Working Group noted that these indirect markers have been shown to be activated even in the absence of PPAR α , i.e. in PPAR α -null mice exposed to peroxisome proliferators ([Rosen et al., 2008](#)); thus, the results from these indirect assays should be interpreted with caution if no evidence of PPAR α activity is available.]

(i) *Humans*

No study assessed markers of PPAR α activation by DEHP. Studies are available from people who may have been exposed to DEHP and other agents that leached from plastics used in medical devices. 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 1 month of dialysis. However, in a liver biopsy from one 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 DEHP occurs in dialysis patients ([Huber et al., 1996](#)). [The Working Group noted that these data should be interpreted with extreme caution.]

(ii) *Experimental systems*

In-vivo studies

[Pugh et al. \(2000\)](#) evaluated the effects of DEHP in young adult male cynomolgus monkeys. Groups of four monkeys received DEHP (500 mg/kg bw per day) or vehicle (0.5% methyl cellulose, 10 mL/kg bw per day) by intragastric intubation for 14 consecutive days. Clofibrate (250 mg/kg bw per day), a hypolipidaemic drug used for cholesterol reduction in human patients, was used as a reference substance. None of the test substances had any effect on body weight or liver weights. Histopathological examination of tissues from these animals revealed no distinctive treatment-related effects in the liver, kidney or testes. There were also no changes in any of the hepatic markers for peroxisomal proliferation, including peroxisomal β -oxidation.

[Tomonari et al. \(2006\)](#) treated male and female marmosets daily with 0, 100, 500 or 2500 mg/kg bw DEHP by gavage for 65 weeks from weaning

(3 months of age) to sexual maturity (18 months) and examined several enzyme activities. The ages at commencement of treatment were 90–115 days and body weights on the day of initiation of the dosing varied significantly (range, 95–180 g for males and 116–188 g for females). Five to six animals per group were examined for hepatic enzyme activity. No change in cyanide-insensitive palmitoyl coenzyme A β oxidation was observed in males but a large variability was seen in females especially at the 500-mg/kg dose, although the 95% increase reported was not statistically significant. For CAT, there was a large variability in control levels and across dose groups, especially in females. Carnitine palmitoyltransferase activity showed a similar pattern of variability in activity for the 2500-mg/kg group with a SD similar in magnitude to the mean. For lauric acid ω -1-hydrolase activity, females had dose-related increases compared with controls that were statistically significant at 500 mg/kg. Males showed an increase in every dose group that was not statistically significant (~40% increase).

[Reddy et al. \(1986\)](#) fed diets containing 0.25–2.0% DEHP to young male F344 rats for 30 days. Dose-related increases in relative liver weight, cyanide-insensitive palmitoyl-coenzyme A oxidation activity and peroxisome volume density were observed. In this study, a 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-coenzyme A oxidation is a good marker for peroxisome proliferation in rodent liver.

[Wong & Gill \(2002\)](#) treated male C57BL/6 mice with 1.0% DEHP in the diet for 13 weeks. Microarray analysis identified 51 DEHP-regulated genes involved in peroxisome proliferation, xenobiotic detoxification, oxidative stress response, immune function, steroid hormone

metabolism, testis development and pheromone transport.

[Currie et al. \(2005\)](#) dosed male B6C3F₁ mice ($n = 6$) with DEHP (10 mL/kg bw) by gavage every 24 hours for 3 days (1150 mg/kg bw per day) or with an equivalent volume of corn oil. Gene expression levels were measured 2, 8, 24 and 72 hours after the first exposure. DEHP treatment induced a statistically significant increase in liver weight 48 hours and 72 hours after the first exposure. Histological analysis revealed an increase in eosinophilic staining of the smooth endoplasmic reticulum in centrilobular hepatocytes at both these times (indicative of peroxisome proliferation) and hypertrophy of these cells. There was an increased rate of hepatocyte DNA synthesis 48 hours and 72 hours after the first dosing, consistent with an increase in S-phase progression in the periportal area of livers of mice exposed to DEHP. There was a considerable increase in *Cyp4a10* expression from the earliest time-point sampled. As well as a coordinate induction of genes involved in fatty acid metabolism confirmed by the over-representation of Gene Ontology terms, Protein Analysis Through Evolutionary Relationships (PANTHER) and Gen Map Annotator and Pathway Profiler (GenMAPP) pathways involved in the metabolism of lipids (e.g. acyl-coenzyme A metabolism, fatty acid β -oxidation, pantothenate and coenzyme A biosynthesis, or coenzyme metabolism).

[Yamazaki et al. \(2009\)](#) examined the effects of 0.5% DEHP in the diet for 7 days on peroxisomal β -oxidation and several acyl transferases including 1-acyl-2-lysophospholipid acyl transferase, 1-acylglycerophosphoethanolamine acyl transferase, 1-acylglycerophosphoinositol acyl transferase, 1-acylglycerophosphoserine acyl transferase and 1-acylglycerophosphocholine acyl transferase) in the kidneys and livers of male Wistar rats (5 weeks of age). Exposure to DEHP considerably increased the activities of the acyl

transferases and peroxisomal β -oxidation in the microsomes of rat kidneys and livers.

In-vitro studies

Hepatocytes isolated from male Wistar rats and Sprague-Dawley rats (180–250 g) were treated with 0.2 mM [55.7 $\mu\text{g}/\text{mL}$] MEHP or 1 mM 2-EH [130.2 $\mu\text{g}/\text{mL}$] for 48 hours (Gray *et al.*, 1982, 1983). Both DEHP metabolites increased CAT activity about 6–15 fold.

The effects of MEOHP, a DEHP metabolite, on mitochondrial β -oxidation were investigated (Grolier & Elcombe, 1993). In isolated rat hepatocytes, MEOHP inhibited long-chain fatty acid oxidation and had no effect on the ketogenesis of short-chain fatty acids, suggesting that the inhibition occurred at the site of carnitine-dependent transport across the mitochondrial inner membrane. In rat liver mitochondria, MEOHP inhibited CAT I competitively with the substrates palmitoyl-coenzyme A and octanoyl-coenzyme A. An analogous treatment of mouse mitochondria produced a similar competitive inhibition of palmitoyl-coenzyme A transport whereas exposure of guinea-pig and human liver mitochondria with MEOHP revealed little or no effect. The addition of clofibric acid, nafenopin or methylclofenopate revealed no direct effects upon CAT I activity. Inhibition of transferase activity by MEOHP was reversed in mitochondria that had been solubilized with octyl glucoside to expose the latent form of CAT II, suggesting that the inhibition was specific for CAT I. The authors concluded that, *in vitro*, MEOHP inhibits fatty acid oxidation in rat liver at the site of transport across the mitochondrial inner membrane with a marked species difference and support the idea that induction of peroxisome proliferation could be due to an initial biochemical lesion of the fatty acid metabolism.

(iii) *Species differences*

Elcombe & Mitchell (1986) made species comparisons of hepatic peroxisomal proliferation in primary hepatocyte cultures. Hepatocytes isolated from Wistar-derived rats, male Alderley Park guinea-pigs, male marmosets and three human liver samples (renal transplant donors) were treated with 0–0.5 mM MEHP for 72 hours. While there was a concentration-dependent induction of cyanide-insensitive palmitoyl-coenzyme A 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 [MEOHP] induced cyanide-insensitive palmitoyl-coenzyme A 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-coenzyme A oxidation activity. Similarly, lauric acid hydroxylase activity was not induced in marmoset or human hepatocytes treated with 0–2.0 mM metabolite VI.

Goll *et al.* (2000) examined the effects of various peroxisome proliferators including DEHP on peroxisomal enzyme activities in rat FaO and human HepG2 hepatoma cell lines. Both growing and confluent cultures were treated with peroxisome proliferators (250 μM) for 48 or 72 hours. In accordance with previous observations in peroxisome proliferator-treated primary hepatocyte cultures of rat and human origin, the various peroxisome proliferators increased peroxisomal enzyme activities in rat FaO cells but not in human HepG2 cells.

Rat hepatocytes treated *in vitro* with MEHP (250, 500 and 750 μM) exhibited increased peroxisomal β -oxidation. In contrast, there was no response of human hepatocytes to 250, 500 or 750 μM MEHP (Hasmall *et al.*, 2000a).

(c) *Effects of DEHP on PPAR-independent receptor-mediated events*

(i) *Humans*

The estrogenic activities of phthalates were investigated in competitive ligand-binding assays, yeast and mammalian gene expression assays and a uterotrophic assay. DEHP did not compete for estrogen receptors or induce luciferase activity in MCF-7 cells transiently transfected with the Gal-4 human estrogen receptor (ER) construct or the Gal4-regulated luciferase reporter gene or HeLa cells stably transfected with the Gal4-human ER construct ([Zacharewski et al., 1998](#)).

Phthalate esters have been tested for their ability to interact with sex hormone receptors. DEHP and its active metabolite, MEHP, do not bind to the human androgen receptor (AR) in the monkey kidney cell line, COS, transiently transfected with a human AR vector ([Parks et al., 2000](#)).

[Ghisari & Bonefeld-Jorgensen \(2009\)](#) investigated the in-vitro estrogenic activities of a range of widely used plasticizers and phenols, including DEHP, in human MVLN cells, derived from the breast cancer MCF-7 cell line stably transfected with an ER element luciferase reporter vector. Furthermore, the combined effect of a multicomponent mixture of six plasticizers was evaluated for its estrogenic activities. DEHP antagonized the 17 β -estradiol (E2)-induced ER function at concentrations of $\geq 10 \mu\text{M}$. The chemicals were tested in the ER trans-activation assay alone and after co-treatment with 25 pM E2 (corresponding to the half maximum effect concentration[EC_{50}]). No significant agonistic ER activity was observed for DEHP.

Transfection assays were performed with a human pregnane X receptor (PXR; also called steroid-xenobiotic receptor) expression plasmid and a reporter plasmid containing the xenobiotic response elements (XREs) in the *CYP3A4* gene promoter in HepG2 cells. DEHP activated

human PXR-mediated transcription on the XREs. The study indicated that DEHP may be an inducer of the *CYP3A4* gene through PXR, and may influence the metabolism of endogenous steroids, drugs and other xenobiotics ([Takeshita et al., 2001](#)).

[Takeuchi et al. \(2005\)](#) characterized the activities of human ER α , ER β and AR in the presence of 22 phthalates, including three of their metabolites, using reporter gene assays in host Chinese hamster ovary cells. Of the 22 compounds tested, several phthalate diesters with alkyl chains ranging in length from C3 to C6 exhibited not only human ER α -mediated estrogenic activity, but also human ER β -mediated anti-estrogenic activity in a dose-dependent manner. DEHP but not MEHP activated ER α . Neither DEHP nor MEHP activated ER β . DEHP but not MEHP had antagonistic effects on ER β . Neither DEHP nor MEHP was antagonistic towards human AR.

[Takeshita et al. \(2006\)](#) tested the hypothesis that leaching of DEHP during parenteral chemotherapy for cancer patients may facilitate PXR-mediated multidrug resistance 1 (*MDR1*) expression in various tissues, including cancer cells, which may promote drug resistance. DEHP was studied in the human colon adenocarcinoma-derived cell line, LS174T, which endogenously expresses PXR, and increased PXR-mediated transcription of the *MDR1* gene in luciferase-reporter assays. The induction by DEHP was abrogated when a reporter plasmid containing a mutated DR+4 motif in the XRE was used. In a mammalian two-hybrid assay, DEHP recruited steroid receptor co-activator-1 to the ligand-binding domain of PXR. Using real-time reverse transcriptase-polymerase chain reaction (RT-PCR), DEHP increased *MDR1* gene expression in a dose-dependent manner. The data support the activation of the *MDR1* gene by DEHP through PXR.

DEHP activated human PXR in stably transfected HGPXR cells, that are derived from HeLa cells and express luciferase under the control of a

chimeric human PXR at an EC_{50} value of 2.5 μ M. DEHP also induced CYP3A4 and -2B6 expression in two preparations of primary cultured human hepatocytes at 10 μ M (eight- and 38-fold for CYP3A4 and two- and fourfold for CYP2B6). The activation of CYP2B6 could also indicate the activation of the CAR ([Mnif *et al.*, 2007](#)).

[Krüger *et al.* \(2008\)](#) determined the effect of several chemicals, including DEHP, alone or in mixtures of selected compounds, on the human aryl hydrocarbon receptor (AhR) and human AR function using chemically activated luciferase gene expression (CALUX) bioassays in recombinant mouse hepatoma Hepa1.12cR cells (AhR-CALUX) or in transiently transfected Chinese hamster ovary cells (AR-CALUX). Weakly induced AhR activities were observed for DEHP, reaching 1.75-fold above the solvent control at the highest concentration tested (100 μ M). DEHP did not affect AR activation in this assay.

DEHP and MEHP induced PXR-mediated transcription of the CYP3A4 promoter in a dose-dependent fashion in HepG2 cells. Co-exposure to either MEHP or DEHP and dexamethasone resulted in enhanced CYP3A4 promoter activity. This induction was abrogated by both the glucocorticoid receptor antagonist, RU486, and glucocorticoid receptor small interfering RNA. Dexamethasone induced PXR protein expression in human hepatocytes and a liver-derived rat cell line, H4IIE-C3. CYP3A4 protein was strongly induced by co-administration of dexamethasone and DEHP in human primary hepatocyte cultures. Enhanced 6β -hydroxytestosterone formation in human primary hepatocytes co-treated with dexamethasone and DEHP or MEHP confirmed CYP3A4 enzyme induction. Concomitant exposure to glucocorticoids and phthalates resulted in enhanced metabolic activity of CYP3A4, which may play a role in the altered efficacy of pharmaceutical agents ([Cooper *et al.*, 2008](#)).

[DeKeyser *et al.* \(2009\)](#) examined a novel CAR — CAR2 — that, unlike the constitutively active reference form of the receptor, is a ligand-activated receptor that comprises approximately 30% of the reference transcript levels in human hepatocytes. CAR2 transcripts are not generated in mice, rats or marmosets. CAR2 was activated in cells transfected with the receptor at an EC_{50} of 211 nM [0.085 μ g/mL] DEHP. The authors reported that MEHP was a weak CAR2 activator at 10 μ M [2.8 μ g/mL] in their in-vitro reporter assays and concluded that the parent compound DEHP is the most active modulator of CAR2. Primary human hepatocyte cultures from three donors, cultured in such a way as to preserve differentiation, had very different responses to CAR-responsive enzyme (CYP2B6 and CYP3A4) mRNA induction. The concentrations of DEHP that induced these enzymes were 0.039 μ g/mL, 0.39 μ g/mL and 19.5 μ g/mL for the three donors. [Cytotoxicity was not noted.]

Induction of CYP2B6 gene expression, indicative of CAR activation in primary human hepatocyte cultures [culture conditions not specified], was increased in cultures from two patients exposed to 19.5 μ g/mL DEHP ([Eveillard *et al.*, 2009](#)). [Cytotoxicity was not noted.]

[Rotroff *et al.* \(2010\)](#) examined the ability of DEHP to activate several response elements in a multiplex transcription factor assay in HepG2 cells. DEHP significantly activated at the following elements indicative of CAR and/or PXR activation (EC_{50} values in μ M): PXRE_CIS (37), PXR_TRANS (38), CAR_TRANS (50) ([Martin *et al.*, 2010](#); <http://actor.epa.gov/actor/faces/ToxCastDB/Assay.jsp>).

[Rotroff *et al.* \(2010\)](#) also examined the ability of DEHP to activate several genes associated with the activation of PPAR α , CAR, PXR, farnesoid X receptor and AhR. Usually one preparation of primary hepatocytes from one human donor was used. Cells were treated with a range of doses and harvested after 6, 24 and 48 hours. DEHP significantly increased the expression of the

CAR-responsive gene *CYP2B6* only at all three time-points.

[DeKeyser et al. \(2011\)](#) examined the interaction of alternatively spliced human CARs and PXR with a range of suspected endocrine disruptors, including phthalates. Transactivation studies in COS-1 cells revealed that DEHP had EC₅₀ values for the activation of CAR2 and PXR of 0.1 μM and 3.8 μM, respectively. DEHP does not activate the CAR3 isoform. Studies with primary human hepatocytes showed DEHP induced *CYP2B6* and *CYP3A4* expression. Mutation analysis of CAR2, in-silico modelling and ligand docking studies suggested that the SPTV amino acid insertion of CAR2 creates a unique ligand-binding pocket, and that this alternative gene splicing results in variant CARs that selectively recognize phthalates.

(ii) Experimental systems

Transgenic mice with hepatocyte-specific constitutively active *Ppara* in the absence of ligand were generated and observed for up to 11 months of age ([Yang et al., 2007](#)). In absence of treatment, these transgenic mice exhibited various responses that mimic wild-type mice treated with peroxisome proliferators, including a significant decrease in serum fatty acids, and numerous liver effects: hepatomegaly, hepatocyte hypertrophy, increased rate of cell proliferation, marked induction of *Ppara* target genes encoding fatty acid oxidation enzymes and increased accumulation of triglycerides. Although these phenotypic changes were similar (and of comparable magnitude) to those induced in wild-type mice by the potent peroxisome proliferator WY-14 643 (0.1% w/w diet), no liver tumours were detected in untreated transgenic mice by 11 months of age whereas all treated wild-type mice developed liver tumours.

Two additional mouse models have been created to evaluate the role of PPARα in responses to peroxisome proliferators. One study used hepatocyte transplantation to generate chimeric

livers composed of *Ppara*-null and -positive hepatocytes in *Ppara*-null or -positive mice ([Weglarz & Sandgren, 2004](#)). The relationship between *Ppara* status and the ability of hepatocytes to proliferate in response to WY-14 643 was examined *in vivo*. When treated with WY-14 643 for 7 days, both *Ppara*-null and -positive hepatocytes in chimeric livers displayed elevated DNA synthesis regardless of host receptor status, as long as at least some hepatocytes contained the receptor. These findings suggest that the mitogenic response to peroxisome proliferators does not require the presence of active PPARα in all hepatocytes.

A transgenic mouse line that overexpresses human *Ppara* in a *Ppara*-null mouse was used in subchronic studies with WY-14 643 and fenofibrate ([Cheung et al., 2004](#)) and a chronic feeding study with WY-14 643 ([Morimura et al., 2006](#)). In these studies, PPARα-humanized mice did not exhibit hepatocellular proliferation, hepatomegaly or liver tumours when treated with peroxisome proliferators; however, typical markers of fatty acid β-oxidation were induced. [These mouse models have not been evaluated with DEHP and no toxicity markers were evaluated in the reports.]

Groups of 18–22-week-old male *Ppara*-null mice and corresponding wild-type mice (C57BL/6J strain) were treated with DEHP (0, 20 or 200 mg/kg bw per day) by gavage for 21 days ([Eveillard et al., 2009](#)). While this study did not report on liver weight or liver histopathology, gene expression profiling was performed on liver tissues, and the authors reported that several prototypic Car target genes were induced by DEHP in *Ppara*-null mice. There is only weak evidence that DEHP activates mouse AhR, although *Cyp1a1*, a marker gene for AhR, was increased in *Ppara*-null mice but not wild-type mice in this study.

[Ren et al. \(2010\)](#) identified PPARα and CAR as targets of DEHP indirectly through meta-analysis of transcript profiles of livers from rats

treated with nuclear receptor activators, and directly through transcriptional analysis in wild-type mice and mice nullizygous for these nuclear receptors. Microarray analysis showed an overlap in the profiles of DEHP-, valproic acid- and clofibrate-treated rats with a classical activator of CAR (phenobarbital) and, to a lesser extent, an activator of PXR (pregnenolone-16- α -carbonitrile). The overlapping genes included *CYP* gene families that are often considered to be signature genes for nuclear receptor activation. In addition, groups of wild-type, *Ppara*-null and *Car*-null mice were treated with 0, 200 or 1150 mg/kg bw DEHP in corn oil by gavage daily for 4 days and liver gene expression was compared. A microarray gene expression comparison of DEHP-treated wild-type and *Ppara*-null mice revealed that PPAR α was required for ~94% of all transcriptional changes in wild-type mice. The remaining 6% of the genes were dominated by those involved in xenobiotic metabolism and are known target genes of CAR or PXR ([Stanley et al., 2006](#); [Timsit & Negishi, 2007](#)), and those involved in cholesterol biosynthesis and are regulated by several transcription factors including the retinoid X receptor ([Anderson et al., 2004a](#)). Xenobiotic metabolism enzymes, including *Cyp2b10*, *Cyp3a11* and *Cyp3a41a*, as well as metallothionein-1 (*Mt1*) were induced by DEHP partially or completely, depending on CAR, but not PPAR α as determined in wild-type mice and mice nullizygous for *Ppara* or *Car*. The expression of the *Car* gene itself was increased by DEHP in *Ppara*-null but not in wild-type mice. Several putative CAR and PXR targets exhibited PPAR α - and CAR-independent induction, including *Cyp8b1*, GST M4 (*Gstm4*) and *Gstm7*. It was concluded that DEHP requires CAR for the induction of a small subset of genes (compared with PPAR α) and that some liver transcriptional effects may be PPAR α -independent.

[Kim et al. \(2010\)](#) examined the effects of DEHP on nuclear receptor expression and phospholipase D (PLD), an enzyme that

catalyses the hydrolysis of phosphatidyl choline to generate phosphatidic acid and choline. PLD is believed to play an important role in cell proliferation, survival signalling, cell transformation and tumour progression. DEHP (500 mg/kg bw per day) was administered orally to prepubertal rats (4 weeks of age) for 1, 7 or 28 days. Protein expression levels of PLD1/2, PPAR and CYP were determined by Western blot analysis using specific antibodies. Liver weight was significantly increased in the DEHP-treated groups. A significant rise in PLD1/2 expression was observed in the liver of DEHP-exposed rats after 7 days. The authors stated that PPAR α , CAR, PXR and CYP2B1 protein expression levels were markedly elevated in DEHP-treated groups. [The Working Group noted that no quantitation of these data was performed.]

[Hurst & Waxman \(2004\)](#) investigated the effects of phthalates on the PXR, which mediates the induction of enzymes involved in steroid metabolism and xenobiotic detoxification. The ability of phthalate monoesters to activate PXR-mediated transcription was assayed in a HepG2 cell reporter assay following transfection with mouse PXR (mPXR), human PXR (hPXR) or the *hPXR* allelic variants V140M, D163G and A370T. MEHP increased the transcriptional activity of both mPXR and hPXR (five- and 15-fold, respectively) with EC₅₀ values of 7–8 μ M. hPXR-V140M and hPXR-A370T exhibited patterns of phthalate responses similar to the wild-type receptor. In contrast, hPXR-D163G was unresponsive to all phthalate monoesters tested.

[Baldwin & Roling \(2009\)](#) performed CAR transactivation assays using mouse CAR in HepG2 cells using a variety of environmental chemicals, steroid hormones and bile acids at 10 μ M. 1,4-Bis-2-(3,5-dichloropyridyloxy)benzene activated dihydroandrosterone-repressed CAR activity nearly fourfold. MEHP also increased CAR activity more than 3.9-fold at 100 μ M.

[Ghisari & Bonefeld-Jorgensen \(2009\)](#) investigated the thyroid hormone-like activities *in vitro* of a range of widely used plasticizers and phenols, including DEHP. Thyroid hormone-disrupting potential was determined by the effect on the proliferation of thyroid hormone-dependent rat pituitary GH3 cells using the T-screen assay. All of the compounds tested, including DEHP, significantly affected GH3 cell proliferation at concentrations below levels that were cytotoxic.

(iii) *Species differences*

[DeKeyser et al. \(2009\)](#) evaluated whether mice, rats and marmosets could generate the *Car2* transcript that encodes the DEHP-responsive isoform of CAR. Protein sequences were retrieved from the National Center for Biotechnology Information database and aligned. The results showed that the rhesus monkey sequence included a four-amino acid insertion very similar to that of human CAR2, suggesting that the splice variant is conserved across multiple species. To determine whether other species could generate similar transcripts, a genomic alignment was performed for three species of rodents and six primates using the University of California Santa Cruz genome browser with mouse CAR as the reference sequence. Although the splice acceptor site for CAR1 was conserved across each species, the CAR2 site was not conserved in marmosets, mice or rats, indicating that these species are incapable of generating a CAR2-like, four-amino acid insertion protein.

(d) *Effects of DEHP on PPAR-independent metabolism*

(i) *Humans*

Primary human hepatocytes, cultured in such a way as to preserve differentiation, had increased CYP3A4 protein levels after exposure for 3 days to 2.0 µg/mL (5 µM) DEHP. This effect was greatly enhanced by co-incubation with 0.01 µM dexamethasone. Testosterone 6β-hydroxylase activity, a measure of CYP3A4

activity, was only slightly increased by exposure to 195 µg/mL (500 µM) DEHP; in the presence of 0.1 µM dexamethasone, however, this activity was considerably increased at 19.5 µg/mL (50 µM). Similarly, testosterone hydroxylase activity was not increased by 14 µg/mL (50 µM) MEHP in primary cultures of human hepatocytes but was increased by 1.4 µg/mL MEHP (5 µM) in the presence of dexamethasone. Cytotoxicity was not noted at these concentrations ([Cooper et al., 2008](#); see Section 4.2.2(c)).

The ability of DEHP to activate CAR-dependent target genes was examined in primary hepatocyte cultures derived from two patients. DEHP dose-dependently increased the expression of CYP2B6, a human homologue and a CAR target, at 50 and 100 µM ([Eveillard et al., 2009](#)).

[Kang & Lee \(2005\)](#) reported increased expression of ERβ in MCF7 human breast-cancer cells exposed to DEHP (3.9 µg/mL; 10⁻⁵ M).

Expression of *MDR1* in the human colon adenocarcinoma-derived LS174T cell line was increased after a 24-hour exposure to DEHP at 0.39 µg/mL (10⁻⁶ M) ([Takeshita et al., 2006](#)).

Increased expression of matrix metalloproteinases -2 and -9, and reduced tissue inhibitor of matrix metalloproteinase-2 mRNA and protein expression were observed in human neuroblastoma SK-N-SH cells treated with 50 µM DEHP. DEHP-induced phosphorylation at Ser473 of the serine/threonine kinase protein was also noted. Cytotoxicity was not reported ([Zhu et al., 2010](#)).

[Turan et al. \(2008\)](#) reported the results of a genome-wide gene-expression analysis based on steady-state mRNA levels in human medulloblastoma TE671 cells exposed for 24 hours to DEHP (0.2 µg/mL; 0.5 µM) *in vitro*. In a microarray of 47 000 transcripts and variants, DEHP caused alterations in 6.2% of transcripts with an overall reduction in mRNAs from genes associated with cell proliferation/survival, signal transduction, development/growth and various other categories, including those associated with

oxidation resistance, histone deacetylase 3 and human α -catenin. RT-PCR confirmed the reduction of gene expression for several selected genes. Increased gene expression was only observed for ER-60 protease, which was confirmed by RT-PCR. Cytotoxicity was not reported.

(ii) *Experimental systems*

[Tomonari et al. \(2006\)](#) treated male and female marmosets daily by gavage with 0, 100, 500 or 2500 mg/kg bw DEHP for 65 weeks from weaning (3 months of age) to sexual maturity (18 months) and examined serum hormone levels and several enzyme activities. No obvious treatment-related changes in testosterone levels were found in any treatment group, but there were large variations in individual values (serum testosterone in males varied over 100-fold). Significantly elevated levels of E2 were recorded in all female marmosets by week 65 in the 500-mg/kg group. Increased ovarian and uterine weights were observed in the two highest-dose groups of females (500 and 2500 mg/kg bw). No treatment-related differences in glutathione (GSH) content, or in the activities of sorbitol dehydrogenase, γ -glutamyl transpeptidase and GSH peroxidase were observed in the testis. GST activity and zinc content in the 100- and 500-mg/kg bw treatment groups were significantly reduced. Testosterone 6 β -hydroxylase activity (CYP3A) in the liver was significantly higher in females of the 2500-mg/kg bw group, but was not statistically significantly increased in males.

Administration of 2000 mg/kg bw DEHP for a period of 7 or 15 days to rats, mice, guinea-pigs and rabbits produced differential effects, as judged by alterations in body weight gain, liver weight and activities of mixed-function oxidases. Exposure to DEHP for 7 days caused an increase in the activities of aniline hydroxylase, arylhydrocarbon hydroxylase (guinea-pigs only) and ethylmorphine *N*-demethylase in rats, mice and guinea-pigs, but caused a decrease in these activities in rabbits. However, exposure for 15

days caused a (smaller) increase in the activity of ethylmorphine *N*-demethylase and aniline hydroxylase in rats and mice and produced a decrease in the activity of these two enzymes in guinea-pigs. The activity of arylhydrocarbon hydroxylase was decreased in all three species [data in rabbits not shown] ([Parmar et al., 1988](#)).

In mice, [Poole et al. \(2001\)](#) examined the expression of the non-specific carboxylesterases (EC.3.1.1.1), a large group of enzymes that play an important role in the metabolism of xenobiotics and endogenous lipids, including activators of PPAR α . After dietary exposure of male SV129 wild-type and *Ppara*-null mice to DEHP (0.6% in the diet) for 3 weeks, the expression of carboxylesterases ES-4 and ES-10 was downregulated in the kidney, but not in the liver of wild-type mice. The decrease in carboxylesterase expression in the kidney was not observed in *Ppara*-null mice, which demonstrates the involvement of PPAR α in these changes. These studies show that carboxylesterase ES-protein expression is under the complex control of peroxisome proliferators.

Decreased liver lysosomal enzyme activity was eliminated in C3H mice fed a diet containing 2% DEHP for 3 weeks. After 2 weeks, vacuolar H⁺-adenine triphosphatase (V-ATPase) was decreased and at 3 weeks the liver lysosomal compartment was completely negative for this enzyme, as shown by immunoblot analysis. Enzyme cytochemical staining showed that acid phosphatase was present in lysosomes; the number of late autophagosomes containing this enzyme was increased after DEHP treatment. These data suggest that the DEHP-induced reduction in V-ATPase in the lysosomal compartment of the liver may result in an inability to degrade excess cell organelles ([Wang et al., 2001](#)).

Nicotinamide adenine dinucleotide phosphate (NADPH)-CYP oxidoreductase (P450R) is an often rate-limiting component in CYP-dependent reactions. In male wild-type SV129 mice, P450R mRNA levels increased in the liver after exposure to DEHP. This induction

of mRNA was not observed in the liver of mice that had no functional PPAR α . In wild-type male mice, P450R protein was decreased ninefold in the liver after treatment with DEHP, but increased twofold in *Ppara*-deficient mice. This study demonstrates the complex regulation of P450R expression by DEHP at two different levels, both of which are dependent upon PPAR α : upregulation of mRNA transcript levels and downregulation of protein levels (Fan *et al.*, 2003).

Fan *et al.* (2004) reported that, in male SV129 wild-type and *Ppara*-deficient mice fed 0.6% DEHP in the diet for 3 weeks, the expression of the 6 α -testosterone hydroxylase *Cyp3a11* gene was increased in the liver. The increase caused by DEHP was PPAR α -independent.

ICR mice (6 weeks of age) were fed a niacin-free, 20%-casein diet supplemented with DEHP (0, 0.1, 0.5, 1.0 or 2%) for 21 days. The mice showed increased urinary excretion of quinolinic acid and of lower metabolites of the tryptophan-niacin pathway. This urinary excretion increased with the dose of DEHP (statistically significant at 0.5, 1.0 and 2.0%) (Ohta *et al.*, 2004).

Takashima *et al.* (2008) conducted a follow-up to the study of the carcinogenic effects of DEHP in wild-type and *Ppara*-null male mice exposed to 0.01 or 0.05% DEHP in the feed for 22 months (Ito *et al.*, 2007a); transcript profiling and RT-PCR were used to examine gene expression in the tumours from the two types of mice. Microarray analysis by RT-PCR showed the expression of five genes involved in carcinogenesis. Significant increases were observed in the expression of the growth arrest and DNA damage 45 α and apoptotic protease-activating factor 1 genes in tumour tissue versus control tissue in wild-type mice. In *Ppara*-null mice, cyclin B2 and myeloid-cell leukaemia sequence 1 genes were significantly increased in tumour tissue versus control tissue. [The Working Group noted that the differences are difficult to interpret because they could be due to treatment with DEHP or to differences between the tumours and

surrounding tissue, or both. The increase in liver tumours could be related to the higher incidence of spontaneous liver tumours in control *Ppara*-null mice compared with control wild-type mice (Howroyd *et al.*, 2004).]

Eveillard *et al.* (2009) examined whole-liver gene expression in wild-type and *Ppara*-null C57BL/6 mice exposed by gavage to 0, 20 or 200 mg/kg bw DEHP daily for 21 days ($n = 10/\text{group}$). Fatty acid homeostasis and xenobiotic metabolism were the most represented pathways in terms of altered gene expression. A total of 56 transcripts were differentially expressed between wild-type and *Ppara*-deficient control mice. DEHP (high dose) altered the expression of 49 transcripts in wild-type mice, and of 16 genes in *Ppara*-null livers. The four genes that were similarly altered in both strains encoded acyl-coenzyme A oxidase 1, ALDH family 1 subfamily 1a1, aminolevulinic acid synthase 1 and *Cyp2c29*. Thus, many of the genes were altered by DEHP in wild-type, but not in *Ppara*-deficient mice.

Male F344 rats fed diets containing 1.2% DEHP for 4, 8 or 16 weeks had significantly increased E2 concentrations in serum, but ER activity in the liver was strongly reduced. These rats also showed a significant loss of hepatic activity of the male estrogen-metabolizing enzyme, estrogen 2-hydroxylase, and of the male-specific estrogen-sequestering protein. In contrast, the expression of mRNAs for ER and for the oncogene *fos* (but not *myc* or *ras*) increased significantly after exposure to DEHP (8 and 16 weeks only), as did the expression of the proliferating cell nuclear antigen (Eagon *et al.*, 1994).

Seo *et al.* (2004) examined the differences in oxidative damage caused by DEHP and three other phthalates and correlations between changes in Phase-I and Phase-II enzymes in groups of 8–10 male Sprague-Dawley rats given daily doses of 50, 200 or 1000 mg/kg bw in corn oil for 14 days. The peroxisomal markers, cyanide-insensitive palmytoyl-coenzyme A oxidation and CAT, were significantly increased at all doses.

Malondialdehyde concentrations in the liver were substantially increased at all doses, while oxidative damage in DNA (8-hydroxydeoxyguanosine; 8-OH-dG) was moderately increased at the highest dose only. CYP1A2 and -3A4, UGT and GST activities were decreased at the two highest doses, with no correlation between inhibitory effects on metabolizing enzymes and peroxisome proliferation. DEHP-induced effects on xenobiotic metabolizing enzymes may thus be independent of peroxisomal proliferation and oxidative stress.

Prepubertal Wistar male rats (4 weeks of age) received an oral dose of DEHP (100 or 1000 mg/kg bw) daily for 5 days. This treatment induced a significant reduction in the activity of cytosolic phospholipase A₂, the rate-limiting enzyme in the synthesis of arachidonic acid and eicosanoids, in the testis. There was increased expression of 12-lipoxygenase in the testis, but no change in the activity of cyclo-oxygenase-2. DEHP increased CYP4A1 expression in the testis in a dose-dependent manner. A dose-related decrease in serum testosterone was statistically significant at 1000 mg/kg bw. Concentrations of arachidonic acid in serum were significantly decreased at both doses. Testicular atrophy (62% reduction in testis weight) was observed at the 1000-mg/kg bw dose with no significant change in body weight ([Kim et al., 2004a](#)).

Changes in expression of several proteins that regulate the homeostasis of essential fatty acids were studied in female Sprague-Dawley rats treated with oral doses of DEHP (750 or 1500 mg/kg bw per day) on gestational days 0–19. Expression of PPAR α , PPAR γ , fatty acid translocase, fatty acid transport protein 1, heart cytoplasmic fatty acid-binding protein and CYP4A1 were upregulated in the placenta while cyclo-oxygenase-2 was downregulated ([Xu et al., 2008](#)).

[van Ravenzwaay et al. \(2010\)](#) examined the metabolite profile in plasma of fasted male and female Wistar rats exposed to DEHP (3000 ppm in the diet) in 28-day studies. Induction

of cyanide-insensitive palmitoyl-coenzyme A oxidation per gram of liver was greater in males than in females.

(iii) *Species differences*

The ability of MEHP to induce CYP1A1, a known AhR target gene, was demonstrated in human CaCo-2 (derived from colon adenocarcinoma), HepG2 (hepatoma) and A549 (lung adenocarcinoma) cell lines, as well as primary human keratinocytes ([Sérée et al., 2004](#)). The induction specifically involved PPAR α and required two PPRE sites that were located within the CYP1A1 promoter. Whether this also occurs in normal human hepatocytes has yet to be established. CYP1A1 is a major CYP isoform that is responsible for the bioactivation of many environmental pro-carcinogens. Compared with hepatocytes from control rats, those isolated from rats treated with DEHP and other peroxisome proliferators showed enhanced DNA-adduct formation when incubated with benzo[*a*]pyrene-7,8-dihydrodiol, a proximate carcinogenic metabolite of benzo[*a*]pyrene. This enhanced adduct formation was the result of the higher activity of CYP1A1 in DEHP-treated animals ([Voskoboinik et al., 1997](#)). Thus, the fact that the regulation of CYP1A1 by MEHP is mediated by PPAR α in human cells may have significant implications; however, other studies have shown that another peroxisome proliferator, clofibrate, inhibits expression of CYP1A1 and CYP1A2 in the liver of treated rats ([Shaban et al., 2004](#)).

(e) *Cell proliferation*

(i) *Humans*

No data on the effects of DEHP on cell proliferation in the human liver were available to the Working Group.

Studies with cultured human hepatocytes failed to produce evidence of increased peroxisome proliferation after exposure to DEHP. Some of these studies directly compared the results in human hepatocytes exposed to comparable

doses of DEHP ([Goll et al., 1999](#); [Hasmall et al., 1999](#); [Roberts, 1999](#)).

[The Working Group noted that only a limited number of human donors were sampled in these studies, which may have affected the significance levels of the results due to presumably higher genetic variability in the human population. In addition, the unknown condition of the livers available for the isolation of hepatocytes may be considered as a confounding variable.]

(ii) Experimental systems

Groups of four cynomolgus monkeys received di-isononyl phthalate (DINP; 500 mg/kg bw), DEHP (500 mg/kg bw) or vehicle (0.5% methyl cellulose, 10 mL/kg bw) by intragastric intubation daily for 14 consecutive days. Clofibrate (250 mg/kg bw per day) was used as a reference substance, because of its peroxisome proliferation-related effects in the liver of rodents ([Doull et al., 1999](#)). There were no changes in any of the hepatic markers for peroxisomal proliferation, including peroxisomal β -oxidation and replicative DNA synthesis. None of the test chemicals – including the reference substance – produced any toxicologically important changes in urine analysis, haematology or clinical chemistry ([Pugh et al., 2000](#)).

DEHP (1.2% in the diet) and WY-14 643 (0.1% in the diet) were fed to male F344 rats for up to 365 days. At the end of this period, all rats fed WY-14 643 had numerous grossly visible nodules in the liver, while no nodules were seen in DEHP-fed animals or in controls. Despite this difference, both DEHP and WY-14 643 increased the peroxisomal volume density (percentage of cytoplasm occupied by peroxisomes) four- to sixfold during the treatment. Activities of enzymes involved in peroxisome proliferation and β -oxidation were increased eightfold by both DEHP and WY-14 643 during the first 18 days of treatment. These enzyme activities remained about 25% higher in the livers of WY-14 643-fed rats than in rats that received DEHP. DEHP or

WY-14 643 increased absolute liver weights by 50–75% above those of controls. Labelling of the hepatocyte nuclei with a [^3H]thymidine pulse given 2 hours before the animals were killed revealed a rapid increase in DNA replication in both groups of rats, with a labelling index that reached a maximum on day 2 for the DEHP-treated group and on day 1 for the WY-14 643-treated group. The labelling index returned to control levels by day 4 in both groups. Implantation of 7-day osmotic pumps containing [^3H] thymidine showed a five- to 10-fold increase in replicative DNA synthesis in rats receiving WY-14 643 for 39–365 days. DEHP induced a four- to fivefold increase in hepatocyte proliferation on day 8. The ability of these two agents to induce a persistent increase in replicative DNA synthesis correlated with their relative strengths as hepatocarcinogens in rodents ([Marsman et al., 1988](#)).

A diet containing 6000 ppm DEHP was given to male B6C3F₁ mice for 7 days. Pulse-labelling with [^3H]thymidine resulted in a sevenfold increase in replicative DNA synthesis in hepatocytes, while no increase was seen when the animals had been fed for 14 or 28 days ([Smith-Oliver & Butterworth, 1987](#)).

Male F344 rats (7–9 weeks of age) were fed a diet containing 1.2% DEHP for various periods up to 365 days. Over the entire treatment period, DEHP produced a sustained stimulation of peroxisome proliferation, as demonstrated by cyanide-insensitive palmitoyl-coenzyme A oxidation activity and peroxisome morphometry. DEHP caused a threefold increase in the level of lipofuscin — a marker of oxidative stress — after 39 days of treatment; this level decreased during the rest of the treatment period but remained higher than that in the controls ([Conway et al., 1989](#)).

Flow cytometry was used to investigate the effects of DEHP, chlorendic acid and 1,4-dichlorobenzene on hepatocyte ploidy, nuclearity and labelling-index distribution. Male F344 rats received 12 000 ppm DEHP in the diet for 7 days. The dose and route of administration

corresponded with those used in the NTP cancer bioassays. DEHP increased the mean hepatic labelling index ($23 \pm 3\%$ compared with $1.4 \pm 0.4\%$ in controls). This index was increased in all hepatocyte ploidy/nuclearity classes except the binucleated tetraploid cells and was highest in the mononucleated octoploid population ($49 \pm 14\%$ versus $1.3 \pm 0.4\%$ of octoploid hepatocytes for DEHP and controls, respectively). DEHP tended to induce DNA synthesis in a greater proportion of diploid and binucleated tetraploid cells ([Hasmall & Roberts, 1997](#)).

Oral administration of DEHP (1150 mg/kg bw per day for 2 days) in corn oil to male B6C3F₁ mice resulted in a 2.4-fold increase in replicative DNA synthesis, measured by immunochemical detection of bromodeoxyuridine incorporated into newly synthesized DNA. In a similar experiment in male F344 rats that were given 950 mg/kg bw DEHP for 2 days, the increase in replicative DNA synthesis was more than 13-fold ([James et al., 1998a](#)).

Male and female F344 rats and B6C3F₁ mice were fed a diet containing DEHP for up to 13 weeks ([David et al., 1999](#)). In rats that received 12 500 ppm DEHP, there was an increase in hepatocyte replicative DNA synthesis (measured after continuous bromodeoxyuridine administration for 3 days before sampling) after 1 week (but not after 2 or 13 weeks) and an increase in hepatic peroxisomal β -oxidation (palmitoyl-coenzyme A oxidation) activity after 1, 2 and 13 weeks of treatment. In mice fed 10 000 and 17 500 ppm DEHP, there was no increase in hepatocyte replicative DNA synthesis at any time-point. However, at these two dose levels, there was an increase in hepatic peroxisomal β -oxidation activity after 1, 2 and 13 weeks, which was not observed at the 1000-ppm dose.

The response of mice transgenic for hepatocyte-specific expression of a constitutively activated form of PPAR α (VP16PPAR α) was compared with that in wild-type mice that were treated with WY-14 643, a PPAR α ligand.

Expression of VP16PPAR α in the transgenic animals led to increases in hepatocyte proliferation in the absence of non-parenchymal cell proliferation. In contrast, treatment with WY-14 643 led to increased replication of both hepatocytes and non-parenchymal cells. Importantly, chronic activation of VP16PPAR α did not increase the incidence of liver tumours in transgenic mice ([Yang et al., 2007](#)). [The Working Group noted that these results indicate that non-parenchymal cell activation is important for hepatocarcinogenesis and that PPAR α -mediated hepatocyte proliferation by itself is not sufficient to induce liver cancer.]

Daily oral doses of 500 mg/kg bw DEHP were given to prepubertal rats (4 weeks of age, weighing approximately 70–90 g) for 1, 7 or 28 days. Liver weight was significantly increased in the DEHP-treated groups compared with controls. Immunohistochemical analysis demonstrated that DEHP caused strong staining of proliferating cell nuclear antigen after 28 days of exposure, suggestive of hepatocyte proliferation ([Kim et al., 2010](#)). [The Working Group noted the lack of quantification of the antigen staining.]

Rat hepatocyte cultures were treated with various peroxisome proliferators at 100–500 μ M for 72 hours. Dependent on the agent used, there was an increase in acyl-coenzyme A oxidase and CAT activities, markers of peroxisome proliferation, with the following potencies: ciprofibrate = nafenopin > bezafibrate > clofibrate > DEHP (negative for acyl-coenzyme A oxidase). DEHP induced a concentration-dependent increase in DNA synthesis (measured as incorporation of bromodeoxyuridine) and a decrease in spontaneous apoptosis after 48 hours of treatment, with no dose-response. The reduced apoptosis was also observed morphologically. Furthermore, DEHP inhibited apoptosis induced by transforming growth factor β (TGF β) but not that induced by tumour necrosis factor α / α amanitine (TNF α / α Ama) ([Goll et al., 1999](#)).

[Hasmall et al. \(1999\)](#) examined differences in the response of rat and human hepatocytes to MEHP – the principal metabolite of DEHP – and DINP *in vitro*. In rat hepatocytes, both DINP and MEHP caused a concentration-dependent induction of DNA synthesis and suppression of both spontaneous apoptosis and apoptosis induced by TGF β 1. Similarly, both compounds caused a concentration-dependent induction of peroxisomal β -oxidation, although the response to DINP was weaker. None of these effects were seen in human hepatocytes.

[The Working Group noted that peroxisome proliferators have not been shown to exhibit a marked effect on replicative DNA synthesis *in vitro* in purified hepatocyte cultures. The magnitude of such a response is much lower than that seen in rat and mouse liver *in vivo* and it has been hypothesized that other cells in the liver (e.g. Kupffer cells) may play an important role in potentiating the proliferative response of the hepatocytes by producing mitogenic cytokines. It was also noted that the human liver may be more refractory than the rodent liver to mitogenic stimuli.]

(iii) Species differences

Male F344 rats and male Dunkin-Hartley guinea-pigs were given 950 mg/kg bw per day DEHP by gavage for 4 days ([Hasmall et al., 2000b](#)). Significant increases in liver weight, hepatic β -oxidation activity and hepatocyte DNA replication, and reductions in hepatocyte apoptosis were observed in rats but not guinea-pigs.

(f) Apoptosis

(i) Humans

Cultured human hepatocytes are non-responsive to the anti-apoptotic activities of DEHP, its principal metabolite MEHP and DINP ([Hasmall et al., 1999](#)). DINP and MEHP did not cause induction of β -oxidation, stimulation of DNA synthesis or suppression of apoptosis in cultured human hepatocytes obtained from three

separate donors. These effects had been observed in rat hepatocytes. The lack of an effect in the human cells was consistent for both spontaneous and cytokine-induced (TGF β 1 or TNF α / α -Ama) apoptosis. [The Working Group noted that the human hepatocyte cultures used in these experiments may have been contaminated with up to 5% of non-parenchymal cells ([Goll et al., 1999](#)).]

Viability of human monocytic leukaemia U937 cells was decreased after 20 hours of exposure to MEHP (range, 150–1000 μ M), with concurrent increases in DNA fragmentation ([Yokoyama et al., 2003](#)). Treatment with MEHP increased caspase-3 activity, which was diminished by 50% by pretreatment with selective PPAR γ inhibitors and antagonists (BADGE, GW9662) but was increased twofold by pretreatment with a PPAR γ ligand (rosiglitazone). A PPAR α antagonist (MK886) had no effect on MEHP-induced caspase-3 activity. In these MEHP-treated U937 cells, the mRNA levels were decreased for B-cell lymphoma-2 (Bcl-2) protein and increased for Bcl-2-associated X (BaX) protein, resulting in a decrease in the Bcl-2/BaX protein ratio.

(ii) Experimental systems

Mice were given 1150 mg/kg bw DEHP per day by gavage in corn oil for 2 days. The treatment resulted in a significant reduction in both spontaneous and TGF β 1-induced apoptosis and a strong induction of DNA synthesis ([James et al., 1998a](#)). In the same study, rats were given 950 mg/kg bw DEHP per day by gavage in corn oil for 2 days. The treatment induced DNA synthesis and suppressed both spontaneous and TGF β 1-induced apoptosis in the hepatocytes. Apoptosis was reduced to undetectable levels in three of five animals. The same authors reported that MEHP was able to suppress apoptosis and induce DNA synthesis in mouse hepatocytes. Those effects were comparable with those observed in rat hepatocytes ([James et al., 1998a](#)).

MEHP (500 μ M) or exogenous TNF α (5000 U/mL) induced hepatocyte proliferation and

suppressed apoptosis in mouse primary hepatocytes ([Hasmall et al., 2002](#)).

[Kim et al. \(2004b\)](#) reported that exposure to DEHP increased the proliferation of MCF-7 (ER-positive) cells but not MDA-MB-231 (ER-negative) cells at the same concentration. DEHP mimicked estrogen in the inhibition of tamoxifen-induced apoptosis in MCF-7 cells, measured by the TUNEL assay. Bcl-2/BaX ratios were decreased by treatment with tamoxifen. Pre-incubation of the MCF-7 cells with DEHP before exposure to tamoxifen reduced this decrease in Bcl-2/BaX ratio.

The survival of human cultured NCTC 2554 keratinocytes was decreased by DEHP-induced necrosis (not apoptosis) at 97 µg/mL after 4 hours of exposure and 20 µg/mL after 24 or 48 hours of exposure ([Martinasso et al., 2006](#)). Necrosis was inhibited by the addition of an antisense oligonucleotide against PPARβ showing the involvement of PPARβ in this effect. Western blot analysis showed decreased expression of the proteins pErk1, Erk2 and c-myc and increased the expression of PPARβ with increasing doses of DEHP. After an initial increase, PPARα expression was reduced at higher doses of DEHP.

(g) Oxidative stress

(i) Humans

No data were available to the Working Group.

(ii) Experimental systems

Several studies have been carried out in rats given DEHP in which end-points indicative of oxidative damage in the liver were measured. These include increases in lipofuscin ([Rao et al., 1982, 1987](#); [Hinton et al., 1986](#); [Cattley et al., 1987](#); [Lake et al., 1987](#); [Conway et al., 1989](#)) and malondialdehyde ([Seo et al., 2004](#)).

Male Sprague-Dawley rats were fed a diet containing 2% DEHP for 2 years ([Lake et al., 1987](#)). Levels of conjugated dienes indicative of lipid peroxidation were increased in liver homogenates, and morphological examination

of liver sections revealed increased lipofuscin deposition in non-nodular but not in nodular areas of the liver.

[Takagi et al. \(1990a, b\)](#) investigated the relationship between hepatic peroxisome proliferation and levels of 8-OHdG in hepatic DNA. Male F344 rats (6 weeks of age) were fed 1.2% DEHP in the diet for periods of 1–12 months. Treatment with DEHP resulted in sustained stimulation of cyanide-insensitive palmitoyl-coenzyme A activity and produced up to a twofold increase in levels of 8-OHdG in hepatic DNA.

[Rusyn et al. \(2001\)](#) used the spin-trapping technique and electron spin resonance spectroscopy to provide evidence of oxidative stress in liver of rats administered DEHP acutely. The spin trapping agent α-(4-pyridyl-1-oxide)-*N*-tert-butyl nitron (POBN) was also administered and bile samples were collected for 4 hours. Under these conditions, the intensity of the six-line radical adduct signal increased to a maximum value of 2.5-fold 2 hours after administration of DEHP, before peroxisomal oxidases were induced. Furthermore, DEHP given with [¹³C₂] dimethyl sulfoxide produced a 12-line electron spin resonance spectrum, providing evidence that DEHP stimulates hydroxyl radical formation *in vivo*. Furthermore, when rats were pretreated with dietary glycine, which inactivates Kupffer cells, DEHP did not increase radical signals. Moreover, similar treatments were performed in knockout mice deficient in NADPH oxidase (p47^{phox} subunit). Importantly, DEHP increased oxidant production in wild-type but not in NADPH oxidase-deficient mice. These data provide evidence for the hypothesis that the molecular source of free radicals induced by peroxisome proliferators is NADPH oxidase in Kupffer cells. Radical adduct formation was not affected in *Ppara* knockout mice. These observations represent in-vivo evidence that DEHP increases free radicals in liver before peroxisomal oxidases are induced.

In a follow-up study, [Woods et al. \(2007\)](#) hypothesized that continuous treatment with peroxisome proliferators will cause a sustained formation of POBN radical adducts in the liver. Mice were fed diets containing either WY-14 643 (0.05% w/w) or DEHP (0.6% w/w) for up to 3 weeks. Liver-derived radical production was assessed in bile samples by measuring POBN radical adducts using electron spin resonance. WY-14 643 caused a sustained increase in POBN radical adducts in mouse liver and this effect was greater than that of DEHP. Free radical production, induced by administration of DEHP, occurred after 2 hours and 3 weeks but not after 3 days or 1 week of exposure. To understand the molecular source of these radical species, NADPH oxidase-deficient (*p47^{phox}*-null) and *Ppara*-null mice were examined after treatment with WY-14 643. No increase in radicals was observed in *Ppara*-null mice that were treated with WY-14 643 for 3 weeks, while the response in *p47^{phox}*-null mice was similar to that in wild-type mice. These results show that PPAR α , but not NADPH oxidase, is critical for a sustained increase in POBN radical production caused by peroxisome proliferators in rodent liver. [Therefore, the production of POBN radicals induced by peroxisome proliferators in Kupffer cells may be limited to an acute response to these compounds in mouse liver.]

Male rats were administered peroxisome proliferators (DEHP, DBP and *n*-butylbenzyl phthalate: 50, 200 and 1000 mg/kg bw per day; clofibrate: 100 mg/kg bw per day) orally for 14 days, and the activities of metabolizing enzymes and peroxisomal enzymes were investigated. Oxidative damage was measured as 8-OHdG in the DNA and as 4,4'-methylenedianiline level in the liver. The four peroxisome proliferators significantly increased the relative liver weights, cyanide-insensitive palmitoyl-coenzyme A oxidation and activity of CAT. DEHP was found to be the most potent peroxisome proliferator among the three phthalates. A dramatic and dose-dependent increase in hepatic levels of

4,4'-methylenedianiline was observed in clofibrate-, DEHP- (≥ 50 mg/kg), DBP- and *n*-butylbenzyl phthalate- (≥ 200 mg/kg) treated groups. However, the level of 8-OHdG in hepatic DNA was increased only in DEHP- (1000 mg/kg) and clofibrate-treated groups ([Seo et al., 2004](#)). The authors indicate that 8-OHdG positively correlates with the carcinogenic potential of peroxisome proliferators, but other factors, as well as peroxisomal peroxidase could be involved in the generation of 8-OHdG and the carcinogenesis of peroxisome proliferators.

A chronic (22-month) feeding study with DEHP was conducted in wild-type (Sv/129 strain) and *Ppara*-null mice ([Ito et al., 2007a](#)). Groups of mice were fed diets containing 0, 0.01 or 0.05% DEHP. No significant effect on the body or liver weight, or serum alanine aminotransferase activity was observed in either dose or genotype group at the commencement of the study. Treatment-related induction of oxidative DNA damage indicated by an increase in 8-OHdG and expression of the inflammatory marker, nuclear factor κ B, were observed in both groups, although the changes were more pronounced in *Ppara*-null mice exposed to DEHP.

Primary monolayer cultures of hepatocytes isolated from male F344 rats were incubated in medium containing one of three different peroxisome proliferators and examined for the induction of peroxisomal coenzyme A oxidase activity and lipid peroxidation. The latter parameter was determined by measuring levels of conjugated dienes in lipid fractions extracted from harvested cells. The peroxisome proliferators used in these studies were nafenopin and clofibric acid (two hypolipidaemic drugs) and MEHP. The relative specific activity of peroxisomal acyl coenzyme A oxidase was increased by about 300% after incubation for 44 hours with 200 μ M (62 μ g/mL) nafenopin; lower levels of induction were observed with clofibric acid or MEHP. Relative to controls, the level of conjugated dienes was increased approximately

twofold after incubation with 200 μM (62 $\mu\text{g}/\text{mL}$) nafenopin; there was no apparent increase in conjugated dienes after incubation with up to 200 μM (55 $\mu\text{g}/\text{mL}$) MEHP or 400 μM (86 $\mu\text{g}/\text{mL}$) clofibric acid ([Tomaszewski et al., 1990](#)).

(h) *Gap-junctional intercellular communication*

(i) *Humans*

No data were available to the Working Group.

(ii) *Experimental systems*

[Pugh et al. \(2000\)](#) investigated the effects of the peroxisome proliferators DINP (500 mg/kg bw per day) and DEHP (500 mg/kg bw per day) or vehicle (0.5% methyl cellulose, 10 mL/kg) administered to male cynomolgus monkeys by intragastric intubation for 14 consecutive days. *In situ* dye transfer studies using fresh liver slices revealed that DINP and DEHP had no effect on gap-junctional intercellular communication.

[Isenberg et al. \(2000\)](#) reported inhibition of dye transfer *in situ* for liver strips from male B6C3F₁ mice treated with 500 ppm DEHP at 2 weeks and 6000 ppm at 4 weeks of exposure, and from male F334 rats treated *in vivo* with DEHP (6000 ppm in the diet) from 1 to 6 weeks. [Food consumption was not recorded and the rats and mice were fed *ad libitum*; therefore the dose in milligrams per kilogram of body weight per day could not be calculated.]

Dietary administration of DEHP at a dose of 20 000 mg/kg diet to male F344 rats for 2 weeks decreased gap-junctional intercellular communication (67% of control) and enhanced replicative DNA synthesis (4.8-fold the control). Elevation of the relative liver weight and the induction of peroxisomal β oxidation were also observed following treatment with DEHP. Following administration of DEHP to F344 rats and B6C3F₁ mice at a dose of 6000 mg/kg diet for 18 months, inhibition of gap-junctional intercellular communication persisted, and the relative liver weight and induction of peroxisomal β oxidation remained elevated in both

species. In recovery studies in which DEHP was administered to male F344 rats for 2 weeks and then withdrawn, the relative liver weight, rate of peroxisomal β oxidation, increase in replicative DNA synthesis and inhibition of gap-junctional intercellular communication returned to control values within 2–4 weeks after cessation of treatment. Recovery studies with phenobarbital produced similar results. The primary active metabolite of DEHP, MEHP, was detected in the livers of rats and mice treated with DEHP for more than 2 weeks. However, it could not be detected after withdrawal of DEHP from the diet after 2 weeks. This study demonstrated that inhibition of gap-junctional intercellular communication, together with indicators of peroxisomal proliferation, including increased relative liver weight and enhanced peroxisomal β oxidation, persist while DEHP treatment continues but are reversed when treatment is stopped. Studies with phenobarbital produced a similar pattern of response ([Isenberg et al., 2001](#)).

Inhibition of gap-junctional intercellular communication (inhibition of metabolic cooperation) in Chinese hamster V79 lung fibroblast cells was reported after exposure to DEHP *in vitro*. [Elmore et al. \(1985\)](#) reported a positive response following exposure to DEHP (5 $\mu\text{g}/\text{mL}$) in one of three experiments that did not also lead to cytotoxicity. In a limited report, [Malcolm et al. \(1983\)](#) stated that DEHP (4 $\mu\text{g}/\text{mL}$) gave positive results for this end-point. In a subsequent study, [Malcolm & Mills \(1989\)](#) reported that DEHP (1, 10, 15 $\mu\text{g}/\text{mL}$) gave a positive response in two experiments. Other studies reported positive results at higher concentrations in Chinese hamster V79 cells.

Inhibition of gap-junctional intercellular communication (inhibition of dye transfer) was also studied in SHE cells exposed to DEHP. [Mikalsen & Sanner \(1993\)](#) reported that normal SHE cells exposed to DEHP (77 μM [30 $\mu\text{g}/\text{mL}$]) for 24 hours gave positive results in the assay and that 5 days of exposure of transformed SHE

cells to DEHP (30 µg/mL) also gave a positive response. [Cruciani et al. \(1997\)](#) reported transient inhibition of dye transfer in SHE cells after exposure to DEHP (9.7 µg/mL). Therefore, metabolic cooperation in Chinese hamster V79 cells appeared to be more sensitive than dye transfer in SHE cells as a metric of gap-junctional intercellular communication after exposure to DEHP.

The only available study of gap-junctional intercellular communication following exposure to MEHP is that of [Cruciani et al. \(1997\)](#) who reported inhibition of metabolic cooperation and inhibition of dye transfer in exposed Chinese hamster V79 cells. The concentrations tested that inhibited metabolic cooperation (28, 56 and 112 µg/mL [100, 200 and 400 µM]) also decreased cell survival which was already at 60%. Cell survival was not noted in the study of inhibition of dye transfer of MEHP but 112 µg/mL (400 µM) MEHP was reported to cause this effect in V79 cells. [Cruciani et al. \(1997\)](#) also studied inhibition of dye transfer in SHE cells and reported a transient inhibition at 7 µg/mL (25 µM) MEHP.

(i) *Liver toxicity*

(i) *Humans*

The subchronic toxicity of DEHP was evaluated in 28 term infants with respiratory failure, 18 of whom received 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, although the rate of administration ranged up to 2 mg/kg bw DEHP 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 DEHP ([Karle et al., 1997](#)).

One study compared cholestasis in premature and newborn infants who received parenteral nutrition through PVC-containing or PVC-free infusion systems ([von Rettberg et al., 2009](#)). A retrospective analysis, before and after changing

from PVC-containing to PVC-free infusion systems, was conducted on two groups of 30 and 46 patients, respectively. It was found that the use of PVC-containing lines correlated strongly with the development of cholestasis ($P = 0.0004$) and that the incidence of cholestasis decreased from 50 to 13% after PVC-containing infusion systems were discontinued.

(ii) *Experimental systems*

Subchronic liver toxicity has been reported in non-human primates that were subjected to chronic transfusions through PVC tubing containing DEHP. Abnormal liver function (e.g. bromosulphophthalein clearance) and cholestasis have been reported in rhesus monkeys in chronic experiments that mimicked conditions of patients undergoing repeated blood or platelet transfusions through PVC-containing tubing, an effect that was absent when polyethylene containers were used ([Jacobson et al., 1977](#)). The average cumulative amount of DEHP infused in 1 year was 69.3 mg (or 21.3 mg/kg bw) which the authors found to be comparable or even lower than that in humans on chronic transfusion therapy.

A subsequent study in rhesus monkeys by the same group evaluated hepatic function and liver histology up to 26 months after cessation of transfusions ([Kevy & Jacobson, 1982](#)). It was reported that abnormal liver function tests and histological abnormalities (e.g. disturbances of hepatic architecture, the presence of round-cell infiltration and multinucleated giant cells) in liver biopsies persisted throughout transfusion and the follow-up period. [While these studies implicate DEHP as a potential toxic ingredient of plastic medical devices, these associations need to be verified further. Similar hepatotoxic effects of DEHP do not appear to be observed in rodents and may be a phenomenon of route of exposure (intravenous versus dietary) or may comprise another set of important species differences.]

The effects of the peroxisome proliferators DINP and DEHP were evaluated in young adult male cynomolgus monkeys, with emphasis on the detection of hepatic and other effects seen in rats and mice after treatment with high doses of phthalates. Groups of four monkeys received DINP (500 mg/kg bw per day), DEHP (500 mg/kg bw per day) or vehicle (0.5% methyl cellulose, 10 mL/kg bw) by intragastric intubation for 14 consecutive days. Clofibrate (250 mg/kg bw per day), a hypolipidaemic drug used for cholesterol reduction in human patients, was used as a reference substance. None of the test substances had any effect on body weight or liver weights. Histopathological examination of tissues from these animals revealed no distinctive treatment-related effects in the liver, kidney or testes ([Pugh et al., 2000](#)).

Diets containing 2% DEHP were fed to male Crlj:CD1(ICR) mice for 10 days (daily dose of DEHP was 0.90 ± 0.52 mg/mouse). The testes, livers, kidneys and pancreata were examined for the presence of MEHP and nitrogen oxides (NO_x) produced by the peroxidation of nitric oxide with free radicals, and lipid peroxidation induced by the chain reaction of free radicals. Histological observations and serum analyses showed the presence of liver dysfunction and dehydration. Unexpectedly, the concentration of MEHP in the testes was extremely low compared with that in the liver. However, the concentration of the NO_x in the testes was as high as the hepatic concentration. Furthermore, free radical-induced lipid peroxidation was detected histochemically in the testes but not in the liver ([Miura et al., 2007](#)). [The Working Group noted that this study did not report data on controls for NO_x . The authors state that NO_x concentrations in all four organs in control mice were less than 10 pmol/10 μL , and that those in the liver of treated mice were ~ 45 pmol/10 μL but that it was not possible to determine the extent of the increase.]

[González et al. \(2009\)](#) examined the effects of exposure to peroxisome proliferators on rat

$\alpha 2$ -macroglobulin, an important acute-phase protein, of which normal adult rats present low serum levels but pregnant rats display high amounts. To determine whether the effects were mediated by *Ppara*, wild-type mice and *Ppara*-null mice were used and treated with WY-14 643 or DEHP. WY-14 643, but not DEHP, reduced $\alpha 2$ -macroglobulin and γ -fibrinogen (another acute-phase protein) expression in the livers of wild-type mice, but had no effect in *Ppara*-null mice. WY-14 643 or DEHP did not affect expression of complement C3 protein, another acute-phase response protein.

[Anderson et al. \(1999b\)](#) examined the expression of acute-phase proteins in mouse liver after exposure to peroxisome proliferators. Mice treated with either WY-14 643 or DEHP for 3 weeks had decreased hepatic $\alpha 1$ antitrypsin expression but increased expression of ceruloplasmin and haptoglobin. *Ppara*-null mice showed no hepatic acute-phase protein gene alteration after treatment with peroxisome proliferators but had higher basal expression than did wild-type controls. It was concluded that *Ppara* activation by different peroxisome proliferators leads to dysregulation of hepatic acute-phase protein gene expression in mice.

A chronic (22-month) study was conducted in groups of wild-type (Sv/129 strain) and *Ppara*-null mice ([Ito et al., 2007a](#)) fed diets containing 0, 0.01 or 0.05% DEHP. No significant effect on the body or liver weights, or serum alanine aminotransferase activity was observed in either dose or genotype group.

A study by [Ward et al. \(1998\)](#) showed that the mean liver weight of DEHP-treated (12 000 ppm for up to 24 weeks) wild-type mice was significantly greater than that of untreated wild-type mice at all time-points. The mean liver weight of treated *Ppara*-null mice did not differ from that of untreated controls at any time-point. Livers from wild-type mice fed DEHP had marked diffuse hepatocytomegaly and cytoplasmic granular hepatocyte eosinophilia, the severity of

which was time-related. These lesions were not present in DEHP-treated *Ppara*-null mice at any time-point. Glycogen deposits were present in hepatocytes in untreated wild-type mice, while treated and untreated *Ppara*-null mice had much less glycogen present. *Ppara*-null mice on control diet developed moderate centrilobular fatty changes beginning at week 8, while no fatty changes were observed in DEHP-treated *Ppara*-null mice.

[The Working Group noted that although no studies have evaluated cholestasis due to DEHP in rats or mice, other peroxisome proliferators are known to have a protective effect against cholestasis. For example, cholestasis was observed in bezafibrate-fed *Ppara*-null, but not wild-type mice ([Hays et al., 2005](#)).]

In a 104-week feeding study of DEHP (100, 500, 1500 or 6000 ppm) in B6C3F₁ mice, an increase in the following signs of liver damage were reported: hepatocyte pigmentation, increased cytoplasmic eosinophilia and chronic inflammation were observed in all high-dose males and females. No signs of these histopathological changes were found in control and lower-dose groups ([David et al., 2000a](#)).

[Nair et al. \(1998\)](#) evaluated the systemic toxicity of DEHP (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 -glutamyl transpeptidase, lactate dehydrogenase, alanine aminotransferase and alkaline phosphatase). No evidence of toxicity was observed.

Young male and female Sprague-Dawley rats (10 per sex per group) were fed diets containing 5, 50, 500 or 5000 ppm DEHP for 13 weeks ([Poon et al., 1997](#)). Mean DEHP 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 141 and 120% of control values in male and female rats, respectively, were observed only in animals given 5000 ppm DEHP.

In a 104-week feeding study in F344 rats, significant increases in the incidence of Kupffer cell/hepatocyte pigmentation was reported in male and female rats fed 12 500 ppm DEHP and of spongiosis hepatitis in male rats fed 2500 and 12 500 ppm compared with controls ([David et al., 2000b](#)).

The hepatic effects of low and high concentrations of DEHP (1000 and 6000 ppm) were examined in male Syrian golden hamsters (refractory to peroxisome proliferator-induced tumorigenicity). A slight increase in the relative liver weight, and peroxisomal β -oxidation activity and replicative DNA synthesis in the liver was observed. However, these effects were not of the same magnitude or consistency as those observed in rats or mice. Furthermore, DEHP had no effect on gap-junctional intercellular communication in hamster liver at any of the time-points examined (2 and 4 weeks). No changes in markers of liver damage were reported ([Isenberg et al., 2000](#)).

The ability of DEHP to induce hepatic microsomal carboxylesterase isozymes in hamsters was studied by measuring hydrolase activities and by immunoblot analysis using specific antibodies. Animals were given 2% (w/w) DEHP in the diet for 7 days. No changes in markers of liver damage were reported ([Hosokawa et al., 1994](#)).

After in-vitro exposure of male Wistar rat primary hepatocytes to DEHP ([Ghosh et al., 2010](#)), decreased cell viability and increased intracellular reactive oxygen species were observed at 39 μ g/mL DEHP. The decreased cell viability was reversed by the addition of catalase. Apoptosis but not necrosis was associated with decreased cell survival at exposure to 9.8 μ g/mL DEHP and was consistent with increased caspase-3 activity and changes in mitochondrial membrane potential.

4.3.2 Non-parenchymal cell-specific events

[Rose et al. \(1999\)](#) tested the hypothesis that Kupffer cells are activated directly by peroxisome proliferators, including DEHP and MEHP. Kupffer cell superoxide production after 30 minutes of exposure was measured following treatment *in vitro*. WY-14 643 increased superoxide production in a dose-dependent manner (0.1 and 50 μM) with half-maximal stimulation at 2.5 μM . DEHP and its metabolite, 2-EH, did not increase superoxide production even at doses 50 times higher than those of WY-14 643; however, its key metabolite, MEHP, activated superoxide production as effectively as WY-14 643 with half-maximal stimulation at 5 μM . In-vivo treatment of rats with WY-14 643 in the diet (0.1%) for 21 days caused a twofold increase in Kupffer cell superoxide production while treatment with DEHP (1.2%) did not. Pretreatment of Kupffer cells with staurosporine (0.01–10 pM), an inhibitor of protein kinase C, completely blocked generation of superoxide demonstrating that protein kinase C is a prerequisite. Moreover, WY-14 643 increased Kupffer cell calcium-dependent protein kinase C activity threefold. Pretreatment of Kupffer cells with the amino acid glycine (0.01–3 mM), which blunts calcium signalling, inhibited both WY-14 643-stimulated protein kinase C activity and superoxide production completely. The authors stated that these data are consistent with the hypothesis that potent peroxisome proliferators (WY-14 643 and MEHP) activate Kupffer cell production of oxidants directly via mechanisms that involve protein kinase C.

Evidence for Kupffer cell-mediated increased oxidant free radical production *in vivo* after treatment with DEHP was obtained using a spin-trapping technique and electron spin resonance spectroscopy ([Rusyn et al., 2001](#)). Specifically, when rats were given DEHP acutely for 2 hours, a radical adduct signal was detected. No increase in the radical signal due to DEHP was observed

when Kupffer cells were inactivated *in vivo* with glycine pretreatment, or in NADPH oxidase-deficient mice (*p47^{phox}*-null mice). The authors suggested that the molecular source of free radicals induced by peroxisome proliferators is NADPH oxidase in Kupffer cells. The rapid DEHP-induced production of free radicals *in vivo* occurred long before peroxidase-generating enzymes in peroxisomes were induced, and was not dependent on PPAR α status.

A gene expression profiling study examined transcriptional changes induced by DEHP in mouse liver ([Currie et al., 2005](#)). In addition to many genes that have traditionally been associated with hepatocyte-specific responses to peroxisome proliferators, several known components of the TNF/interleukin-1 (IL-1) signalling pathways, including the IL-1 receptor-associated kinase-like 2, myeloid differentiation primary response gene 88 (inhibitor of nuclear factor κB kinase gamma) and other genes were induced very early (2 hours) and declined at later times (24 hours) after acute treatment with DEHP, consistent with other studies showing a time-course of Kupffer cell activation.

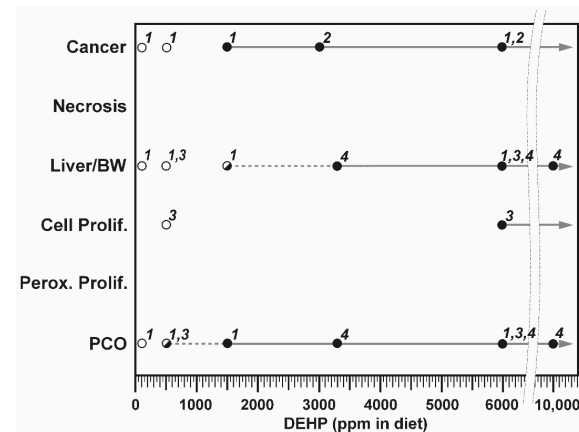
4.3.3 Dose–response for the mechanistic endpoints

Exposure to DEHP caused increases in the incidence of liver tumours in male and female B6C3F₁ mice and F344 rats ([NTP, 1982](#); [Cattley et al., 1987](#); [David et al., 1999, 2000a, b](#)). Dose–response characteristics for liver tumour induction are summarized in Fig. 4.2 and 4.3 for mice and rats, respectively. In male and female mice, consistent induction of liver tumours was observed at doses of 1500 ppm DEHP in the diet and higher ([NTP, 1982](#); [David et al., 1999](#)), and a small increase in liver tumour incidence occurred in male but not female mice at 500 ppm in one study ([David et al., 1999](#)). In rats, consistent induction of liver tumours was observed at 6000 ppm and higher ([NTP, 1982](#); [Cattley et al., 1987](#); [David](#)

et al., 1999). A minimal increase in the incidence of liver tumours occurred in female but not male rats at 100 ppm, no increase occurred in either sex at 500 ppm and an increase was observed in males but not females at 2500 ppm (David *et al.*, 1999). In a separate study, liver tumours were observed at 12 000 ppm but not at 350 ppm or 1000 ppm (Cattley *et al.*, 1987). DEHP also increased the incidence of pancreatic acinar-cell tumours in male rats but only at the highest dose (12 500 ppm) (David *et al.*, 2000b).

Markers of PPAR α activation have been quantitated in mice and rats after exposure to DEHP and compared with increases in the incidence of liver tumours (Fig. 4.2 and 4.3). There was a good correlation between liver tumour induction and several well characterized indicators of PPAR α activation. These indicators include: 1) increases in the size and/or numbers of peroxisomes; 2) increases in acyl-coenzyme A oxidase encoding a peroxisomal palmitoyl-coenzyme A oxidase (a marker of peroxisome proliferation and the rate-limiting enzyme of fatty acid β -oxidation); 3) increases in the levels of CYP4A protein, a ω -lauric acid hydroxylase; and/or 4) increases in the levels of CAT (also known as carnitine palmitoyl transferase) involved in fatty acid transport into the mitochondria. In mice and rats, the induction of the markers of PPAR α occurred at doses coincident with or usually lower than those that induce liver cancer, as predicted if a PPAR α -dependent mechanism is operational. The data indicated that a relatively good correlation exists between liver tumour induction and induction of markers of PPAR α activation in mice and rats, supporting a role for PPAR α in liver tumour induction by DEHP.

Fig. 4.2 Dose-dependent relationships between liver cancer and end-points associated with peroxisome proliferator-activated receptor α activation after exposure to di(2-ethylhexyl) phthalate (DEHP) in mice



End-points evaluated in this figure include those associated with typical peroxisome proliferation including peroxisome proliferation (measured as increases in volume or number) and increases in palmitoyl-coenzyme A oxidase (PCO) activity. Also shown are the dose-response data for end-points relevant to the mode of action of several liver carcinogens: increases in liver to body weights, hepatocyte proliferation and hepatocyte necrosis. The times in parentheses after the literature citation refer to the time of exposure relevant for measurement of changes in cell proliferation. Figures show the doses at which the measured end-point has (filled circles) or has not (open circles) exhibited statistically significant increases. Half-filled circles indicate conditions where there are conflicting studies in which the end-point was or was not observed at the same dose. Solid lines indicate doses that consistently induce the indicated end-point whereas dotted lines indicate conditions that result in inconsistent effects. Data were taken from feeding studies at the indicated concentrations.

BW, body weight

From Corton (2008). © Informa Healthcare. Adapted with permission of Informa Healthcare.

¹ From David *et al.* (1999)

² From NTP (1982)

³ From Isenberg *et al.* (2000) (2, 4 weeks)

⁴ From Ochs *et al.* (1992)

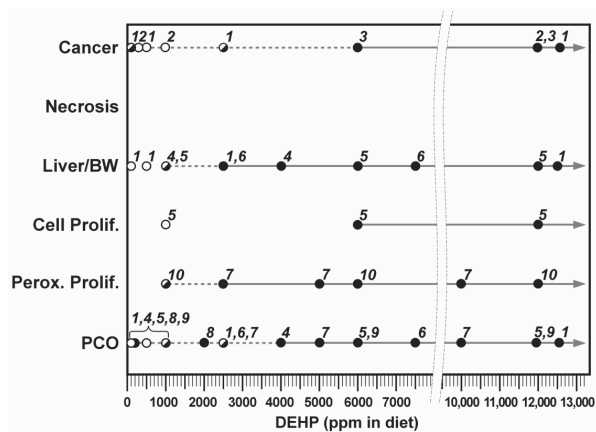
4.3.4 Other relevant data

(a) Acute toxicity

(i) Humans

Dermal application of DEHP was moderately irritating, but only slightly sensitizing to human skin (Shaffer *et al.*, 1945; Mallette & Von Haam, 1952). Two adults given single oral doses of either 5 or 10 g DEHP did not show adverse effects other

Fig. 4.3 Dose-dependent relationships between liver cancer and end-points associated with peroxisome proliferator-activated receptor α activation after exposure to di(2-ethylhexyl) phthalate (DEHP) in rats



End-points evaluated in this figure include those associated with typical peroxisome proliferation including peroxisome proliferation (measured as increases in volume or number) and increases in palmitoyl-coenzyme A oxidase (PCO) activity. Also shown are the dose-response data for end-points relevant to the mode of action of several liver carcinogens: increases in liver to body weights, hepatocyte proliferation and hepatocyte necrosis. The times in parentheses after the literature citation refer to the time of exposure relevant to measurement of changes in cell proliferation. Figures show the doses at which the measured end-point has (filled circles) or has not (open circles) exhibited statistically significant increases. Half-filled circles indicate conditions where there are conflicting studies in which the end-point was or was not observed at the same dose. Solid lines indicate doses that consistently induce the indicated end-point whereas dotted lines indicate conditions that give inconsistent data. Data were taken from feeding studies at the indicated concentrations. BW, body weight

From [Corton \(2008\)](#). © Informa Healthcare. Adapted with permission of Informa Healthcare.

¹ From [David et al. \(1999\)](#)

² From [Cattley et al. \(1987\)](#)

³ From [NTP \(1982\)](#)

⁴ From [Mitchell et al. \(1985\)](#)

⁵ From [Isenberg et al. \(2000\)](#) (1, 2, 4 weeks)

⁶ From [Dirven et al. \(1990\)](#)

⁷ From [Reddy et al. \(1986\)](#)

⁸ From [Ganning et al. \(1990\)](#)

⁹ From [Short et al. \(1987\)](#)

¹⁰ From [Barber et al. \(1987\)](#)

than mild gastric disturbances and moderate diarrhoea at the higher dose ([Shaffer et al., 1945](#)).

Few data on the effects of occupational exposure specifically to DEHP are available ([WHO, 1992](#)). In a study involving workers at a Swedish PVC-processing factory, 54 workers exposed to DEHP and other phthalate diesters

(0.02–2 mg/m³) were studied clinically. 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 the results of conventional lung function tests showed no relation with exposure ([Nielsen et al., 1985](#)). Occupational exposure to DEHP was associated with asthma in one worker at a PVC-processing plant ([WHO, 1992](#)).

Several surgical procedures involve the use of PVC-containing tubing that comes into contact with patients' blood. This was shown to result in peri-operative exposure to various plasticizers, including a seven- to 10-fold increase in blood concentrations of DEHP in infants undergoing corrective operations for congenital defects, and in adults after cardiopulmonary bypass surgery ([Barry et al., 1989](#)).

PVC-containing tubing is also used in artificial ventilation devices. DEHP was measured in the inspired air of five mechanically ventilated pre-term infants; exposures ranged from 1 to 4200 mg DEHP/h (estimated from water traps in the ventilators). In three of the five cases studied, signs of advanced hyaline membrane lung disease were detected. One patient died of pneumothorax soon after being ventilated (DEHP was detected in the lung tissue of this patient), and the other two recovered after the PVC tubing was replaced with ethylene vinyl acetate tubing ([Roth et al., 1988](#)).

(ii) Experimental systems

Acute oral median lethal dose values for DEHP ranged from 26 to 34 g/kg bw in rats, mice, guinea-pigs and rabbits. Median lethal dose values after intraperitoneal administration were 30.7 g/kg bw in rats and 14–38 g/kg bw in mice ([Shaffer et al., 1945](#); [Calley et al., 1966](#); [Lawrence et al., 1975](#); [IARC, 1982](#)).

Acute inhalation exposure to MEHP was studied in BALB/c mice. The breathing pattern

of these animals was monitored during exposure to 0.3–43.6 mg/m³ MEHP for 60 minutes, and inflammatory effects were studied by analysis of bronchoalveolar lavage fluid. Lower airway irritation was reported with a no-observed-effect level (NOEL) of 0.3 mg/m³. The NOEL for inflammatory markers in bronchoalveolar lavage fluid was 1.7 mg/m³, and the number of macrophages in this fluid reached a maximum about 16 hours after exposure (Larsen *et al.*, 2004).

The effects of intravenous injection of up to 300 mg/kg bw DEHP were studied in male Wistar rats. The DEHP was solubilized in aqueous solutions of several Tween surfactants. Dose-dependent lethality was observed with death generally occurring within 90 minutes after injection. The DEHP:Tween-treated animals had enlarged and generally darkened lungs, and in some cases showed haemorrhagic congestion. Histological examination of the lungs revealed an oedematous swelling of the alveolar wall and a marked infiltration of polymorphonuclear leukocytes. The effects were observed at doses as low as 50 mg/kg bw. Intravenous administration of aqueous Tween solutions alone did not cause any adverse effects (Schulz *et al.*, 1975).

The systemic toxicity of DEHP was investigated in male Wistar rats that received 0–7.5 mg/kg bw in six intraperitoneal injections on alternate days. Animals were evaluated by organ weight (liver and testis), light microscopy (liver, heart, brain and testis) and plasma clinical chemistry (γ -glutamyl transpeptidase, lactate dehydrogenase, alanine aminotransferase and alkaline phosphatase). No evidence of toxicity was observed (Nair *et al.*, 1998).

The acute testicular toxicity of MEHP was investigated in 28-day-old male Wistar rats 3, 6 and 12 hours after a single oral dose (by gavage) of 400 mg/kg bw. Detachment and sloughing of germ cells was reported (Dalgaard *et al.*, 2001).

The acute cardiac effects of increasing doses of MEHP were studied in anaesthetized rats injected via the femoral artery. There was a steady

and significant decrease in heart rate, beginning after a total dose > 20 mg, and a decline in blood pressure after a total dose > 40 mg (Rock *et al.*, 1987).

Female F344 rats treated with a single oral dose (up to 5000 mg/kg bw) of DEHP showed no neurobehavioural effects, as evaluated by a functional observational test battery and by motor-activity testing (Moser *et al.*, 1995).

(b) *Subchronic and chronic toxicity*

(i) *Humans*

Kidney

The relationship between exposure to DEHP and uraemic pruritus was studied in patients undergoing regular continuous (4 hours) ambulatory peritoneal dialysis, during which exposure to DEHP and related compounds may occur. The post-dialysis serum concentrations of DEHP, MEHP and 2-EH were significantly higher than the corresponding values before dialysis. There was no relationship between severity of pruritus and post-dialysis serum concentrations of DEHP, MEHP, phthalic acid or 2-EH. Furthermore, the serum concentrations of these compounds did not differ significantly between patients with or without uraemic pruritus (Mettang *et al.*, 1996b). [The Working Group noted potential difficulties in exposure assessment of phthalates in biological fluids. Additional details are provided in Section 4.1].

Lung

A cross-sectional human study assessed the association between phthalate exposure — in terms of phthalate metabolite concentrations in urine — and four pulmonary function parameters (forced vital capacity, forced expiratory volume in one second, peak expiratory flow and maximum mid-expiratory flow) among 240 adult participants in the Third National Health and Nutrition Examination Survey (Hoppin *et al.*, 2004). Concentrations of MEHP in urine were not associated with any of the pulmonary

function parameters evaluated. [The Working Group noted potential difficulties in exposure assessment of phthalates in biological fluids. Additional details are provided in Section 4.1].

Immune function (in-vitro studies)

The human epithelial A549 cell line was exposed to 15.6–2000 µg/mL MEHP and concentrations of the pro-inflammatory cytokines IL-6 and IL-8 were measured in the cell culture supernatant. A concentration-dependent increase in cytokine production was observed at the lower (100–200 µg/mL) concentrations, whereas cytokine production was suppressed at higher (~1000 µg/mL) concentrations (Jepsen *et al.*, 2004). Similar observations have been reported for other phthalate esters (Larsen *et al.*, 2002).

A study in human peripheral blood mononuclear cells (containing 0.1–1% basophils) was aimed to assess a possible effect of plasticizers on immunoglobulin (Ig) E and IgG release in the basophil histamine-release assay, which models the inflammatory part of allergic disease (Glue *et al.*, 2005). Concentrations of 5, 50 and 500 µM MEHP and DEHP did not induce histamine release *per se*. However, when cross-binding of the high-affinity IgE receptor (FcεRI) was performed by stimulation with anti-IgE antibody, an increased dose-dependent histamine release was obtained.

(ii) Experimental systems

General toxicity

Young male and female Sprague-Dawley rats (10 per sex per group) were fed diets containing 5, 50, 500 or 5000 ppm DEHP for 13 weeks (Poon *et al.*, 1997). Mean DEHP 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 1.4- and 1.2-fold the control values in male and female rats, respectively, were

observed only in animals given 5000 ppm DEHP. Relative testis weight was significantly reduced in male rats fed 5000 ppm. 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, and mild Sertoli-cell vacuolation in male rats fed 500 ppm DEHP.

Subchronic (13-week) feeding studies were conducted in F344 rats and B6C3F₁ mice (NTP, 1982). Diets containing 0, 1600, 3100, 6300, 12 500 or 25 000 ppm DEHP were fed to male and female rats, and male and female mice received diets containing 0, 800, 1600, 3100, 6300 or 12 500 ppm. A reduction in mean body-weight gain of male and female rats (29% and 53%, respectively) was observed in the 25 000-ppm group. Testicular atrophy was observed in all male rats fed 25 000 ppm and was present, but less pronounced, in rats fed 12 500 ppm. No other compound-related histopathological effects were observed. In mice, a reduction in mean body-weight gain of 10% or more was observed in males fed 3100, 6300 or 12 500 ppm, and in all treated females except those fed 1600 ppm. No other compound-related effects were observed.

Two-year cancer bioassays (feeding studies) were conducted in F344 rats and B6C3F₁ mice (NTP, 1982). Diets containing 0, 6000 or 12 000 ppm DEHP were fed to male and female rats, and male and female mice received diets containing 0, 3000 or 6000 ppm. In male rats, pituitary hypertrophy and testicular atrophy were observed in the 12 000-ppm group. In male mice, chronic kidney inflammatory changes and testicular degeneration were reported in the 6000-ppm group. No general toxicity was reported in female mice.

The effects of DEHP were studied in male Sv/129 mice (6 weeks of age at the beginning of the feeding study) that were homozygous wild-type or *Ppara*-null and fed 12000 ppm for up to 24 weeks (Ward *et al.*, 1998). General toxicity, evident from high lethality and considerably retarded body weight gain, was most evident in

treated wild-type mice. No animal in this group survived past 16 weeks. In treated *Ppara*-null mice, the weight gain became retarded only from week 16 and the mice began to lose weight by week 24. Throughout this study, the mean kidney weight of DEHP-treated wild-type mice was significantly greater than that of untreated control mice. In treated *Ppara*-null mice, kidney weight did not differ from that of untreated controls at any time-point. On necropsy, DEHP-treated wild-type mice had developed nephropathy, the severity of which was time-related. After 4 weeks of feeding, focal tubular degeneration, atrophy and regenerative tubular hyperplasia were observed, which became more diffuse with age. In DEHP-treated mice that died between 8 and 16 weeks, severe cystic renal tubules were seen, which were especially prominent in the straight portion of the proximal tubules. In the stomach, lung, heart or bone, no lesion indicative of renal failure was noted in this group. DEHP-treated *Ppara*-null mice also showed focal renal tubular lesions after 4–8 weeks, that were less pronounced than those in wild-type mice. Severe diffuse lesions that were morphologically similar to those found earlier in DEHP-treated wild-type mice were observed by week 24 in the *Ppara*-null animals.

A study in which rats were exposed to DEHP was designed to mimic the dose to which humans on haemodialysis would be subjected during each dialysis session. Three groups of rats were administered (by gavage three times a week for 3, 6, 9 and 12 months) sesame seed oil (control), 2.14 mg/kg bw (150 mg/70 kg) DEHP or 2.14 mg/kg bw (150 mg/70 kg) of a leachate obtained from a phthalate-containing plastic artificial kidney. Body weight, serum creatinine and kidney histopathology were evaluated. No significant weight loss was found in any of the groups at any time point. Significantly reduced kidney function, denoted by reduced clearance of creatinine, was found in the DEHP-treated group at 12 months. In both treated groups, a

statistically significant increase in focal cysts in the kidneys was observed at 12 months ([Crocker et al., 1988](#)).

In a 104-week feeding study, B6C3F₁ mice received DEHP at 0, 100, 500, 1500 or 6000 ppm in the diet. At 104 weeks, mean relative kidney weights were significantly lower in males in the three highest-dose groups compared with controls. Chronic progressive nephropathy was seen in males in all dose groups, including the controls. In female mice, chronic progressive nephropathy was significantly increased at 78 and 104 weeks in the 1500- and 6000-ppm groups. Similar nephrotoxic effects were observed in a 104-week feeding study in F344 rats. In males, there was an increase in mean relative kidney weight, which was significant at 2500 and 12 500 ppm, while in females this increase was found to be significant only at the highest dose. In males, a significantly higher incidence of mineralization of the renal papilla was found in the 500-, 2500- and 12 500-ppm groups at 104 weeks ([David et al., 2000a, b](#)).

Immune function

Several studies have been performed to assess whether various phthalates, including DEHP and MEHP, act as sensitizers. These have been reviewed ([Jaakkola & Knight, 2008](#)) and some are discussed below.

Subcutaneous injection, not a probable route of exposure in humans yet an important mode of administration in studies on immune function, was performed in several studies. Ovalbumin was frequently used as the model antigen and was injected subcutaneously into the neck region of BALB/cJ mice, with or without the test substance. MEHP was shown ([Larsen et al., 2001](#)) to have an immunosuppressive effect, measured as a statistically significant reduction in IgE and IgG1 antibody production at a dose of 1000 µg/mL, and an adjuvant effect, measured as a statistically significant increase in IgE antibody concentration at a dose of 10 µg/mL. In a follow-up study,

the adjuvant effect of DEHP, defined as a statistically significant increase in IgG₁ concentration, was observed at a dose of 2000 µg/mL ([Thor Larsen et al., 2001](#)).

In a study that tested whether PPARα may be involved in the adjuvant effect of DEHP, *Ppara*-deficient 129/Sv mice were exposed intraperitoneally to a mixture of ovalbumin and DEHP (2000 µg/mL), and the ovalbumin-specific IgE, IgG1 and IgG2a responses were compared with the corresponding responses in wild-type mice. Compared with mice only given ovalbumin, DEHP induced a strong increase in ovalbumin-specific IgG1 and IgG2a, both in the wild-type and in the *Ppara*-null mice, indicating that DEHP is a mixed T-helper cell adjuvant and that its activity as an adjuvant is mediated through a *Ppara*-independent mechanism ([Larsen & Nielsen, 2007](#)).

[The Working Group noted that the findings that DEHP can act as an adjuvant have been challenged in a study ([Dearman et al., 2008](#)) in which topical administration of DEHP had no impact on antibody responses, regardless of whether the compound was applied locally or distant to the site of immunization with ovalbumin. Several methodological differences from the original studies have been identified in the latter work by the group who originally reported the findings ([Hansen et al., 2008](#)).]

The immuno-modulatory effects of MEHP on the immune response of BALB/c mice to ovalbumin were studied after inhalation exposure (60 minutes; 0.3–43.6 mg/m³). A concentration-dependent decrease in tidal volume and an increased number of alveolar macrophages were reported, but no changes in the numbers of neutrophils, lymphocytes, eosinophils or epithelial cells in bronchoalveolar lavage fluid were observed ([Larsen et al., 2004](#)).

A study in female B6C3F₁ mice aimed to assess the potential of DEHP to cause general and respiratory sensitization following topical administration. The positive (25% trimellitic

anhydride) and reference (1% dinitrochlorobenzene) control animals were initiated by dermal application of DEHP (50 µL/flank; 25%, 50% and 100% solutions). The challenge dose which was applied 7 days later involved application to both ears at a dose of 25 µL/ear. Seven days later, the animals were killed and IgE was determined. This study showed no significant increases in IgE or cytokines after exposure to DEHP ([Butala et al., 2004](#)).

The effects of DEHP (50–500 µM) on antigen-induced degranulation of rat basophilic leukaemia (RBL-2H3) cells was evaluated. Without antigen stimulation, DEHP did not cause any significant increase in degranulation; however, it significantly potentiated the release of β-hexosaminidase induced by dinitrophenylated bovine serum albumin ([Nakamura et al., 2002](#)).

Lung

A 28-day toxicity study of DEHP was carried out in 9-week-old male and female Wistar rats that were exposed via head-nose inhalation to aerosols for 6 hours per day on 5 days per week for 4 weeks; target concentrations in air were 0, 0.01, 0.05 or 1.0 mg/L and estimated doses were 2.30, 11 or 230 and 3.6, 18 or 360 mg/kg bw per day in males and females, respectively ([Klimisch et al., 1992](#)). No general toxicity, based on clinical investigation and blood-chemistry parameters, was observed. A statistically significant (16%) increase in relative lung weights, accompanied by increased foam-cell proliferation and thickening of the alveolar septi, was reported in the highest-dose males.

In a 104-week feeding study, B6C3F₁ mice received 0, 100, 500, 1500 and 6000 ppm DEHP in the diet. At 104 weeks, mean relative lung weights were significantly increased in highest-dose males (6000 ppm). A similar effect was observed at the end of a 104-week feeding study in male F344 rats fed 2500 and 12 500 ppm DEHP ([David et al., 2000a, b](#)).

Brain and neurobehavioural effects

Female F344 rats treated with repeated doses (up to 1500 mg/kg bw per day for 14 days) of DEHP showed no neurobehavioural effects, as evaluated by a functional observational test battery and by motor-activity testing ([Moser et al., 1995](#)).

In the 104-week feeding studies mentioned above, DEHP caused an increase in mean relative brain weights in male B6C3F₁ mice at the highest dose (6000 ppm). A similar effect was observed in male and female F344 rats at the highest dose of 12 500 ppm ([David et al., 2000a, b](#)).

(c) Reproductive and developmental effects

(i) Humans

While no human studies were available at the time of the previous evaluation ([IARC, 2000](#)), several studies of reproductive and developmental effects of DEHP have been published since then. [The Working Group noted that the human studies detailed below, which showed either positive or negative associations, relied exclusively on concentrations of MEHP in biological fluids as a biomarker of exposure to DEHP and should be interpreted with caution. Assessment of oxidative metabolites may be necessary to evaluate in-vivo exposures to DEHP fully. Additional details are provided in Section 4.1.]

Developmental toxicity

A study of 84 newborns in Perrino Hospital, Brindisi, Italy, evaluated in-utero exposures to DEHP and gestational age. DEHP and MEHP were detectable in 88% of the samples of serum (cord blood). This finding was interpreted as evidence of in-utero exposure to DEHP in humans. Furthermore, the authors compared gestational age between MEHP-positive [the difference in levels was not taken into account] and -negative newborns and found shorter pregnancies in the MEHP-positive group ([Latini et al., 2003](#)).

A possible association between pre-term birth and exposure to phthalates was investigated in a Mexican birth cohort study in which third-trimester urinary concentrations of phthalate metabolites in 30 women who delivered pre-term (< 37 weeks of gestation) were compared with those of 30 controls (≥ 37 weeks of gestation). Pre-term birth cases had significantly higher odds ratios for the presence of urinary DEHP metabolites — MEHP (OR, 3.5; 95%CI: 1.0–12.9), MEHHP (OR, 4.6; 95%CI: 1.3–16.7) and MEOHP (OR, 7.1; 95%CI: 1.9–26.5) — and for the sum of four DEHP metabolites (OR, 5.0; 95%CI: 1.4–18.0). After correction of metabolite concentrations by creatinine, only the association with the four DEHP metabolites remained significant (OR, 4.1; 95%CI: 1.0–17.5) ([Meeker et al., 2009a](#)).

A study of 201 newborn–mother pairs from Shanghai, China, were investigated in a nested case–control study of in-utero exposure to phthalates, including DEHP, and birth outcomes. Of the newborns, 88 had low body weight and 113 had normal body weight at birth. Higher levels of phthalates in maternal and cord blood were found in low-body weight cases compared with controls. While some associations were found between prenatal exposures to phthalates and low body weight and body length at birth, these were not significant for DEHP after adjustment for potential confounders ([Zhang et al., 2009](#)).

A cross-sectional study investigated the relationship between the levels of prenatal exposure to phthalate esters and birth outcomes among 149 pregnant Japanese women. Urinary concentrations of nine phthalate ester metabolites — monomethyl phthalate (MMP), monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MnBP), MBzP, MEHP, MEHHP, MEOHP, mono-iso-nonyl phthalate and mono-*n*-octyl phthalate — were analysed in spot urine samples collected from the pregnant women. The health outcomes evaluated were birth weight, birth length, head circumference and gestational age,

and the relationships between prenatal exposure to phthalate esters and these outcomes were not significant ([Suzuki et al., 2010](#)).

Data from a prospective Danish-Finnish cohort study on cryptorchidism collected from 1997 to 2001 were used to analyse individual breast milk samples collected 1–3 months postnatally as additive aliquots ($n = 130$; 62 cryptorchid/68 healthy boys) for phthalate monoesters, including MEHP. Serum samples (obtained from 74% of all boys) were analysed for gonadotropins, sex-hormone binding globulin, testosterone and inhibin B. No association was found between phthalate monoester levels and cryptorchidism. MEHP concentrations in breast milk did not show significant associations with any of the hormones evaluated ([Main et al., 2006](#)).

A study examined anogenital distance (AGD), an end-point associated with impaired testicular function, and other genital measurements in 134 boys 2–36 months of age ([Swan et al., 2005](#)). Shortening of the AGD has been shown in male rodents to be related to prenatal exposure to phthalates ([Parks et al., 2000](#)). [Swan et al. \(2005\)](#) reported that AGD was significantly correlated with penile volume ($R = 0.27$; $P = 0.001$) and the proportion of boys with incomplete testicular descent ($R = 0.20$; $P = 0.02$). Urinary concentrations of four phthalate metabolites (MEP, MnBP, MBzP and mono-iso-butyl phthalate [MiBP]) were measured and correlated with the anogenital index (AGI) [AGI = AGD/weight (mm/kg)]. Comparing boys with prenatal MnBP concentrations in the highest quartile with those in the lowest quartile, the odds ratio for a smaller than expected AGI was 10.2 (95%CI: 2.5–42.2). The corresponding odds ratios for MEP, MBzP and MiBP were 4.7, 3.8, and 9.1, respectively (all P -values < 0.05). The age-adjusted AGI decreased significantly (P -value for slope = 0.009) with increasing summary phthalate score (joint exposure to the four phthalate metabolites). The authors concluded that the associations between

male genital development and exposure to phthalates seen here are consistent with the phthalate-related syndrome of incomplete virilization that has been reported in prenatally exposed rodents, which supports the hypothesis that prenatal exposure to phthalates at environmental levels can adversely affect male reproductive development in humans.

However, another similar study found no association between in-utero exposure to phthalates and either the AGD or AGI in male newborns ([Huang et al., 2009](#)). The authors evaluated the association between maternal urinary excretion of phthalates, exposure of the fetus to phthalates in amniotic fluid and the health of newborns. Amniotic fluid and urine samples from pregnant women were collected and five phthalate monoesters, including MEHP, were measured. The birth weight, gestational age and AGD of newborns were also recorded. No effects of MEHP were found. However, a significantly negative correlation between MnBP in amniotic fluid, AGD ($R = -0.31$; $P < 0.06$) and AGI adjusted by birth weight ($R = -0.32$; $P < 0.05$) was found only in female newborns. The authors concluded that in-utero exposure to phthalates may have anti-androgenic effects on the fetus.

Male reproductive toxicity

A randomized controlled study of 21 infertile men (low sperm counts and abnormal sperm morphology) and 32 control men (normal semen quality and evidence of conception) investigated the relationship between seminal plasma concentrations of phthalate esters and semen quality ([Rozati et al., 2002](#)). Concentrations of phthalate esters in infertile men were higher than those in controls. Sperm quality parameters (total motile sperm count) were inversely proportional to concentrations of phthalates.

A study conducted in Shanghai, China, investigated semen concentration of phthalates, including DEHP, and semen quality in men. A positive significant association was reported

between semen concentrations of all three phthalates tested and liquefaction time of semen. No correlations were found for other sperm quality parameters, such as sperm density or viability. No adjustment for confounders was performed in this study ([Zhang et al., 2006](#)).

A study of 220 male partners of subfertile couples, aged between 20 and 54 years, investigated the relationship between sperm movement characteristics (straight-line velocity, curvilinear velocity and linearity) and urinary levels of phthalates ([Duty et al., 2004](#)). Urinary concentrations of the phthalate monoesters — MEP, MBzP, MnBP, MEHP and MMP — were measured. While no consistent and statistically significant associations were found between urinary levels of phthalates and sperm motility end-points, negative trends were reported for MEHP.

A study of 234 young Swedish men (normal population) investigated the relationship between urinary concentrations of five phthalates, including MEHP, serum levels of reproductive hormones and male reproductive end-points (semen volume, sperm concentration and motility) ([Jönsson et al., 2005](#)). No significant associations were found between any of the reproductive biomarkers and urinary levels of phthalates.

A study of 463 men who were partners in subfertile couples included detailed semen analysis (sperm concentration, motility and morphology) and single spot urinary concentrations of three DEHP metabolites ([Hauser et al., 2006](#)). No relationships were found between DEHP metabolites and semen parameters.

Sperm DNA damage and urinary phthalate metabolites were assessed in male subjects ($n = 379$) who were patients at an infertility clinic ([Hauser et al., 2007](#)). DNA damage was assessed with the neutral comet assay and it was reported that sperm DNA damage was associated with urinary MEHP after adjusting for DEHP oxidative metabolites; however, this study did not include control subjects (with no infertility).

A study of 349 men who were partners in subfertile couples included detailed semen analysis (sperm concentration, motility and morphology) and single spot urinary concentrations of four DEHP metabolites: MEOHP, MEHHP, MECPP and MEHP ([Herr et al., 2009](#)). The evaluation of human reproductive parameters in this study (from semen analyses) did not show significant associations with concentrations of DEHP metabolites determined in spot urine sampled at the day of andrological examination.

A study of 74 male workers exposed to phthalates at the workplace (production of unfoamed PVC-containing flooring) and a matched group of 63 construction workers (no known occupational exposure to phthalates) investigated urinary concentrations of MnBP and MEHP and serum concentrations of gonadotropin, luteinizing hormone, follicle-stimulating hormone, free testosterone and E2 ([Pan et al., 2006](#)). In exposed workers, urinary levels of both phthalates were significantly higher and serum free testosterone was significantly lower than those in controls. Phthalate concentrations were significantly negatively correlated with free testosterone.

A study of 408 infertile men assessed urinary levels of MEHP and DEHP and serum concentrations of free thyroxine and total tri-iodo thyronine. Urinary MEHP concentration was found to be inversely correlated with serum free thyroxine and total tri-iodo thyronine ([Meeker et al., 2007](#)).

In a study of 425 men recruited through an infertility clinic in the USA, urinary concentrations of MEHP and several other phthalate monoester metabolites were measured, together with serum levels of testosterone, E2, sex hormone-binding globulin, follicle-stimulating hormone, luteinizing hormone, inhibin B and prolactin. The authors reported that, after adjusting for potential confounders, urinary levels of MEHP were inversely associated with levels of testosterone and E2 and the free

androgen index (molar ratio of total testosterone to sex hormone-binding globulin). The ratio of testosterone to E2 was positively associated with MEHP concentration ($P = 0.07$) and MEHP percentage (proportion of DEHP metabolite) ($P = 0.007$), which was interpreted as suggestive of a potential relationship with aromatase suppression ([Meeker et al., 2009b](#)).

A study of 13 healthy male adolescents (14–16 years of age) who received ECMO as neonates, a procedure performed with medical devices that contain high levels of phthalates, investigated sexual development (testicular volume, phallic length and serum levels of sex hormones), as well as thyroid, liver and renal function ([Rais-Bahrami et al., 2004](#)). All subjects were found to have normal growth percentile for age and sex, had normal internal organ function and exhibited sexual development (including serum hormone levels) appropriate for the stage of pubertal maturity.

Female reproductive toxicity

A study of 41 subjects with premature breast development, defined as growth of mammary tissue in girls under 8 years of age with no other manifestations of puberty, and 35 age-matched control female subjects assessed serum concentrations of phthalates ([Colón et al., 2000](#)). In 28 of 41 subjects, compared with one of 35 controls, DEHP and MEHP were detectable. This effect was challenged, however, due to the possible contamination of samples ([McKee, 2004](#)).

A study of six female adolescents (14–16 years of age) who received ECMO as neonates, a procedure performed with medical devices that contain high levels of phthalates, investigated sexual development (serum levels of sex hormones), as well as thyroid, liver and renal function ([Rais-Bahrami et al., 2004](#)). All female subjects were found to have a normal growth percentile for age and sex, had normal internal organ function and exhibited sexual development (including serum

hormone levels) appropriate for their stage of pubertal maturity.

A study of 55 women with endometriosis and 24 age-matched control women investigated concentrations of DEHP and MEHP in plasma and peritoneal fluid ([Cobellis et al., 2003](#)). Significantly higher plasma levels of DEHP were observed in women with endometriosis, and both phthalates were detected in the peritoneal fluid in > 90% of cases with endometriosis. However, there was no association between phthalate levels and stage of the disease or time of diagnosis.

A prospective case-control study of 49 infertile women with endometriosis, 38 age-matched women without endometriosis but with infertility related to tubal defects, fibroids, polycystic ovaries, idiopathic infertility and pelvic inflammatory diseases diagnosed by laparoscopy and 21 age-matched women with proven fertility and no evidence of endometriosis or other gynaecological disorders was conducted in Hyderabad, Andhra Pradesh state, India ([Reddy et al., 2006](#)). Serum concentrations of di(n-butyl) phthalate, butyl benzyl phthalate, di(n-octyl) phthalate and DEHP were measured. Significant differences in the serum concentrations of phthalates were observed between women with and without endometriosis. A significant correlation between serum concentrations of phthalates, including DEHP, and different levels of severity of endometriosis was observed.

A cross-sectional study of urinary concentrations of phthalate metabolites obtained from the US National Health and Nutrition Examination Survey (1999–2004) examined their relation to self-reported history of endometriosis and uterine leiomyomata among 1227 women 20–54 years of age ([Weuve et al., 2010](#)). Four phthalate metabolites, including MEHP, were examined. Eighty-seven (7%) and 151 (12%) women reported diagnoses of endometriosis and leiomyomata, respectively. After comparing the highest versus lowest three quartiles of urinary MEHP, there were no significant associations

with endometriosis or leiomyomata. A significant inverse association (OR, 0.59; 95%CI: 0.37–0.95) was found for both conditions combined.

A prospective case–control study of 97 women with advanced-stage endometriosis and 169 control women was performed in the Republic of Korea (Kim *et al.*, 2011). Plasma concentrations of MEHP and DEHP were determined using liquid chromatography–tandem mass spectrometry. The study reported that the concentrations of MEHP and DEHP were significantly higher in women with advanced-stage endometriosis.

(ii) Experimental systems

The teratogenicity and reproductive toxicity of DEHP have been reviewed (Huber *et al.*, 1996; Lyche *et al.*, 2009). It was noted that DEHP impairs fertility in both sexes of adult rats at doses above 100 mg/kg bw per day, and that several studies indicate that DEHP is embryotoxic and teratogenic in rodents. DEHP was associated with a reduction in relative testis weight, decreases in sperm production and a depletion of testicular zinc. The testicular response appears to be somewhat species-specific (among the experimental animal systems tested), but is not consistent with the reported species sensitivity to peroxisome proliferation (e.g. guinea-pigs were more sensitive to the testicular effects than Syrian hamsters). The metabolite MEHP was judged to be more potent in causing both teratogenicity and reproductive toxicity. Both embryotoxic and testicular effects in adults were 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 prenatal exposure to DEHP and exposure 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). Detailed summaries of most of the studies published before 2000 were included in the previous evaluation (IARC, 2000) and are

not repeated here (unless a particular study was omitted previously).

Developmental toxicity

Gestational exposure to DEHP was reported to lead to decreased maternal and fetal body weight, fetal resorptions and decreased viability of the pups (Singh *et al.*, 1972; Tyl *et al.*, 1988; Narotsky & Kavlock, 1995; Hellwig *et al.*, 1997; NTP, 1997). Some but not all studies reported an increase in the incidence of abnormalities (e.g. haemangiomas of the legs) (Singh *et al.*, 1972; Narotsky & Kavlock, 1995) and retarded development (renal pelvic dilatations) (Merkle *et al.*, 1988). A comparative study of the teratogenicity of DEHP, 2-EH and 2-EHA in rats showed that all three chemicals induced malformations (hydro-nephrosis, laevocardia, septal defects, short and kinky tails, ectrodactyly, misplaced digits and bowed radius), and 2-EHA was the most potent (Ritter *et al.*, 1987).

DEHP and several other phthalates were administered orally to pregnant rats at 750 mg/kg bw from gestational day 14 to postnatal day 3. None of the phthalates induced overt maternal toxicity or reduced litter sizes. DEHP treatment reduced maternal weight gain by about 15 g during the entire dosing period, reduced pregnancy weight gain to gestational day 21 by 24 g and reduced pup weight at birth (15%). Male pups from the DEHP-treated group displayed shortened AGDs (about 30%) and reduced testis weights (about 35%). As infants, males in the DEHP-treated group displayed female-like areolas/nipples (87%; $P < 0.01$) and a significant incidence of reproductive malformations (82%; $P < 0.0001$). The authors concluded that DEHP altered sexual differentiation in rats (Gray *et al.*, 2000).

Female Sprague-Dawley rats were given oral doses of 0, 500 or 1000 mg/kg bw per day DEHP on gestation days 7–18. Fetal deaths were recorded at gestation days 12, 14, 16, 18 and 20. At all of these time-points, fetal deaths (20–36%)

were detected in the highest-dose group. Multiple abnormalities in male germ cells in fetal testis were also reported in DEHP-treated groups. In a follow-up experiment, lower doses (125, 250 and 500 mg/kg bw per day) were tested using the same protocol. In the 250- and 500-mg/kg groups, adverse effects on male testis development were observed; however, in the 125-mg/kg group, no effect was detected ([Shirotta *et al.*, 2005](#)).

DEHP was administered orally to female Sprague-Dawley rats from gestation day 3 to postnatal day 21 at doses of 0, 375, 750 or 1500 mg/kg bw per day ([Moore *et al.*, 2001](#)). Male offspring were investigated for end-points of reproductive health and sexual behaviour at postnatal days 21, 63 or 105–112. Dose-related adverse effects in male sex organ development and sperm quality were found, as well as low sexual activity. These effects were most pronounced in groups exposed prenatally to 750 and 1500 mg/kg bw per day.

Lactational exposures to DEHP were shown to result in a decrease in body weight, as well as alterations in liver weight and function in rat pups ([Parmar *et al.*, 1985](#); [Dostal *et al.*, 1987](#)). [Parmar *et al.* \(1985\)](#) showed that lactational exposure is indicated by a significant quantity of DEHP in the liver of pups, which shows that DEHP can be transferred through the mother's milk and is not hydrolysed as it is in adults ([Parmar *et al.*, 1985](#)). Exposure to DEHP during early life through mother's milk causes biochemical alterations which may affect the functional development of the testis ([Tandon *et al.*, 1990](#)).

Exposure of newborn rats to DEHP induced both general toxicity (e.g. reduced body weight and body weight gain) and hepatic effects (e.g. hepatomegaly and induction of peroxisomal enzymes) regardless of the route of exposure or age ([Greener *et al.*, 1987](#); [Cimini *et al.*, 1994](#)). Male Sprague-Dawley rats (3–5 days of age) were administered DEHP by intravenous injections (0, 60, 300 or 600 mg/kg bw per day) or gavage (0, 300 or 600 mg/kg bw per day) for 21 days.

Organ weights and reproductive organ toxicity were evaluated at the end of treatment or after rats reached sexual maturity (90 days of age). No effects were observed in the low-dose/intravenous group. Abnormal histology was reported in testis in all other treated groups after the 21-day dosing period regardless of the route of administration. A dose-dependent increase in the severity of testicular lesions was observed and the effect was modestly stronger in rats exposed orally. No effects on sperm count, sperm morphology or sperm motility were observed in treated rats at 90 days of age ([Cammack *et al.*, 2003](#)).

Adverse effects on testicular development in male rat offspring after exposure of female rats to DEHP during gestation and suckling have been reported ([Tandon *et al.*, 1991](#); [Arcadi *et al.*, 1998](#)). The pathological effects in the testis (e.g. decreased AGD, retained nipples and high levels of testicular and epididymal abnormalities, including atrophy and agenesis) induced by DEHP differed from those induced by other known androgen-receptor antagonists (i.e. vinclozolin, procymidone and 1,1-dichloro-2,2-bis(*para*-chlorophenyl)ethylene) ([Wolf *et al.*, 1999](#)).

Groups of Long-Evans rats were administered DEHP (0 or 10 mg/kg bw per day) orally from postnatal day 21 (weaning) to postnatal day 120 and showed no signs of overt toxicity, although Leydig-cell hyperplasia, reduced Leydig-cell testosterone production *ex vivo*, and increased levels of serum luteinizing hormone, testosterone and E2 were observed ([Akingbemi *et al.*, 2004](#)). The rise in testosterone levels found in this study contrasts with the decrease in testosterone observed in studies of prenatal exposure to DEHP. In a follow-up study ([Ge *et al.*, 2007](#)), a wider dose-range of DEHP (0, 10, 500 or 750 mg/kg bw per day) was administered to male rats starting at postnatal day 21 for 28 days. Pubertal onset was significantly decreased in the 10-mg/kg group, while it was significantly delayed in the 750-mg/kg group compared with controls. Furthermore,

similar bi-modal effects of the low- (10 mg/kg bw per day) versus high- (750 mg/kg bw per day) dose effects were seen for serum testosterone, as well as testis and body weight. The authors tested the effects of various concentrations of the DEHP metabolite MEHP *in vitro* in Leydig cell cultures. MEHP at 100 μ M (27.8 μ g/mL) increased luteinizing hormone-stimulated testosterone production, while 10 mM (2780 μ g/mL) was inhibitory.

Gestational effects of DEHP similar to those observed in rats have been also reported in mice ([Yagi et al., 1980](#); [Shiota & Nishimura, 1982](#); [Tomita et al., 1982b](#); [Shiota & Mima, 1985](#); [Tyl et al., 1988](#)).

Groups of 10–13 pregnant female homozygous wild-type or *Ppara*-null mice were administered DEHP by gavage at 0 or 1000 mg/kg bw on days 8–9 of gestation. Offspring were evaluated on gestational days 10 and 18. Similar developmental toxicity (resorptions, growth retardation and incidence of malformations) was seen in mice of both genotypes, suggesting that the developmental effects are not PPAR α -mediated. Additional analysis showed that DEHP induced maternal hepatic *CYP4A1* mRNA in the wild-type females only; both genotypes showed DEHP-induced metallothionein-1 and zinc levels in the maternal livers and reduced zinc concentration in maternal serum and in the fetus after exposure ([Peters et al., 1997](#)).

Reproductive toxicity

Exposure of adolescent male rats to DEHP has been shown to lead to the reduction in absolute and relative testicular weights, seminiferous tubular atrophy and cessation of spermatogenesis, leading to smaller litters ([Gray et al., 1977](#); [Agarwal et al., 1986](#); [Parmar et al., 1986](#); [Dostal et al., 1988](#); [Ganning et al., 1990](#); [Siddiqui & Srivastava, 1992](#); [Poon et al., 1997](#)). These effects appear to persist even after cessation of exposure ([Oishi, 1985](#); [Sjöberg et al., 1985c](#)).

A study of female reproductive function following exposure to DEHP reported prolonged

estrous cycles, suppressed or delayed ovulation and smaller pre-ovulatory follicles, reduced pre-ovulatory granulosa-cell estrogen production, with secondary increases in follicle-stimulating hormone and insufficient luteinizing hormone surge for ovulation ([Davis et al., 1994](#)).

In a 104-week feeding study of DEHP in male and female F344 rats, a statistically significant decrease in mean relative testes weight was observed with the highest dose tested (12 500 ppm). Bilateral aspermatogenesis was observed at 78 weeks in the 12 500-ppm group and at 104 weeks in the 500-, 2500- and 12 500-ppm groups. No effect on mean relative uterine weight was observed in this study ([David et al., 2000b](#)).

A dose-response study following in-utero and lactational exposure to DEHP was conducted to investigate effects on androgenic status, developmental landmarks and testicular histology in male offspring rats. Female Wistar rats were treated daily by gavage with DEHP from gestation day 6 to lactation day 21 at doses of 0.015, 0.045, 0.135, 0.405 or 1.215 mg/kg bw per day (low doses) and 5, 15, 45, 135 or 405 mg/kg bw per day (high doses). Nipple retention and reduced AGD were observed in male offspring exposed *in utero* and during lactation to the highest dose (405 mg/kg bw per day). Delayed preputial separation was observed in animals exposed to 15 mg/kg bw per day or higher doses. The presence of bi- and multinucleated gonocytes and reduced germ-cell differentiation in seminiferous tubules were observed during histopathological examination of the testis on postnatal days 1 and 22 at doses of 135 and 405 mg/kg bw per day ([Andrade et al., 2006a](#)).

A companion dose-response study following in-utero and lactational exposure to DEHP was conducted in rats to investigate reproductive toxicity in female offspring. The study design and dose range were identical to those detailed above ([Andrade et al., 2006a](#)). No effects on organ (liver, kidney, spleen, thymus, thyroid, ovary and uterus) or body weights were detected in female

offspring, which presented a normal pattern of estrous cyclicity with no hormonal alterations (serum E2 and progesterone). An increase in the number of ovarian atretic tertiary follicles (observed only at the highest dose of 405 mg/kg bw per day) was the only effect observed in adult female offspring exposed to DEHP *in utero* and during lactation ([Grande et al., 2007](#)).

Evidence for the toxicity of DEHP in both male (e.g. testicular effects) and female (e.g. effects on the uterus and hormone levels) mice, as well as reductions in litter size have been reported ([NTIS, 1988](#); [Lamb et al., 1987](#); [Agarwal et al., 1989](#); [Jain & Joshi, 1991](#)).

In a 104-week feeding study of DEHP in B6C3F₁ mice ([David et al., 2000a](#)), a decrease in mean relative testes weight was reported in male mice fed doses of 500, 1500 or 6000 ppm. Significant increases in immature/abnormal epididymal sperm and bilateral hypospermia of the testes were observed in male mice that received 6000 ppm at 78 weeks. Similar effects were observed at 104 weeks in male mice treated with 1500 or 6000 ppm. A significant reduction in mean relative uterus weight was observed in female mice at the highest dose tested (6000 ppm).

In a 13-week study, groups of four mature male marmosets were given daily doses of 0, 100, 500 or 2500 mg/kg bw DEHP. 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](#)).

In a 65-week study, groups of male and female marmosets were given daily oral doses of 0, 100, 500 or 2500 mg/kg bw DEHP by gavage throughout the pre- and peri-adolescent period, from weaning (3 months of age) through to sexual maturity (18 months). No significant effect of DEHP on male organ weights was observed, and no microscopic changes were found in male gonads or secondary sex organs. The

authors evaluated sperm head counts, zinc levels, GSH levels and testicular enzyme activities. Furthermore, electron microscopic examination revealed no treatment-related abnormalities in Leydig, Sertoli or spermatogenic cells. Histochemical examination of the testis after 3 β -hydroxysteroid dehydrogenase staining did not reveal any alterations in steroid synthesis in the Leydig cells. In females, increased ovarian and uterine weights and elevated blood E2 levels were observed in groups treated with 500 and 2500 mg/kg bw. In addition, the activity of several liver enzymes involved in the biosynthesis of sex hormones (CYP contents, testosterone 6 β -hydroxylase and lauric acid ω -1-hydroxylase) was increased in males and/or females of either the mid- or high-dose groups, but no consistent dose-related trend was evident ([Tomonari et al., 2006](#)).

Mechanistic-based reproductive toxicity

[Ward et al. \(1998\)](#) observed that the mean testis weight in DEHP-treated (12 000 ppm for up to 24 weeks) wild-type and *Ppara*-null Sv/129 mice was significantly lower than that of controls after 4 and 8 weeks of feeding. Focal tubular degenerative lesions were found in all wild-type mice fed DEHP. Spermatogenesis was also diminished and giant cells were found within the epididymis in this group by 8–16 weeks. DEHP-fed *Ppara*-null mice had primarily normal testes after 4–8 weeks except for a few tubules on the outer portion of the testis that lacked normal indicators of spermatogenesis. However, after 24 weeks, most DEHP-treated *Ppara*-null mice had severe tubular lesions. Tubules of untreated wild-type and *Ppara*-null mice were normal.

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 follicle-stimulating hormone reactivity. None of these

factors alone appears to account for the observed testicular effects (reviewed in [Boekelheide, 1993](#)).

The mode of cell death in the testicular toxicity of MEHP has been suggested to be germ-cell apoptosis ([Richburg & Boekelheide, 1996](#); [Lee et al., 1997](#)).

[Gray & Butterworth \(1980\)](#) and [Sjöberg et al. \(1986a\)](#) found age-dependent induction of testicular atrophy in rats; younger rats were more sensitive to DEHP than older rats. However, this difference may be related to changes in absorption, metabolism and distribution rather than to changes in tissue sensitivity ([Heindel & Powell, 1992](#)).

To determine which compound was responsible for the testicular damage after oral administration of DEHP, [Sjöberg et al. \(1986b\)](#) administered DEHP and five of its major metabolites (MEHP, 2-EH and three identified metabolites of MEHP — MEHHP, MEOHP and MECPP) for 5 days. No testicular damage was observed following oral doses of DEHP or 2-EH. The number of degenerated spermatocytes and spermatids was increased in rats receiving MEHP; no such effects were seen in animals given the MEHP-derived metabolites.

To investigate whether the anti-androgenic action of DEHP occurs through the inhibition of testosterone production or inhibition of androgenic action by binding to the AR, maternal treatment with DEHP (750 mg/kg bw per day from gestational day 14 to postnatal day 3) was investigated ([Parks et al., 2000](#)). Exposure to DEHP caused a reduction in testosterone production, and reduced testicular and whole-body testosterone levels in fetal and neonatal male rats from gestational day 17 to postnatal day 2. AGD on postnatal day 2 was reduced by 36% in exposed male but not female offspring. By gestational day 20, DEHP treatment also reduced testis weight. Testes in the DEHP-treated group displayed enhanced 3β -hydroxysteroid dehydrogenase staining and increased numbers of multifocal areas of Leydig-cell hyperplasia as

well as multinucleated gonocytes compared with controls at gestational day 20 and postnatal day 3. Neither DEHP nor MEHP displayed affinity for the human AR at concentrations up to 10 μ M (3.9 μ g/mL) *in vitro*. The authors concluded that DEHP disrupts male rat sexual differentiation by reducing testosterone in the fetus to female levels during a critical stage of reproductive tract differentiation.

Suppression of aromatase activity, an enzyme that catalyses the conversion of testosterone to E2 and plays a critical role in brain sexual differentiation, has been suggested as a mechanism for the interference of DEHP with estrogen metabolism ([Andrade et al., 2006b](#)). Wistar rat dams were treated daily with DEHP (0.015, 0.045, 0.135, 0.405 or 1.215 mg/kg bw per day, low doses; and 5, 15, 45, 135 or 405 mg/kg bw per day, high doses) by gavage from gestation day 6 to lactation day 21. Aromatase activity was determined in hypothalamic/preoptic area brain sections from male and female pups on postnatal days 1 and 22. In males on postnatal day 1, aromatase activity was inhibited at low doses and increased at high doses resulting in a non-monotonic dose-response profile that resembled a J-shaped curve. Inhibition was statistically significant at 0.135 and 0.405 mg/kg bw per day, while statistically significant increased activity was observed at 15, 45 and 405 mg/kg bw per day. In contrast to findings on postnatal day 1, aromatase activity at weaning (postnatal day 22) was more strongly affected in females than in males. An increase in aromatase activity was observed at only one dose in males (0.405 mg/kg bw per day) while an increase in activity was observed at all doses in the females except for 0.045 and 5 mg/kg bw per day.

4.4 Susceptibility

4.4.1 Genetic polymorphisms

The human PPAR α is indistinguishable from the rodent *Ppara* in overall structure (Sher *et al.*, 1993; Mukherjee *et al.*, 1994; Tugwood *et al.*, 1996), but several allelic variants of human PPAR α have been isolated which possess properties that differ from those of the originally cloned human PPAR α . The L162V variant that contains an amino acid change in the DNA-binding domain is found at an allelic frequency of ~0.025–0.073 in ethnically diverse populations (Flavell *et al.*, 2000; Lacquemant *et al.*, 2000; Tai *et al.*, 2002). In North Indians, this allele is found at high frequencies (0.745) (Sapone *et al.*, 2000). The human PPAR α L162V variant exhibits no response to low doses of WY-14 643 but greater ligand-induced activity (up to ~four-fold) at higher doses compared with the wild-type receptor (Flavell *et al.*, 2000; Sapone *et al.*, 2000). Humans carrying this variant exhibit greater decreases in total serum cholesterol after treatment with the hypolipidaemic, bezafibrate (Flavell *et al.*, 2000). Three different Asian populations carry a human PPAR α variant (V227A) within the hinge region between the DNA-binding and ligand-binding domains at frequencies of 0.003–0.051 (Yamakawa-Kobayashi *et al.*, 2002; Chan *et al.*, 2006). This allele has been associated with decreases in serum cholesterol and triglycerides in a Japanese population (Yamakawa-Kobayashi *et al.*, 2002) and in Chinese women (Chan *et al.*, 2006). Because of increased interactions with the nuclear receptor corepressor, this variant exhibits decreased responsiveness to PPAR α activators (Liu *et al.*, 2008). The human PPAR α -6/29 variant containing four amino acid substitutions is a dominant negative that binds to a PPRE but cannot be activated by PPAR α activators (James *et al.*, 1998b). The human PPAR α -6/29 variant is probably very rare, because it was not detected in any of the 173 human subjects investigated in

two studies (Roberts, 1999; Sapone *et al.*, 2000). Overall, some PPAR α allelic heterogeneity exists in human populations, but no variants have been identified that are more sensitive to low, environmentally-relevant doses of PPAR α activators than the 'wild-type' human receptor. This topic would benefit from a contiguous comparison of wild-type and human PPAR α variants in trans-activation assays to determine dose–response relationships of PPAR α activators.

4.4.2 Identification of groups or subpopulations with an enhanced susceptibility to DEHP with a focus on fetal and neonatal responses

Cimini *et al.* (1994) treated F344 rat dams with 1 g/kg bw per day DEHP by gavage for up to 21 days from the day of delivery through to lactation. Pups were killed on days 14, 21 or 35 following a 14-day recovery period (of treatment withdrawal). Relative liver weights increased 1.65-fold in the dams at weaning and 1.47-fold in 14- and 21-day pups. At day 21, palmitoyl coenzyme A oxidase activity in the liver increased 9.3-fold in dams, while it increased sixfold in the nursing pups at 14 days and 4.85-fold at 21 days. However, this activity was substantially lower in the pups than in the dams treated with DEHP (pups, 1.2 mU/mg protein at 14 days; dams, 34.4 mU/mg protein at 21 days). Dihydroxyacetone phosphate acyltransferase in the liver was increased about twofold in 14- and 21-day neonates, but levels were unaffected in DEHP-treated dams. Catalase activity was increased about twofold in 14-day and 21-day neonates and adults. Following 14 days of recovery, most enzyme levels returned to normal in the dams and pups, although catalase activity remained slightly higher.

In a separate study, pregnant lactating F344 rat dams were given 1 g/kg bw per day DEHP by gavage for 21 days beginning on the day of delivery until weaning, and the pups nursed by treated dams were killed after 2–3 weeks or

following a 14-day recovery period ([Stefanini et al., 1995](#)). The numerical density or volume density of peroxisomes was increased marginally (less than twofold) relative to controls in both pup groups. Dams treated for 21 days with DEHP showed a more pronounced increase in the volume density of peroxisomes (about twofold), but the numerical density of peroxisomes was increased in the dams to the same degree as that in the 2–3-week-old pups. The increases in volume density or numerical density of peroxisomes did not decline to control levels in the 3-week-old pups after a 14-day recovery period. Volume density of peroxisomes apparently declined to near control levels after a recovery period of 8 days in dams treated for 3 weeks, but there was no apparent decline in the numerical density of peroxisomes. Relative liver weights were increased about equally in 2–3-week-old pups and dams (1.5–1.6-fold).

A study designed to investigate the effects of a PPAR α agonist on neonatal rats of different ages was conducted by [Dostal et al. \(1987\)](#). Male Sprague-Dawley rats that were 6, 14, 16, 21, 42 or 86 days of age were given daily doses of 0, 10, 100, 1000, or 2000 mg/kg bw DEHP by gavage for 5 days; 24 hours after they were killed, the activities of hepatic peroxisomal enzymes, palmitoyl coenzyme A oxidase and CAT were determined. Administration of 1000 mg/kg bw per day caused significant decreases in body weight and caused mortality (66–70%) in pups 14–18 days of age, and administration of 2000 mg/kg bw per day caused mortality in virtually all pups of these ages. At a non-lethal dose level of 100 mg/kg bw per day, increases in absolute liver weight relative to the controls were 0, 17, 3, 10 and 14% for 6–10-, 14–18-, 21–25-, 42–46- and 86–89-day old pups and adults, respectively. At this dose level, measurements of palmitoyl coenzyme A activity showed that there was a greater increase only in the 14–18-day-old pups compared with 86–90-day-old adults (6.9-fold versus 3.98-fold). A greater increase in CAT also was shown at

this dose level only for 14–18-day-old pups compared with 86–90-day-old adults (7.8-fold versus 4.4-fold). The data on increased liver weights and peroxisomal enzyme activities from this study indicated that neonatal or young adult rats do not differ greatly from adult rats in their response to treatment with DEHP, although the palmitoyl coenzyme A and CAT activities were higher in the 14–18-day-old pups than in adults.

4.5 Mechanistic considerations

4.5.1 Effects on the liver

The effects of DEHP on the liver — a primary target organ for the pleiotropic effects of DEHP and other peroxisome proliferators in rodents — have been the focus of scientific debate for the last three decades. In the liver of rodents, parenchymal cells (also called hepatocytes) are the major cell type that is responsive to DEHP; however, other cells, such as resident hepatic macrophages (called Kupffer cells) may also play an important role. The increase in the number and size of peroxisomes in hepatocytes — peroxisome proliferation that results in elevation of fatty acid metabolism — is a hallmark response to DEHP and other peroxisome proliferators in the liver of susceptible species. A link between peroxisome proliferation and liver tumour response has been a predominant theory to explain the cause of a hepatocarcinogenic effect, although the experimental data are not unequivocal. Other molecular events, such as the induction of cell proliferation, decreased apoptosis, oxidative DNA damage and selective clonal expansion of the initiated cells, have also been proposed to be critically involved.

Overall, it is believed that the events that occur relative to DEHP-induced liver carcinogenesis in rodents involve the following, whereby the combination of the molecular signals and multiple pathways rather than a single hallmark event (such as activation of PPAR α , peroxisome

proliferation or cell proliferation) contribute to the formation of tumours: (1) rapid metabolism of the parent compound to primary and secondary bioactive metabolites that are readily absorbed and distributed throughout the body; (2) receptor-independent activation of hepatic macrophages and production of oxidants; (3) activation of PPAR α in hepatocytes and sustained increases in the expression of peroxisomal and non-peroxisomal metabolism-related genes; (4) enlargement of many hepatocellular organelles (e.g. peroxisomes, mitochondria); (5) a rapid but transient increase in cell proliferation and a decrease in apoptosis; (6) sustained hepatomegaly; (7) chronic low-level oxidative stress and accumulation of DNA damage; (8) selective clonal expansion of initiated cells; (9) appearance of preneoplastic nodules; and (10) development of adenomas and carcinomas.

Despite the wide use of phthalates, including DEHP, which leads to appreciable exposure of the general population, only limited data are available for consideration of the possible adverse health effects of DEHP in human populations. The majority of experimental human data comes from in-vitro studies in cultured human liver cells; however, results of these studies suggest that human cells do not respond to DEHP or its metabolites in manner that parallels responses observed in cultured rodent liver cells. At the same time, even rodent liver cells do not replicate many of the events observed in rodent livers *in vivo*.

Major differences in the metabolism and molecular signalling events elicited by DEHP in the liver, such as the activation of PPAR α , have been observed between species. A previous evaluation ([IARC, 2000](#)) considered DEHP and concluded that, in rodents, peroxisome proliferators exercise their pleiotropic effects in the liver due to the activation of PPAR α and that this process is essential for liver hypertrophy and hyperplasia and eventual hepatocarcinogenesis. This conclusion was based on data from a

variety of studies that considered the molecular biology of PPAR α signalling in different species, transactivation potency of PPAR α from different species, in-vivo studies in non-human primates and studies in genetically modified mice.

One of the key pieces of evidence reviewed previously ([IARC, 2000](#)) was the chronic feeding study with the peroxisome proliferator WY-14 643 in wild-type and *Ppara*-null mice that showed that the null mice were completely refractory to liver carcinogenesis. Although this study made one of the most significant contributions to mechanistic research on peroxisome proliferators, the large dose of the agent used, a duration of exposure of less than 2 years and the relatively small numbers of animals evaluated somewhat limit the utility of these data. Several additional studies that used the same and other genetically engineered mice have been completed during the past 4 years, and these provide important additional data key for consideration of the relevance of the PPAR α mode of action to rodent and human liver carcinogenesis. These include, but are not limited to, studies in *Ppara*-null mice, PPAR α humanized transgenic mice and hepatocyte-specific constitutively activated *Ppara* transgenic mice ([Yang et al., 2007](#)). The data from these animal models suggest that, although the activation of PPAR α and the subsequent downstream events mediated by this transcription factor represent one key mechanism of action, it is evident that several additional molecular signals and multiple pathways in several cell types in the liver, rather than a single molecular event, contribute to the formation of liver tumours in rats and mice.

Furthermore, it should be noted that although important species differences in the activation of PPAR α or its signalling network by peroxisome proliferators exist, human cells express PPAR α and are not devoid of transactivation responses to many peroxisome proliferators, including MEHP. Important interindividual differences in PPAR α expression have been reported, suggesting

that the differences in expression between species may need to be verified using larger samples of both humans and animal strains. Thus, although quantitative differences between species may well exist, qualitative similarities cannot be ignored, especially because DEHP and other PPAR α activators are known to induce molecular responses independent of PPAR α activation. It remains a possibility that these pathways contribute to human risk in ways that differ somewhat from those postulated for liver cancer in rats and mice.

Overall, the mechanisms for the induction of cancer by DEHP have not been established entirely and are certainly complex. In the following section, some of the diverse effects that could contribute are summarized. Although it is becoming more evident that the complex molecular events that lead to cancer may not always lend themselves to unequivocal assignment to one of the stages of carcinogenesis (i.e. initiation, promotion or progression), this established paradigm provides a useful framework for a discussion of most of the mechanistic data on DEHP that are relevant to the liver.

(a) *Tumour initiation*

(i) *Genotoxic and related events elicited by DEHP or its metabolites*

DEHP and its metabolites generally give negative results in bacterial mutagenicity assays in the presence or absence of metabolic activation. However, several recent studies in human and other mammalian primary cells or established cell lines showed that in-vitro exposures to DEHP or its primary metabolite, MEHP, produced DNA strand breaks detectable in the Comet assay or induced cell transformation. One mutation study in transgenic mice has shown evidence for the in-vivo genotoxicity of DEHP, while another gave negative results. In one study, DEHP did not exhibit initiation activity. It is not clear whether these effects that indicate genotoxicity are a result of a direct reaction of DEHP

or its metabolites with DNA or could be due to secondary oxidative stress or other events.

(ii) *Oxidative stress*

It is widely accepted that in-vivo exposure of rats or mice to DEHP leads to increased oxidative stress in the liver. The induction of peroxisomal and microsomal enzymes, a pathway largely dependent on the activation of PPAR α , contributes to an increase in reactive oxygen species formed in the hepatocytes. Oxidative DNA damage, generation of lipid peroxidation products that can form DNA adducts and the induction of base-excision DNA repair genes (presumably in response to increased oxidative DNA damage) in the liver has been observed in both rats and mice. An alternative PPAR α -independent mechanism for increased oxidative stress has been shown to involve the activation of resident liver macrophages (Kupffer cells) which have been shown to be activated by MEHP *in vitro* to generate oxidants. Also, several studies *in vivo* in both rats and mice have shown that Kupffer cells produce oxidants that, in turn, may damage DNA. There may be important differences in the cellular source of oxidants in the liver that are dependent on the duration of exposure to DEHP. Although the peroxisomal- and microsomal-derived oxidants may be generated continuously throughout treatment, the role of Kupffer cells is best established after only short-term or acute exposures to DEHP. Although the peroxisome proliferator-induced activation of Kupffer cells has been shown to generate oxidants in rodent cells, no data are available to determine whether this PPAR α -independent mechanism is also operative in human cells.

(iii) *Effects on toxicokinetics of other carcinogens*

DEHP has a major effect on liver metabolism. Although most of the effects pertain to lipid biotransformation, several studies have shown that drug metabolism enzymes other than the CYP4A family are also affected by exposure to

DEHP. These effects occur in both rodents and humans, and PPAR α -independent effects on metabolism genes have been observed.

(b) *Tumour promotion*

(i) *Cell proliferation*

The rate of hepatocellular proliferation is markedly increased even after administration of a single dose of peroxisome proliferators, including DEHP, to rats or mice. There is clear evidence that DEHP causes an acute increase in hepatocellular proliferation in both rats and mice. The elevation in cell replication rate in the liver has been implicated in the mechanism of carcinogenesis, because it may result in higher levels of mutation by increasing the frequency of replicative DNA synthesis as well as the number of hepatocytes at risk.

A cell-proliferation response has not been observed in non-human primates or in some rodent species exposed to DEHP, but no studies in humans have addressed this potential mechanism. The acute cell-proliferation response in the liver of rats and mice *in vivo* is a whole-liver phenomenon, and has not been observed in rodent hepatocytes *in vitro* even when the cells were properly purified, suggesting that factors produced by other cell types in the whole liver are also a prerequisite. Although there appears to be a relationship between the activation of PPAR α in rats and mice and an increase in cell proliferation, the molecular mechanisms of this association are not well understood.

Cell-cycle genes do not appear to be under the transcriptional control of PPAR α . Rather, several indirect mechanisms have been proposed, including the involvement of microRNAs, the activation of p38 mitogen-activated kinase or the activation of *Ras*-like proto-oncogenes via post-translational modification. None of these mechanisms has been investigated with regard to DEHP in susceptible species, and their relevance to human hazard has yet to be elucidated.

In addition, one study showed that, although constitutive activation of PPAR α in mouse liver (through genetic means and without chemical treatment) leads to increased cell proliferation, it does not lead to hepatocarcinogenesis.

Numerous studies that used an initiation–promotion protocol — with NDEA or another agent as an initiator and DEHP or phenobarbital as a promoter — showed that DEHP can promote genotoxic carcinogenesis; however, it is not clear whether this effect is due to an effect of DEHP on carcinogen metabolism, the induction of cell proliferation or other events. In addition, because the increase in cell proliferation in rat and mouse liver caused by DEHP is an acute phenomenon, its relevance to DEHP-induced liver carcinogenesis needs further study.

(ii) *Suppression of apoptosis*

Suppression of apoptosis in the liver has been suggested as an additional mechanism through which the normal balance of cell turnover may be impaired to create conditions that promote neoplastic growth. *In vivo*, little evidence exists to support this hypothesis, even in rats and mice. In cultured hepatocytes, several peroxisome proliferators, including DEHP, have been shown to inhibit pro-apoptotic signalling events. This has not been observed in cultured human hepatocytes, but the database on human studies is limited to a single report. The potential role for this mechanism is further complicated by the observation of an increase in apoptotic signalling after continuous exposure to WY-14 643.

(iii) *Activation of nuclear receptors other than PPAR α*

Recent studies showed that DEHP and other peroxisome proliferators can induce several drug-metabolizing genes that are not known PPAR α targets. In human primary hepatocytes and cancer cell lines, some studies have shown that DEHP can increase activity and/or expression of the CYP3A, CYP2B and CYP1A family of

enzymes. In mice, comparative analysis of gene expression profiles in the liver of DEHP-treated wild type animals with those in DEHP-treated *Ppara*-null animals showed that, although PPAR α -dependent events are a dominant transcriptional response, the induction of other genes, many of which are homologous to those affected in human cells, was also observed. There is evidence in both mice and humans that other nuclear receptors are targets for DEHP including CAR and PXR.

4.5.2 Effects on testes

Many rodent studies have observed that chronic administration of DEHP leads to testicular toxicity. Various pre- and postnatal study designs showed that DEHP is a reproductive and developmental toxicant in rats and mice. It has also been demonstrated that the testicular toxicity of DEHP is a PPAR α -independent phenomenon, because identical, although slightly delayed, effects were observed in *Ppara*-null mice. Two bioassays (103–104 weeks in duration) in F344 rats and B6C3F₁ mice did not show evidence of testicular tumours although consistent observations of testicular toxicity were reported. Life-long (up to 159 weeks) exposure to DEHP in a different strain of rats (Sprague-Dawley) showed a significant increase in testicular (as well as liver) tumours, and it was noted that latency for the testicular tumours was even shorter than that for liver tumours. In humans, the database of the reproductive and developmental effects of phthalates has been growing rapidly over the past decade, and numerous studies reported data suggestive of an association between exposure to DEHP and/or other phthalates and adverse effects on both male and female reproduction.

4.5.3 Pancreatic tumours

One chronic bioassay found that DEHP can induce acinar-cell adenoma of the exocrine pancreas of F344 rats. No mechanistic or other follow-up studies are available to determine whether evidence in animals may be relevant to humans.

5. Summary of Data Reported

5.1 Exposure data

Di(2-ethylhexyl) phthalate has been produced since the 1930s by the reaction of 2-ethylhexanol with phthalic anhydride. It is widely used as a plasticizer to soften polyvinyl chloride plastics (used in medical devices, tubing, footwear, food packaging, wire and cable coverings and toys) and, to a lesser extent, non-polymers (used in dielectric fluids, paints, adhesives and inks). Occupational exposure to di(2-ethylhexyl) phthalate generally occurs by inhalation during its manufacture and use as plasticizer of polyvinyl chloride. Exposure of the general population to di(2-ethylhexyl) phthalate occurs from the use of medical devices, such as blood bags and medical tubing, its presence as a contaminant of food and, to a lesser extent, its presence in the environment (air, water and soil).

Human intake of di(2-ethylhexyl) phthalate can be estimated by measurement of di(2-ethylhexyl) phthalate and its total metabolites in blood and urine; particularly high concentrations of urinary metabolites were reported in neonates in intensive care.

5.2 Human carcinogenicity data

The only analytical epidemiological study that measured exposure to di(2-ethylhexyl) phthalate specifically was a case-control study of female breast cancer and exposure to phthalates, including di(2-ethylhexyl) phthalate. Phthalate

metabolites were measured in the urine and an increased risk for breast cancer was identified for one of the four di(2-ethylhexyl) phthalate metabolites evaluated (mono(2-ethyl-5-carboxypentyl) phthalate). No association was found for the other three di(2-ethylhexyl) phthalate metabolites: mono(2-ethylhexyl) phthalate, mono(2-ethyl-5-hydroxyhexyl) phthalate and mono(2-ethyl-5-oxohexyl) phthalate.

One cohort study and one nested case-control study assessed pancreatic cancer in workers potentially exposed to di(2-ethylhexyl) phthalate. In the nested-case-control study, a large excess of pancreatic cancer was observed among men who had worked for more than 16 years in vinyl and polyethylene production areas, where di(2-ethylhexyl) phthalate was used. However, this excess was based on five exposed cases only. One small study of workers in a di(2-ethylhexyl) phthalate production plant did not show any excess mortality from cancer. However, this cohort study did not have adequate power to detect a potential excess risk. Only eight deaths from any cause occurred and the one observed cancer death was from pancreatic cancer.

Two of three case-control studies of testicular cancer reported a statistically significant association with occupational exposure to polyvinyl chloride; although there was no positive exposure-response relationship in one study. The third study was limited by the small number of men exposed to polyvinyl chloride for more than 1 year. These workers were potentially exposed to phthalate plasticizers, including the most common (i.e. di(2-ethylhexyl) phthalate), but none of these studies evaluated exposure to di(2-ethylhexyl) phthalate specifically.

In a cohort study of polyvinyl chloride processing workers, moderate and high cumulative exposure to plasticizers was associated with an increased risk for respiratory cancer but no statistically significant positive exposure-response was observed. Di(2-ethylhexyl) phthalate was the main plasticizer used in this study.

In a population-based case-control study, the risk for multiple myeloma increased with increasing duration of probable occupational exposure to phthalates.

5.3 Animal carcinogenicity data

Di(2-ethylhexyl) phthalate was tested for carcinogenicity by oral administration in the diet in male and female mice in two studies, in male and female rats in two studies, and in one study in male rats. The incidence of hepatocellular adenoma and hepatocellular carcinoma was consistently increased in both species. In one study in rats, a significant increase in the incidence of pancreatic acinar-cell adenoma was observed in males. In another study in rats, the incidence of benign Leydig-cell tumours was increased, and was dose-related with early onset.

In two initiation-promotion studies in mice, exposure to di(2-ethylhexyl) phthalate following administration of *N*-nitrosodiethylamine enhanced the incidence of hepatocellular adenoma or hepatocellular carcinoma. In several initiation-promotion studies in rats and in two such studies in hamsters, in general, no promoting activity of di(2-ethylhexyl) phthalate was demonstrated but, in one initiation-promotion study in rats with *N*-ethyl-*N*-hydroxyethylnitrosamine, the incidence and multiplicity of renal tubule adenoma or carcinoma (combined) were increased by di(2-ethylhexyl) phthalate.

Tumours of the exocrine pancreas are rare spontaneous neoplasms in experimental animals.

5.4 Other relevant data

In humans and rodents, di(2-ethylhexyl) phthalate is metabolized by lipases, ubiquitous enzymes in various tissues, to mono(2-ethylhexyl) phthalate, which is then oxidatively metabolized to several compounds that are subsequently excreted in the urine. Pancreatic lipase plays a major role, especially after oral exposure. Species

differences in lipase activity between tissues have been identified and may play a role in species differences in the effects of di(2-ethylhexyl) phthalate. In humans, urinary metabolites include mono(2-ethyl-5-hydroxyhexyl) phthalate, mono(2-ethyl-5-oxohexyl) phthalate, mono(2-ethylhexyl) phthalate, mono(2-ethyl-5-carboxypentyl) phthalate and mono(2-carboxymethyl)hexyl phthalate. The major metabolites excreted in the urine are mono(2-ethyl-5-oxohexyl) phthalate, mono(2-ethyl-5-carboxypentyl) phthalate and mono(2-ethyl-5-oxohexyl) phthalate. Due to the complexity of secondary metabolites formed, no single metabolite has been identified as a good biomarker for exposure to di(2-ethylhexyl) phthalate. The forms of cytochrome P450 or other enzymes responsible for oxidative metabolism are not well understood.

Studies of the absorption, distribution, excretion and metabolism of di(2-ethylhexyl) phthalate are hampered by its ubiquitous presence in the environment and laboratory equipment, and that it can be hydrolysed under abiotic conditions. Studies of the radiolabelled compound provide the most accurate indications of the absorption and distribution of di(2-ethylhexyl) phthalate and its metabolites. Variation in urinary metabolites of di(2-ethylhexyl) phthalate in humans is large and may reflect differences in exposure, as well as its absorption, distribution, excretion and metabolism between subjects.

Di(2-ethylhexyl) phthalate and its metabolites have been extensively tested in bacterial mutagenicity assays in the presence or absence of metabolic activation. The results of these studies have been generally negative. Studies in human and other mammalian primary cells or established cell lines provide evidence that in-vitro exposure to di(2-ethylhexyl) phthalate or its primary metabolite, mono(2-ethylhexyl) phthalate, may result in DNA strand breaks or induce cell transformation. It is not clear whether these effects are a result of the direct reaction of di(2-ethylhexyl) phthalate or its metabolites with DNA

or could be due to secondary oxidative stress or other events. Studies of in-vivo mutagenicity in two different transgenic mouse models have been conducted, but the results are conflicting, which confounds the interpretation of these findings.

The molecular events associated with the reproductive and developmental effects of di(2-ethylhexyl) phthalate and other phthalates are not well characterized, but many studies suggest that the effects of phthalates on metabolism and other cellular functions lead to disruption of steroidogenesis, increased oxidative stress, increased apoptosis and other events. Data also suggest that both Sertoli and Leydig cells are targets for the toxicity of di(2-ethylhexyl) phthalate.

Since the previous evaluation, important additional mechanistic information has become available, including, but not limited to, subacute, subchronic and chronic studies with di(2-ethylhexyl) phthalate in peroxisome proliferator-activated receptor α -null mice, as well as findings from several transgenic (peroxisome proliferator-activated receptor α -humanized and hepatocyte-specific constitutively activated peroxisome proliferator-activated receptor α mouse lines. Activation of peroxisome proliferator-activated receptor α and the subsequent downstream events mediated by this transcription factor represent an important mechanism of action for di(2-ethylhexyl) phthalate in rats and mice. However, additional data from animal models and studies in humans exposed to di(2-ethylhexyl) phthalate from the environment suggest that multiple molecular signals and pathways in several cell types in the liver, rather than a single molecular event, contribute to the induction of cancer in rats and mice. Thus, the relevance to human cancer of the molecular events that lead to cancer elicited by di(2-ethylhexyl) phthalate in several target tissues (e.g. the liver and testis) in rats and mice cannot be ruled out.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

Di(2-ethylhexyl) phthalate is *possibly carcinogenic to humans (Group 2B)*.

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BENZOPHENONE

1. Exposure Data

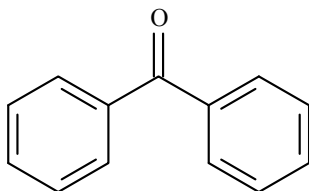
1.1 Chemical and physical data

From [IUCLID \(2000\)](#), [IPCS-CEC \(2005\)](#), [NTP \(2006\)](#), [GESTIS \(2010\)](#), and [Repertoire Toxicologique \(2010\)](#), unless otherwise specified

1.1.1 Nomenclature

Chem. Abstr. Services Reg. No.: 119-61-9
Chem. Abstr. Name: Benzene, benzoyl-; benzoylbenzene, phenyl ketone; diphenylketone; diphenyl ketone; diphenylmethanone; ketone, diphenyl; methanone, diphenyl-; α -oxodiphenylmethane; α -oxoditane
RTECS No.: DI9950000
EINECS No.: 204-337-6

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{13}H_{10}O$

Relative molecular mass: 182.22

1.1.3 Chemical and physical properties of the pure substance

Description: Colourless crystalline solid with geranium- or rose-like odour

Boiling-point: 305.4 °C

Melting-point: 48.5 °C (α form) and 26 °C (β form)

Density: 1.111 at 18 °C

Vapour pressure: 1.93×10^{-3} mm Hg at 25 °C

Refractive index: 1.6077 at 19 °C

Solubility: Practically insoluble in water, but soluble in organic solvents such as alcohol, acetone, ether, acetic acid, chloroform and benzene.

Flash-point: > 110 °C

Stability: Decomposes on heating to produce toxic gases; reacts with strong oxidants.

Octanol/water partition coefficient: $\log K_{ow}$, 3.18 ([LOGKOW, 2010](#))

Henry's law constant: 1.9×10^{-6} atm.m³/mol at 25 °C

1.1.4 Technical products and impurities

No data were available to the Working Group.

1.1.5 Analysis

(a) Air

One method has been reported by the Occupational Safety and Health Administration of the United States of America (OSHA PV2130) regarding the possibility of measuring benzophenone in air using a tube filled with chromosorb 106 (100/50-mg sections, 60/80 mesh) at a recommended maximum volume of 48 L and a maximum flow rate of 0.2 L/min. An analytical solvent (99:1 carbon disulfide:*N,N*-dimethylformamide) is used to desorb the chromosorb, and the substance is then measured by gas chromatography with flame ionization detection.

(b) Food

Analysis of benzophenone in breakfast cereals has been reported using ultrasonic extraction in combination with gas chromatography-tandem mass spectrometry ([Van Hoeck et al., 2010](#)).

1.2 Production and use

1.2.1 Production

A 66% yield of benzophenone can be obtained by Friedel-Crafts acylation of benzoyl chloride with an excess of benzene in the presence of anhydrous aluminium chloride ([NTP, 2006](#)). Benzophenone is also produced by atmospheric oxidation of diphenylmethane in the presence of metal catalysts, such as copper naphthenate ([HSDB, 2010](#)).

According to the US Environmental Protection Agency, it was classified in 2003 as a high volume chemical, with an annual production exceeding 1 million pounds [453 000 kg], in the USA ([NTP, 2006](#)).

1.2.2 Use

Benzophenone is used as a flavour ingredient, a fragrance enhancer, a perfume fixative and an additive for plastics, coatings and adhesive formulations; it is also used in the manufacture of insecticides, agricultural chemicals, hypnotic drugs, antihistamines and other pharmaceuticals ([HSDB, 2010](#)). Benzophenone is used as an ultraviolet (UV)-curing agent in sunglasses, and to prevent UV light from damaging scents and colours in products such as perfumes and soaps. Moreover, it can be added to plastic packaging as a UV blocker, which allows manufacturers to package their products in clear glass or plastic rather than opaque or dark packaging. It is also used in laundry and household cleaning products ([NTP, 2006](#); [HSDB, 2010](#)).

Benzophenone is widely used as a photoinitiator for inks and varnishes that are cured with UV light. In addition to being a drying catalyst, benzophenone is an excellent wetting agent for pigments; it can also be used in printing to improve the rheological properties and increase the flow of inks by acting as a reactive solvent.

[No data were available to the Working Group on the use of benzophenone in sunscreens, whereas data were available on the use of one of its derivatives (3-benzophenone, 2-hydroxy-4-methoxybenzophenone) in such products.]

1.3 Occurrence

1.3.1 Natural occurrence

Benzophenone has been reported to occur naturally in food (see Section 1.3.3).

1.3.2 Occupational exposure

Benzophenone can be absorbed into the body by inhalation, through the skin and by ingestion ([IPCS-CEC, 2005](#)).

Industrial sectors that entail risks of occupational exposure are painting (paints,

varnishes and lacquers), the manufacture of plastic composites and the manufacture and use of glues and adhesives. The National Institute for Occupational Safety and Health conducted the National Occupational Exposure Survey in 1981–1983, which estimated that, among the 4490 establishments surveyed in the USA (522 industry types, employing approximately 1 800 000 workers), 41 516 workers (18 162 women) were potentially exposed to benzophenone ([NIOSH, 1990](#)).

1.3.3 Occurrence in food and dietary exposure

Dietary sources of exposure to benzophenone include its natural occurrence in food, its presence as a contaminant in drinking-water, its migration from food packaging and its addition to food as a flavouring.

(a) Food

Benzophenone was reported to occur naturally in wine grapes (*Vitis vinifera* L.) at concentrations of 0.08–0.13 ppm [mg/kg] ([TNO, 2010](#)). According to the [Council of Europe \(2000\)](#), it mainly occurs in muscat grapes. Benzophenone has been detected quantitatively in passiflora species at 0.045 ppm ([TNO, 2010](#)) and qualitatively in black tea, cherimoya (*Annona cherimola*), mountain papaya (*Carica pubescens*) and soursop (*Annona muricata* L.) ([Burdock, 2005](#)). Concentrations in mountain papaya (*C. pubescens* and *C. candamarcensis*) were reported to be lower than 0.01 ppm ([TNO, 2010](#)).

Based on its concentration in muscat grapes, the Working Group estimated that consumption of 200 g grapes would result in exposure to approximately 20 µg benzophenone, i.e. 0.3 µg/kg body weight (bw) for a 60-kg adult.

(b) Drinking-water

The data on benzophenone in drinking-water are limited. Levels of 8.8 ppb [µg/L] were found in tap-water in Japan ([Shinohara et al., 1981](#)) and

0.26 µg/L in finished drinking-water in a water filtration plant in the USA in 2001–02 ([Loraine & Pettigrove, 2006](#), see Section 1.3.4).

To assess exposure to contaminants through drinking-water, the WHO uses a default consumption value of 2 L of drinking-water per capita per day for a typical adult of 60-kg bw ([WHO, 2008](#)), based on the assumption that total water consumption is 3 L per capita per day, including water present in food, which represents a conservative estimate ([WHO, 2003](#)). However, such a default assumption is not appropriate for all populations and climates. Reference hydration values under average conditions are 0.75 L in infants (5 kg), but, for physically active persons in areas with higher temperatures, could reach 4.5 L for men, women and children, 4.8 L for pregnant women and 5.5 L for lactating women ([WHO, 2003](#)).

The available data on concentrations of benzophenone in drinking-water were used by the Working Group to assess dietary exposure in adults and infants (60-kg and 5-kg bw, respectively), assuming a consumption of 2 and 0.75 L of drinking-water, respectively, i.e. 33 and 150 mL/kg bw, respectively. The infant scenario (in mL/kg bw) would correspond to a consumption of 9 L of drinking-water per day in a 60-kg adult and would therefore encompass any possible scenario of physically active persons in high-temperature areas. Hence, the estimated dietary exposure to benzophenone through the drinking-water of a standard 60-kg adult would range from 0.52 to 17.6 µg per day, i.e. 9–290 ng/kg bw per day, and that of a 5-kg infant would range from 0.2 to 6.6 µg per day, i.e. 40–1320 ng/kg bw per day.

(c) Migration from food packaging

The main source of exposure to benzophenone through food packaging is related to its wide use as photo-initiator in UV-cured inks on the external face of paperboard packaging. Benzophenone is neither totally exhausted during the printing process nor removed thereafter, and

is nor irreversibly bound into the print film layer (Koivikko *et al.*, 2010). It may thus migrate to food from paperboard, either by direct contact or through the vapour phase. Substances present on the external face of the packaging may contaminate the internal face when the carton is rolled and compressed, which is a common practice in the food packaging industry, and thus contaminate food through direct contact. Benzophenone may also contaminate food through the vapour phase, even from the secondary packaging. Internal plastic bags that are used as a barrier against moisture are not always effective (EFSA, 2009). Benzophenone is known to migrate easily through polypropylene film, whereas aluminium and multilayer materials inhibit migration efficiently (Nerín & Asensio, 2007; Pastorelli *et al.*, 2008).

Under low-temperature conditions (-20 °C), benzophenone migrates from cartonboard to food during frozen storage, even when there is no direct contact between the packaging and the food or when the packaging is polyethylene-coated (Johns *et al.*, 2000). Moreover, the most commonly used raw material for paperboard is recycled, and the product therefore often contains photo-initiators, including benzophenone. Recycled board is commonly used in direct contact with dry foodstuffs, such as flour and pasta, but also with fast-food items, i.e. foodstuffs with a short duration of contact, such as pizzas. Normally, a functional barrier, e.g. plastic or aluminium foil, is used between fatty or aqueous foodstuffs and the recycled material to avoid direct contact.

Analytical data are available on the concentrations of benzophenone in food packagings and in foods. In particular, in a comprehensive survey performed by the United Kingdom Food Standards Agency (UK FSA, 2006), benzophenone was detected in four of 115 samples of foodstuff packaged in printed plastic (maximum concentration, 0.15 mg/kg), in 60/296 samples packaged directly or indirectly in printed paper

or board that contained 0.05–3.3 mg benzophenone/dm² at a concentration of 0.035–4.5 mg/kg (mean concentration, 0.9 mg/kg) and in one of 54 foodstuffs to which a printed sticky label had been attached (at 0.029 mg/kg). In this survey, a high percentage of products tested positive for benzophenone among the categories of frozen foods (18/35), ‘jelly’ (3/5) and ‘savoury snacks’ (15/40). A lower percentage of products tested positive in the categories of ‘sweets, chocolate biscuits and crisps’ (5/35), ‘bakery products’ (8/35) and ‘cereals’ (4/25). Only one ‘ready meal’ of 20 and none of 10 ‘desserts’ tested positive.

According to the United Kingdom Food Standards Agency (UK FSA, 2006), potential dietary exposure to benzophenone in high-level consumers is 1.2–1.5 µg/kg bw for adults. These estimates were calculated by combining a high level of consumption of foods that may contain benzophenone (449 g/day, the 97.5th percentile in the United Kingdom national survey of adults) with two average levels of its occurrence therein (160 and 200 µg/kg), depending on different assumptions of the values below the limit of quantification (45 µg/kg), for a 60-kg bw adult.

More recent but limited data on benzophenone concentrations in food products are available in other countries.

Samples of milk packaged in cartons on the market in the People’s Republic of China were tested: skimmed milk, whole milk and partially skimmed milk. Benzophenone was detected in the packaging of all products at a concentration of 0.94–1.37 µg/dm², and in five of six of the milk products. Higher levels were found in milk with a higher fat content and ranged from 2.84 to 18.35 µg/kg (Shen *et al.*, 2009).

The migration of benzophenone into five selected dry foods (cake, bread, cereals, rice and pasta) sampled in a supermarket in Spain was assessed by Rodríguez-Bernaldo de Quirós *et al.* (2009). The highest concentration of benzophenone was found in cake (12 mg/kg). Migration levels were positively correlated with both

porosity and fat content. These results correlated well with those reported by [Anderson & Castle \(2003\)](#), who analysed 71 food samples selected at random from a total of 143 items packaged in printed cartonboard, in which benzophenone had been detected. The highest value (7.3 mg/kg) was found in a high-fat chocolate packaged in direct contact with cartonboard.

In a study by [Sanches-Silva et al. \(2008\)](#), samples of 36 commercial beverages (fruit and vegetable juices, wine and soft drinks) were collected in Italy, Portugal and Spain in 2005 and 2006, all of which were packaged in multi-material multilayer boxes or aluminium cans. Benzophenone was detected in four samples of packaging (one under the limit of quantification of 1.7 µg/dm² and three ranging from 3.6 to 12.3 µg/dm²), and samples of the beverages contained therein were analysed. None of the extracts yielded positive results. However, according to the authors, although fruit juices contain low amounts of fat, photo-initiators can migrate and be adsorbed by juice fibres (fibre content, 0.2%) and thus contaminate the beverage.

In a study conducted by [Koivikko et al. \(2010\)](#) in the European Union (EU), samples of printed board used for secondary packaging were collected from supermarkets, together with the food contained therein ($n = 22$), and some were acquired from industrial production lines before the introduction of foodstuffs ($n = 24$). In addition, samples were taken of recycled paperboard collected from a supplier to evaluate the background level of benzophenone and other derivatives therein ($n = 19$). The most abundant photo-initiator found in the non-recycled products was benzophenone, which was detected in 61% of samples. Traces of the compound were also found in 42% of the samples of recycled unprinted board. The content of benzophenone in these samples varied from 0.57 to 3.99 mg/m².

Benzophenone migrated into 95% ethanol from recycled paperboard used for contact

with food in Japan, but not from virgin paper. Migration ranged from 1.0 to 18.9 ng/mL in eight of the 21 samples of recycled paperboard collected ([Ozaki et al., 2006](#)).

In 2009, high levels of 4-methylbenzophenone (another photo-initiator) detected in some breakfast cereal products (chocolate crunch muesli) were notified under the EU Rapid Alert System for Food and Feed (RASFF) ([European Commission, 2009](#)). Further analysis performed by the producer demonstrated high concentrations of this substance and up to 4210 µg/kg benzophenone in these products ([CS AFSCA Belgium, 2009](#)).

(d) Addition to foods as a flavouring

In the USA, the average reported levels of use of benzophenone as an additive range from 0.57 ppm [mg/kg] in non-alcoholic beverages to 1.57 ppm in baked goods, and maximum reported levels range from 1.28 ppm in non-alcoholic beverages to 3.27 ppm in frozen dairy products. Other reported uses are in soft candy, gelatins and puddings ([Burdock, 2005](#)).

Maximum levels reported by the Council of Europe are 0.5 mg/kg in beverages and 2 mg/kg in foods in general, with no exception ([Council of Europe, 2000](#)). Benzophenone is listed in the EU register of chemically defined flavourings. In the European Food Safety Authority (EFSA) Flavouring Group Evaluation 69 ([EFSA, 2008](#)), dietary exposure to benzophenone in the EU, based on poundage data provided by industry, was estimated to be 23 µg per capita per day, assuming that consumers represent 10% of the population. On the same basis, dietary exposure is estimated to be 11 µg per capita per day in the USA ([EFSA, 2008](#)).

As a flavouring of threshold of toxicological concern class III, evaluated by the EFSA on the basis of a Joint FAO/WHO Expert Committee on Food Additives evaluation, refined, surveyed levels of additive use were provided by industry to the European Commission ([IOFI-DG SANCO,](#)

2008). The single portion exposure technique was developed by the Joint FAO/WHO Expert Committee on Food Additives to estimate dietary exposure from the consumption of one standard portion per day of flavoured food or beverages containing the flavouring substance at its average level of use (Leclercq *et al.*, 2009). Using this technique, the Working Group calculated that estimated exposure to benzophenone is 6 µg per person per day when applied to IOFI-DG SANCO (2008) data, 40 µg per person per day when applied to data from the Council of Europe (2000) and 170 µg per person per day when applied to data from the USA reported by Burdock (2005).

1.3.4 Environmental occurrence

Benzophenone is harmful to aquatic organisms (IPCS-CEC, 2005). Benzophenones in general have the environmentally critical properties of high lipophilicity and persistence, and are known to have adverse effects on the reproduction and hormonal functions of fish (Parks, 2009). According to Brooks *et al.* (2009), benzophenone is persistent, bioaccumulative and toxic (PBT).

Because of its high octanol:water partition coefficient and its insolubility in water, benzophenone partitions in soil and sediment (US EPA, 1984, cited by NTP, 2006), and its adsorption to soil is proportional to the organic content therein (OHMTADS, 1991, cited by NTP, 2006).

(a) Water and sediments

Benzophenone is among the pharmaceuticals and personal care products that are known to occur in drinking-water and in reclaimed wastewater when water sources are impacted by sewage treatment plant effluent (Loraine & Pettigrove, 2006). The removal of these compounds during wastewater-treatment processes is not fully effective, and effluent-dominated streams represent 'worse-case scenarios' for studying personal care

products and other organic wastewater contaminants. In these streams, even compounds with relatively short environmental half-lives, such as benzophenone, may act as 'pseudo-persistent' compounds. Due to their continuous introduction from wastewater-treatment plants, these compounds are continuously released into the environment. As a result, aquatic organisms are exposed over their entire life cycle (Pedrouzo *et al.*, 2010).

Another route by which benzophenone enters the aquatic environment is from municipal solid-waste landfill leachates. In a study by Pitarch *et al.* (2010), benzophenone was qualitatively identified in wastewater samples from the municipal solid-waste treatment plant at Reciplasa (Castellón province, Spain) between March 2007 and February 2009. Samples of water were collected before and after reverse osmosis treatment, which is performed before the release of water into the environment. Benzophenone was detected in 38% of treated samples and in 55% of raw leachates. In a study by Trzcinski and Stuckey (2010), submerged anaerobic membrane bioreactors were fed a simulated feedstock of the organic fraction of municipal solid waste, and benzophenone was found among contaminants in the permeate of the leachate.

In a study by Yoon *et al.* (2010) of surface waters from sampling sites on the river and in effluent-dominated creeks along the Han River (Seoul, Republic of Korea), benzophenone was detected (limit of detection, 50 ng/L) in two of four river samples (mean, 52 ng/L; maximum, 59 ng/L) and in all four effluent-dominated creek samples (mean, 102 ng/L; maximum, 130 ng/L) as a result of wastewater outfall. Benzophenone was detected in surface water at Ozark Plateau of northeastern Oklahoma (USA) at a site downstream from the outfall of a municipal wastewater-treatment plant and in a hydrologically linked cave (Bidwell *et al.*, 2010). It was present in wastewater effluent from the main sewer of the city of Zagreb (Croatia), which received no

treatment at the time of the survey and comprised a mixture of effluent from domestic and industrial sources ([Grung et al., 2007](#)).

Benzophenone was detected qualitatively in water from the Baltic Sea ([Ehrhardt et al., 1982](#)) and from Hamilton harbour, Bermuda ([Ehrhardt, 1987](#)), and was determined in two water samples from the Tama river in Japan at concentrations of 21.0 and 22.8 ng/L ([Kawaguchi et al., 2006](#)). It has been detected at concentrations of < 2.6–1040 ng/L in water samples from Venice lagoon and San Francisco estuary ([Oros et al., 2003](#), [Pojana et al., 2004](#); [Pojana et al., 2007](#)) and of 14–200 µg/kg in sediment samples ([Burkhardt et al., 2005](#); [Pojana et al., 2007](#)).

Benzophenone was detected in all 11 samples of bluegill fish collected from a regional effluent-dominated stream, i.e. about 650 m downstream from the effluent discharge of the Pecan Creek Water Reclamation Plant, in Denton County, TX, USA, at a mean concentration of 57 ng/g wet weight (standard deviation, 18 ng/g) and a range of 37–90 ng/g. The mean concentration in three samples of bluegill fish in Clear Creek (Denton County, TX, USA), a stream that experiences limited, if any, anthropogenic influence, was 24 ng/g ([Mottaleb et al., 2009](#)). A survey of water, sediment and biota (Mediterranean mussel, *Mytilus galloprovincialis*) in the Venice lagoon, a highly urbanized coastal water ecosystem that receives both industrial and municipal wastewater effluents, detected concentrations of benzophenone in lagoon sediments of 14–110 µg/kg ([Pojana et al., 2007](#)).

Benzophenone was detected by gas chromatography–mass spectrometry at a level of 8.8 ppb [µg/L] in tap-water from the Kitakyushu Municipal Institute in Japan ([Shinohara et al., 1981](#)). A survey of raw and treated drinking-water from four water filtration plants in San Diego County (CA, USA) conducted in 2001–02 showed large seasonal variations in benzophenone concentrations, with higher levels in the summer than in the winter, probably because

sunscreens are used more frequently during the summer months ([Loraine & Pettigrove, 2006](#)). Benzophenone was detected in one of 15 samples of finished drinking-water at a concentration of 0.26 µg/L, and in four of six samples of reclaimed wastewater at a concentration of 0.99 µg/L (range, 0.56–1.35 µg/L).

Benzophenone has been used as a model hydrophobic contaminant ([Brooks et al., 2009](#)). Due to their hydrophobic nature, PBT contaminants move out of the water phase and become associated with sediments. Animals that reside in or on these sediments are therefore at risk of bioaccumulating PBT compounds, and acting as vectors in their transfer to predators that may otherwise have limited direct contact with contaminated sediments. Predator species accumulate benzophenone from their prey, and exposure to narcotic organic contaminants, such as benzophenone, results in hypoactivity which may alter their ability to capture such animals successfully ([Brooks et al., 2009](#)).

Benzophenone was identified in surface sediment samples from the Havel and Spree Rivers (Germany), which are characterized by high inputs of anthropogenic contaminants into their eutrophic to hypertrophic riverine system with very slow flowing conditions. In the sedimentary records from 1979/80 up to 1995, benzophenone was detected and quantified in 10 out of 11 samples at concentrations ranging from 0.5 to 4 ng/g dry matter ([Ricking et al., 2003](#)).

(b) Air

Benzophenone was identified qualitatively in the atmosphere of a 45-year-old spruce forest located in North Rhine-Westfalia (Germany) in 1988 at a height of 1 m, where severe forest damage had been observed ([Helmig et al., 1989](#)). [Leary et al. \(1987\)](#) found that benzophenone was a component of emissions from a standard residential oil burner. Although benzophenone has been identified in the atmosphere, it is difficult to determine whether its presence is due to its being

a direct product of combustion or a secondary product of atmospheric degradation ([Helmig et al., 1989](#)).

Within an indoor-air monitoring survey conducted by the Japanese Ministry of Environment, benzophenone was detected in 67/68 samples analysed ([The Japanese Ministry of Environment, 2006](#)). Human exposure through inhalation should therefore be taken into account.

[The Working Group noted that there is no consensus in relation to the potential for bioaccumulation of benzophenone in the environment, nor for its persistence or pseudo-persistence.]

1.3.5 Other occurrence

Because of its use as an additive in fragrances, cosmetics, toiletries, pharmaceuticals, insecticides, and laundry and household cleaning products, exposure to benzophenone through dermal contact may be significant. The percutaneous absorption of benzophenone was determined *in vivo* in monkeys, and was approximately 70% of the dose applied to occluded skin within 24 hours. Under unoccluded conditions, skin penetration was reduced to 44%, presumably because of evaporation from the site of application ([Bronaugh et al., 1990](#)).

Many dentures are commonly prepared through a polymerization reaction that uses benzoyl peroxide as the initiator, of which benzophenone is a decomposition product that was found to be eluted in artificial saliva from four commercial soft denture liners (two plasticized acrylates and two silicone elastomers) ([Brożek et al., 2008](#)).

1.3.6 Total human exposure

Benzophenone ingested by humans is excreted in the urine as metabolites, such as benzhydrol ([Kawaguchi et al., 2009](#)), and the measurement of its derivatives in urine may therefore provide

an indication of overall human exposure to benzophenone. In a study conducted by [Ito et al. \(2009\)](#) in 14 healthy volunteers, benzophenone derivatives were detected in all urine samples. The concentration of benzhydrol ranged from 0.27 to 10.0 ng/mL, but the parent compound was not detected in any sample.

1.4 Regulations and guidelines

The current American Industrial Hygiene Association workplace environmental exposure level for benzophenone is 0.5 mg/m³ ([AIHA, 2009](#)).

The EU Standing Committee for the Food Chain and Animal Health endorsed a limit of 0.6 mg/kg for the sum of benzophenone and 4-methylbenzophenone ([European Commission, 2009](#)). In its conclusions, the Committee stated that the European Printing Ink Association, as well as the European Carton Board Manufacturers, advised their members that printing inks containing 4-methylbenzophenone and benzophenone are not suitable for printing of food packaging unless a functional barrier is present that blocks their transfer into food and also via the gas phase. Examples of functional barriers are aluminium, poly(ethylene terephthalate)/silicon oxide or an equivalent layer.

According to the EU Directive 2002/72/EC, benzophenone may be used in the EU as an additive in plastics materials, with a specific migration limit of 0.6 mg/kg ([European Commission, 2002](#)).

Benzophenone has been listed by the Council of Europe in category B (flavouring substances for which further information is required before the Committee of Experts is able to offer a firm opinion on their safety in use; these substances can be used provisionally in foodstuff) ([Council of Europe, 2000](#)).

The United Kingdom authorities have so far judged benzophenone as a 'class B volatile

organic compound' within the context of integrated pollution control ([IUCLID, 2000](#)).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Oral administration

The results of carcinogenicity studies of oral administration of benzophenone are summarized in [Table 3.1](#).

3.1.1 Mouse

In a 2-year carcinogenicity study, groups of 50 male and 50 female B6C3F₁ mice, 8 weeks of age, were fed diets containing 0, 312, 625 or 1250 ppm benzophenone (> 99.5% pure; equivalent to average daily doses of approximately 40, 80 or 160 and 35, 70 or 150 mg/kg bw for males and females, respectively) for 105 weeks. Feed consumption of exposed males and females was similar to that of controls, but 1250-ppm females weighed 14% less than controls at the end of the study. A positive trend in the incidence of hepatocellular adenoma was observed in males; the incidence in the 625- and 1250-ppm groups was significantly greater than that in controls and exceeded the historical control ranges (12–30%) for feed studies. Hepatoblastomas were also observed in treated males, and, although the incidence in the 1250-ppm group (3/50, 6%) was not statistically significant, it exceeded the historical control range for feed studies (0–2%). The incidence of hepatocellular adenoma in 625- and 1250-ppm female mice was increased, but the difference from controls was not significant. A positive trend in the incidence of histiocytic sarcoma of the liver, lung, ovary, uterus, spleen,

adrenal gland, kidney, urinary bladder and multiple lymph nodes was observed in female mice; the incidence in the 625-ppm group was significantly increased, and that in the 625- and 1250-ppm groups exceeded the historical control range for feed studies (0–2%) ([NTP, 2006](#); [Rhodes *et al.*, 2007](#)). [The Working Group noted that hepatoblastomas and histiocytic sarcomas are rare neoplasms in mice.]

3.1.2 Rat

In a 2-year carcinogenicity study, groups of 50 male and 50 female F344/N rats, 6 weeks of age, were fed diets containing 0, 312, 625 or 1250 ppm benzophenone (> 99.5% pure; equivalent to average daily doses of approximately 15, 30 or 60 and 15, 30 and 65 mg/kg bw for males and females, respectively) for 105 weeks. Feed consumption of 1250-ppm males was lower than that of controls after week 70, and that of 1250-ppm females was generally lower than that of controls throughout the study. Survival of 1250-ppm males was significantly shorter than that of control group, which was attributed to the increased severity of chronic progressive nephropathy in the kidney. In the standard (single sections) and extended (step-sections) evaluations of the kidney, the incidence of renal tubule adenoma was increased in male rats exposed to 625 or 1250 ppm, and the combined incidence (single and step-sections) of renal tubule adenoma in males was also increased in these groups; the incidence in the 1250-ppm group was significantly greater than that in controls. A renal tubule carcinoma and a transitional epithelium carcinoma of the renal pelvis also occurred in 625-ppm males. Male rats exposed to 312 or 625 ppm had a significantly increased incidence of mononuclear-cell leukaemia, whereas the incidence in 1250-ppm males was slightly decreased compared with controls. This incidence and that in all groups of treated females exceeded the range for historical controls from feed studies (30–68% for males,

Table 3.1 Carcinogenicity studies of exposure to benzophenone in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mouse, B6C3F ₁ (M, F) 105 wk NTP (2006) , Rhodes et al. (2007)	Oral (feed) 0, 312, 625 or 1 250 ppm (M, F) 50 animals/group	Liver (hepatocellular adenoma): M ^a –11/50 (22%), 15/50 (30%), 23/50 (46%), 23/50 (46%) F ^b –5/50 (10%), 4/50 (8%), 10/50 (20%), 8/50 (16%) Liver (hepatocellular carcinoma): M–8/50 (16%), 5/50 (10%), 6/50 (12%), 6/50 (12%) F–0/50, 0/50, 1/50 (2%), 0/50 Liver (hepatocellular adenoma or carcinoma): M–18/50 (36%), 20/50 (40%), 25/50 (50%), 27/50 (54%) F–5/50 (10%), 5/50 (10%), 10/50 (20%), 9/50 (18%) Liver (hepatoblastoma, multiple): M ^c –0/50, 1/50 (2%), 1/50 (2%), 3/50 (6%) All organs (histiocytic sarcoma) F ^d –0/50, 0/50, 5/50 (10%), 3/50 (6%)	<i>P</i> = 0.01 (mid- and high-dose M) <i>P</i> = 0.006 (trend in M) <i>P</i> = 0.027 (high-dose M) <i>P</i> = 0.013 (trend in M) <i>P</i> = 0.03 (mid-dose) <i>P</i> = 0.032 (trend)	The incidence of non-neoplastic hepatocellular lesions was significantly increased including hepatocyte necrosis, cystic degeneration, centrilobular hypertrophy, multinucleated hepatocytes and chronic active inflammation in male mice and centrilobular hypertrophy in female mice; > 99.5% pure
Rat, F344 (M, F) 105 wk NTP (2006) , Rhodes et al. (2007)	Oral (feed) 0, 312, 625 or 1 250 ppm (M, F) 50 animals/group	Kidney (renal tubule adenoma, standard evaluation): M ^e – /50 (2%), 1/50 (2%), 2/50 (4%), 4/50 (8%) F ^f –0/50, 0/50, 2/50 (4%), 1/50 (2%) Kidney (renal tubule carcinoma, standard evaluation): M–0/50, 1/50 (2%), 0/50, 0/50 Kidney (renal tubule adenoma, extended evaluation): M–1/50 (2%), 1/50 (2%), 5/50 (10%), 4/50 (8%) F–3/50 (6%), 0/50, 2/50 (4%), 1/50 (2%) Kidney (renal tubule carcinoma, extended evaluation): M–0/50, 1/50 (2%), 0/50, 0/50 Kidney (renal tubule adenoma, standard + extended evaluations): M ^e –2/50 (4%), 2/50 (4%), 7/50 (14%), 8/50 (16%) F ^f –3/50 (6%), 0/50, 2/50 (4%), 1/50 (2%) Kidney (renal tubule carcinoma, standard + extended evaluations): M–0/50, 1/50 (2%), 0/50, 0/50	<i>P</i> = 0.046 (trend in M) <i>P</i> = 0.034 (trend in M) <i>P</i> ≤ 0.017 (high-dose M) <i>P</i> = 0.006 (trend in M)	Survival of the 1 250-ppm males was significantly lower than that of controls (<i>P</i> < 0.001). The incidence of renal tubule hyperplasia was significantly increased (<i>P</i> ≤ 0.01) in all treated groups. The incidence of renal pelvis transitional epithelial hyperplasia was significantly increased (<i>P</i> ≤ 0.01) in treated males. In male and female rats, the severity of chronic nephropathy increased significantly (<i>P</i> ≤ 0.05) with increasing exposure concentration; > 99.5% pure.

Table 3.1 Carcinogenicity (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence and/or multiplicity of tumours (%)	Significance	Comments
Rat, F344 (M, F) (contd)		Kidney (transitional epithelial carcinoma of the renal pelvis): M–0/50, 0/50, 0/50, 1/50 (2%) (M) Haematopoietic (mononuclear-cell leukaemia): M ^g –27/50 (54%), 41/50 (82%), 39/50 (78%), 24/50 (48%) F ^h –19/50 (38%), 25/50 (50%), 30/50 (60%), 29/50 (58%) All organs (histiocytic sarcoma): F ⁱ –0/50, 0/50, 1/50 (2%), 2/50 (4%)	<i>P</i> = 0.003 (low-dose M) <i>P</i> = 0.005 (mid-dose M) <i>P</i> = 0.048 (mid-dose F)	
Mouse, Swiss (F) 120 wk Stenbäck & Shubik (1974)	0% (vehicle), 5%, 25% or 50% in acetone Dermal application twice/wk for 120 wk 50 animals/group	Skin (squamous-cell papilloma): 2/50 (4%), 2/50 (4%), 0/50, 0/50 Skin (squamous-cell carcinoma): 0/50, 0/50, 1/50 (2%), 0/50 Lung (adenomas): 9/50 (18%), 3/50 (6%), 3/50 (6%), 6/50 (12%) Liver (haemangioma): 2/50 (4%), 1/50 (2%), 1/50 (2%), 2/50 (4%) Haematopoietic (lymphoma): 12/50 (24%), 15/50 (30%), 11/50 (22%), 6/50 (12%) Haematopoietic (thymoma): 0/50, 1/50 (2%), 1/50 (2%), 0/50	NS	Squamous-cell papillomas in the 5% group occurred at the site of application; the squamous-cell carcinoma in the 25% group occurred on the lip; papillomas in the control occurred on the tail and ear; purity not specified.

^a Historical incidence (mean ± SD) for 2-year feed studies in mice: 90/460 (20.0 ± 7.1%), range 12–30%

^b Historical incidence (mean ± SD) for 2-year feed studies in mice: 40/457 (9.6 ± 2.4%), range 6–12%

^c Historical incidence (mean ± SD) for 2-year feed studies in mice: 1/460 (0.2 ± 0.6%), range 0–2%

^d Historical incidence (mean ± SD) for 2-year feed studies in mice: 2/459 (0.3 ± 0.8%), range 0–2%

^e Historical incidence (mean ± SD) for 2-year feed studies in rats: 1/459 (0.3 ± 0.8%), range 0–2%

^f Historical incidence (mean ± SD) for 2-year feed studies in rats: 1/460 (0.1 ± 0.4%), range 0–1%

^g Historical incidence (mean ± SD) for 2-year feed studies in rats: 231/460 (49.1 ± 11.9%), range 30–68%

^h Historical incidence (mean ± SD) for 2-year feed studies in rats: 112/460 (24.6 ± 9.5%), range 12–38%

ⁱ Historical incidence for 2-year feed studies in rats: 0/460

F, female; M, male; NS, not significant; SD, standard deviation; wk, week or weeks

12–38% for females). A low incidence of histiocytic sarcoma occurred in both 625- and 1250-ppm female rats (1/50 and 2/50, respectively). Histiocytic sarcomas have not been observed in historical controls in feed studies and in only 1/1209 historical controls in studies by all routes of administration during the time these studies were conducted ([NTP, 2006](#); [Rhodes *et al.*, 2007](#)).

3.2 Dermal application

3.2.1 Mouse

Groups of 50 female Swiss mice received dermal applications of 0, 5, 25 or 50% benzophenone [purity unspecified] dissolved in acetone twice a week for 120 weeks. Dermal application of benzophenone was not carcinogenic in the skin of mice ([Stenbäck & Shubik, 1974](#)).

4. Other Relevant Data

4.1 Absorption and metabolism

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

After dermal application of [¹⁴C]benzophenone, approximately 70% was absorbed in rhesus monkeys within 24 hours ([Bronaugh *et al.*, 1990](#)). Benzophenone was rapidly absorbed from the gastrointestinal tract of Sprague-Dawley rats that were administered a single dose (100 mg/kg bw) by gavage in corn oil ([Jeon *et al.*, 2008](#)).

The metabolism of benzophenone in rabbits was originally shown to involve reduction of the keto group to produce benzhydrol, which was excreted in the urine as a glucuronide conjugate ([Robinson, 1958](#)). In a subsequent study, 4-hydroxybenzophenone was isolated from the urine of Sprague-Dawley rats that had been

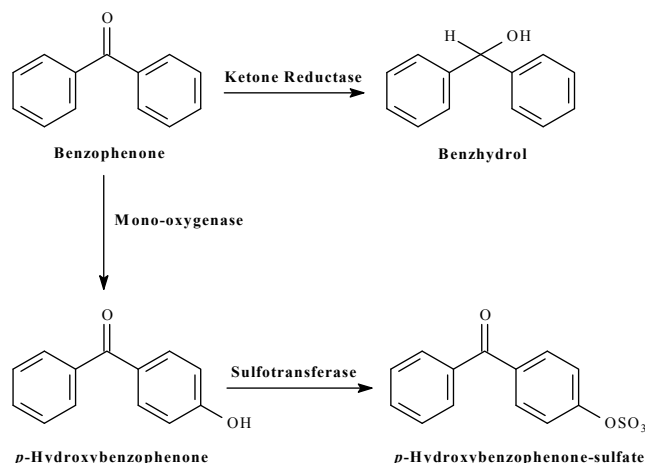
administered benzophenone in corn oil by gavage ([Stocklinski *et al.*, 1980](#)), and accounted for about 1% of the administered dose. It was isolated after treatment of the urine samples with a β -glucuronidase/aryl sulfatase preparation. A schema for the metabolism of benzophenone is shown in Fig. 4.1.

Twenty-four hour plasma time courses for benzophenone, benzhydrol and 4-hydroxybenzophenone were determined in Sprague-Dawley rats administered benzophenone by gavage in corn oil ([Jeon *et al.*, 2008](#)). 4-Hydroxybenzophenone, a product of aromatic hydroxylation, was identified after hydrolysis of the isolated metabolite with sulfatase. No dihydroxybenzophenone metabolites were identified in this study. Peak levels of benzophenone and its metabolites were reached approximately 4 hours after dosing, and the elimination half-life of the parent compound was approximately 19 hours. In toxicokinetic studies, the plasma elimination half-life of benzophenone in F344 rats was approximately 4 hours after intravenous injection and 8 hours after administration by gavage in corn oil; the plasma elimination half-life in B6C3F₁ mice was approximately 1 hour after intravenous injection and 1.5 hours after gavage in corn oil ([NTP, 2006](#)).

Benzophenone was metabolized to 4-hydroxybenzophenone, its sulfate conjugate, and benzhydrol in isolated F344 rat hepatocytes. Pretreatment of the hepatocyte suspension with 2,6-dichloro-4-nitrophenol, a sulfotransferase inhibitor, resulted in increased concentrations of free 4-hydroxybenzophenone ([Nakagawa *et al.*, 2000](#)).

Exposure of an aqueous solution of benzophenone to UV or sunlight irradiation produced two-ring hydroxylated derivatives — 3-hydroxybenzophenone and 4-hydroxybenzophenone — with concomitant generation of hydrogen peroxide, and the formation of 4-hydroxybenzophenone by UV irradiation was enhanced by the addition of hydrogen peroxide. The authors

Fig. 4.1 Proposed metabolism of benzophenone



Adapted from [Nakagawa & Tayama \(2001\)](#)

suggested that benzophenone might act as a photosensitizer that generates a reactive oxygen species which can cause aromatic ring hydroxylation ([Hayashi et al., 2006](#)).

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

Benzophenone was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535 or TA1537 in the presence or absence of metabolic activation systems. It did not increase the frequency of micronucleated polychromatic erythrocytes in samples of bone marrow obtained from male B6C3F₁ mice administered three intraperitoneal injections of benzophenone (200 to 500 mg/kg bw), or the frequency of micronucleated normochromatic erythrocytes in the peripheral blood of male or female B6C3F₁ mice administered benzophenone (1250 to 20 000 ppm) in the diet (estimated daily dose range, 200–4200 mg/kg bw) for 14 weeks ([NTP, 2006](#)).

Neither benzophenone nor its metabolites — benzhydrol or 4-hydroxybenzophenone — induced *umu* gene expression in *S. typhimurium* strain TA1535 in the presence or absence of rat or mouse liver microsomes. However, *umu* gene expression, which can be caused by DNA damaging agents, was elicited when *Escherichia coli* membranes expressing recombinant human cytochrome P450 (CYP) 2A6, 1A1, 1A2 or 1B1 were added to the incubation medium of *Salmonella*. The metabolite(s) responsible for this genotoxic effect were not identified ([Takemoto et al., 2002](#)).

4.3 Toxic effects

4.3.1 Humans

No data were available to the Working Group.

4.3.2 Experimental systems

In a 2-year feed study, treatment with benzophenone increased the severity of chronic nephropathy and the incidence of renal tubule hyperplasia and hepatocellular hypertrophy in rats, and the incidence of nephropathy, metaplasia of the olfactory epithelium and hyperplasia

of splenic lymphoid follicles in mice ([NTP, 2006](#)). [The animals were 6 weeks (rats) or 8 weeks (mice) of age when treatment began and, consequently, any potential endocrine-related effects associated with perinatal exposure were not captured in these studies.]

4.4 Endocrine-disrupting effects

4.4.1 *In-vitro* effects

The benzophenone metabolite, 4-hydroxybenzophenone, induced proliferation of MCF-7 cells (an estrogen-responsive human breast cancer cell line) when cultured in estradiol-free medium; this effect was also produced by 17 β -estradiol, but not by benzophenone or benzhydrol ([Nakagawa et al., 2000](#)).

4-Hydroxybenzophenone competed with 17 β -estradiol to bind to human recombinant estrogen receptor α (ER α) coated on 96-well plates (50% inhibitory concentration, $\sim 5 \times 10^{-5}$ M), but neither benzophenone nor benzhydrol demonstrated such competition ([Nakagawa & Tayama, 2001](#)). The competitive potency of 4-hydroxybenzophenone was approximately three orders of magnitude lower than that of diethylstilbestrol.

The two-ring hydroxylated compounds (3- and 4-hydroxybenzophenone) that are produced during exposure of benzophenone to sunlight competitively inhibited the binding of 17 β -estradiol to human recombinant ER α and elicited ER-mediated transcriptional activity in yeast cells ([Hayashi et al., 2006](#)).

Certain derivatives of benzophenone that have been widely used as UV screens also have estrogenic activity: benzophenone-2 (2,2',4,4'-tetrahydroxybenzophenone) also competed with 17 β -estradiol to bind to ER α and ER β ([Seidlová-Wuttke et al., 2004](#)).

Moreover, benzophenone-3 (2-hydroxy-4-methoxybenzophenone) elicited anti-androgenic activity in a human breast carcinoma cell

line (MDA-kb2) by inhibiting dihydrotestosterone-induced activation of androgen receptor, but showed no evidence of agonistic activity for this nuclear receptor ([Ma et al., 2003](#)). It transcriptionally activated human ER α and ER β in transfected human embryonic kidney cells (HEK293) and was antagonistic to the transcriptional activation of the androgen receptor by dihydrotestosterone and the progesterone receptor by a synthetic progestin (ORG 2058) in a transfected human osteosarcoma cell line (U2-OS) ([Schreurs et al., 2005](#)).

Benzophenone and its metabolite, 4-hydroxybenzophenone, elicited estrogenic activity in MCF-7 cells and anti-androgenic activity in transfected rat fibroblast NIH3T3 cells. In both assays, 4-hydroxybenzophenone was more potent than benzophenone but less potent than benzophenone-2 ([Suzuki et al., 2005](#)).

4.4.2 *In-vivo* effects

The *in-vivo* estrogenic activity of benzophenone was confirmed in the uterotrophic assay. Subcutaneous injection of 4-hydroxybenzophenone (once a day for 3 days at doses of 100, 200 or 400 mg/kg bw) to immature (21-day-old) female Sprague-Dawley rats produced a dose-related increase in absolute and relative uterine weights ([Nakagawa & Tayama, 2001](#)). Morphological evaluation showed that the treatment increased the luminal epithelial height and the thickness of the stromal layer of the uterus due to proliferation of uterine luminal epithelial cells, and increased the thickness and induced cornification of the vaginal epithelium. The same uterotrophic effects were observed in ovariectomized Sprague-Dawley rats administered benzophenone at doses of 100 and 400 mg/kg bw for 3 consecutive days by gavage in corn oil ([Nakagawa & Tayama, 2002](#)). Uterine weights were also increased in ovariectomized female F344 rats that received intraperitoneal injections of benzophenone (300 mg/kg bw) for 3 days

(Suzuki *et al.*, 2005). [The estrogen-like effects of benzophenone in the female reproductive tract appear to be due to metabolism to 4-hydroxybenzophenone, which binds to ER α .]

3- and 4-Hydroxybenzophenone induced increases in uterine weights in immature female Sprague-Dawley rats exposed subcutaneously for 3 consecutive days (Hayashi *et al.*, 2006). The effect on uterine weight was suppressed by pretreatment with the anti-estrogen ICI 182 780 (Faslodex). Thus, estrogenic products of benzophenone can also be generated by photochemical activation. [This observation is important because benzophenone has been used as a UV filter in cosmetics.]

The same uterotrophic effects as those described for 4-hydroxybenzophenone were observed in ovariectomized Sprague-Dawley rats fed benzophenone-2 in the diet for 3 months (Seidlová-Wuttke *et al.*, 2004). In addition to uterotrophic effects, the expression of ER-related receptor 1 in the uterus and ER β expression in the thyroid was increased and ER α expression in the uterus was decreased in ovariectomized Sprague-Dawley rats administered benzophenone-2 by gavage in olive oil for 5 days (Schlecht *et al.*, 2004). At similar exposures, benzophenone-3 did not increase uterine weight, but did decrease ER α expression in the pituitary and ER β expression in the uterus. Apart from the induction of estrogen-like effects by benzophenone-2 in multiple organs (including increased expression of insulin growth factor 1 in the vagina, decreased expression of insulin growth factor 1 in the liver, reduced luteinizing hormone synthesis by the pituitary gland, and a reduction of serum cholesterol high- and low- density lipoproteins), the 5-day treatment caused a reduction in serum thyroxine and triiodothyronine levels through a non-ER-mediated process (Jarry *et al.*, 2004). The latter effect of benzophenone-2 appears to be due to interference of thyroid hormone biosynthesis by inhibiting or inactivating thyroid peroxidase (Schmutzler *et al.*, 2007).

Among 17 benzophenone derivatives that were evaluated for anti-androgenic activity *in vitro*, the most potent (2,4,4'-trihydroxybenzophenone) also significantly suppressed the effect of testosterone on the weight gains of prostate and seminal vesicle in castrated male F344 rats (Hershberger assay), confirming the *in-vivo* anti-androgenic effect of this chemical (Suzuki *et al.*, 2005). Benzophenone-2 — an estrogenic chemical — also induced hypospadias in male C57BL/6 mice that were treated by gavage from gestational day 12 through to gestational day 17 (Hsieh *et al.*, 2007). The authors concluded that this effect was dependent on ER signalling because co-administration with an ER antagonist (EM-800) prevented the induction of hypospadias by benzophenone-2.

Benzophenone was also shown to induce an interaction between the pregnane X receptor and the steroid receptor coactivator 1 *in vitro*, and to induce the expression of *CYP2B1/2*, *-2C11* and *-3A1* genes in the liver of male Sprague-Dawley rats that had been administered intraperitoneal doses of 50, 100 or 250 mg/kg bw per day for 3 days (Mikamo *et al.*, 2003). [The increased expression of *CYP2B1* suggests the involvement of the constitutive androstane receptor.] Thus, benzophenone can also disrupt normal endocrine function by transcriptionally activating the pregnane X receptor and upregulating the expression of genes that code for enzymes involved in the metabolism of endogenous steroid hormones.

The above studies indicate that endocrine-active, benzophenone-derived chemicals may alter normal development and affect endocrine regulation in multiple organs by multiple mechanisms.

4.5 Mechanisms of carcinogenesis

Although the mechanisms of tumour induction by benzophenone are not fully known, they may be complex. The effects may include the generation of reactive oxygen species, or

endocrine disruption through multiple receptors, which include the induction of estrogen-like effects as a result of binding of benzophenone to ER α , alteration of the metabolism of endogenous steroid hormones, antagonism of transcriptional activation of the androgen receptor, and possible activation of nuclear constitutive androstane and pregnane X receptors. [The Working Group noted that aromatic hydroxylation of benzophenone to a transcriptionally active metabolite is likely to occur in humans.]

5. Summary of Data Reported

5.1 Exposure data

Benzophenone is produced by the acylation of benzoyl chloride with an excess of benzene. It may also be formed by atmospheric oxidation of diphenylmethane. Benzophenone is used as an ultraviolet curing agent, flavour ingredient, fragrance enhancer and perfume fixative, and as an additive for plastics, coatings and adhesive formulations. Benzophenone is also used as a screen to prevent ultraviolet light-induced damage to cosmetics. It is used in laundry and household cleaning products, and in the manufacture of pharmaceuticals, insecticides and agricultural chemicals. Benzophenone enters the environment after having been washed from skin and clothes through wastewater and from municipal solid-waste landfill leachates, and is ubiquitous in water, sediment and biota.

Occupational exposure may occur through inhalation and dermal contact in the manufacture of products that contain benzophenone. Dietary exposure to benzophenone occurs as a result of its natural occurrence in food or addition to food as a flavouring agent, its presence in drinking-water as a contaminant, and through its migration from food packaging, printing inks or recycled paperboard. Exposure may also occur through the inhalation of fragrances used

in indoor air and dermal contact with household cleaning and personal care products.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Benzophenone was tested for carcinogenicity by oral administration in the diet in one study in mice and rats and by dermal application in one study in mice. Oral administration of benzophenone significantly increased the incidence of hepatocellular adenoma, and hepatocellular adenoma, hepatocellular carcinoma and hepatoblastoma (combined) in male mice and histiocytic sarcoma in female mice. It increased the incidence of mononuclear-cell leukaemia in male and female rats (not statistically significant in females), renal tubule adenoma in male rats and histiocytic sarcoma in female rats (not statistically significant). Dermal application of benzophenone did not induce tumours in mice.

Tumours of the kidney, histiocytic sarcomas and hepatoblastomas are rare spontaneous neoplasms in experimental animals.

5.4 Other relevant data

No data were available on the toxicokinetics of benzophenone in humans. Benzophenone is absorbed in monkeys after dermal application, and is rapidly absorbed from the gastrointestinal tract of rodents. It is metabolized by reduction to benzhydrol or by oxidation to 4-hydroxybenzophenone. The latter compound can also be formed by ultraviolet or sunlight irradiation of benzophenone.

Benzophenone was not mutagenic in *Salmonella* and did not induce micronuclei in mice. Benzophenone and its metabolites induced *umu* gene expression, an indication of

DNA damage, in *Salmonella* in the presence of *Escherichia coli* membranes expressing recombinant human cytochrome P450s.

The benzophenone metabolite, 4-hydroxybenzophenone, elicits estrogenic activity and anti-androgenic activity *in vitro*, and the *in-vivo* estrogenic activity of benzophenone has been confirmed in multiple uterotrophic assays. Benzophenone may alter endocrine signalling through multiple effects on receptors.

The mechanistic evidence for tumour induction by benzophenone is weak, but the relevance of the tumour response in experimental animals to humans cannot be excluded.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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METHYL ISOBUTYL KETONE

1. Exposure Data

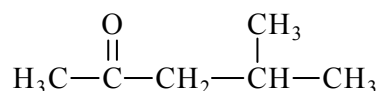
1.1 Chemical and physical data

From [IPCS \(1990, 1997\)](#), [Toxicological Index \(2005\)](#), and [HSDB \(2008\)](#) unless otherwise specified.

1.1.1 Nomenclature

Chem. Abstr. Services Reg. No.: 108-10-1
Chem. Abstr. Name: 4-Methylpentan-2-one
Synonyms: Hexone; isobutyl methyl ketone; isopropylacetone; ketone, isobutyl methyl; methyl *i*-butyl ketone; methyl-2-oxopentane; methylpentan-2-one; 2-methyl-4-pentanone; 4-methyl-2-pentanone; 4-methylpentan-2-one; 2-methylpropyl methyl ketone; MIBK; MIK; 2-pentanone, 4-methyl
RTECS No.: SA9275000
EINECS No.: 203-550-1
United Nations TDG number: 1245

1.1.2 Structural and molecular formulae and relative molecular mass



Relative molecular mass: 100.16

1.1.3 Chemical and physical properties of the pure substance

Description: Colourless liquid with a sweet odour

Boiling-point: 117–118 °C

Melting-point: -84 °C ([Lide, 2005](#));
-80.26 °C

Relative density (water = 1): 0.80 at 20 °C ([Lewis, 2001](#))

Vapour pressure: 19.9 mm Hg at 25 °C

Solubility: Soluble in water (1.91 g/100 mL at 20 °C); miscible with most organic solvents; soluble in chloroform

Relative vapour density (air = 1): 3.45

Flash-point: 14 °C

Autoignition: 460 °C

Octanol/water partition coefficient: log P, 1.31 ([LOGKOW, 2010](#))

Water/air partition coefficient: 79 ([Sato & Nakajima, 1979](#))

Blood/air partition coefficient: 90 ([Sato & Nakajima, 1979](#))

Oil/air partition coefficient: 926 ([Sato & Nakajima, 1979](#))

Henry's law constant:

1.38×10^{-4} atm.m³/mol at 25 °C

Conversion factor at 25°C and 760 mm/Hg:
1 ppm = 4.09 mg/m³; 1 mg/m³ = 0.245 ppm

1.1.4 Technical products and impurities

No data were available to the Working Group.

1.1.5 Analysis

More than 15 methods to measure methyl isobutyl ketone in different environments are available. Although sampling and extraction techniques differ, all methods involve gas chromatography.

The Environmental Protection Agency of the United States of America (US EPA) has published at least three different methods for the analysis of waste material (EPA methods 8015, 8015A and 8240A), two methods for water (EPA-NERL 524.2 and EPA-OSW 8015C) and one method for different environmental matrices (EPA-OSW 8260B).

The National Institute of Occupational Safety and Health (NIOSH) has also developed methods for the analysis of methyl isobutyl ketone in air. Both methods (1300, Issue 2 and 2555, Issue 1) use gas chromatography with a flame ionization detector. The American Society for Testing and Materials (ASTM D5790) uses gas chromatography/mass spectrometry to measure methyl isobutyl ketone in water ([HSDB, 2008](#)).

1.2 Production and use

1.2.1 Production

More than 60% of methyl isobutyl ketone is produced by aldol condensation of acetone and its derivative intermediates, diacetone alcohol and mesityl oxide. Acetone is treated with barium hydroxide to yield diacetone alcohol, which is dehydrated to mesityl oxide, which in turn is hydrogenated to saturate the double bond and produce methyl isobutyl ketone. Another method is the hydrogenation of mesityl oxide over nickel at 160–190 °C. Methyl isobutyl ketone can also be prepared by reacting sodium acetoacetic ester with isopropyl bromide and treating the resulting 2-isopropyl acetoacetic ester with diluted acid to saponify the ester and decarboxylate the resulting keto acid ([NTP, 2007](#)).

In 1995 and 1996, the USA produced 80 000 tonnes of methyl isobutyl ketone ([NTP, 2007](#)). In 2003, the industrial production capacity in the USA was 195 million pounds [88 000 tonnes] per year ([HSDB, 2008](#)). Sources indicate that methyl isobutyl ketone was produced by three companies in the USA ([HSDB, 2008](#)). According to [IUCLID \(2000\)](#), nine companies in Europe produced methyl isobutyl ketone in 2002: three in France, two in the Netherlands, and one each in Belgium, Germany, Denmark, and the United Kingdom.

1.2.2 Use

Methyl isobutyl ketone is used primarily as a denaturant and solvent in cosmetic products, in denatured alcohol, and as an excipient in drugs. It is also used as a component of synthetic flavouring substances and adjuvants, and as a component of adhesives that are present in articles intended for use in packaging, transporting or holding food ([HSDB, 2008](#)).

Methyl isobutyl ketone is also considered to be an excellent solvent for resins used in the production of surface coating and is widely used in rubber chemicals for the production of tyres. ([HSDB, 2008](#)). Methyl isobutyl ketone is also used as a solvent in paint and lacquers ([IPCS, 1990](#)).

1.3 Occurrence and exposure

1.3.1 Natural occurrence

Methyl isobutyl ketone occurs naturally in food (see Section 1.3.3).

1.3.2 Occupational exposure

The most probable routes of exposure in the workplace are by inhalation of vapours and by skin and eye contact during the production and use of methyl isobutyl ketone and products in which it is a constituent. In the National

Occupational Exposure Survey ([NIOSH, 1990](#)) conducted from 1981 to 1983, the number of workers potentially exposed to methyl isobutyl ketone in the USA was estimated as 48 000.

Exposure to methyl isobutyl ketone during spray painting was found to be 0.6 ppm (time-weighted average; TWA) ([Whitehead et al., 1984](#)). Concentrations of methyl isobutyl ketone at three locations in a poly(vinyl) chloride plastic waste recycling plant in Taiwan, China, ranged from 1517 to 11 324 $\mu\text{g}/\text{m}^3$. In the same study, concentrations at nine locations in polyethylene-polypropylene recycling plants ranged from 12 to 72 $\mu\text{g}/\text{m}^3$ ([Tsai et al., 2009](#)). In a study of workers in two factories in Taipei, mean concentrations of methyl isobutyl ketone in the air samples of solvent-exposed workers were 2 ppm (range, 0–68 ppm), while the mean exposure of spray painters in painting booths was 76 ppm (range, 8–369 ppm) ([Chen et al., 1991](#)). In a study among solvent-exposed workers, a mean exposure of 16.7 ppm was noted in an unidentified factory ([Ogata et al., 1995](#)). Among a group of 27 furniture-makers exposed to a mixture of methyl isobutyl ketone, methyl ethyl ketone, acetone, toluene, xylene, ethylbenzene, butyl acetate, and isobutyl acetate, the TWA concentration of each solvent was below the corresponding occupational exposure limit. Arithmetic mean exposure to methyl isobutyl ketone was 1.8 ppm (range, 0.1–15.1 ppm). A linear relationship between exposure and urinary concentration was found ([Kawai et al., 2003](#)). [Hänninen et al. \(1976\)](#) reported a mean TWA concentration of 7 mg/m^3 (range, 4–160 mg/m^3) [1.7 ppm; range, 1–39 ppm] in the breathing zone of spray painters in car repair shops.

Methyl isobutyl ketone may occur as a contaminant in environments near spacecraft, where it has been detected at levels of < 0.005–0.02 mg/m^3 ([IPCS, 1990](#)). It has been identified as a volatile degradation product of polypropylene at temperatures of 220–280 °C and under reduced pressure ([Frostling et al., 1984](#)).

1.3.3 Occurrence in food and dietary exposure

The most probable routes of exposure to methyl isobutyl ketone by the general population are ingestion of contaminated drinking-water and dermal contact with consumer products of which it is a constituent ([Johnson, 2004](#)).

Dietary sources of exposure are: natural occurrence in food, addition to food as a flavouring, and migration into food from food packaging. Methyl isobutyl ketone has also been detected in human breast milk ([Pellizzari et al., 1982](#)), and traces have also been detected in tap-water in the USA ([IPCS, 1990](#)).

(a) Natural occurrence in food

Methyl isobutyl ketone was reported to occur naturally in orange and lemon juice, grapes, vinegar, baked potatoes, papaya, ginger, wheat bread, cheeses, milk, cooked eggs, roast chicken, cooked beef, lamb fat, pork liver, hop oil, beer, cognac, coffee, tea, plumcot, plum brandy, mushrooms, trassi, sesame seed, buckwheat, wort, elder flowers, Bourbon vanilla, clary and red sage, crabs, clams, and Chinese quince ([Burdock, 2005](#)). The following levels have been reported ([IPCS, 1990](#)): papaya, 8 $\mu\text{g}/\text{kg}$; beer, 10–120 $\mu\text{g}/\text{kg}$; and coffee, 6.5 mg/kg .

(b) Flavouring agent

Methyl isobutyl ketone is permitted as a flavouring agent in the USA, where it is considered as safe at current levels of intake. Usual reported levels ranged from 2.6 mg/kg in meat products to 12.3 mg/kg in soft candy; maximum reported levels were 25 mg/kg in frozen dairy and non-alcoholic beverages; other reported uses are in baked goods, gelatines and puddings ([Burdock, 2005](#)).

The Council of Europe reported maximum levels of 11 mg/kg in beverages and 1 mg/kg foods in general ([Council of Europe, 2000](#)).

Table 1.1 International limit values for methyl isobutyl ketone

	Limit value – 8 h		Limit value – short-term	
	ppm	mg/m ³	ppm	mg/m ³
Austria	20	83	50	208
Belgium	20	83	50	208
Canada – Québec	50	205	75	307
Denmark	20	83	40	166
European Union	20	83	50	208
France	20	83	50	208
Germany (AGS)	20	83	40 (1)	166 (1)
Germany (DFG)	20	83	40	166
Hungary		83		208
Italy	20	83	50	208
Japan	50			
the Netherlands		104		208
Poland		83		200
Spain	20	83	50	208
Sweden	25	100	50	200
Switzerland	20	82	40	164
USA – NIOSH	50	205	75 (1)	300 (1)
USA – OSHA	100	410		
United Kingdom	50	208	100	416
Remarks				
European Union	Bold type: indicative occupational exposure limit values and limit values for occupational exposure			
France	Bold type: restrictive statutory limit values			
Germany (AGS)	(1) 15 min average value			
Germany (DFG)	15 min average value			
USA – NIOSH	(1) 15 min average value			

AGS, German Committee on Hazardous Substances (Ausschuss für Gefahrstoffe); DFG, German Research Foundation (Deutsche Forschungsgemeinschaft); h, hour or hours; NIOSH, National Institute of Occupational Safety and Health; OSHA, Occupational Safety and Health Administration

From [GESTIS \(2011\)](#)

Per-capita exposure to methyl isobutyl ketone, estimated by the FAO/WHO Expert Committee on Food Additives based on poundage data provided by industry, is 7 µg per capita per day in Europe (based on a reported volume of 50 kg/year) and 2 µg per capita per day in the USA (based on a reported production volume of 8 kg/year) ([FAO/WHO, 1999](#)). More recently, individual intake was estimated at 0.02 µg/kg per day ([Burdock, 2005](#)).

(c) Migration from food packaging

Methyl isobutyl ketone is used in packaging materials that come into contact with food. Levels reported in foods from packaging are: baked goods, 10.9 mg/kg; frozen dairy products, 11.5 mg/kg; meat products, 2.6 mg/kg; soft candy, 12.3 mg/kg; gelatins and puddings, 10.9 mg/kg; and beverages, 10.2 mg/kg ([IPCS, 1990](#)).

1.3.4 Environmental occurrence

Methyl isobutyl ketone may be released into the environment in effluent and emissions from its manufacture and use, in exhaust gas from motor vehicles ([Hoshika & Takata, 1976](#)) and from land disposal of waste that contains this compound ([Verschueren, 2009](#)). Release of methyl isobutyl ketone into the atmosphere may occur during its production through fugitive emissions and incomplete removal of vapours from reaction gases before they are vented or disposed of in a scrubber. In addition, methyl isobutyl ketone has frequently been identified in leakages from landfills and could potentially contaminate groundwater ([Francis *et al.*, 1980](#); [IPCS, 1990](#)). Another source of environmental contamination is the release of methyl isobutyl ketone during the discharge of spent scrubbing water from industrial production processes ([IPCS, 1990](#)).

1.4 Regulations and guidelines

Methyl isobutyl ketone has been listed by the Council of Europe in category B (flavouring substances for which further information is required before the Committee of Experts is able to offer a firm opinion on the safety of their use; these substances can be used provisionally in foodstuff) ([Council of Europe, 2000](#)). Methyl isobutyl ketone is listed in the European register of chemically defined flavourings (FLAVIS number 07.017), and no further evaluation is needed from a legal point of view according to the European Union (EU) evaluation programme ([European Commission, 2009](#)). Methyl isobutyl ketone is listed in the EU database for cosmetic ingredients with the following functions: denaturant, solvent and perfume ([European Commission, online](#)). Based on the Commission Regulation (EC) No 3199/93, methyl isobutyl ketone is permitted for use to denature alcohol in all EU countries ([European Commission, 1993](#)).

Short-term and 8-hour limit values for methyl isobutyl ketone are given in [Table 1.1](#). Both the American Conference of Governmental Industrial Hygienists (ACGIH) in the USA and the Maximale Arbeitsplatz-Konzentrations Commission in Germany provide guidelines for methyl isobutyl ketone in the workplace environment ([Table 1.1](#)).

2. Studies in Humans

No data were available to the Working Group

3. Cancer in Experimental Animals

The carcinogenicity studies reviewed below are limited to those of inhalation exposure of mice and rats to methyl isobutyl ketone that were adequately conducted by the National Toxicology Program ([NTP, 2007](#); [Stout *et al.*, 2008](#)), the results of which are summarized in [Table 3.1](#).

3.1 Inhalation

3.1.1 Mouse

Groups of 50 male and 50 female B6C3F₁ mice were exposed by whole-body inhalation to methyl isobutyl ketone (> 99% pure) at concentrations of 0, 450, 900 and 1800 ppm for 6 hours plus T₉₀ (time required to reach 90% of the target concentration within the exposure chamber; 12 minutes) per day on 5 days a week for 105 weeks. Treatment-related increases in the incidence of liver tumours (hepatocellular adenoma and carcinoma combined) were observed in males and females, together with concurrent treatment-related increases in the incidence of eosinophilic foci in the liver.

Table 3.1 Carcinogenicity studies of inhalation exposure to methyl isobutyl ketone (> 99% pure) in experimental animals

Species, strain (sex) Duration	Dosing regimen Animals/group at start	Incidence of tumours	Significance (poly-3 test)	Comments
Mouse, B6C3F ₁ (M) 105 wk	0, 450, 900 or 1 800 ppm, 6 h plus T ₉₀ (12 min)/d, 5 d/wk 50 animals/group	Liver (hepatocellular adenoma or carcinoma) ^a : 27/50, 34/50, 28/50, 37/50 Liver (hepatocellular adenoma) ^a : 17/50, 25/50, 23/50, 34/50 Liver (hepatocellular carcinoma) ^a : 12/50, 12/50, 10/50, 9/50	<i>P</i> = 0.019 (high dose) <i>P</i> = 0.028 (trend) <i>P</i> < 0.001 (high dose) <i>P</i> < 0.001 (trend) NS	Survival: 40/50, 42/50, 35/50, 37/50 Liver (eosinophilic foci): 3/50, 4/50, 5/50, 8/50
Mouse, B6C3F ₁ (F) 105 wk	0, 450, 900 or 1 800 ppm, 6 h plus T ₉₀ (12 min)/d, 5 d/wk 50 animals/group	Liver (hepatocellular adenoma or carcinoma) ^b : 17/50, 17/50, 22/50, 27/50 Liver (hepatocellular adenoma) ^b : 13/50, 15/50, 20/50, 23/50 Liver (hepatocellular carcinoma) ^b : 6/50, 5/50, 6/50, 11/50	<i>P</i> = 0.035 (high dose) <i>P</i> = 0.013 (trend) <i>P</i> = 0.033 (high dose) <i>P</i> = 0.016 (trend) NS	Survival: 35/50, 37/50, 39/50, 38/50 Liver (eosinophilic foci): 4/50, 11/50 ^g , 10/50, 14/50 ^f
Rat, F344/N (M) 104 wk	0, 450, 900 or 1 800 ppm, 6 h plus T ₉₀ (12 min)/d, 5 d/wk 50 animals/group	Kidney (renal tubule adenoma or carcinoma) ^{c,d} : 2/50, 4/50, 3/50, 11/50 Kidney (renal tubule adenoma) ^d : 2/50, 3/50, 3/50, 10/50 Kidney (renal tubule carcinoma) ^{c,d} : 0/50, 1/50, 0/50, 2/50 <i>Single section alone</i> Kidney (renal tubule adenoma): 0/50, 0/50, 2/50, 3/50 Kidney (renal tubule carcinoma): 0/50, 1/50, 0/50, 2/50 <i>Step section evaluation alone (3–4 sections per kidney):</i> Kidney (renal tubule adenoma): 2/50, 3/50, 1/50, 7/50	<i>P</i> = 0.004 (high dose) <i>P</i> < 0.001 (trend) <i>P</i> = 0.009 (high dose) <i>P</i> = 0.002 (trend) NS	Survival: 32/50, 28/50, 25/50, 19/50 Kidney (renal tubule hyperplasia) ^e : 1/50 (2.0), 14/50 ^f (2.9), 7/50 ^f (2.0), 21/50 ^g (2.5) ^h Kidney (nephropathy): 42/50 (2.0), 45/50 (2.6), 47/50 (2.4), 50/50 ^f (3.1) Kidney (papilla, mineralization): 1/50 (1.0), 6/50 ^f (1.2), 22/50 ^g (1.6), 29/50 ^g (1.5) Kidney (pelvis, transitional epithelium hyperplasia): 1/50 (1.0), 5/50 (1.8), 6/50 ^f (1.2), 19/50 ^g (1.4)

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen Animals/group at start	Incidence of tumours	Significance (poly-3 test)	Comments
Rat, F344/N (F) 104 wk	0, 450, 900 or 1 800 ppm, 6 h plus T ₉₀ (12 min)/d, 5 d/wk 50 animals/group	Kidney (malignant mesenchymal tumour ⁱ): 0/50, 0/50, 0/50, 2/50	<i>P</i> = 0.043 (trend)	Survival: 35/50, 34/50, 26/50, 32/50 Kidney (nephropathy): 19/50 (1.4), 35/50 ^f (1.5), 38/50 ^f (1.5), 44/50 ^f (1.9)

^a Historical incidence in male B6C3F₁ mice for 2-year inhalation studies with chamber controls given NTP-2000 diet (mean ± standard deviation): hepatocellular adenoma or carcinoma, 196/350 (56.0 ± 6.2%), range 50–68%; hepatocellular adenoma, 134/350 (38.3 ± 6.3%), range 30–46%; hepatocellular carcinoma, 85/350 (24.3 ± 4.8%), range 18–32%

^b Historical incidence in female B6C3F₁ mice for 2-year inhalation studies with chamber controls given NTP-2000 diet (mean ± standard deviation): hepatocellular adenoma or carcinoma, 108/347 (31.1 ± 6.8%), range 22–39%; hepatocellular adenoma, 78/347 (22.5 ± 8.1%), range 12–35%; hepatocellular carcinoma, 37/347 (10.7 ± 1.8%), range 8–12%

^c No additional renal tubule carcinomas were identified in the step section evaluation.

^d Historical incidence in male F344/N rats for 2-year inhalation studies with chamber controls given NTP-2000 diet for single section evaluations (mean ± standard deviation): renal tubule adenoma, 3/399 (0.8 ± 1.0%), range 0–2%; renal tubule carcinoma, 1/399 (0.3 ± 0.7%), range 0–2%; renal tubule adenoma or carcinoma, 4/399 (1.0 ± 1.1%), range 0–2%

^e Based on combined single section and step section evaluations. Single sections alone: 1/50 (2.0), 11/50^f (3.2), 3/50 (2.0), 18/50^f (2.7); Step section evaluation alone: 0/50, 3/50 (2.0), 4/50 (2.0), 6/50^g (2.3)

^f Significantly different (*P* ≤ 0.05) from the chamber control group by the poly-3 test

^g *P* ≤ 0.01

^h Numbers in parentheses indicate average grade of severity of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

ⁱ Historical incidence in female F344/N rats for 2-year inhalation studies with chamber controls given NTP-2000 diet: 0/396

d, day or days; F, female; h, hour or hours; M, male; min, minute or minutes; NS, not significant; wk, week or weeks

From [NTP \(2007\)](#), [Stout et al. \(2008\)](#)

3.1.2 Rat

Groups of 50 male and 50 female F344/N rats were exposed by whole-body inhalation to methyl isobutyl ketone (> 99% pure) at concentrations of 0, 450, 900 and 1800 ppm for 6 hours plus T_{90} (12 minutes) per day on 5 days a week for 104 weeks. Treatment-related increases in the incidence of kidney tumours were observed in males and females (renal tubule adenoma and carcinoma combined in males and two malignant mesenchymal tumours in high-dose females), together with concurrent treatment-related increases in the incidence of renal tubule hyperplasia and papillary mineralization (which had a linear pattern) in males.

[The Working Group noted that kidney tumours are rare spontaneous neoplasms in experimental animals.]

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

[Johnson \(2004\)](#) reviewed the available pharmacokinetic data for methyl isobutyl ketone. Primary data in humans were described for eight male volunteers (18–35 years of age) exposed to 2.4, 24.4 or 48.8 ppm (10, 100 or 200 mg/m³) for 2 hours on three different occasions during light physical exercise ([Hjelm et al., 1990](#)). The relative pulmonary uptake was ~60%, which increased with increasing dose (0.2 mmol at 10 mg/m³, 1.7 mmol at 100 mg/m³ and 3.2 mmol at 200 mg/m³). Levels in the blood rose rapidly after the onset of exposure, levelled off and did not reach a plateau for 2 hours. At the end of the exposure, blood concentrations increased linearly with dose, with no tendency for saturation kinetics. The terminal elimination half-life was increased with dose,

but exposure concentration did not impact the apparent rate of blood clearance (1.6 L/h/kg). Blood concentrations measured at the end of the exposures indicated linear kinetics (dose-proportional blood concentration) of methyl isobutyl ketone at the three doses tested. Only 0.04% of the total dose was eliminated unchanged in the urine within 3 hours after dosing, but the concentration in the urine was higher than that in arterial blood at 0.5 and 3 hours after the end of exposure.

After exposure, methyl isobutyl ketone was reported to be eliminated via a rapid and slow phase. [Saghir & Rick \(2008\)](#) cited another study that reported biphasic urinary elimination of methyl isobutyl ketone from human volunteers ([Ogata et al., 1995](#)). However, the major route of elimination was exhalation.

Human volunteers (98 men and women) were exposed to 100 ppm (410 mg/m³) methyl isobutyl ketone for 4 hours in an environmental chamber ([Dick et al., 1990](#)). Steady-state blood concentrations of methyl isobutyl ketone were attained after 2 hours of exposure. Blood and breath samples collected 90 minutes after exposure indicated that most of the absorbed compound had been eliminated from the body.

[Bellanca et al. \(1982\)](#) reported that methyl isobutyl ketone was detected in the brain, liver, lung, vitreous fluid, kidney, and blood in two workers who died after exposure to several organic solvents during spray painting. One died from a fall and the other died of cerebral oedema 9 hours later. Tissue concentrations (mg/100 g) were reported to be: case 1 — brain, 0.25; liver, 0.49; lung, 0.43; vitreous fluid, 0.52; kidney, 0.24; and femoral blood, 0.14; and case 2 — brain, 0.06; liver, 0.22; lung, 0.11; vitreous fluid, 0.02; kidney, 0.08; and heart blood, 0.04.

[Dowty et al. \(1976\)](#) reported methyl isobutyl ketone in human maternal blood samples collected immediately after delivery, indicating the potential for the compound to enter the umbilical cord and cross the placenta. Methyl

isobutyl ketone is readily soluble in blood and has a high affinity for fat. In-vitro partition coefficients of 70–90 between blood and air and 926 between water and oil were reported ([Sato & Nakajima, 1979](#)).

[Saghir and Rick \(2008\)](#) used the data of [Hjelm et al. \(1990\)](#) on single-dose inhalation exposure to simulate the repeated-dose kinetics of methyl isobutyl ketone in humans. The physiologically based pharmacokinetic model predicted the kinetics and accumulation of methyl isobutyl ketone after repeated exposures for 8, 12 and 24 hours per day for 7 days to the current ACGIH exposure threshold limit value-TWA of 50 ppm, and followed a two-compartment model using inhalation-chamber data. Elimination of methyl isobutyl ketone was assumed to occur primarily through exhalation, because only 0.04% of the total dose was reported to be eliminated through the urine ([Hjelm et al., 1990](#)). The model did not account for elimination from the blood/body via other routes of elimination, e.g. as carbon dioxide, or metabolic incorporation into tissues. Measured blood concentrations were used to derive kinetic parameters that were then used to predict blood concentrations following different exposure scenarios at 50 ppm. [The model was not validated using an independent data set.] The model output was then used to assess the probable effects of various conditions of blood concentration, potential for accumulation and TWA blood concentrations. Kinetic rates were calculated using the methodology of [Hjelm et al. \(1990\)](#). Berkeley-Madonna modelling software was used that can simulate a simple one- or two-compartment situation. The model made no attempt to predict the levels of exhaled methyl isobutyl ketone. Therefore, the amount exhaled was not fed back into the exposure concentration and the exposure concentration defined in the model was fixed over a defined exposure period. The model was optimized by fitting all three blood concentration time-course concentrations of methyl isobutyl ketone. The model ([Saghir](#)

[& Rick, 2008](#)) correctly simulated the experimental data measured after single exposures and demonstrated a rapid rise in blood concentration to 1.06 µg/mL within 1 hour, which approached steady-state levels of 1.37 µg/mL at 4 hours of exposure and 1.47 µg/mL at the end of exposure. Methyl isobutyl ketone was predicted to be rapidly eliminated from blood after cessation of exposure, reaching 0.53 µg/mL and 0.13 µg/mL within 0.5 and 2 hours after cessation, respectively. It was concluded that methyl isobutyl ketone is not likely to accumulate in workers exposed to 50 ppm.

4.1.2 Experimental systems

Methyl isobutyl ketone was rapidly absorbed following oral administration to or inhalation exposure of male Sprague-Dawley rats ([Duguay & Plaa, 1995](#)). The compound was rapidly absorbed and was detected in the lung, liver and plasma after inhalation or within 1 hour after an oral dose. In CD-1 mice, an intraperitoneal injection was quickly distributed and eliminated ([Granvil et al., 1994](#)). Clearance time was 6 hours and the half-life in serum was 66 minutes in guinea-pigs that received a single intraperitoneal dose of methyl isobutyl ketone ([DiVincenzo et al., 1976](#)).

Male Sprague-Dawley rats were exposed to methyl isobutyl ketone orally (0.5, 3 or 6 mmol/kg body weight (bw)) or by inhalation (200, 400 or 600 ppm) ([Duguay & Plaa, 1995](#)). The parent compound and two products — 4-hydroxymethyl isobutyl ketone and 4-methyl-2-pentanol — were identified in the plasma, liver and lung following inhalation. After oral administration, the parent compound and the hydroxylated product were detected in these tissues, but not 4-methyl-2-pentanol. These results were consistent with the metabolism of methyl isobutyl ketone via alcohol dehydrogenase and cytochrome P450 (CYP) mono-oxygenases ([Vézina et al., 1990](#)). In CD-1 mice that received an intraperitoneal injection of

5 mmol/kg bw methyl isobutyl ketone ([Granvil et al., 1994](#)), the major metabolites detected in the blood and brain were 4-methyl-2-pentanol and 4-hydroxy-4 methyl-2-pentanone.

In a review, [Stout et al. \(2008\)](#) indicated that the metabolism of methyl isobutyl ketone in guinea-pigs entails the reduction of the carbonyl group to a secondary alcohol (4-methyl-2-pentanol) and oxidation at the ω -1 carbon atom to form a hydroxylated ketone (4-hydroxymethyl isobutyl ketone, also known as diacetone alcohol). 4-Methyl-2-pentanol may be further conjugated with sulfate or glucuronic acid, may undergo intermediary metabolism and be eliminated as carbon dioxide or may be incorporated into tissues.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

[Johnson \(2004\)](#) reviewed the genetic toxicology of methyl isobutyl ketone and found that it is generally not genotoxic in a variety of systems. An effort was made to test this volatile compound in closed systems in some of the assays, including *Salmonella* and the mouse lymphoma thymidine kinase locus $Tk^{+/-}$, but the compound still gave either negative or equivocal results. In studies in *Salmonella* ([Brooks et al., 1988](#); [Zeiger et al., 1992](#)), methyl isobutyl ketone was not mutagenic in the presence or absence of metabolic activation in a variety of strains (TA98, TA100, TA1535, TA1537 and TA1538) in the pre-incubation assay in closed tubes. Similar negative results were also observed in the *S. typhimurium* assay with TA102 and TA104 ([Zeiger et al., 1992](#)). Equivocal results were found in the L5178Y mouse lymphoma $Tk^{+/-}$ assay which was

also performed in closed tubes ([O'Donoghue et al., 1988](#)).

Methyl isobutyl ketone gave negative results for unscheduled DNA synthesis in rat hepatocytes, for micronuclei in the bone marrow of CD-1 mice (after intraperitoneal injection), for cell transformation in BALB/3T3 mouse embryo cells ([O'Donoghue et al., 1988](#)), for mitotic gene conversion ([Brooks et al., 1988](#)) and mitotic chromosome loss ([Zimmermann et al., 1989](#)) in yeast and for chromosome damage in rat liver cells *in vitro* ([Brooks et al., 1988](#)).

4.3 Toxic effects

4.3.1 Humans

No data were available to the Working Group.

4.3.2 Experimental systems

(a) Liver and kidney

Methyl isobutyl ketone, its metabolites and other ketones are known to potentiate liver necrosis induced by haloalkanes (e.g. carbon tetrachloride and chloroform ([Vézina et al., 1990](#))), and cholestasis induced by cholestatic agents (e.g. tauroolithocholate ([Plaa & Ayotte, 1985](#); [Dahlström-King et al., 1990](#); [Duguay & Plaa, 1997](#)) or manganese-bilirubin ([Vézina & Plaa, 1987](#); [Duguay & Plaa, 1997](#))). There is no evidence, however, that methyl isobutyl ketone or its metabolites have adverse effects on the liver when administered alone either orally (up to 7.5 mmol/kg) or by inhalation (up to 600 ppm) under conditions of a single or repeated (3 days) exposure ([Joseph et al., 1992](#); [Duguay & Plaa, 1997](#)).

Although methyl isobutyl ketone showed no toxic effect on the liver in acute or subacute exposures, it has been shown ([Vézina et al., 1990](#)) to increase the total amount of CYP in the rat liver 24 hours after a single dose (lowest effective dose tested, 7.5 mmol/kg bw in corn oil vehicle

by gavage) or after a 5-day treatment regime (lowest effective dose, 5 mmol/kg bw). While no individual CYP enzymes were evaluated in this study, the authors tested for the activity of aniline hydroxylase (CYP2E activity), ethoxycoumarin *O*-deethylase (CYP1A and -2E activity) and aminopyrine *N*-demethylase (CYP2B activity) and reported increased activity after treatment with methyl isobutyl ketone for all three enzymes at both time-points with minimal effective doses identical to that required to increase total CYP content.

The induction of liver CYPs has been associated with the 'potentiating' effect of methyl isobutyl ketone on prior hepatotoxic treatments (Vézina *et al.*, 1990). However, this mechanism does not appear to be exclusive because it also affects the toxicity of bile acid, and it has been suggested that methyl isobutyl ketone may also reduce the bile salt pool and/or affect hepatic secretion of bile acids (Joseph *et al.*, 1992).

A similar effect on the induction of total CYP, as well as aniline hydroxylase (CYP2E activity) and aminopyrine *N*-demethylase (CYP2B activity), was observed in the rat kidney after oral (3 days; minimal effective dose, 13.6 mmol/kg bw) administration of methyl isobutyl ketone (Raymond & Plaa, 1995a). Interestingly, while total CYP, CYP2E and -2B activities in the liver were much greater than those in the kidney, the induction of total CYP was 2–3 times greater in the kidney than in the liver. In the same study, covalent binding of [¹⁴C]carbon tetrachloride to renal proteins was potentiated by methyl isobutyl ketone, similarly to the observation in the liver. It was also shown that methyl isobutyl ketone potentiates chloroform-induced kidney toxicity, albeit to a lesser degree than that of carbon tetrachloride (Raymond & Plaa, 1995b). Both studies concluded that enzyme induction by methyl isobutyl ketone played a major role in the potentiation of toxicity of the two nephrotoxics which are known to require metabolic activation.

Subchronic (≥ 90 days) exposure to methyl isobutyl ketone by inhalation was shown to increase liver weights, characterized by hepatocellular hypertrophy, in both rats and mice (Phillips *et al.*, 1987; Nemeč *et al.*, 2004). A comparison of the effects of exposure by inhalation to 410 mg/m³ methyl isobutyl ketone for 90 days in male rats, beagle dogs and *Macaca mulatta* monkeys (MacEwen *et al.*, 1971) revealed no pathological effects in dogs or monkeys in any of the major organs examined at necropsy. Statistically significant increases in liver and kidney weights were observed only in male rats. Hyaline droplets were detected in the proximal tubules of all exposed male rats within 15 days of exposure (some rats were necropsied after 2, 3, 4, 10, or 12 weeks). A study that investigated oral administration of methyl isobutyl ketone (1.04 g/kg per day in the drinking-water for 120 days) to female rats found a significant increase in absolute and relative kidney (but not liver or other organ) weight, and one of five rats tested had renal tubule-cell hyperplasia (US EPA, 2003). Another subchronic toxicity study compared male and female rats administered methyl isobutyl ketone daily by oral gavage (59, 250 or 1000 mg/kg bw) for 13 weeks (IPCS, 1990). Nephrotoxicity and increased liver and kidney weights were observed in both males and females at the highest dose. The no-adverse-effect dose was 50 mg/kg bw.

(b) Other studies of toxicity

Rats, mice, guinea-pigs, cats and dogs were studied for the acute toxicity of methyl isobutyl ketone after oral, dermal, inhalational, intravenous or intraperitoneal administration. These studies have been reviewed extensively (Johnson, 2004). No species differences in acute toxic effects were observed. Methyl isobutyl ketone was shown to be neurotoxic and irritating (to the upper respiratory tract and lungs after inhalation) at the highest concentrations tested. Little evidence of hepatic or renal toxicity was reported even at doses that were lethal.

Two studies of the reproductive and developmental toxicity of methyl isobutyl ketone were available. [Tyl *et al.* \(1987\)](#) exposed F344 rats and CD-1 mice to methyl isobutyl ketone (up to 3000 ppm) by inhalation on gestational days 6–15. Maternal toxicity (death, increases in absolute and relative liver weights) were observed in the 3000-ppm groups of rats and mice. Fetal toxicity (decrease in body weight, retardation of ossification) was observed in the offspring of both rats and mice in the 3000-ppm group. [Nemec *et al.* \(2004\)](#) exposed Sprague-Dawley rats to up to 2000 ppm methyl isobutyl ketone (whole-body inhalation) for 70 days before mating. F0 and F1 females were exposed from mating to gestational day 20 and then from postnatal day 5; F2 litters were maintained through postnatal day 21. Most adverse effects were observed only in the 2000-ppm group. Specifically, a sedative effect (central nervous system depression) was observed in the pups. Increased liver weight was observed in the F0 and F1 generation males and females. In F0 generation males, increased kidney (in the 500- and 1000-ppm groups) and seminal vesicle weights were observed. In F0 females, an increase in the weight of ovaries and adrenal glands was observed. Nephropathy, characterized by basophilic tubules with variable inflammation and thickening of the tubular basement membrane, was reported in F0 and F1 males exposed to 1000 or 2000 ppm methyl isobutyl ketone. No effects on sexual maturation or reproductive end-points were observed at any dose tested.

4.4 Mechanistic considerations

4.4.1 *Tumours of the kidney*

The development of kidney tumours in male rats in association with chemically induced α 2u-globulin nephropathy is mechanism that is not considered to be a predictor of carcinogenic risk to humans by the IARC or the US EPA ([US EPA, 1991](#); [Swenberg & Lehman-McKeeman,](#)

[1999](#)). The lack of relevance of the α 2u-globulin mechanism for the evaluation of carcinogenic risk is based on the absence of the production of an analogous protein in humans. Strict scientific criteria have been outlined to establish the role of α 2u-globulin-associated nephropathy in renal carcinogenesis in male rats ([Swenberg & Lehman-McKeeman, 1999](#)), and were used to determine the plausibility of an α 2u-globulin-associated nephropathy based on a limited number of studies that have been carried out with subchronic and chronic exposures to methyl isobutyl ketone.

Criterion 1 is evidence of a lack of genotoxic activity (agent and/or metabolite) based on an overall evaluation of in-vitro and in-vivo data. As reviewed in Section 4.2, there was little, if any evidence that methyl isobutyl ketone was genotoxic. Two of its metabolites — 4-hydroxymethyl isobutyl ketone and 4-methyl-2-pentanol — that are found in male Sprague-Dawley rat liver, serum and lung after exposure to methyl isobutyl ketone have not been evaluated for genotoxicity. Thus, this criterion appears to be met.

Criterion 2 is the specificity in male rats for nephropathy and renal tumorigenicity; criterion 6 is the induction of sustained increased cell proliferation in the renal cortex; and criterion 7 mentions the observed similarities in the dose-response relationship of the tumour outcome with the histopathological end-points (protein droplets, α 2u-globulin accumulation and cell proliferation). [Stout *et al.* \(2008\)](#) evaluated the potential of methyl isobutyl ketone to induce toxic and carcinogenic effects following chronic exposure. Groups of 50 male and 50 female F344 rats were exposed to concentrations of 0, 450, 900 or 1800 ppm by inhalation for 6 hours per day on 5 days a week for 2 years. Survival was decreased in 1800-ppm males, and body weight gains were decreased in 900- and 1800-ppm males. In males, but not females, increased mineralization of the renal papilla was observed at all exposure concentrations. The incidence of chronic progressive

nephropathy was increased at 1800 ppm and its severity was increased in all exposed groups of males. The incidence of chronic progressive nephropathy was increased in all treated groups of females, the severity of which was increased with the highest dose. In male, but not females, increases in incidence of renal tubule hyperplasia were observed at all exposure concentrations, and in that of adenoma and adenoma or carcinoma (combined) at 1800-ppm. α 2u-Globulin levels were not evaluated in this study. This chronic study provided dose-response consistency and male specificity of mineralization, sustained increases in cell proliferation in the renal cortex and the induction of combined adenomas and carcinomas in the kidney. However, the study found that chronic progressive nephropathy was not male-specific.

Criterion 3 is the induction of the characteristic sequence of histopathological changes in shorter-term studies, of which protein droplet accumulation is obligatory. The [Bushy Run Research Center \(1982\)](#) exposed three groups of F344 rats (six males and six females) to methyl isobutyl ketone at concentrations of 101 ppm [413 mg/m³], 501 ppm [2050 mg/m³] and 1996 ppm [8180 mg/m³] for 6 hours per day for 9 days. A fourth group served as the untreated control. The first 5 days and the remaining 4 days of exposure were separated by a 2-day non-treatment period. In the highest-dose group (1996 ppm), a significant increase in both absolute and relative kidney weights was noted in male and female rats. Epithelial regeneration of the proximal convoluted tubules was also noted at 1996 ppm [male and female specificity was indicated in the summary]. In the 501-ppm exposure group, a non-significant increase in kidney weight was observed in male but not female rats. In both 501- and 1996-ppm exposure groups, hyaline droplet formation was observed in the kidneys of male rats. No microscopic abnormalities were noted in rats exposed to 101 ppm methyl isobutyl

ketone ([Bushy Run Research Center, 1982](#), cited by [US EPA, 2003](#)).

Male and Female F344 rats and B6C3F₁ mice were exposed to 0, 100, 500 or 2000 ppm methyl isobutyl ketone for 6 hours per day for 2 weeks. At 2000 ppm, a slight increase in male rat liver weight (absolute and relative) was observed. The only changes observed histologically were increases in regenerative tubular epithelia and hyaline droplets in the kidneys of male but not female rats exposed to 500 or 2000 ppm [Phillips et al. \(1987\)](#). Exposure levels for a subchronic study were 0, 50, 250 or 1000 ppm methyl isobutyl ketone vapour for 6 hours per day on 5 days a week for 14 weeks. The 14-week exposure had no adverse effect on the clinical health or growth of the rats. The only microscopic change observed was an increase in the incidence and extent of hyaline droplets within the proximal tubular cells of the kidneys of male but not female rats exposed to 250 and 1000 ppm. These two studies indicate the induction of hyaline protein droplets in shorter-term studies. Protein droplets were also identified and characterized further in the study described below ([Borghoff et al., 2009](#)).

Another criterion is the identification of protein accumulation in tubule cells as α 2u-globulin. [Borghoff et al. \(2009\)](#) administered corn oil (vehicle control), *d*-limonene (positive control, 300 mg/kg bw) or methyl isobutyl ketone (1000 mg/kg bw) to male and corn oil (vehicle control) or methyl isobutyl ketone to female F344 rats by oral gavage for 10 consecutive days. Methyl isobutyl ketone caused an increase in protein droplets, accumulation of α 2u-globulin and renal cell proliferation in male but not female rats. It produced histopathological changes in the male rat kidney identical to those of *d*-limonene, an acknowledged inducer of α 2u-globulin-mediated nephropathy, except that the grade of severity tended to be slightly lower with methyl isobutyl ketone. Methyl isobutyl ketone did not induce any effects in female rats. The authors found α 2u-globulin

accumulation in tubule cells after exposure in males. However, the experimental design did not allow for the evaluation of a dose–response relationship in the increases in α 2u-globulin accumulation; moreover, treatment-related increases in the incidence and average severity of chronic progressive nephropathy were observed in both males and females, which suggests that an alternative mechanism may also be involved. Renal tubule neoplasms probably arose via the male rat-specific α 2u-globulin-mediated mechanism ([NTP, 2007](#)).

The last criterion is the reversible binding of the chemical or metabolite to α 2u-globulin, which was not shown direct in any of the studies. One study showed reversibility of the adverse effects in the kidney after withdrawal of methyl isobutyl ketone. The Wright-Patterson Air Force Base Aerospace Medical Research Laboratory ([MacEwen *et al.*, 1971](#)) conducted a subchronic inhalation toxicity study in male Wistar albino rats that were exposed to 410 mg/m³ methyl isobutyl ketone vapour [100 ppm] for 90 days in an altitude chamber. The untreated control group was maintained in a separate altitude chamber. Statistically significant increases in liver and kidney weights and organ-to-body weight ratios for these tissues were noted in exposed rats. Microscopic examination of the kidneys revealed hyaline droplet degeneration of the proximal tubules (with occasional foci of tubular necrosis) in all of the exposed rats, including those that were removed from the inhalation chamber after 15, 22, 28, 71 and 85 days. The authors noted a trend towards a linear progression of hyaline droplet degeneration during exposure, but this pattern was not seen in all treatment groups. Moreover, the hyaline droplets appeared to increase in size with time. This observation was thought to have resulted from the coalescence of smaller droplets. Microscopic examination of rat kidneys removed after 15 days of exposure indicated a gradual reversion of tubular damage with time. Kidney damage was completely reversed in rats

observed up to 60 days after exposure. Recovery from methyl isobutyl ketone-induced kidney lesions was also noted in rats that were serially killed to study reversibility after 90 days of exposure. However, recovery was not as rapid as that noted in animals exposed for shorter periods. A weakness of the study was the exclusion of female rats. The study showed the reversibility of effects that could be attributed to α 2u-globulin nephropathy.

Kidney tumours were induced in male but not female rats. Mechanistic studies provide evidence that some of the criteria that denote an α 2u-globulin mode of action were met. [These include male rat-specific nephropathy, dose–response associations of end-points and dose-related increases in cell proliferation.] However, in a review of the linkages between end-points that are typically considered to support an α 2u-globulin mode of action ([Doi *et al.*, 2007](#)), recent NTP studies demonstrated inconsistencies with this proposed mechanism, including, in some cases, kidney tumour responses that were far weaker than expected based on the extent of α 2u-globulin nephropathy. The review revealed no, or at best weak, associations between tumour responses and renal α 2u-globulin concentrations, indices of cell turnover or microscopic evidence of α 2u-globulin nephropathy in pre-chronic studies. While tumour responses corresponded to some extent with a measure of cumulative α 2u-globulin nephropathy (linear mineralization of the papilla) at the end of the 2-year studies, the severity of chronic nephropathy generally correlated best with the pattern of tumour response. These results suggest that, while α 2u-globulin nephropathy may contribute to the renal tumour response, the critical component(s) of the nephropathy most closely associated with the development of tumours has not been identified. Thus, the strength of the evidence that male rat kidney tumours arose through a α 2u-globulin nephropathy mechanism is weak. The relevance

of the tumour response to humans cannot be excluded.

4.4.2 Other sites

There is little, if any, evidence that rodent tumours arose through a genotoxic mechanism. However, two metabolites — 4-hydroxymethyl isobutyl ketone and 4-methyl-2-pentanol — found in male Sprague-Dawley rat liver, serum and lung after exposure to methyl isobutyl ketone have not been evaluated for genotoxicity. Methyl isobutyl ketone induced liver tumours in male and female mice, but there was no evidence that the tumours arose from a cytotoxic-regenerative cell proliferation mechanism as no overt toxicity occurred in the livers of exposed mice. Only weak evidence exists that the tumours arose through a receptor-mediated mechanism, resulting from the induction of enzymes (CYP1A1 and CYP2B) that have been considered to be typical targets of the aryl hydrocarbon receptor and the constitutive activated receptor, respectively ([Nebert *et al.*, 2000](#); [Zelko & Negishi, 2000](#)). The strength of evidence that male and female liver tumours arose through a nuclear receptor mechanism is weak. The relevance of the tumour response to humans cannot be excluded.

5. Summary of Data Reported

5.1 Exposure data

Methyl isobutyl ketone is used as an industrial solvent, a fragrance, denaturant or solvent in cosmetic products, to denature alcohol and as an excipient in drugs. Methyl isobutyl ketone is naturally present in food and may be added as a flavouring ingredient. It may be released into the environment as emissions from its manufacture and use, and from leakage from landfills. Exposure in the workplace occurs by inhalation of the vapours and skin contact. Routes

of exposure to methyl isobutyl ketone by the general population are ingestion through food and dermal contact with consumer products.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

In a 2-year inhalation study in male and female mice and rats, methyl isobutyl ketone increased the incidence of hepatocellular adenoma and hepatocellular adenoma and carcinoma combined in male and female mice, and that of renal tubule adenoma and renal tubule adenoma and carcinoma combined in male rats, and caused two rare renal malignant mesenchymal tumours in high-dose female rats.

Kidney tumours are rare spontaneous neoplasms in experimental animals.

5.4 Other relevant data

Toxicokinetic data for methyl isobutyl ketone indicate that pulmonary uptake and blood concentrations of this chemical increase linearly with the dose in human volunteers who were exposed via inhalation. Steady-state blood levels were attained after 2 hours of exposure. The major route of elimination was exhalation, and only a tiny fraction was excreted in the urine. Analysis of blood and breath samples collected after exposure indicated that most of the absorbed methyl isobutyl ketone had been eliminated from the body within 2 hours. The compound was detected in the brain, liver, lung, vitreous fluid, kidney and blood in autopsy samples of two workers who had been exposed to organic solvents. There is evidence that methyl isobutyl ketone may enter the umbilical cord and cross the placenta.

Data from single-dose inhalation exposure studies were used to simulate the repeated-dose kinetics of methyl isobutyl ketone in humans. The two-compartment pharmacologically based pharmacokinetic model predicted the kinetics and accumulation for repeated exposures. It correctly simulated the experimental data measured after single exposures and demonstrated a rapid rise in blood concentration within 1 hour and rapid elimination from the blood after cessation of exposure. On the basis of these results, methyl isobutyl ketone is not likely to accumulate in workers exposed to 50 ppm. Methyl isobutyl ketone was rapidly absorbed after oral administration to or inhalation exposure of male rats. It was detected in the lung, liver and plasma within 1 hour after an oral dose. In mice, methyl isobutyl ketone administered by intravenous injection was quickly distributed and eliminated. A clearance time of 6 hours and a half-life in serum of about 1 hour were measured in guinea-pigs after a single intraperitoneal dose of methyl isobutyl ketone.

No data were available on the metabolism of methyl isobutyl ketone in humans. In rats, the parent compound and two metabolites — 4-hydroxymethyl isobutyl ketone and 4-methyl-2-pentanol — were identified in the plasma, liver and lung following inhalation. After an oral dose, the parent compound and the hydroxylated product were detected in these tissues, but not 4-methyl-2-pentanol. These data are consistent with metabolism that involves alcohol dehydrogenase and cytochrome P450 mono-oxygenases. Similar patterns of metabolism were seen in mice and guinea-pigs.

Methyl isobutyl ketone was generally not genotoxic in a variety of systems. Also, when this volatile compound was tested in closed systems, no indication of genotoxicity was found. Methyl isobutyl ketone was not mutagenic in bacterial assays. It did not induce unscheduled DNA synthesis in rat hepatocytes, micronuclei mouse bone marrow, cell transformation in

BALB/3T3 mouse embryo cells, mitotic gene conversion or mitotic chromosome loss in yeast or chromosome damage in rat liver cells *in vitro*. There is little, if any, evidence that methyl isobutyl ketone-induced tumours in rodents arise through a genotoxic mechanism, although its two metabolites (4-hydroxymethyl isobutyl ketone and 4-methyl-2-pentanol) have not been evaluated for genotoxicity.

Methyl isobutyl ketone and its metabolites potentiate liver necrosis induced by haloalkanes and enhance cholestasis induced by cholestatic agents or manganese-bilirubin. There is no evidence that acute administration of methyl isobutyl ketone or its metabolites has adverse effects on the liver when given alone, either orally or via inhalation. It increased the total activity of cytochrome P450s 1A, 2B and 2E in the liver and kidney of rats. The induction of these isozymes by methyl isobutyl ketone has been associated with the potentiating effect mentioned above. It has been suggested that methyl isobutyl ketone also reduces the bile-salt pool and affects the secretion of bile acids by the liver.

Subchronic inhalation exposure to methyl isobutyl ketone was shown in one study to result in increased liver weight, characterized by hepatocellular hypertrophy, in both rats and mice, but statistically significant increases in both liver and kidney weights were observed only in male rats. Hyaline droplets were detected in the proximal tubules of all exposed male rats. However, another subchronic toxicity study that compared male and female rats demonstrated nephrotoxicity and increased liver and kidney weights in both male and female rats.

In various animal species, methyl isobutyl ketone was shown to be neurotoxic and irritating to the upper respiratory tract and lungs after inhalation at high concentrations.

The IARC scientific criteria to determine the plausibility of α_2 -globulin-associated nephropathy as the underlying mechanism of kidney tumorigenesis were considered based on the

limited number of studies on subchronic and chronic exposure to methyl isobutyl ketone, and were not completely fulfilled. The strength of the evidence that the kidney tumours in male rats arise through an α 2u-nephropathy-associated mechanism is weak.

There is no evidence that liver tumours in mice arise from a cytotoxic-regenerative cell-proliferation mechanism, because no overt liver toxicity has been demonstrated. There is only weak evidence that the tumours arise through a receptor-mediated mechanism.

The relevance of the tumour response in mice and rats to humans cannot be excluded.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of methyl isobutyl ketone.

6.3 Overall evaluation

Methyl isobutyl ketone is *possibly carcinogenic to humans* (Group 2B).

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1. Exposure Data

1.1 Chemical and physical data

From [IPCS \(1999\)](#), [European Commission \(2001\)](#), [IPCS-CEC \(2004\)](#), and [HSDB \(2005\)](#), unless otherwise specified

1.1.1 Nomenclature

Chem. Abstr. Services Reg. No.: 98-82-8

Chem. Abstr. Name: (1-Methylethyl) benzene;

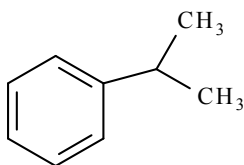
Synonyms: Benzene, isopropyl; cumol; isopropylbenzene; isopropylbenzol; 2-phenylpropane; propane, 2-phenyl

RTECS No.: GR8575000

EINECS No.: 202-704-5

United Nations TDG: 1918

1.1.2 Structural and molecular formulae and relative molecular mass



C_9H_{12}

Relative molecular mass: 120.2

1.1.3 Chemical and physical properties of the pure substance

Description: Colourless liquid with a sharp, penetrating, aromatic odour

Boiling-point: 152 °C

Melting-point: -96 °C

Density: 0.86 g/cm³ at 20 °C

Vapour pressure: 3.2 mm Hg at 20 °C; 4.6 mm Hg at 25 °C

Refractive index: 1.491 at 20 °C

Spectroscopy data: Infrared, ultraviolet, nuclear magnetic resonance and mass spectral data have been tabulated.

Solubility: Slightly soluble in water (50 mg/L at 25 °C); soluble in alcohol and many organic solvents

Flash-point: 31 °C; upper and lower explosive limit, 6.5% and 0.9%, respectively

Stability: Reacts violently with acids and strong oxidants, causing fire and explosions; can form explosive peroxides.

Octanol/water partition coefficient: log P_{ow} , 3.66 ([Sangster Research Laboratories, 2006](#))

Vapour density (air = 1): 4.2

Auto-ignition temperature: 420 °C

Henry's law constant: 1.15×10^{-2} atm.m³/mol at 25 °C

Oil/air partition coefficient: 6215

Water/air partition coefficient: 1.44

Human blood/air partition coefficient: 37

Conversion factor: 1 ppm = 5.2 mg/m³;

1 mg/m³ = 0.19 ppm (calculated

from: mg/m³ = (relative molecular

mass/24.45) × ppm, assuming a temperature of 25 °C and pressure of 101 kPa)

1.1.4 Technical products and impurities

No data were available to the Working Group.

1.1.5 Analysis

(a) Air

To measure cumene in air, Method 1501 of the US National Institute for Occupational Safety and Health (NIOSH) includes the use of a solid sorbent tube (coconut shell charcoal) sampler with gas chromatography/flame ionization detection, the detection limit of which is 0.6 µg/sample (NIOSH, 2003).

(b) Other media

Methods of the United States Environmental Protection Agency (EPA) for detecting cumene in media other than air include the use of gas chromatography with photo-ionization (Method 8021B), which is applicable to nearly all types of sample, regardless of the water content. The detection limit for cumene is 0.05 µg/L, and the applicable concentration range is approximately 0.1–200 µg/L. Another gas chromatographic assay commonly used for volatile compounds, including cumene, is EPA Method 8260B, which has a general estimated quantitation limit of approximately 5 µg/kg wet weight (wt) for soil/sediment samples, 0.5 mg/kg wet wt for wastes and 5 µg/L for groundwater (IPCS, 1999).

1.2 Production and use

1.2.1 Production

Cumene is manufactured from the distillation of coal tar and petroleum fractions, or is produced by the alkylation of benzene with propene using an acidic catalyst (European Commission, 2001).

Production volumes in the European Union (EU) ranged between 850 000 and 4 100 000 tonnes in 1992–93 (IUCLID, 2000; European Commission, 2001), and was 1 793 000 tonnes in 1985, distributed between seven countries and eight companies (IPCS, 1999; European Commission, 2001).

Production in the United States of America in 1977 was 1 200 000 tonnes, and rose to 1 800 000 tonnes in 1987 (HSDB, 2005). In 1998, 12 companies produced cumene in the USA (HSDB, 2005), and, in 2010, 50 producers were reported worldwide: eight in the People's Republic of China, 12 in East Asia, two in India, 18 in Europe, two in South and Central America and nine in the USA (Chemical Economics Handbook, 2010).

1.2.2 Use

Cumene is used primarily (95%) as an intermediate in the production of phenol and acetone. Other uses include: the manufacture of styrene, α -methylstyrene, acetophenone, detergents and di-isopropylbenzene; as a catalyst for acrylic and polyester-type resins; as a thinner for paints, enamels and lacquers; as a solvent for fat and resins; and in printing and rubber manufacture. Minor amounts are used in gasoline blending and as a component of high-octane aviation fuel.

1.3 Occurrence

1.3.1 Natural occurrence

Cumene is a natural constituent of crude oil and occurs naturally in the environment in plants, marsh grasses and foodstuff (see Section 1.3.3; HSDB, 2005). Crude oil typically contains 0.1% wt cumene but may contain up to 1% wt. Concentrations of cumene in petrol range from 0.14 to 0.51% vol, with an average of 0.3% vol. Premium diesel fuel contains 0.86% wt cumene (IPCS, 1999).

Table 1.1 Sources of exposure to cumene

Source/location	Comment	Emission rate
Releases to air		
Production	Controlled	0.08 kg/tonne cumene
	Uncontrolled	0.27 kg/tonne cumene
Use		1.03 kg cumene/tonne phenol
Production and use	Overall release ^a	1.31 kg/tonne
Gasoline engine vehicles	Catalytic converter	0.0002–0.0009 g/km
	No catalytic converter	0.002 g/km
Photocopying machines	Emission rate	140–220 µg/h
Releases to water and soil		
Production and use	Wastewater	1.5 kg/tonne cumene
	Soil	0.02 kg/tonne cumene

^a Includes release to the air from wastewater

h, hour or hours

From [European Commission \(2001\)](#), [HSDB \(2005\)](#)

1.3.2 Environmental occurrence

(a) Release/effluents

Cumene is released into the environment during its manufacture, use and transport. Another major source of pollution is its presence in crude oil and finished fuels; cumene is released from incomplete combustion of fossil fuels from vehicles, oils spills, transportation and distribution of fossil fuels, and evaporation from gasoline stations. Minor sources of release are from its use as a solvent, during paint manufacture and vulcanization of rubber, from building materials, jet engine exhaust and outboard motor operations, during pharmaceuticals production, from textile plants and from tobacco smoke ([IPCS, 1999](#); [HSDB, 2005](#)).

Emission rates from various sources of cumene are provided in [Table 1.1](#); releases rates of cumene in Europe and the USA are provided in [Table 1.2](#). Reported yearly cumene emissions to the air from cumene production were 125 tonnes [417 kg per day] in 1993 and 75 tonnes [250 kg per day] in 1995. Using these values, it was estimated that, during its production and use in the EU, cumene is released into the air at a rate of 1.3 kg/tonne, resulting in a rate of 17 903

kg per day, into water at a rate of 1.5 kg/tonne, resulting in a rate of 20 500 kg per day, and into the soil at a rate of 0.02 kg/tonne resulting in rate of 33.3 kg per day. It was also estimated that 3211 kg of cumene per day are released into the air in the EU from gasoline distribution, and 20 298 kg per day are released from motor vehicles; the total estimated amount released into the air from production, process and disperse sources is 41 412 kg per day ([European Commission, 2001](#)).

It was estimated from modelling that, in Los Angeles, USA, 2 300 kg of cumene per day (for 2 days) were released into the air in 1987 ([Harley & Cass, 1994](#)).

(b) Ambient air

Levels of cumene measured in ambient air are reported in [Table 1.3](#). The highest levels were found near industrial sites, such as an oil refinery (29.4–53.9 µg/m³), followed by urban areas; the lowest levels were found in rural areas. In the USA, cumene was found at 14.7 µg/m³ in urban areas and 2.5 µg/m³ in rural areas. In general, ambient levels of cumene were lower in Europe and Asia than in the USA.

Table 1.2 Daily release rates of cumene

Geographic location	Source	Media	Emission rate (kg/day)	Reference
European Union	Total	Air	41 412	European Commission (2001)
Estimated values	Production and use	Air ^a	17 903	
		Water ^b	20 500	
		Soil ^c	273	
	Disperse sources	Air		
Reported values	Gasoline		3 211 ^d	
	Motor exhaust		20 298 ^e	
	Production only	Air		
	1993		[417] ^f	
	1995		[250] ^f	
Los Angeles, CA Measured 2 d 1987	All sources	Air	2 300	Harley & Cass (1994)
USA, estimated	Total	Air	[26 027] ^g	US EPA (1988)

^a Assumes maximum production of 500 000 tonnes at one site (41 000 000 tonnes/year for the entire European Union) and release factor of 1.31 kg/tonnes (see [Table 1.1](#)).

^b Assumes maximum production of 500 000 tonnes at one site and release factor of 1.5 kg/tonne (See [Table 1.1](#)).

^c Assumes maximum production of 500 000 tonnes at one site and release factor of 0.02 kg/tonne (See [Table 1.1](#)).

^d Assumes 0.2% cumene from hydrocarbon loss, volatile organic compound (VOC) emission factor of 5 kg VOC/gasoline delivered and 117 205 000 tonnes/year of gasoline for the entire European Union.

^e Assumes 0.2% of cumene in motor exhaust, emission of 617 400 tonnes VOC/year and population ratio of 6 in the entire European Union.

^f Reported as 125 and 75 tonnes in 1993 and 1995, respectively.

^g Reported as 9500 tonnes/year

d, day or days

Table 1.3 Environmental occurrence of cumene in ambient air

Country	Location/sample	Concentration ($\mu\text{g}/\text{m}^3$)
Asia		
Nepal	Mount Everest	0.07
Taiwan, China	Urban area – heavy traffic	0.6–0.9
	Urban area – away from heavy traffic	0.5
Europe		
Belgium	Antwerp Craeybeckx tunnel (1991)	0.003–0.009 g/kg carbon-based pollutants
France	Grenoble area (1987)	1.6 (0.9–7.45) ^a
Germany	Urban area	6–9
	Hamburg – major road tunnel	3–3.8
Italy	Rome – urban area	1.1
	Milan – urban area	1.1–1.8
Netherlands	Urban area	0.3
	Rural area	0–5
	Delft	< 0.49–1.96
	Ambient air	0.49–34.79
	Rotterdam and Ede – near homes	0.3
Russian Federation	Leningrad – urban area (1977–79)	8.3 (0.98–11.76) ^a
Sweden	Near factory	4.5 ^a
	Göteborg	0.6 ^a
	Rural area	0.02 ^a
United Kingdom	Urban air	1–20
	Gatwick airport	1.6–12
	Southampton estuary	0.6–410
Americas		
Brazil	Porte Alegre (1996–97)	900

Table 1.3 (continued)

Country	Location/sample	Concentration ($\mu\text{g}/\text{m}^3$)
USA	Urban areas	14.7 ^a
	Rural areas	2.5 ^a
	Miami, FL – urban air	1.11–2.59
	Chicago, IL	0.59–1.1
	Boston, MA	0.1
	Lake Michigan (1976; 2 samples)	0.49
	Los Angeles, CA	
	1966, 136 samples	14.7 ^a , max 144
	8 samples	16.7 (2.45–36) ^a
	1981, 17 samples	ND–9.8
	Dear Park, TX – near Shell Oil Refinery	
	Downwind	29.4
	Upwind	53.9
	Houston, TX – urban and industrial areas (1973–74)	12.15 (ND–24.9) ^a
	Houston, TX (1986)	0.14–0.81
Jones State Forest, near Houston, TX	2.5 (0.11–9.8) ^a	
Rio Blanco Country, CO	1.57	
Great Smoky Mountains, TN (9 samples)	0.25 (< 0.0–0.39) ^a	

^a Mean (range) or mean
max, maximum; ND, not detected

From [IPCS \(1999\)](#), [European Commission \(2001\)](#), [HSDB \(2005\)](#)

(c) Water and soil

Cumene that is released into water is predicted to adsorb to suspended solids and sediment. It is removed from water and water surfaces by volatilization (half-lives of 1.2 hours in a model river and 4.4 days in a model lake) and degradation by hydroxyl radicals (estimated half-life, 107 days) ([HSDB, 2005](#)). Cumene may also be removed by aerobic biodegradation. Results of studies on biodegradation have been mixed, with some reporting between 13 and 86% degradation after 28 days ([European Commission, 2001](#)). Studies of oil spills found that cumene disappeared within 90 minutes of the spill ([HSDB, 2005](#)). Cumene may also bioaccumulate, based on an octanol/water partition coefficient greater than 3. Estimates of its bioaccumulation factor range from 208 to 356 in fish species; a value of 36 has been measured in goldfish ([IPCS, 1999](#); [European Commission, 2001](#)).

In soil, cumene is predicted to have low mobility based on its estimated soil absorption coefficient of 820. Similar to that from water, volatilization of cumene from moist soil (based on a Henry's Law constant of 0.0115 atm.m³/mol) or dry soil (based on its vapour pressure of 4.5 mm Hg) may occur ([HSDB, 2005](#)).

[Table 1.4](#) summarizes concentrations of cumene detected in water and soil. The highest levels in aquatic environments have been found near industrial sites and in industrial effluents, ranging up to 1581 µg/L in groundwater near underground storage tanks ([Botta et al., 1984](#)). High levels were also found in contaminated soil, ranging up to 305 mg/kg for soils contaminated by garage spills. Cumene has been detected at much lower levels (usually less than 1 µg/L) in groundwater and surface waters not adjacent to industry or contaminated by fuel, in some samples of drinking-water and in snow.

1.3.3 Other occurrence

Cumene occurs in cigarette smoke and in food. Levels of cumene in condensates of cigarette smoke ranged from 7 to 14 µg/cigarette and an indoor air concentration of 2 ppb [10 µg/m³] was reported after a single cigarette had been smoked ([IPCS, 1999](#)). The occurrence of cumene in food may result naturally or from environmental contamination. Cumene has been detected in fruits and vegetables (papaya, Sapodilla fruit, tomatoes and grapes), cooked meat (fried chicken, fried bacon and pork), cooked foods (cooked rice and baked potatoes), dairy products (cheese) and other foodstuff, including honey, dried legumes (beans, split peas and lentils), wine, southern pea seeds and plants, including curly parsley, marsh grasses and oakmoss ([IPCS, 1999](#); [HSDB, 2005](#)).

1.4 Human exposure

Exposure to cumene may occur via the workplace, the environment, cigarette smoking and food. The major source of exposure for the general public is through inhalation of contaminated air. Little exposure occurs from consumer use of products that contain cumene.

1.4.1 Occupational exposure

Workers may be exposed to cumene during its production and use, or the use of products that contain cumene. The major route of potential occupational exposure to cumene is inhalation. Dermal exposure may occur but is predicted to be low ([European Commission, 2001](#)).

No current information was found on the number of individuals occupationally exposed to cumene. In 2001, approximately 110–200 cumene-manufacturing workers had potential exposure in the EU ([European Commission, 2001](#)); manufacturing workers exposed to cumene include shift operators, foremen, maintenance fitters, quality control personnel and

Table 1.4 Environmental levels of cumene in water and soil

Country	Industrial site	Location/sample type and size	Concentration (µg/L)
Groundwater or effluents near industrial sites			
Australia	Near dump site	Melbourne	Detected
Denmark	Contaminated with creosote and/or gasoline	Groundwater Holte (3 samples) Fredericia (5 samples)	2–22 ND–3
Germany			0.5–5
Italy	Near underground storage tanks	Milan	Detected
Sweden		Wastewater – Göteborg	0.1–0.8
United Kingdom	Near gasoline storage tank	Groundwater – Great Ouse River basin	9.8 (0.01–30) ^a
	Contaminated Site	Solent estuary Groundwater	0.01–47.3 1–3
	Airfield	Groundwater	1–30
USA	Coal gasification sites	Groundwater – Hoe Creek, WV Wyoming	35 (1–59) ^a 19–54
	Petroleum plants and refineries		5
	Near offshore drilling platform, Gulf of Mexico	Sea water	140
	Around outboard motor operations		700
	Near chemical plants	Groundwater (3 sites)	11, 360, and 1581
Groundwater – other			
USA		50 states and Puerto Rico Ames, IO New York State	< 0.5 Detected Detected
Surface water			
Germany		River Rhine Lake Constance	0.028 0.006–0.028
Japan	Near potential emission sources	Surface water	0.09–0.44
Spain		River Gallego	< 0.001 ng/L

Table 1.4 (continued)

Country	Industrial site	Location/sample type and size	Concentration (µg/L)
United Kingdom		British North Sea	0.001–0.069
		River Lee (2 samples)	< 0.1, > 0.1
USA		Narraganset Bay, RI	Detected
		River Brazos, TX	0.006–0.017
Drinking-water			
Japan		Tap-water	Detected
USA		Terrebonne-Parish, LA	0.01
		9 other cities countrywide	ND
		Cincinnati, OH	0.014
		Drinking-water systems countrywide	< 0.5 mg/L ^b
		New York State	Detected
Snow			
Antarctica		1987/88	0.008
		1988/89	0.016
		1990/91	ND
Sediment			
Japan	Near potential emission source		0.6–11 µg/g
United Kingdom		Southampton	0.25–43.37 µg/g
USA		Strait of Juan de Fuca, Alaska	0.02–5.5 µg/g
		Puget Sound, Washington	2.3 (0.02–19) ^a µg/g
Soil			
Germany	Below building		24 mg/kg
Netherlands	Contaminated sites		0.012–0.02 mg/kg
	Garage oil spills		10–305 mg/kg

^a Mean (range)

^b Detection limit, 0.5 µg/L

ND, not detected

From [IPCS \(1999\)](#), [European Commission \(2001\)](#), [HSDB \(2005\)](#)

Table 1.5 Measured levels of occupational exposure to cumene

Process or work area	No. and type of samples	Concentration (ppm)
8-h TWA		
Manufacture – all job categories	7 European companies	0.1–0.65 ^a (0.05–4.46)
Cumene producing plant – specific jobs: runner, filling station attendant, laboratory co-worker, chemical technology co-worker	Personal air samples	< 1
Manufacture – long-term exposure, 1991	40–50 samples	< 0.1
Offset printing works	17 person-related measurements	0.1–1.3
Printing signs using lacquering machines	2 person-related measurements	0.2
Maintenance printers – 23 different jobs	45 person-related measurements	0–0.81
Short-term (10–20 min or 20–30 min) exposure data		
Car repair work	8 person-related measurements	1.9–6.7
Rubber manufacturing process		
Shoe sole factory ^b	13	0.012–0.05
Tyre retreading ^b	6	0.0004–0.04
Tyre retreading ^c	6	0–0.002
Electrical cable insulation plant	10	ND
1 h duration of exposure – 90% value		
Production of paints	125	0.8
Surface treatment, manual (painting, paint rolling)	255	3.4
Surface treatment, manual (spraying)	300	1.01
Surface treatment, mechanical	84	0.8
Other monitoring data		
Cumene producers and processors	NR	
Distillation		0.45 (0.0001–3.35)
Oxidation		0.93 (0.0001–5.58)
Laboratory		0.39 (0.34–0.44)
Repair		1.33 (0.16–2.50)
Recovery		0.31 (0.001–1.20)
Cumene unit		0.19 (0.078–0.62)

Table 1.5 (continued)

Process or work area	No. and type of samples	Concentration (ppm)
Cumene-exposed workers, 1973–84	1457 air samples	
	6 samples	4–30
	4 samples	3–4
	25 samples	1–2
	Remainder	< 1
Exposure from solvents, United Kingdom		Up to 0.6
Gasoline delivery truck drivers		< 0.01–0.04

^a Range of means

^b Vulcanization area

^c Extrusion area

h, hour or hours; min, minute or minutes; ND, not detected; NR, not reported; TWA, time-weighted average

From [IPCS \(1999\)](#), [European Commission \(2001\)](#), [HSDB \(2005\)](#)

Table 1.6 Cumene levels from non-occupational exposure

Workers	Cumene concentration (mean [range] in ng/L)		
	Environmental (8-h)	Alveolar	Blood
27 chemical workers	38.9 (1–279)	12 (1–81)	762 (43–3352)
33–40 hospital workers	9.6 (2–36)	4.7 (1–22)	176 (31–929)

h, hour or hours

From [Brugnone et al. \(1989\)](#)

others, such as delivery drivers. The National Occupational Exposure Survey, conducted in 1981–83, estimated that 14 268 workers, of whom 2760 were women, were potentially exposed to cumene in the USA. The major occupations were janitors and cleaners, maids and housemen, machine operators, including laundering, dry cleaning and unspecified, and vehicle washers and equipment cleaners ([NIOSH, 1990](#)). An industrial survey by the EPA reported that approximately 739 workers were occupationally exposed to cumene in the USA ([US EPA, 1988](#)). [The Working Group noted the large discrepancy in numbers reported by the two sources.]

Cumene is typically produced using a closed system. The [European Commission \(2001\)](#) reported that one manufacturing company stated that contact with cumene is limited to work activities that involve the collection of samples for analysis, loading tanks, and cleaning and maintenance. [Table 1.5](#) lists exposure levels reported in the cumene-manufacturing industry and industries that use cumene. The mean 8-hour

time-weighted average levels for seven cumene-manufacturing companies in the EU ranged from 0.1 to 0.65 ppm for all activities (range of data, 0.05–4.46 ppm). The Workplace Exposure Model predicts that inhalation exposure from the use of a closed system would be in the range of 0–0.1 ppm. It also predicts that dermal exposure for all activities would be 0–0.1 mg/cm² per day.

According to the [European Commission \(2001\)](#), the manufacture of phenol and acetone at cumene production sites is also carried out in closed systems; thus, the EU assumed that exposure levels for this industry would be similar to those observed for cumene-manufacturing. In general, exposure levels for most other uses of cumene (such as printing and rubber manufacture) were less than 1.5 ppm, although somewhat higher levels were found for short-term exposure among workers involved in car repairs (see [Table 1.5](#)). Exposure levels were less than 1 ppm in nearly all 1487 air samples evaluated in an industrial survey of cumene-exposed workers by the EPA ([US EPA, 1988](#)).

Table 1.7 Estimated human daily intake of cumene

Source	Regional intake (mg/kg bw per day)
Air	1.43×10^{-5}
Drinking-water	4.87×10^{-9}
Fish	9.8×10^{-8}
Leaf crops	7.9×10^{-8}
Root crops	3.24×10^{-8}
Meat	3.23×10^{-9}
Milk	1.91×10^{-9}
Total	1.45×10^{-5}

From [European Commission \(2001\)](#)

Table 1.8 Regulations and guidelines concerning occupational exposure to cumene

	TWA – 8 h		Short-term – 10 minutes		Note
	ppm	mg/m ³	ppm	mg/m ³	
Australia	25	125	75	375	
Austria	20	100	50	250	
Belgium	20	100	50	250	
Canada – Ontario	50				
Canada – Québec	50	246			
Denmark	20	100	40	200	
European Union ^a	20	100	50	250	
France ^b	20	100	50	250	
Germany (AGS)	20	100	50 ^c	250 ^c	
Germany (DFG)	50	250	200 ^c	1000 ^c	
Hungary		100		250	sk
Italy	20	100	50	250	sk
Netherlands	20	100	50	250	
New Zealand	25	125	75	375	
Poland		100		250	
Singapore	50	246			
Spain	20	100	50	250	
Sweden	25	120	35	170	
Switzerland	50	245	200	980	
USA – NIOSH					
TLV	50	245			
REL	50	245			
IDLH			900 (30 min)		
TWA	50				
USA – OSHA	50	245			
USA – ACGIH	50	245			
United Kingdom	25	120	75	375	

^a Indicative occupational exposure limit values and limit values for occupational exposure

^b Restrictive statutory limit values

^c 15-minute average value

ACGIH, American Conference of Governmental Industrial Hygienists; AGS, Ausschuss für Gefahrstoffe; DFG, Deutsche Forschungsgemeinschaft; h, hour or hours; IDLH, immediately dangerous to life or health; NIOSH, National Institute of Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; REL, recommended exposure limit; sk, skin; TLV, threshold limit value; TWA, time-weighted average

From [ACGIH \(2010\)](#) and [GESTIS \(2011\)](#)

Table 1.9 Acute exposure guideline levels for cumene in the USA

	10 minutes	30 minutes	1 hour	4 hours	8 hours
AEGL-1	50	50	50	50	50
AEGL-2	550	380	300	190	130
AEGL-3	1300	920	730	460	300

AEGL, acute exposure guideline levels
From [US EPA \(2007\)](#)

1.4.2 Environmental exposure

[Brugnone et al. \(1989\)](#) measured non-occupational exposure to cumene in the breath (alveolar) and blood from workers at a benzene chemical plant (no direct exposure to cumene) and at a hospital infirmary ([Table 1.6](#)). Environmental exposure to cumene was also measured in air (8-hour work shift) at the workplace. Mean levels of exposure to cumene for all three exposure metrics was higher in 27 chemical workers than in 33–40 hospital workers, and significantly so for blood levels. Alveolar levels correlated with environmental levels at both workplaces, and blood levels correlated with environmental levels and alveolar levels in chemical workers.

1.4.3 Estimated human intake

The [European Commission \(2001\)](#) developed a model to predict total human intake from various sources of environmental exposure. The regional environment represents a highly industrial area (200 km × 200 km with 20 million inhabitants). Inhalation of air accounted for 97% of intake. Other sources of exposure were various food items and, to a lesser degree, drinking-water (see [Table 1.7](#)). The concentration of cumene in food was predicted from its concentration in air, water and soil and its bioaccumulation. A total daily intake of cumene of 1.45×10^{-5} mg/kg bw per day was estimated.

1.5 Regulations and guidelines

Some country-specific regulatory guidelines that are presented in [Table 1.8](#) and [Table 1.9](#), give more detailed guidelines for short-term exposures.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

Carcinogenicity studies of inhalation exposure of mice and rats to cumene and one of its metabolites been conducted ([NTP, 2007, 2009](#)), the results of which are summarized in [Table 3.1](#).

3.1 Inhalation exposure

3.1.1 Mouse

Groups of 50 male and 50 female B6C3F₁ mice were exposed by whole-body inhalation to 0, 125 (females only), 250, 500 or 1000 (males only) ppm cumene (> 99% pure) for 6 hours plus T₉₀ (the time taken to reach 90% of the target concentration within the exposure chamber; 12 minutes) per day on 5 days per week for 105 weeks. Dose-related increases in the incidence of alveolar/bronchiolar adenoma and carcinoma were observed in both males and females. Treatment-related increases in the incidence of

Table 3.1 Carcinogenicity studies of inhalation exposure of experimental animals to cumene and α -methylstyrene

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Results Incidence (%) and/or multiplicity of tumours	Significance (poly-3 test)	Comments
Cumene				
Mouse, B6C3F ₁ (M) 105 wk NTP (2009)	0, 250, 500 or 1 000 ppm 6 h plus T ₉₀ (12 min)/d, 5 d/wk 50 animals/group	Lung (alveolar/bronchiolar adenoma): 13/50 (26%), 31/50 (62%), 31/50 (62%), 29/50 (58%) Lung (alveolar/bronchiolar carcinoma): 9/50 (18%), 19/50 (38%), 32/50 (64%), 33/50 (66%) Lung (alveolar/bronchiolar adenoma or carcinoma): 19/50 (38%), 38/50 (76%), 42/50 (84%), 43/50 (86%) Spleen (haemangiosarcoma ^a): 0/50, 0/50, 0/49, 4/50 (8%) All organs (haemangiosarcoma ^b): 0/50, 1/50 (2%), 2/50 (4%), 4/50 (8%) Thyroid gland (follicular-cell adenoma ^c): 0/50, 0/50, 0/49, 3/50 (6%)	$P < 0.001$ (all doses) $P < 0.001$ (trend) $P < 0.001$ (high dose) $P < 0.001$ (mid dose) $P = 0.014$ (low dose) $P < 0.001$ (trend) $P < 0.001$ (all doses) $P < 0.001$ (trend) $P = 0.045$ (high dose) $P = 0.002$ (trend) $P = 0.01$ (trend)	99.9% pure Survival: 38/50, 34/50, 30/50, 23/50* Thyroid gland (follicular-cell hyperplasia): 7/50 (1.9) ⁱ , 7/50 (2.4), 7/49 (1.7), 11/50 (1.9)
Mouse, B6C3F ₁ (F) 105 wk NTP (2009)	0, 125, 250 or 500 ppm 6 h plus T ₉₀ (12 min)/d, 5 d/wk 50 animals/group	Lung (alveolar/bronchiolar adenoma): 1/50 (2%), 26/50 (52%), 36/50 (72%), 38/50 (76%) Lung (alveolar/bronchiolar carcinoma): 3/50 (6%), 16/50 (32%), 20/50 (40%), 34/50 (68%) Lung (alveolar/bronchiolar adenoma or carcinoma): 4/50 (8%), 31/50 (62%), 42/50 (84%), 46/50 (92%) Liver (hepatocellular adenoma): 18/50 (36%), 23/50 (46%), 27/50 (34%), 29/50 (58%) Liver (hepatocellular carcinoma): 10/50 (20%), 7/50 (14%), 6/50 (12%), 12/50 (24%) Liver (hepatocellular adenoma or carcinoma): 25/50, 26/50, 29/50, 36/50	$P < 0.001$ (all doses) $P < 0.001$ (trend) $P < 0.001$ (all doses) $P < 0.001$ (trend) $P < 0.001$ (all doses) $P < 0.001$ (trend) $P = 0.046$ (high dose) $P = 0.04$ (trend) $P = 0.043$ (high dose) $P = 0.024$ (trend)	99.9% pure Survival: 37/50, 36/50, 39/50, 35/50

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Results Incidence (%) and/or multiplicity of tumours	Significance (poly-3 test)	Comments
Rat, F344 (M) 105 wk NTP (2009)	0, 250, 500 or 1 000 ppm 6 h plus T ₉₀ (12 min)/d, 5 d/wk 50 animals/group	Nose (respiratory epithelial adenoma): 0/50, 7/50 (14%), 18/49 (37%), 10/50 (20%) Kidney (renal tubule adenoma): 1/50 (2%), 4/50 (8%), 5/50 (10%), 4/50 (8%) Kidney (renal tubule carcinoma): 1/50 (2%), 1/50 (2%), 3/50 (6%), 3/50 (6%) Kidney (renal tubule adenoma or carcinoma ^d): 2/50 (4%), 5/50 (10%), 8/50 (16%), 7/50 (14%) Testis (interstitial-cell adenoma): 36/50 (72%), 38/50 (76%), 40/50 (80%), 46/50 (92%)	<i>P</i> < 0.001 (high dose) <i>P</i> < 0.001 (mid dose) <i>P</i> = 0.006 (low dose) <i>P</i> = 0.004 (trend) <i>P</i> = 0.044 (mid dose) <i>P</i> = 0.007 (high dose) <i>P</i> = 0.006 (trend)	99.9% pure Survival: 26/50, 23/50, 27/50, 24/50 Nose (olfactory epithelial basal-cell hyperplasia): 0/50, 19/50** (1.1), 27/49** (1.1), 26/50** (1.0) Nose (respiratory epithelial hyperplasia): 0/50, 15/50** (2.0), 16/49** (2.9), 23/50** (2.7) Kidney (renal tubule hyperplasia): 0/50, 3/50 (3.3), 8/50** (2.6), 6/50* (2.2) Kidney (papilla mineralization): 5/50 (1.0), 35/50** (1.7), 44/50** (2.1), 41/50** (2.1) Kidney (pelvic transitional epithelial hyperplasia): 3/50 (1.7), 5/50 (1.8), 14/50** (2.4), 15/50** (2.0) Kidney (nephropathy): 47 (2.3), 47 (2.6), 47 (2.9), 50 (2.7)
Rat, F344 (F) 105 wk NTP (2009)	0, 250, 500 or 1 000 ppm 6 h plus T ₉₀ (12 min)/d, 5 d/wk 50 animals/group	Nose (respiratory epithelial adenoma ^e): 0/50, 5/48 (10%), 4/50 (8%), 3/50 (6%)	<i>P</i> = 0.03 (low dose)	99.9% pure Survival: 21/50, 27/50, 31/50, 32/50 Nose (olfactory epithelial basal-cell hyperplasia): 0/50, 14/48** (1.0), 25/50** (1.0), 31/50** (1.1) Nose (respiratory epithelial hyperplasia): 0/50, 0/48, 4/50 (3.0), 6/50* (2.3)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Results Incidence (%) and/or multiplicity of tumours	Significance (poly-3 test)	Comments
α-Methylstyrene				
Mouse, B6C3F ₁ (M) 105 wk NTP (2007)	0, 100, 300 or 600 ppm 6 h plus T ₉₀ (12 min)/d, 5 d/wk 50 animals/group	Liver (hepatocellular adenoma or carcinoma): 28/50 (56%), 36/50 (72%), 33/50 (66%), 37/50 (74%) Liver (hepatocellular adenoma): 24/50 (48%), 27/50 (54%), 27/50 (54%), 25/50 (50%) Liver (hepatocellular carcinoma): 10/50 (20%), 12/50 (24%), 11/50 (22%), 17/50 (34%)	$P = 0.035$ (high dose) $P = 0.031$ (low dose)	99.5% pure Survival: 35/50, 32/50, 40/50, 36/50
Mouse, B6C3F ₁ (F) 105 wk NTP (2007)	0, 100, 300 or 600 ppm 6 h plus T ₉₀ (12 min)/d, 5 d/wk 50 animals/group	Liver (hepatocellular adenoma): 10/50 (20%), 20/50 (40%), 21/50 (42%), 23/50 (46%) Liver (hepatocellular carcinoma): 3/50 (6%), 9/50 (18%), 6/50 (12%), 18/50 (36%) Liver (hepatocellular adenoma or carcinoma): 13/50 (26%), 26/50 (52%), 24/50 (48%), 33/50 (66%)	$P = 0.005$ (high dose) $P = 0.007$ (mid dose) $P = 0.018$ (low dose) $P = 0.014$ (trend) $P < 0.001$ (high dose) $P < 0.001$ (trend) $P < 0.001$ (high dose) $P = 0.012$ (mid dose) $P = 0.004$ (low dose) $P < 0.001$ (trend)	99.5% pure Survival: 39/50, 38/50, 37/50, 37/50
Rat, F344 (M) 105 wk NTP (2007)	0, 100, 300 or 1 000 ppm 6 h plus T ₉₀ (12 min)/d, 5 d/wk 50 animals/group	Kidney (renal tubule adenoma ^{f, g}): 1/50 (2%), 2/50 (4%), 2/50 (4%), 5/50 (10%) Kidney (renal tubule adenoma or carcinoma ^h): 1/50 (2%), 2/50 (4%), 3/50 (6%), 7/50 (14%) <i>Single sections only</i> Kidney (renal tubule carcinoma): 0/50, 0/50, 1/50 (2%), 2/50 (4%) Kidney (renal tubule adenoma or carcinoma): 0/50, 0/50, 2/50 (4%), 2/50 (4%) <i>Step section evaluation alone (3–4 sections per kidney)</i> No additional renal tubule carcinomas were identified Haematopoietic (mononuclear-cell leukaemia ⁱ): 26/50 (52%), 32/50 (64%), 29/50 (58%), 38/50 (76%)	$P = 0.026$ (high dose) $P = 0.006$ (trend) $P = 0.016$ (high dose) $P = 0.018$ (trend)	99.5% pure Survival: 27/50, 32/50, 23/50, 22/50 Kidney (papillary mineralization): 12/50 (1.1), 16/50 (1.0), 10/50 (1.0), 33/50** (1.4) Kidney (nephropathy): 41/50 (2.2), 46/50 (2.3), 46/50 (2.4), 45/50 (2.4) Kidney (renal tubule hyperplasia ^k): 1/50 (1.0), 0/50, 1/50 (1.0), 4/50 (2.3)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Results Incidence (%) and/or multiplicity of tumours	Significance (poly-3 test)	Comments
Rat, F344 (F) 105 wk NTP (2007)	0, 100, 300 or 1 000 ppm 6 h plus T ₉₀ (12 min)/d, 5 d/wk 50 animals/group	No significant results		99.5% pure Survival: 27/50, 24/50, 36/50, 26/50

* Significantly different ($P \leq 0.05$) from the chamber control group by the poly-3 test

** $P \leq 0.01$

^a Historical incidence in male B6C3F₁ mice in 2-year inhalation studies using chamber controls (mean \pm standard deviation): 6/444 (1.4% \pm 1.5%), range 0–4%; all routes: 24/1483 (1.7% \pm 1.2%), range 0–4%

^b Historical incidence in male B6C3F₁ mice in 2-year inhalation studies using chamber controls (mean \pm standard deviation): 21/450 (4.7% \pm 3.7%), range 0–12%; all routes: 76/1499 (5.2% \pm 3.2%), range 0–12%

^c Historical incidence in male B6C3F₁ mice for 2-year inhalation studies using chamber controls (mean \pm standard deviation): 5/441 (1.1% \pm 2.0%), range 0–6%; all routes: 21/1483 (1.4% \pm 1.8%), range 0–6%

^d Historical incidence in male F344/N rats in 2-year inhalation studies using chamber controls (mean \pm standard deviation): kidney (renal tubule adenoma or carcinoma): 6/449 (1.3% \pm 1.4%), range 0–4%; all routes: 10/1436 (0.7% \pm 1.0%), range 0–4%; kidney (renal tubule adenoma): 4/449 (0.9% \pm 1.0%), range 0–2%; all routes: 8/1436 (0.6% \pm 0.8%), range 0–2%; kidney (renal tubule carcinoma): 2/449 (0.4% \pm 0.9%), range 0–2%; all routes: 2/1436 (0.1% \pm 0.5%), range 0–2%

^e Historical incidence in female F344/N rats in 2-year inhalation studies using chamber controls: 0/496; all routes: 0/1343

^f This incidence is based on the combined single section and step section evaluations. Single sections alone – renal tubule adenoma: 0/50, 0/50, 1/50, 0/50; step section evaluation alone (3–4 sections per kidney) – renal tubule adenoma: 1/50, 2/50, 1/50, 5/50 ($P = 0.033$ for trend).

^g Historical incidence in male F344/N rats in 2-year inhalation studies using chamber controls for single section evaluations (mean \pm standard deviation): kidney (renal tubule adenoma): 3/399 (0.8% \pm 1.0%), range 0–2%

^h Historical incidence in male F344/N rats in 2-year inhalation studies using chamber controls for single section evaluations (mean \pm standard deviation): kidney (renal tubule carcinoma): 1/399 (0.3% \pm 0.7%), range 0–2%; kidney (renal tubule adenoma or carcinoma): 4/399 (1.0% \pm 1.1%), range 0–2%

ⁱ Historical incidence in male F344/N rats in 2-year inhalation studies with chamber control groups (mean \pm standard deviation): 188/399 (47.1% \pm 10.3%); range 32–66%
d, days or days; min, minute or minutes; N/A, not applicable; wk, week or weeks

^j Numbers in parentheses indicate average grade of severity of the lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

^k This incidence is based on the combined single and step section evaluations. In the single section evaluation, no renal tubular hyperplasia was identified (all such lesions were diagnosed in the step section evaluation).

haemangiosarcoma of the spleen in male mice and of hepatocellular adenoma of the liver in female mice also occurred ([NTP, 2009](#)).

3.1.2 Rat

Groups of 50 male and 50 female F344 rats were exposed to 0, 250, 500 or 1000 ppm cumene (> 99% pure) for 6 hours plus T₉₀ (12 minutes) per day on 5 days per week for 105 weeks. Treatment-related increases were observed in the incidence of nasal tumours (respiratory epithelial adenoma) in both males and females, and kidney tumours (renal tubule adenoma or carcinoma) in males, with a dose-related increase in the incidence of nasal tumours in males, with a concurrent increase in renal tubule hyperplasia and papillary mineralization in males, which had a linear pattern. Furthermore, in a subchronic study in rats with five exposure groups (62.5, 125, 250, 500 or 1000 ppm), dose-related increases in the severity of proximal tubular hyaline droplet accumulation and regeneration occurred, together with increases in the incidence of medullary granular casts and in the levels of α 2u-globulin in males. Moreover, males had a treatment-related increase in the incidence of testicular tumours (interstitial-cell adenoma) ([NTP, 2009](#)).

[Tumours of the nasal cavity and kidney and splenic haemangiosarcomas are rare spontaneous neoplasms in experimental animals.]

3.2 Carcinogenicity of metabolites

3.2.1 α -Methylstyrene

α -Methylstyrene, a major metabolite of cumene, has been identified, together with its derivatives, in the exhaled air and urine of rats and mice exposed to cumene ([Chen et al., 2011](#)).

(a) Mouse

Groups of 50 male and 50 female B6C3F₁ mice, 6 weeks of age, were exposed by whole-body inhalation to 0, 100, 300 or 600 ppm α -methylstyrene (99.5% pure) for 6 hours plus T₉₀ (12 minutes) per day on 5 days per week for 105 weeks. Treatment-related increases in the incidence of hepatocellular adenoma or carcinoma (combined) in both males and females and of hepatocellular adenomas and carcinomas (separately) in females were observed ([NTP, 2007](#)).

(b) Rat

Groups of 50 male and 50 female F344/N rats, 6 weeks of age, were exposed by whole-body inhalation to 0, 100, 300 or 1000 ppm α -methylstyrene (99.5% pure) for 6 hours plus T₉₀ (12 minutes) per day on 5 days per week for 105 weeks. Dose-related increases in the incidence of renal tubule adenoma and renal tubule adenoma or carcinoma (combined) were observed in males ([NTP, 2007](#)).

4. Other Relevant Data

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 or intravenous administration of radiolabelled cumene ([¹⁴C]isopropylbenzene) to rats and mice, 16 metabolites were identified in the expired air, urine, bile and microsomes; 2-phenyl-2-propanol glucuronide was the major urinary metabolite ([Chen et al., 2011](#)). The volatile organic compounds in the expired air comprised mainly cumene and up to 4% α -methylstyrene. There were some marked parallels between the

sex, species and organs in which maximum levels of radiolabel were observed (Chen *et al.*, 2011) and those in which carcinogenic effects were observed (NTP, 2009). For example, the highest levels of radiolabel in rats were found in the adipose tissue, liver and kidney; and male, but not female, rats developed kidney tubule adenomas. In mice, the highest concentrations of radiolabel were found in the liver, kidney and lung 24 hours after a single administration; after repeated dosing, radiolabel was found in the same tissues as well as in the blood, brain, heart, muscle and spleen. Cumene-treated mice had an increased incidence of tumours in the lung, spleen and liver.

A proposed metabolic pathway of cumene (Chen *et al.*, 2011) is the formation of α -methylstyrene (Morgan *et al.*, 1999) and its conversion by cytochrome P450 (CYP) to α -methylstyrene oxide, which can then be either conjugated to glutathione by glutathione S-transferase or converted to a glycol by epoxide hydrolase. Cumene was converted to α -methylstyrene more efficiently by mouse than by rat lung microsomes *in vitro*, which may account for the excess of radiolabelled compound found in mouse lung following multiple doses of cumene (Chen *et al.*, 2011). Also, Morgan *et al.* (1999) showed that α -methylstyrene was more lethal to female mice than to male mice or male and female rats; however, no enzymatic studies were performed (Morgan *et al.*, 1999) to clarify the metabolic pathways. Collectively, these data suggest that cumene is metabolized differentially in mice and rats, resulting in potentially higher levels of α -methylstyrene, and possibly α -methylstyrene oxide, in the lungs of mice than in those of rats.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

(a) Mutations

Cumene itself was generally not mutagenic; however, some mutagenic metabolites were identified, including α -methylstyrene oxide (Chen *et al.*, 2011), which is mutagenic in *Salmonella* (Rosman *et al.*, 1986).

Cumene was not mutagenic in the *Salmonella* mutagenicity assay in a variety of strains in the presence or absence of metabolic activation (Florin *et al.*, 1980; NTP, 2009). A summary of several unpublished reports noted that cumene was not mutagenic in *Salmonella*, yeast or Chinese hamster ovary cells (hypoxanthine (guanosine) phosphoribosyltransferase assay) in the presence or absence of metabolic activation, and gave negative or equivocal results for the induction of unscheduled DNA synthesis in rat primary hepatocytes or cell transformation in BALB/3T3 cells (US EPA, 1997).

(b) Chromosomal effects

Intraperitoneal injection (up to 1 g/kg body weight) of cumene induced small, but significant, increases in micronuclei in the bone marrow of male F344 rats in two independent trials; however, cumene did not induce micronuclei in erythrocytes in the peripheral blood of male or female B6C3F₁ mice exposed by inhalation to up to 1000 ppm for 6 hours per day on 5 days per week for 3 months (NTP, 2009).

(c) Alterations in oncogenes and suppressor genes in tumours

Analysis of mutations in the cumene-induced lung tumours in mice from the NTP (2009) study found that 87% had *K-ras* mutations, predominantly G to T transversions in exon 1 codon 12 and A to G transitions in exon 2 codon 61. Mutations in *Tp53* were found in 52% of the cumene-induced tumours, and were predominantly G to A transitions in exon 5 codon 155 and C to T transitions in exon 5 codon 133; 56%

of the cumene-induced tumours overexpressed p53 protein. Loss of heterozygosity was found on chromosome 4 near the *p16* gene in 13%, and on chromosome 6 near the *K-ras* gene in 12%. In contrast, among spontaneous tumours, none had *Tp53* mutations, only 14% had *K-ras* mutations and none had loss of heterozygosity (Hong *et al.*, 2008). Based on previous studies (reviewed in Hoenerhoff *et al.*, 2009), the authors suggested that this pattern of mutations indicated that DNA damage and genomic instability probably contribute to cumene-induced lung cancer in mice (Hong *et al.*, 2008). No additional mutational analyses were performed.

Analysis of changes in global gene expression showed that the lung tumours could be separated into groups with regard to *K-ras* mutations (with or without), but not based on *Tp53* mutations (Wakamatsu *et al.*, 2008). Expression of genes associated with the extracellular signal-regulated kinase-mitogen activated protein kinase signalling pathway was altered in tumours with *K-ras* mutations compared with those with no such mutations or normal lung tissue. Also, cumene-induced tumours with *K-ras* mutations had greater malignant potential than those without. The authors concluded that most cumene-induced mouse lung tumours contained *K-ras* mutations that probably resulted in increased extracellular signal-regulated kinase-mitogen activated protein kinase signalling and modification of histones (Wakamatsu *et al.*, 2008). No additional gene expression analyses were performed.

4.3 Mechanistic data

4.3.1 Effect on cell physiology

Cumene induced renal tubule adenomas, which might involve an α 2u-globulin mechanism, in male rats. However, one of the mutagenic metabolites of cumene, α -methylstyrene oxide, could play a role in the initiation of such tumours.

In a subchronic study in rats, dose-related increases in proximal tubular hyaline droplet accumulation and the levels of α 2u-globulin were observed in males (NTP 2009). Exposure to α -methylstyrene also resulted in increased accumulation of hyaline droplets in the renal tubules of male rats (Morgan *et al.*, 1999). Hyaline droplets, which contain α 2u-globulin, can lead to granular casts and single-cell necrosis, increased cell division and tubule hyperplasia, and finally renal tubule adenoma and carcinoma (Rodgers & Baetcke, 1993).

The development of kidney tumours in male rats in association with chemically induced α 2u-globulin nephropathy is one mechanism that is not considered to be a predictor of carcinogenic risk to humans by the IARC or the EPA (US EPA, 1991; Swenberg & Lehman-McKeeman, 1999). The lack of relevance of the α -2u-globulin mechanism for the evaluation of carcinogenic risk is based on the absence of the production of an analogous protein in humans. Strict scientific criteria have been outlined to establish the role of α 2u-globulin-associated nephropathy in male rat renal carcinogenesis (Swenberg & Lehman-McKeeman, 1999; see also Section 4.4 of the *Monograph* on Methyl isobutyl ketone in this Volume). Because these criteria are not met, the data do not support a mechanism that involves α 2u-globulin-associated nephropathy in the development of these kidney tumours.

4.3.2 Structure–activity relationships

The two main tumour types induced by cumene are nasal adenomas in rats and lung tumours in mice. The genes that are mutated and have altered expression in cumene-induced mouse lung tumours are similar to those that are mutated and have altered expression in tumours induced in rodents by other related compounds, as well as to those found in human lung tumours (Hong *et al.*, 2008; Wakamatsu *et al.*, 2008; Hoenerhoff *et al.*, 2009).

[Cruzan *et al.* \(2009\)](#) compared the metabolism of cumene and some structurally related compounds (coumarin, naphthalene, styrene and ethylbenzene) that produce a similar tumour profile, i.e. bronchiolar/alveolar lung tumours in mice and nasal tumours in rats. They concluded that metabolism of the compounds in the Clara cells of mouse lung by CYP2F2 and in the nasal tissues of rats by CYP2F4 results in the production of cytotoxic metabolites that produce the respective tumours. Rat lung, human lung and human nasal turbinates also have the orthologous isozymes (CYP2F4 in rats and CYP2F1 in humans) that allow them to produce the necessary cytotoxic and mutagenic metabolites. These enzymes are polymorphic in humans. A detailed modelling of CYP2F substrates among various species was performed ([Lewis *et al.*, 2009](#)) that showed that the CYP2F subfamily of enzymes exists in a variety of species; however, differences exist between humans and rodents in the activities of this enzyme subfamily. Although [Cruzan *et al.* \(2009\)](#) argue for a cytotoxicity-driven model, consistent with the lack of mutagenicity of cumene itself, a mutagenic metabolite of cumene, α -methylstyrene oxide, could provide the basis for a genotoxicity-driven model both in rodents and humans — especially because the necessary enzymes are present in humans.

4.4 Mechanisms of carcinogenesis

At least one mutagenic metabolite of cumene, α -methylstyrene oxide, has been found in rats and mice. Moreover, mouse lung tumours had an elevated frequency of mutations in *K-ras* and *Tp53*, and exhibited a variety of changes in gene expression that involve pathways that are well known in both murine and human carcinogenesis. Comparisons among a group of compounds that are related structurally to cumene showed that the enzymes that probably produce mutagenic/carcinogenic metabolites in rodent lung and nose are also present in humans. Thus, a

mutational mechanism is possibly the means by which cumene could produce lung or nasal tumours in both rodents and humans. The data do not support a mechanism that involves α 2u-globulin in the development of tumours of the kidney.

5. Summary of Data Reported

5.1 Exposure data

Cumene is produced from the distillation of coal tar and petroleum fractions or by the alkylation of benzene with propene using an acidic catalyst. It is used almost exclusively to produce phenol and acetone. Cumene occurs naturally in crude oil, and is found in the environment in plants and foodstuff.

Cumene is primarily released into the environment during its production and use, and from emissions from gasoline engines. It can also be released during the transportation and distribution of fossil fuels or accidental spills of fuel. Cumene has also been detected in cigarette smoke. The major source of exposure of the general public is through inhalation of contaminated air. Occupational exposure, primarily via inhalation, occurs during its production and use, or the use of products that contain cumene. Cumene is typically produced under closed conditions and most reported levels of occupational exposure are low.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

Exposure of male and female mice and rats to cumene by whole-body inhalation increased the incidence of tumours of the respiratory tract in

rats (nasal adenoma in males and females) and mice (alveolar/bronchiolar adenoma and carcinoma in males and females), of the kidney (renal tubule adenoma and carcinoma) in male rats, of the spleen (haemangiosarcoma) in male mice and of the liver (hepatocellular adenoma) in female mice. Exposure by inhalation to α -methylstyrene, a probable major metabolite of cumene, resulted in an increased incidence of hepatocellular adenoma or carcinoma (combined) in male mice, hepatocellular adenoma, carcinoma and adenoma or carcinoma (combined) in female mice and renal tubule adenoma or carcinoma (combined) in male rats.

5.4 Other relevant data

No data on the toxicokinetics of cumene in humans were available. In rats and mice exposed to radiolabelled cumene, more than a dozen metabolites are formed; 2-phenyl-2-propanol glucuronide is the major urinary metabolite.

Cumene itself is generally not mutagenic, but its metabolite, α -methylstyrene oxide, is mutagenic in bacteria. Intraperitoneal injection of cumene induced micronuclei in the bone marrow of male rats, but no micronuclei were observed in erythrocytes in the peripheral blood of mice exposed by inhalation.

At least one mutagenic metabolite of cumene, α -methylstyrene oxide, has been found in rats and mice. The mouse lung tumours induced by cumene had an elevated frequency of mutations in *K-ras* and *p53*, and showed a variety of changes in the expression of genes that are involved in the pathways of carcinogenesis in mice and humans. The enzymes that produce α -methylstyrene oxide in rodents are also present in humans. Thus, there is moderate evidence that a mutational mechanism underlies the development of cumene-induced lung or nasal tumours in rodents and possibly in humans.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

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

There is *sufficient evidence* in experimental animals for the carcinogenicity of α -methylstyrene.

6.3 Overall evaluation

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

α -Methylstyrene is *possibly carcinogenic to humans* (Group 2B).

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3-MONOCHLORO-1,2-PROPANEDIOL

There appears to be no general consensus on a common trivial name for this agent: α -chlorohydrin and 3-monochloro-1,2-propanediol are equally used; however, a preference for 3-monochloro-1,2-propanediol, and especially the abbreviation 3-MCPD, was noted in the more recent literature.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

From [Merck Index \(2010\)](#) and [SciFinder \(2010\)](#)

Chem. Abstr. Serv. Reg. No.: 96-24-2

Chem. Abstr. Name:

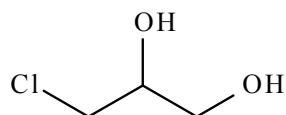
3-Chloro-1,2-propanediol

IUPAC Systematic Name:

3-Chloropropane-1,2-diol

Synonyms: Chlorodeoxyglycerol; 1-chloro-2,3-dihydroxypropane; 3-chloro-1,2-dihydroxypropane; α -chlorohydrin; chloropropanediol; 1-chloropropane-2,3-diol; 3-chloropropanediol; 3-chloro-propylene glycol; 3-chloro-1,2-propylene glycol; 1,2-dihydroxy-3-chloropropane; 2,3-dihydroxypropyl chloride; glyceryl chloride; glycerol chlorohydrin; glycerol 3-chlorohydrin; glyceryl α -chlorohydrin; 3-MCPD; 3-monochloropropane-1,2-diol
EPA Chemical Code: 117101
EINECS No.: 202-492-4

1.1.2 Structural and molecular formulae and relative molecular mass



$C_3H_7ClO_2$

Relative molecular mass: 110.54

1.1.3 Chemical and physical properties of the pure substance

From [Liu et al. \(2005\)](#), [Beilstein \(2010\)](#), [Merck Index \(2010\)](#), and [SciFinder \(2010\)](#)

Description: Liquid with a pleasant odour, and a tendency to turn to a straw colour

Boiling-point: 114–120 °C

Melting-point: Decomposes at 213 °C

Density: $d_4 = 1.3218$ at 20 °C

Refractive index: $n_D = 1.4831$ at 20 °C

Solubility: Soluble in water, alcohol, diethyl ether and acetone

Vapour pressure: 0.195–5.445 mmHg at 50–100 °C

1.1.4 Technical products and impurities

The purity of 3-monochloro-1,2-propanediol (3-MCPD) has been discussed ([Jones & Cooper, 1999](#)). The commercial product, which is racemic (*R,S*)-3-MCPD was shown to contain various impurities such as hydrochloric acid, glycerol, chlorinated acetic acids and chlorinated dioxolanes, indicating that many studies in the past may have been confounded by the impurities.

1.1.5 Analysis

Most methods for the analysis of 3-MCPD focus on the trace analysis at microgram per kilogram levels in various food matrices, which is relatively complicated ([Wenzl *et al.*, 2007](#)). The three main physical characteristics that contribute to these complications have been attributed to the absence of a suitable chromophore, a high boiling-point and a low molecular weight ([Hamlet *et al.*, 2002a](#)). Initial methods that were developed for the determination of chloropropanols without derivatization showed low sensitivity ([Table 1.1](#)). Because of the absence of a chromophore, approaches based on high-performance liquid chromatography with ultraviolet or fluorescence detection cannot be applied, and only one such method with refractive index detection that has been used to study the kinetics of 3-MCPD formation in model systems appears to be unsuitable to determine trace quantities of the compound in food matrices ([Hamlet & Sadd, 2002](#)).

Direct analysis by gas chromatography (GC) without derivatization is also restricted. The low volatility and high polarity of 3-MCPD give rise to unfavourable interactions with components of the GC system that result in poor peak shape and low sensitivity. For example, during GC, 3-MCPD can react with other components of the sample to form hydrochloric acid in the presence of water, and with active sites in the column and non-volatile residues in the column inlet

([Kissa, 1992](#)). Interferences may also arise from the reaction of 3-MCPD with ketones contained in the matrix to form ketals ([Kissa, 1992](#)). Peak broadening and ghost peaks were observed with GC-based methods for the analysis of underivatized 3-MCPD ([Rodman & Ross, 1986](#)).

The low molecular weight of 3-MCPD aggravates detection by mass spectrometry (MS) because diagnostic ions cannot be distinguished reliably from background chemical noise. Due to these apparent limitations, the methods based on direct GC (e.g. [Wittmann, 1991](#); [Spyres, 1993](#)) are more or less obsolete, and, because of their high limits of detection, are unsuitable to control maximum levels of 3-MCPD (see Section 1.4).

[Xing & Cao \(2007\)](#) developed a simple and rapid method applied capillary electrophoresis with electrochemical detection. However, its sensitivity appears to be insufficient to determine contents in the lower microgram per kilogram range found in foods.

None of the methods that use underivatized analytes is of sufficient sensitivity or selectivity to determine low microgram per kilogram levels in foodstuffs, nor is derivatization using silylation with bis(trimethylsilyl)trifluoroacetamide ([Kissa, 1992](#); [Bodén *et al.*, 1997](#)), the detection limits of which were above 0.02 mg/kg using MS.

In combination with GC-MS, the three most common derivatives that give adequate sensitivity and selectivity are: (1) cyclic derivatives from the reaction with *n*-butylboronic acid or phenylboronic acid (PBA) ([Rodman & Ross, 1986](#); [Pesselman & Feit, 1988](#)); (2) heptafluorobutyrate derivatives from heptafluorobutyrylimidazole (HFBI) or heptafluorobutyric anhydride ([van Bergen *et al.*, 1992](#); [Hamlet, 1998](#); [Chung *et al.*, 2002](#)); and (3) cyclic ketal derivatives from ketones ([Meierhans *et al.*, 1998](#); [Dayrit & Niñonuevo, 2004](#); [Rétho & Blanchard, 2005](#)). These methods are summarized in [Table 1.1](#). For further details on the derivatization of 3-MCPD, see [Wenzl *et al.* \(2007\)](#).

Table 1.1 Selected methods for the analysis of 3-monochloro-1,2-propanediol (MCPD) in various matrices

Matrix	Analytes	Pre-treatment	Clean up	Derivatization	Detection	LOD for 3-MCPD (µg/kg)	Reference
Seasonings	2-MCPD, 3-MCPD, 1,3-DCP, 2,3-DCP	Water, pH adjustment	Extrelut	None	GC-MS SIM	100	Wittmann (1991)
HVP	3-MCPD	20% NaCl solution	Extrelut	None	GC-ECD	250	Spyres (1993)
Cereal products	MCPD esters	Ethyl acetate extraction	Preparative TLC	None	GC-MS Scan	-	Hamlet & Sadd (2004)
Model systems	3-MCPD	-	-	None	HPLC-RI	-	Hamlet & Sadd (2002)
Solvents	3-MCPD	-	-	BSTFA	GC-FID	5000	Kissa (1992)
Paper	3-MCPD, 1,3-DCP	Acetonitrile extraction	-	BSTFA	GC-MS SIM	40	Bodén et al. (1997)
Soya sauce	3-MCPD	Dilution with buffer	-	None	CE-ECD	130	Xing & Cao (2007)
Standards	3-MCPD	-	-	PBA	GC-MI-FTIR	-	Rodman & Ross (1986)
Aqueous solutions	3-MCPD	-	-	BBA	GC-ECD	100	Pesselman & Feit (1988)
HVP	3-MCPD	20% NaCl solution	-	PBA	GC-FID	500–1000	Plantinga et al. (1991) , Anon (1995)
Various foods	3-MCPD	20% NaCl solution	-	PBA	GC-MS SIM	3–10	Breitling-Utzmann et al. (2003)
Various foods	3-MCPD	20% NaCl solution	-	PBA	GC-MS/MS MRM (triple quadruple)	5	Kuballa & Ruge (2003)
Various foods	Free and bound 3-MCPD	Fat extraction, interesterification	-	PBA	GC-MS SIM	3	Divinová et al. (2004)
HVP, soya sauce	3-MCPD	Dilution 1:10	HS-SPME	PBA	GC-MS SIM	3.87	Huang et al. (2005)
HVP	2-MCPD, 3-MCPD, 1,3-DCP, 2,3-DCP	5M NaCl solution	Extrelut, two-stage extraction	HFBI	GC-ECD, GC-MS	50–100	van Bergen et al. (1992)
HVP, seasonings	3-MCPD, 2-MCPD	5M NaCl solution	Extrelut	HFBI	GC-MS/MS MRM (ion trap)	5	Hamlet & Sutton (1997)

Table 1.1 (continued)

Matrix	Analytes	Pre-treatment	Clean up	Derivatization	Detection	LOD for 3-MCPD (µg/kg)	Reference
Various foods	3-MCPD, 2-MCPD	5M NaCl solution	Extrelut	HFBI	GC-MS SIM or GC-MS/MS MRM (ion trap)	5–10	Hamlet (1998) , Brereton et al. (2001)
Water	3-MCPD, 1,3-DCP (& bromo-propanediols)	Ethyl acetate extraction	-	HFBA	GC-ECD	0.7	Matthew & Anastasio (2000)
Soya sauce	1,3-DCP, 3-MCPD	5M NaCl solution	Silica gel (60 mesh)	HFBA	GC-MS SIM	5	Chung et al. (2002)
Cereal products	Free and bound 3-MCPD	Enzyme hydrolysis (lipase)	Extrelut	HFBI	GC-MS	-	Hamlet & Sadd (2004)
Model systems	3-MCPD, 2-MCPD	Hexane extraction	ASE	HFBI	GC-MS	5	Bel-Rhliid et al. (2004) , Robert et al. (2004)
Soy sauce, flavouring	2-MCPD, 3-MCPD, 1,3-DCP, 2,3-DCP	5M NaCl solution	Extrelut	HFBA-Et ₃ N	GC-MS EI SIM or NCI SIM	3 (EI), 0.6 (NCI)	Xu et al. (2006)
Various foods	1,3-DCP, 3-MCPD	Saturated NaCl solution	Aluminium oxide	HFBA	GC-MS SIM	1	Abu-El-Haj et al. (2007)
Various foods	3-MCPD, 2-MCPD	Saturated NaCl solution	Extrelut	Acetone	GC-MS SIM	10	Meierhans et al. (1998)
Soya sauce	3-MCPD	Saturated NaCl solution	Extrelut	4-Heptanone	GC-MS Scan	1.2	Dayrit & Niñonuevo (2004)
Various foods	3-MCPD	Pure water extraction	Extrelut	Acetone, filtration over aluminium oxide	GC-MS SIM	2–5	Rétho & Blanchard (2005)
Blood, urine	3-MCPD	Dilution, acidification, (enzymatic pretreatment)	Silica gel (60 mesh)	HFBA	GC-MS NCI SIM	2	Berger-Preiss et al. (2010)
Various foods	3-MCPD and 3-MCPD esters	20% NaCl solution	LLE with MTBE	PBA	GC-MS SIM	1–6	Küsters et al. (2010)

Table 1.1 (continued)

Matrix	Analytes	Pre-treatment	Clean up	Derivatization	Detection	LOD for 3-MCPD (µg/kg)	Reference
Seasoning	3-MCPD, 1,3-DCP, 2,3-DCP	No data	No data	TSIM	GC-MS SIM	0.14	Cao et al. (2009)
Oils	3-MCPD after cleavage of MCPD esters	Cleavage with sodium methoxide	Different LLE steps	PBA	GC-MS SIM	50–150	Weihaar (2008)
Soya sauce	1,3-DCP, 3-MCPD	NaCl addition	HS-SPME	MSTFA	GC-MS SIM	4.62	Lee et al. (2007)
Oils	Bound 3-MCPD	Alkaline release	LLE	PBA	GC-MS SIM	50	Kuhlmann (2010)

BBA, *n*-butylboronic acid; BSTFA, bis(trimethylsilyl)trifluoroacetamide; CE-ECD, capillary electrophoresis with electrochemical detection; DCP, dichloropropanol; EI, electron-impact ionization; Et₃N, triethylamine; GC-ECD, gas chromatography with electron capture detection; GC-FID, gas chromatography with flame ionization detection; GC-MI-FTIR, gas chromatography-matrix isolation-Fourier transform infrared spectroscopy; GC-MS, gas chromatography-mass spectrometry; GC-MS/MS, gas chromatography-tandem mass spectrometry; HFBA, heptafluorobutyric anhydride; HFBI, heptafluorobutyrylimidazole; HPLC-RI, high-performance liquid chromatography with refractive index detection; HS-SPME, headspace solid-phase microextraction; HVP, acid-hydrolysed vegetable protein; LLE, liquid liquid extraction; LOD, limit of detection; MCPD, monochloro-1,2-propanediol; MRM, multiple reaction monitoring; MS, mass spectrometry; MSTFA, *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide; MTBE, methyl *tert*-butyl ether; NaCl, sodium chloride; NCI, negative chemical ionization; PBA, phenylboronic acid; SIM, selected ion monitoring; TLC, thin-layer chromatography; TSIM, 1-trimethylsilylimidazole
 Updated from [Wenzl et al. \(2007\)](#)

Of the different procedures, the PBA derivatization method is the most common. For example, it is used as a German reference method for food ([Anon., 1995](#)). An advantage of PBA derivatization is that no sample clean-up is required because PBA reacts specifically with diols to form non-polar cyclic derivatives that are extractable into *n*-hexane. The disadvantage of PBA is that other chloropropanols, such as 1,3-dichloro-2-propanol (1,3-DCP) cannot be determined simultaneously using this method. Sensitivity can be further improved by the application of triple quadruple MS/MS ([Kuballa & Ruge, 2003](#)). Sample preparation may possibly be improved by headspace solid-phase microextraction ([Huang *et al.*, 2005](#)).

HFBI/heptafluorobutyric anhydride derivatization is also very commonly applied, although it is less selective than that with boronic acids. The procedure has been validated by a collaborative trial ([Brereton *et al.*, 2001](#)). Repeatability ranged from 0.005 to 0.013 mg/kg and reproducibility from 0.010 to 0.027 mg/kg. The validation of the method was judged to be satisfactory and the method was adopted by the Association of Official Analytical Chemists International as an official method ([Brereton *et al.*, 2001](#)). The method was also adopted as European norm EN 14573 ([European Standard, 2005](#)). The HFBI method was found to be more labour-intensive than the PBA method but has the advantage of analysing both 1,3-DCP and 3-MCPD simultaneously during the same GC-MS run. The procedure can also be used with little modification to analyse blood and urine samples of rats in the context of toxicological studies ([Berger-Preiss *et al.*, 2010](#)).

Currently, only a few methods exist to analyse so-called 'bound' 3-MCPD, which is a 3-MCPD ester bound with fatty acids. Unhydrolysed MCPD esters can be analysed directly by extraction into an organic solvent, clean-up by a preparative thin-layer chromatography ([Davídek *et al.*, 1980](#)) and analysis using GC-MS ([Hamlet &](#)

[Sadd, 2004](#)). More commonly, bound 3-MCPD is released from the esters and analysed in free form, through enzyme hydrolysis with a commercial lipase from *Aspergillus* ([Hamlet & Sadd, 2004](#)), interesterification of the sample with sulfuric acid ([Divinová *et al.*, 2004](#)), or cleavage with sodium methoxide ([Weißhaar, 2008](#)) or methanolic sodium hydroxide ([Kuhlmann, 2010](#)).

1.2 Production and use

1.2.1 Production

Chlorohydrins are readily prepared by the reaction of an alkene with chlorine and water ([Richey, 2000](#)). The reaction of allyl alcohol with chlorine and water at 50–60 °C gives a yield of 88% monochlorohydrins and 9% dichlorohydrins ([Liu *et al.*, 2005](#)). A 85–88% yield was reported by the reaction of glycerol with aqueous hydrochloric acid in the presence of a catalytic quantity of acetic acid ([Richey, 2000](#)). An anhydrous procedure that involves the reaction of glycerol and hydrogen chloride gas in the presence of acetic acid has also been described ([Richey, 2000](#)).

3-MCPD is listed in the CHEMCATS database ([SciFinder, 2010](#)) as being available at 96 suppliers worldwide in amounts up to bulk quantities. The commercial market for chlorohydrins has been described as small ([Richey, 2000](#)). 3-MCPD was available from at least three manufacturers in the USA in steel drums (227.3–240 kg net) ([Richey, 2000](#)).

1.2.2 Use

According to the [Merck Index \(2010\)](#), 3-MCPD has been used to lower the freezing-point of dynamite and in the manufacture of dye intermediates. 3-MCPD is one of the few chemosterilants that has been commercialized for rodent control ([Ericsson, 1982](#); [Buckle, 2005](#); [EPA, 2006](#)). It has been used at a dose of 90–100 mg/kg body

weight (bw) to sterilize male Norway rats, and is available as a 1% ready-to-use bait and as a 20% concentrate (trade names: Epibloc, Gametrics). It has been reported that chemosterilants are not widely used in pest control because their effects are often transient and the presence of rodents — sterile or otherwise — is considered to be undesirable ([Buckle, 2005](#)).

3-MCPD may be used as a raw material for the synthesis of guaifenesin, a secretomotoric drug ([Yale et al., 1950](#); [Bub & Friedrich, 2005](#)), and for the synthesis of an intermediate in the production of the statin drug, atorvastatin ([Kleemann, 2008](#)). It was also used in the final step of the synthesis of iohexol, a contrast medium for angiography and urography ([Lin, 2000](#)).

1.3 Occurrence

1.3.1 Natural occurrence

3-MCPD is not known to occur as a natural product.

1.3.2 Occupational exposure

Individuals who are potentially exposed to 3-MCPD include production workers and users of chemosterilants for rodent control. The [EPA \(2006\)](#) considered that the risk of occupational exposure from its use as a rodenticide was unlikely, because the end-use product is packaged in poly-paper sachets, which are placed intact into tamper-resistant (closed loading) systems.

1.3.3 Occurrence in food

Chloropropanols are foodborne contaminants that can be formed during the processing of various foodstuffs ([Wenzl et al., 2007](#)). This class of food contaminant was first recognized at the Institute of Chemical Technology in Prague ([Velíšek et al., 1978](#)) in acid-hydrolysed vegetable protein (HVP), a seasoning ingredient that is

widely used in a variety of processed and prepared foods such as soups, sauces, bouillon cubes and soya sauce ([Calta et al., 2004](#)). 3-MCPD is the most abundant chloropropanol found in foodstuff, while 1,3-DCP generally occurs at lower levels (see the *IARC Monograph* on 1,3-dichloro-2-propanol in this volume). Their isomers — 2-MCPD and 2,3-DCP — are usually found at much lower concentrations ([Wenzl et al., 2007](#)).

During the last decade, renewed interest in chloropropanols and the development of analytical methods of their presence in food matrices other than acid-HVP was triggered by the detection of 3-MCPD in a wide range of foods and food ingredients, notably in thermally processed foods such as malts, cereal products and meat ([Brereton et al., 2001](#); [Hamlet et al., 2002a, b](#); [Breitling-Utzmann et al., 2003](#)). In addition, domestic processing (e.g. grilling and toasting) can produce substantial increases in the 3-MCPD content of bread or cheese ([Crews et al., 2001](#); [Breitling-Utzmann et al., 2003](#)).

Several studies on the mechanism of 3-MCPD formation have been performed ([Hamlet & Sadd, 2002](#); [Hamlet et al., 2003](#); [Velíšek et al., 2003](#); [Calta et al., 2004](#); [Doležal et al., 2004](#); [Hamlet et al., 2004a, b](#); [Robert et al., 2004](#); [Breitling-Utzmann et al., 2005](#); [Hamlet & Sadd, 2005](#); [Muller et al., 2005](#)), and showed that it is formed from glycerol or acylglycerols and chloride ions in heat-processed foodstuffs that contain fat with low water activity. Although the overall levels of 3-MCPD in bakery products are relatively low, the high level of consumption of bread, and its additional formation from toasting, indicate that this staple food alone can be a significant dietary source of 3-MCPD ([Breitling-Utzmann et al., 2003](#)). In malt products, 3-MCPD was only found in coloured malts and at highest levels in the most intensely coloured samples. Additional heat treatments, including kilning or roasting, were judged to be a significant factor in the formation of 3-MCPD in these ingredients ([Hamlet et al., 2002b](#); [Muller et al., 2005](#)). The occurrence

of 3-MCPD in beer, which is generally less than 10 µg/L, was reviewed recently (IARC, 2010).

Concentrations of 3-MCPD above 0.02 mg/kg were recently found in smoked fermented sausages and smoked ham (Kuntzer & Weißhaar, 2006; Jira, 2010), and the smoking process was identified as a major source. In contrast to the formation of 3-MCPD in acid-HVP, soya sauce and bakery products, lipids are not precursors of 3-MCPD in smoked foods. A hypothetical mechanism, in which 3-hydroxyacetone is the precursor, was suggested for the formation of 3-MCPD in wood smoke (Kuntzer & Weißhaar, 2006). Further details on the mechanisms of formation are available in several recent reviews (Hamlet, 2009; Hamlet & Sadd, 2009; Velíšek, 2009).

Data from a large international survey with over 5000 analytical results on the occurrence of 3-MCPD in food have been published (JECFA, 2007), and are summarized in Table 1.2. The average concentration of 3-MCPD in soya sauce and soya sauce-related products was much higher (average, 8 mg/kg) than that in any other food or food ingredient (average, < 0.3 mg/kg). Data from Japan show that soya sauce produced by traditional fermentation contains insignificant average amounts of 3-MCPD (0.003 mg/kg) compared with soya sauce made with acid-HVP (1.8 mg/kg) (JECFA, 2007). Estimated average dietary exposures of the general population from a wide range of foods, including soya sauce and soya sauce-related products, ranged from 0.02 to 0.7 µg/kg bw per day, and those for consumers at the high percentile (95th), including young children, ranged from 0.06 to 2.3 µg/kg bw per day. The exposures were calculated by linking data on individual consumption and body weight from national food consumption surveys with data on mean occurrence obtained from food contamination surveys (JECFA, 2007).

Other exposure estimates have been published since that time. For secondary school students in China, Hong Kong Special Administrative Region, the average exposure

was estimated to be 0.063–0.150 µg/kg bw per day, while that for high consumers was 0.152–0.300 µg/kg bw per day (Yau *et al.*, 2008).

In the Republic of Korea, the mean intake level of 3-MCPD was estimated to range from 0.0009 to 0.0026 µg/kg bw per day and that at the 95th percentile of consumption was 0.005 µg/kg bw per day (Hwang *et al.*, 2009). [The Working Group noted that the exposure estimate of Hwang *et al.* (2009) only included soya sauce and did not consider total food consumption.]

Since the implementation of limits on permissible concentrations (see Section 1.4), actions by industry have reduced the level of contamination by chloropropanols of acid-HVP prepared in Europe (Crews *et al.*, 2002). A recent survey confirmed that the limit was still exceeded in only single samples of soya sauce on the European market (Schlee *et al.*, 2011).

In foodstuffs, 3-MCPD occurs, not only in its free form, but also as esters with higher fatty acids (so-called bound 3-MCPD) (Table 1.3). 3-MCPD esters were found in goats' milk and human milk (Zelinková *et al.*, 2008; Rahn & Yaylayan, 2010). During food processing (especially during oil refining), the formation of process-induced 3-MCPD esters may occur and various mechanisms are currently under investigation that most probably involve a nucleophilic attack by chloride ions (Rahn & Yaylayan, 2010). Evidence has been found that the content of bound 3-MCPD exceeded that of free 3-MCPD by at least five- and up to 396-fold (Svejkovská *et al.*, 2004). Hamlet & Sadd (2004) found MCPD esters in baked cereal products and showed that they might be generated as stable intermediates or by-products of the formation reaction from mono- and diacylglycerol precursors. The esters were also found in food groups that did not contain free 3-MCPD (e.g. coffee creamers, cream aerosols, bouillon cubes; Karšulínová *et al.*, 2007). In refined fats and oils, the highest concentrations were detected in palm oil and palm oil-based fats (Weißhaar & Perz, 2010). This is consistent with findings

Table 1.2 Summary of the distribution-weighted concentration of 3-monochloro-1,2-propanediol in soya sauce and soya sauce-based products, in other foods and in food ingredients from various countries, 2001–06^a

Product	LOQ (mg/kg)	No.	<i>n</i> < LOQ	Mean ^b (mg/kg)	Maximum (mg/kg)
Soya sauce and soya sauce-based products	0.006–5.000	2629	1433	8.39	1779
Dairy products (cheeses)	0.005–0.010	149	138	0.007	0.095
Fat, oils and fat emulsions	0.005–0.010	38	24	0.081	1.5
Nuts, seeds and processed vegetables	0.01	37	14	0.061	0.69
Cereals and cereal products (flours, starch, pasta, noodles and bakery products)	0.005–0.020	577	348	0.023	0.945
Meat and meat products	0.005–0.010	251	170	0.027	0.41
Fish products	0.005–0.010	89	68	0.012	0.191
Salts, spices, soup sauces, salads and protein products	0.01–2.5	454	248	0.286	50.7
Foodstuffs intended for particular nutritional uses	0.005–0.080	137	128	0.007	0.02
Ready-to-eat savouries	0.006–0.020	23	15	0.01	0.041
Composite food	0.01	24	19	0.013	0.113
Coffee, roasted	0.005–0.010	23	23	0.005	0.005
Cocoa paste and chocolate products	0.005–0.080	15	13	0.007	0.005
Beer and malt beverages	0.005–0.080	138	128	0.008	0.03
Confectionery, sugar-based (chewing gum, candy and nougats)	0.01	27	24	0.007	0.023
Food ingredients (including acid-hydrolysed vegetable proteins, meat extracts, malts, modified starches and seasonings)	0.01–1.15	489	262	0.099	2.5

^a Includes data of surveys before intervention to reduce occurrence had been undertaken by government or industry

^b Data below the limit of detection or LOQ have been assumed to be half of those limits and the mean was weighted according to the number of samples per country

LOQ, limit of quantification

Data summarized from [JECFA \(2007\)](#)

Table 1.3 Summary of the concentrations of 3-monochloro-1,2-propanediol esters in foodstuffs quantified as 3-monochloro-1,2-propanediol^a

Product	No.	Mean (mg/kg)	Maximum (mg/kg)	Reference
Bread (toast)	7	0.086	0.16	Hamlet & Sadd (2004)
Coffee	15	0.14	0.39	Doležal et al. (2005)
Oils				
Virgin seed oils	9	0.063	< 0.3	Zelinková et al. (2006)
Virgin olive oils	4	0.075	< 0.3	Zelinková et al. (2006)
Virgin germ oils	2	0.1	< 0.3	Zelinková et al. (2006)
Refined seed oils	5	0.524	1.234	Zelinková et al. (2006)
Refined olive oils	5	1.426	2.462	Zelinková et al. (2006)
Vegetable oil–fat mixes	11	1.534	2.435	Seefelder et al. (2008)
Refined palm oil and palm oil-based fats	12	3.24	5.8	Weißhaar & Perz (2010)
Other refined vegetable oils	57	0.4–1.7 ^b	(no data)	Weißhaar & Perz (2010)
Infant and baby foods	14	0.289	0.588	Zelinková et al. (2009b)
Human breast milk	12	0.036	0.076	Zelinková et al. (2008)
Infant formula and follow-up formula	10	2.568	4.169	BfR (2007)

^a Includes data of surveys before intervention to reduce occurrence had been undertaken by government or industry

^b Range

Data updated from [Hamlet & Sadd \(2009\)](#)

that frying oil is the major source of 3-MCPD fatty acid esters in potato products (French fries and chips) ([Hamlet, 2009](#); [Hamlet & Sadd, 2009](#); [Zelinková et al., 2009a](#)).

These esters should also be treated as food contaminants because 3-MCPD may be released *in vivo* by a lipase-catalysed hydrolysis reaction ([Wenzl et al., 2007](#)). It was assumed that 3-MCPD esters behave similarly to triacylglycerols and undergo similar metabolism and digestion, which could either lead to the release of free 3-MCPD or to its incorporation into lipoprotein particles, depending on the positioning of the 3-MCPD fatty acid ester group on the glycerol backbone ([Schilter et al., 2010](#)). Recent *in-vitro* studies confirmed that 3-MCPD fatty acid esters are probably hydrolysed in the human intestine followed by rapid resorption of free 3-MCPD ([Buhrke et al., 2010](#)). After 4 hours of incubation with small intestine juice containing pancreatic lipase, the release of free 3-MCPD ranged between 25 and 50% from palm oil, and ≈82%

was released from toasted bread ([Schilter et al., 2010](#)). These results indicated that 3-MCPD fatty acid esters substantially increase the amount of 3-MCPD ingested from food ([Buhrke et al., 2010](#)).

[The Working Group noted that, in light of the recent evidence that 3-MCPD occurs in a bound form as 3-MCPD esters, it can be assumed that all of the exposure values mentioned above are probably underestimates.]

1.3.4 Environmental occurrence

3-MCPD can occur as a contaminant in drinking-water from water purification plants that use epichlorohydrin-linked cationic polymer resins or in wastewaters ([Nienow et al., 2009](#)).

1.4 Regulations and guidelines

Regulations and guidelines on permissible concentrations of 3-MCPD in foodstuffs are summarized in [Table 1.4](#). The Scientific Committee on Food of the European

Table 1.4 International maximum concentration limits/specifications for 3-monochloro-1,2-propanediol in foodstuffs

Country/Region	3-MCPD (mg/kg)	Scope
Australia/New Zealand	0.2	Soya/oyster sauces
Canada	1	Soya/oyster sauces
the People's Republic of China	1	Acid-HVP
European Union	0.02	HVP and soya sauces (40% solids)
Republic of Korea	0.3	Soya sauce containing acid-HVP
	1	HVP
Malaysia	0.02	Liquid foods with acid-HVP
	1	Acid-HVP, industrial product
Switzerland	0.2	Savoury sauces
Thailand	1	Hydrolysed soya bean protein
USA	1	Acid-HVP

HVP, hydrolysed vegetable protein; 3-MCPD, 3-monochloro-1,2-propanediol
Adapted from [Hamlet & Sadd \(2009\)](#)

Commission considered that a threshold-based approach for deriving a tolerable daily intake would be appropriate, and determined a value of 2 µg/kg bw ([SCF, 2001](#)). This value was confirmed as a provisional maximum tolerable daily intake ([JECFA, 2007](#)). The European Commission has set a regulatory limit of 0.02 mg/kg for 3-MCPD in HVP and soya sauce ([European Commission, 2001](#)).

2. Cancer in Humans

No data were available to the Working Group.

3. Studies in Experimental Animals

3.1 Oral administration

3.1.1 Mouse

See [Table 3.1](#)

Four groups of 50 male and 50 female B6C3F₁ mice received 0 (control), 30, 100 or 300 ppm 3-MCPD in the drinking-water up to day 100 and 0 or 200 ppm thereafter until 104 weeks (0, 4, 2,

14.3 or 33.0 and 0, 3.7, 12.2 or 31.0 mg/kg bw in males and females, respectively). No neoplasms attributable to treatment with 3-MCPD were observed ([Jeong *et al.*, 2010](#)).

3.1.2 Rat

See [Table 3.2](#)

Groups of 26 male and 26 female Charles River Sprague-Dawley (CD) rats were administered 30 mg/kg bw or 60 mg/kg bw (maximum tolerated dose) 3-MCPD by gavage twice a week. Groups of 20 males and 20 females served as untreated controls. After 10 weeks, the doses were increased to 35 and 70 mg/kg bw, respectively, and treatment was continued until week 72. The study was terminated after 2 years. Three parathyroid adenomas were found in high-dose males, but this finding was not statistically significant compared with the control group. While females showed no signs of toxicity, male rats had a higher mortality rate than controls ([Weisburger *et al.*, 1981](#)).

Groups of 50 male and 50 female Sprague-Dawley rats received drinking-water containing 0, 25, 100 or 400 ppm 3-MCPD for 100 and 104 weeks, respectively. The incidence of renal tubule

Table 3.1 Carcinogenicity studies of 3-monochloro-1,2-propanediol in mice

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance
B6C3F ₁ (M, F) 104 wk Jeong <i>et al.</i> (2010)	Drinking-water 0 (control), 30, 100 and 300 µg/mL until d 100 followed by 200 µg/mL until termination of the experiment 50/group	Liver (hepatocellular carcinoma): M–13/50, 7/49, 4/49, 13/49; F–0/48, 1/50, 3/50, 1/50 Lymphoma (all): M–8/50, 6/50, 6/50, 7/50; F–12/50, 10/50, 17/50, 8/50 Lung (bronchioalveolar adenoma): M–5/50, 5/50, 5/50, 2/50; F–1/48, 3/50, 3/50, 1/50 Lung (bronchioalveolar carcinoma): M–8/50, 2/50, 1/50, 4/50; F–1/48, 3/50, 2/50, 0/50 Kidney (renal tubule adenoma): M–1/50, 1/50, 0/48, 0/49; F–1/45, 0/46, 0/47, 0/47 Kidney (renal tubule adenocarcinoma): M–0/50, 1/50, 0/48, 0/49; F–0/45, 0/46, 0/47, 0/47	NS
ICR/Ha Swiss (F) 580 d Van Duuren <i>et al.</i> (1974)	Subcutaneous injection in the left flank of 0 (control) or 1.0 mg in 0.05 mL tricapyrin once/wk 50/group	Skin (sarcoma): 1/50, 1/50 Skin (squamous-cell carcinoma): 0/50, 0/50 Skin (adenocarcinoma): 0/50, 0/50	NS
ICR/Ha Swiss (F) 580 d Van Duuren <i>et al.</i> (1974)	Dermal application to the interscapular region of 0 (control) or 2.0 mg in 0.1 mL acetone 3 × /wk 50/group	Skin (papilloma): 0/50, 0/50 Skin (carcinoma): 0/50, 0/50	NS

d, day or days; F, female; M, male; NS, not significant; wk, week or weeks

Table 3.2 Carcinogenicity studies of 3-monochloro-1,2-propanediol in rats

Strain (sex) Duration Reference	Dosing regimen Animals/group at start	Tumour incidence	Significance	Comments
CD (M, F) 104 wk Weisburger et al. (1981)	Gavage, twice/wk Group 1: control; Group 2: 30 mg/kg bw for 10 wk, followed by 35 mg/kg bw for 62 wk; Group 3: 60 mg/kg bw for 10 wk, followed by 70 mg/kg bw for 62 wk 20–26/group	Parathyroid (adenoma): M–0/20, 0/26, 3/26; F–0/20, 0/26, 0/26	NS	
Sprague-Dawley (M, F) 100–104 wk Cho et al. (2008)	Drinking-water 0, 25, 100 or 400 ppm 50/group	Kidney (renal tubule adenoma): M–0/50, 0/50, 1/50, 4/50; F–0/50, 0/50, 1/50, 6/50* Kidney (renal tubule carcinoma): M–0/50, 0/50, 0/50, 5/50*; F–1/50, 0/50, 1/50, 3/50 Kidney (renal tubule adenoma or carcinoma): M–0/50, 0/50, 1/50, 7/50*; F–1/50, 0/50, 2/50, 9/50* Testis (Leydig-cell adenoma): M–1/50, 1/50, 4/50, 14/50* Pituitary gland (adenoma): M–25/50, 26/50, 24/50, 13/50*	NS * <i>P</i> < 0.05 (poly-3 test) * <i>P</i> < 0.05 (poly-3 test) NS * <i>P</i> < 0.05 (poly-3 test) * <i>P</i> < 0.05 (poly-3 test) * <i>P</i> < 0.05 (decrease, poly-3 test)	Survival: M–28, 34, 18, and 26%; F–30, 44, 22, and 32%

bw, body weight; F, female; M, male; NS, not significant; wk, week or weeks

carcinoma, renal tubule adenoma or carcinoma (combined) and Leydig-cell adenoma showed dose-related positive trends in male rats, and the incidence of renal tubule carcinoma and Leydig-cell adenoma was significantly increased in high-dose males. The incidence of renal tubule adenoma or carcinoma (combined) showed a positive trend in female rats, and the increase was also significant in the high-dose group ([Cho et al., 2008](#)).

[Kidney tumours are spontaneous neoplasms in experimental animals.]

3.2 Subcutaneous administration

3.2.1 Mouse

See [Table 3.1](#)

Groups of 50 female ICR/Ha Swiss mice received weekly subcutaneous injections of 0 (control) or 1.0 mg 3-MCPD in 0.05 mL tricapylin for 580 days. Median survival time was 487 days. At termination of the study, one 3-MCPD-treated and one vehicle-treated mouse had a sarcoma at the site of injection. No other neoplasms were observed ([Van Duuren et al., 1974](#)).

3.3 Dermal application

3.3.1 Mouse

Two groups of 50 female ICR/Ha Swiss mice received topical applications of 0 (control) or 2.0 mg 3-MCPD in 0.1 mL acetone three times a week for up to 580 days. Throughout the duration of the study, no skin tumours were observed ([Van Duuren et al., 1974](#)).

4. Other Relevant Data

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, distribution and excretion

Most studies appear to have been conducted with racemic 3-MCPD, and only limited information is available on the various toxicological properties of the (*R*)- and (*S*)-isomers of 3-MCPD (e.g. see [Jones & Cooper, 1999](#)).

Available data on the absorption, distribution and excretion of 3-MCPD in experimental systems have been reviewed previously ([JECFA, 2002](#)).

3-MCPD crosses the blood–testis barrier and the blood–brain barrier and is widely distributed in the body fluids ([Edwards et al., 1975](#)). [¹⁴C]3-MCPD has been found to accumulate in the cauda epididymis of rats and, to a lesser extent, in that of mice, as observed by autoradiography ([Crabo & Appelgren, 1972](#)). In contrast, no tissue-specific retention of radiolabel was observed in rats given an intraperitoneal injection of 100 mg/kg bw [³⁶Cl]3-MCPD. The 3-MCPD metabolite β -chlorolactate did not accumulate in the tissues either ([Jones et al., 1978](#)).

Male Wistar rats given a single intraperitoneal injection of 100 mg/kg bw [¹⁴C]3-MCPD exhaled 30% of the dose as [¹⁴C]carbon dioxide and excreted 8.5% unchanged in the urine after 24 hours ([Jones, 1975](#)). After a single intraperitoneal injection of 100 mg/kg bw [³⁶Cl]3-MCPD into rats, 23% of the radiolabel was recovered in the urine as β -chlorolactate ([Jones et al., 1978](#)).

(b) Metabolism

Available data on the metabolism of 3-MCPD in experimental systems have been reviewed previously ([IECFA, 2002](#)).

In rats, 3-MCPD may be detoxified by conjugation with glutathione, yielding S-(2,3-dihydroxypropyl)cysteine and the corresponding mercapturic acid, N-acetyl-S-(2,3-dihydroxypropyl)cysteine ([Jones, 1975](#)). The compound also undergoes oxidation to β -chlorolactic acid and then to oxalic acid ([Jones & Murcott, 1976](#)). An intermediate metabolite, β -chlorolactaldehyde, may also be formed, because traces have been found in the urine of rats ([Jones et al., 1978](#)). [The Working Group noted that the intermediate formation of an epoxide has been postulated but not proven ([Jones, 1975](#)).]

There is evidence that microbial enzymes — halohydrin dehalogenases — can dehalogenate haloalcohols to produce glycidol ([van den Wijngaard et al., 1989](#)), which is a direct-acting alkylating agent that is mutagenic in a wide range of in-vivo and in-vitro test system and was evaluated by IARC as *probably carcinogenic to humans* (Group 2A, [IARC, 2000](#)). [The Working Group noted that insufficient information was available to determine which bacteria were used in these studies.]

In a review of the metabolism of 3-MCPD, it was concluded that the main metabolic route in mammals is the formation of β -chlorolactate and oxalic acid, while many bacteria metabolize 3-MCPD primarily via glycidol ([Lynch et al., 1998](#)).

[The Working Group noted the absence of experimental evidence to propose a definite metabolic pathway of 3-MCPD in mammals, and that the formation of glycidol has yet to be established. The Working Group further noted the absence of specific information on the enzymes involved in its metabolism in mammals.]

Proposed metabolic pathways for 3-MCPD are summarized in Fig. 4.1.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group

4.2.2 Experimental systems

Genotoxicity studies of 3-MCPD *in vitro* and *in vivo* have recently been reviewed ([IECFA, 2002](#)), and the data are summarized in [Table 4.1](#).

In vitro, 3-MCPD induced reverse mutation in various strains of *Salmonella typhimurium*, and DNA strand breaks in the Comet assay with Chinese hamster ovary cells.

No effects of 3-MCPD were observed in studies *in vivo* on micronucleus formation in male Han Wistar rat bone-marrow cells and unscheduled DNA synthesis in male Han Wistar rat hepatocytes ([Robjohns et al., 2003](#)).

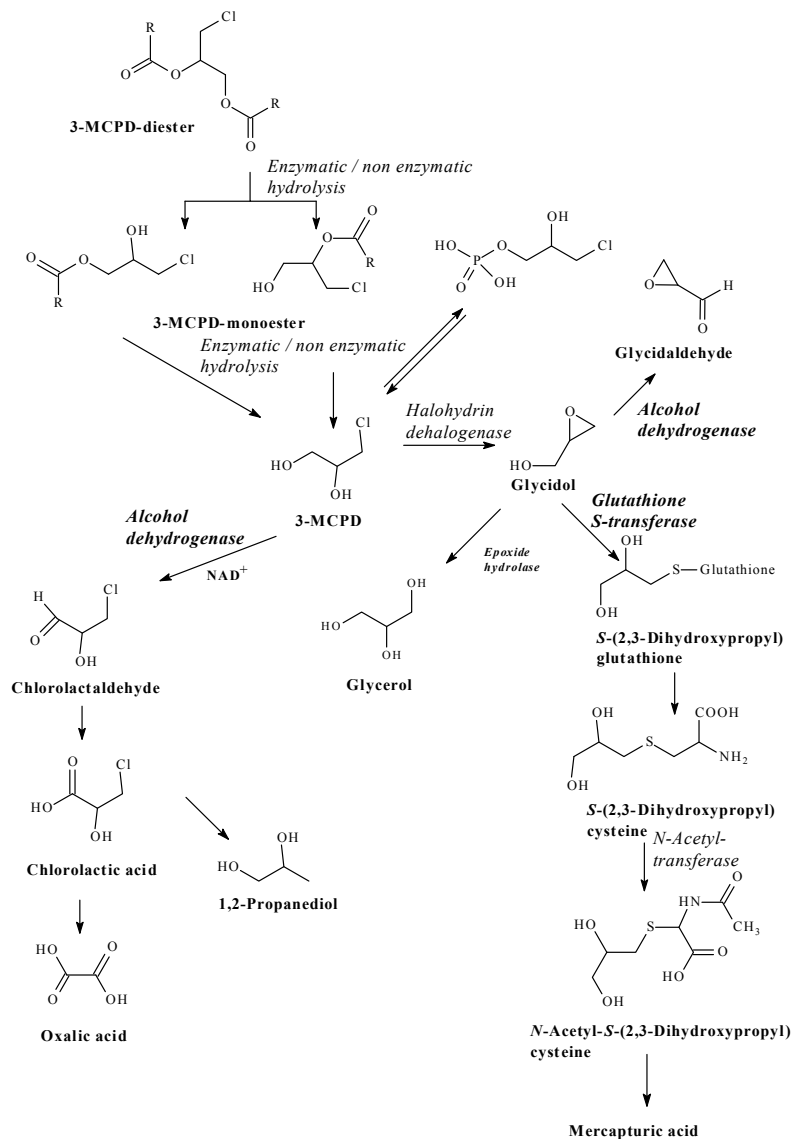
4.3 Mechanistic considerations

4.3.1 Effects on cell physiology and function

Available data on the effects of 3-MCPD on cell function have been reviewed previously ([IECFA, 2002, 2007](#)). The major effects on testicular tissue and kidney are discussed in detail below. Other effects on cell function include immunotoxicity ([Lee et al., 2004, 2005](#); [Byun et al., 2006](#)) and neurotoxicity, which may be mediated, at least in part, through disturbances in the nitric oxide signalling pathway ([Kim, 2008](#)).

In a study in male Crl:HanWistBR rats, a clear reduction in the ratio of polychromatic to normochromatic erythrocytes was observed with the highest dose (60 mg/kg bw per day for 2 days), indicating bone-marrow cytotoxicity and that the substance and/or its metabolites had reached the bone marrow ([Robjohns et al., 2003](#)). This finding is consistent with a study in primates,

Fig. 4.1 Hypothesized metabolic pathways for 3-monochloro-1,2-propanediol based on data from bacterial and putative mammalian pathways



Adapted from Lynch *et al.* (1998), based on data from Jones *et al.* (1978)
3-MCPD; 3-monochloro-1,2-propanediol

Table 4.1 Genetic and related effects of 3-monochloro-1,2-propanediol

Test system	Results		Dose (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Salmonella typhimurium</i> TA100, TA1535, reverse mutation	+	+	40 mg/plate	Stolzenberg & Hine (1979, 1980)
<i>Salmonella typhimurium</i> TA100, reverse mutation	NT	-	NR	Majeska & Matheson (1983)
<i>Salmonella typhimurium</i> TA100, TA1535, reverse mutation	+	+	1.0–3.33 mg/plate	Zeiger et al. (1988)
<i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	0.62 mg/plate	Ohkubo et al. (1995)
<i>Salmonella typhimurium</i> TA1535, reverse mutation	+	+	1 mg/plate	Silhánková et al. (1982)
<i>Salmonella typhimurium</i> TA1537, TA1538, TA98, reverse mutation	-	-	22 mg/plate	Silhánková et al. (1982)
<i>Salmonella typhimurium</i> TA97, reverse mutation	NT	-	10 mg/plate	Zeiger et al. (1988)
<i>Salmonella typhimurium</i> TA98, reverse mutation	(+)	-	110 mg/plate	Stolzenberg & Hine (1979)
<i>Salmonella typhimurium</i> TA98, reverse mutation	+	-	10 mg/plate	Zeiger et al. (1988)
<i>Salmonella typhimurium</i> TA98, reverse mutation	+	-	1.25 mg/plate	Ohkubo et al. (1995)
<i>Salmonella typhimurium</i> TM677, forward mutation	+	-	0.05 mg/plate	Ohkubo et al. (1995)
<i>Escherichia coli</i> WP2, TM930, TM1080, reverse mutation	-	-	22 mg/plate	Silhánková et al. (1982)
<i>Schizosaccharomyces pombe</i> , forward mutation	+	-	11 mg/mL	Rossi et al. (1983)
Mutation, DNA synthesis inhibition, HeLa cells <i>in vitro</i>	-	-	NR	Painter & Howard (1982)
Transformation assay, mouse fibroblasts, M2 clone <i>in vitro</i>	+	NT	0.25 mg/mL	Piasecki et al. (1990)
DNA strand breaks (Comet assay), Chinese hamster ovary cells <i>in vitro</i>	+	NT	2.5 mg/mL	El Ramy et al. (2007)
<i>Drosophila melanogaster</i> , somatic mutation, wing-spot test	-	-	1.1 mg/mL	Frei & Würigler (1997)
ICR/Ha Swiss male mice, dominant lethal mutation	-	-	125 mg/kg bw, ip × 1 or 20 mg/kg bw, po × 5	Epstein et al. (1972)
Male rats, dominant lethal mutation	-	-	10 mg/kg bw, po × 5	Jones et al. (1969)
Male Wistar rats, dominant lethal mutation	-	-	20 mg/kg bw, po × 5	Jones & Jackson (1976)

Table 4.1 (continued)

Test system	Results		Dose (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, male Han Wistar rat bone-marrow cells <i>in vivo</i>	-		60 mg/kg bw, po × 2	Robjohns et al. (2003)
Unscheduled DNA synthesis, male Han Wistar rat hepatocytes <i>in vivo</i>	-		100 mg/kg bw, po x 1	Robjohns et al. (2003)
DNA strand breaks (Comet assay), male Sprague-Dawley rat leukocytes, liver, kidney, testis and bone marrow	-		60 mg/kg bw, po x 2	El Ramy et al. (2007)
DNA strand breaks (Comet assay), male F344 rats leukocytes and testis	-		60 mg/kg bw, po x 2	El Ramy et al. (2007)

+, positive; (+), weakly positive; -, negative; bw, body weight; HID, highest ineffective dose; ip, intraperitoneal; LED, lowest effective dose; NT, not tested; NR, not reported; po, oral

in which three of six male macaque monkeys (*Macaca mulatta*) given 30 mg/kg bw 3-MCPD orally per day for 6 weeks showed haematological abnormalities, including anaemia, leukopenia and severe thrombocytopenia. Two of the affected monkeys died during the study due to bone-marrow depression ([Kirton et al., 1970](#)).

(a) Testicular toxicity

Incubation of ram sperm with 3-MCPD *in vitro* inhibited the glycolysis of spermatozoa ([Brown-Woodman et al., 1975](#)).

The activity of all glycolytic enzymes in the epididymal and testicular tissue of rats was reduced following daily subcutaneous injections of 6.5 mg/kg bw 3-MCPD for 9 days ([Kaur & Guraya, 1981a](#)). It has been suggested that the mechanism involved is the inhibition of glyceraldehyde-3-phosphate dehydrogenase and triose-phosphate isomerase by the 3-MCPD metabolite, β -chlorolactaldehyde ([Jones & Porter, 1995](#); [Lynch et al., 1998](#)).

The inhibition of spermatozoan glycolysis by 3-MCPD (and/or its metabolites) resulted in reduced sperm motility. The inhibition was reversible and has been attributed to the *S*-enantiomer of the substance ([Porter & Jones, 1982](#); [Stevenson & Jones, 1984](#); [Jones & Porter, 1995](#)). In addition, 3-MCPD decreased testosterone secretion in cultured Leydig cells from rats ([Paz et al., 1985](#)). No effect on concentrations of testosterone or luteinizing hormone was detected in the blood of male rats during a 28-day study of reproductive toxicity with doses of up to 5 mg/kg bw per day ([Kwack et al., 2004](#)).

Rats that received 6.5 mg/kg bw 3-MCPD per day for 9 days had significantly decreased ($P < 0.05$) levels of RNA and protein in the testis and epididymis, and these changes were paralleled by increases in the concentrations of proteinase and ribonuclease. The DNA content was unchanged ([Kaur & Guraya, 1981b](#)).

The spermatotoxic effect is mediated by reduced H^+ -adenosine triphosphatase expression in the cauda epididymis ([Kwack et al., 2004](#)).

(b) Renal toxicity

Increased blood urea nitrogen and serum creatinine concentrations, chronic progressive nephropathy and renal tubule-cell lesions — all indicative of overt nephrotoxicity — were generally seen at doses somewhat higher than those that caused testicular and epididymal effects ([JECFA, 2002](#)). The nephrotoxicity was associated with the *R*-enantiomer of 3-MCPD ([Porter & Jones, 1982](#); [Dobbie et al., 1988](#)).

Oxalic acid, a metabolite of 3-MCPD, appeared to play an important role in the development of renal damage ([Jones et al., 1979](#)). Birefringent crystals characteristic of calcium oxalate that were seen in tubules at the corticomedullary junction of rats 1 day after a single subcutaneous injection of 75 mg/kg bw 3-MCPD were considered to be early morphological changes. On day 75, focal tubule necrosis, regeneration and tubule dilatation were observed in the kidneys ([Kluwe et al., 1983](#)).

4.4 Mechanisms of carcinogenesis

A genotoxic mechanism of carcinogenicity was originally assumed for 3-MCPD, based on positive results in several *in-vitro* assays ([SCF, 2001](#)). Following the publication of negative results in *in-vivo* assays for micronucleus formation in rat bone marrow and unscheduled DNA synthesis ([Robjohns et al., 2003](#)), this assessment was questioned. [The Working Group noted that there is no evidence to suggest that 3-MCPD is not genotoxic. Further research appears to be necessary to assess the formation of glycidol as a putative metabolite.]

The kidney tumours observed in Sprague-Dawley rats ([Cho et al., 2008](#)) may have been caused by the cytotoxic, metabolically formed

oxalate (Hwang *et al.*, 2009). A genotoxic mechanism of action may also be involved.

5. Summary of Data Reported

5.1 Exposure data

3-Monochloro-1,2-propanediol is used as intermediate in the synthesis of several drugs and as a chemosterilant for rodent control. The major source of human exposure is its formation as a heat-induced contaminant during food processing. The highest levels of 3-monochloro-1,2-propanediol in free form in food were generally detected in soya sauce and soya sauce-based products (average, 8 mg/kg; maximum levels, up to > 1000 mg/kg), as well as in foods and food ingredients that contain acid-hydrolysed vegetable protein. Staple foods, such as bread (especially when toasted), may contribute to the daily intake of 3-monochloro-1,2-propanediol exposure. Free 3-monochloro-1,2-propanediol is regulated in many jurisdictions and its level of contamination has decreased in recent years. Considerable additional exposure may occur through ingestion of the bound form of esters of 3-monochloro-1,2-propanediol with higher fatty acids in refined vegetable oils, as well as infant formulae. However, no data on exposure to bound 3-monochloro-1,2-propanediol in the form of esters were available.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

In three studies in mice, administration of 3-monochloro-1,2-propanediol in the drinking-water, by subcutaneous injection or by dermal application did not increase the incidence of

tumours. Administration of 3-monochloro-1,2-propanediol in the drinking-water to rats increased the incidence of renal tubule carcinoma, renal tubule adenoma or carcinoma (combined) and Leydig cell adenoma in males, and that of renal tubule adenoma or carcinoma (combined) in females in one study. In another study, administration by gavage to rats did not increase tumour incidence.

Kidney tumours are rare spontaneous neoplasms in experimental animals.

5.4 Other relevant data

3-Monochloro-1,2-propanediol can be metabolized in rodents by alcohol dehydrogenase to chlorolactic acid, which was identified as a major metabolite in the urine. Chlorolactaldehyde is formed as an intermediate, and the chlorolactic acid may be oxidized further to oxalic acid. In bacteria, 3-monochloro-1,2-propanediol can be metabolized by halohydrin dehalogenase, to generate glycidol, which is classified by IARC as *probably carcinogenic to humans (Group 2A)*. A pathway that involves the glycidol intermediate may also be active in mammals, because glycidol can be detoxified further by glutathione S-transferase to form mercapturic acid metabolites — putative products of the reaction — which have been identified *in vivo*.

3-Monochloro-1,2-propanediol is mutagenic *in vitro*, but the limited available data *in vivo* showed negative results. Most of the target tissues of cancer in experimental animals were not tested for genetic effects *in vivo*.

3-Monochloro-1,2-propanediol exhibits nephrotoxicity, immunotoxicity, neurotoxicity, and testicular toxicity in rodents. Inhibition of glycolysis in the cells of the testes has been postulated as the mechanism for the adverse testicular effects. Chronic nephropathy has been proposed as a mechanism for the adverse effects on the kidney. Overall, the mechanistic data for cancer

are weak, but a genotoxic mechanism may be involved.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 3-monochloro-1,2-propanediol.

6.3 Overall evaluation

3-Monochloro-1,2-propanediol is *possibly carcinogenic to humans (Group 2B)*.

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1,3-DICHLORO-2-PROPANOL

1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

From [Merck Index \(2010\)](#) and [SciFinder \(2010\)](#)

Chem. Abstr. Serv. Reg. No.: 96-23-1

Chem. Abstr. Name:

1,3-Dichloro-2-propanol

IUPAC Systematic Name:

1,3-Dichloropropan-2-ol

Synonyms: 1,3-DCP; α -dichlorohydrin;

1,3-dichlorohydrin; 1,3-dichloro-2-

hydroxypropane; 1,3-dichloroisopro-

panol; 1,3-dichloroisopropyl alcohol;

1,3-dichloropropanol; enodrin; glycerol

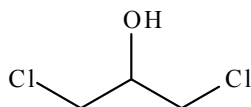
α,γ -dichlorohydrin; 2-glycerol 1,3-dichlo-

rohhydrin; propanol, 1-3-dichloro-;

α -propenyldichlorohydrin; sym-glycerol
dichlorohydrin

EINECS No.: 202-491-9

1.1.2 Structural and molecular formulae and relative molecular mass



$C_3H_6Cl_2O$

Relative molecular mass: 128.99

1.1.3 Chemical and physical properties of the pure substance

From [Beilstein \(2010\)](#), [Merck Index \(2010\)](#), and [SciFinder \(2010\)](#)

Description: Liquid with an ethereal odour

Boiling-point: 174.3 °C at 760 mm Hg

Melting-point: -4 °C

Density: 1.3530–1.3670 g/cm³ at 20 °C

Refractive index: 1.4830 at 20 °C

Solubility: Soluble in water (up to 1:9); miscible with alcohol, ether and acetone

1.1.4 Technical products and impurities

No data were available to the Working Group.

1.1.5 Analysis

A review of the analysis of chloropropanols in general is provided in the *IARC Monograph on 3-monochloro-1,2-propanediol (3-MCPD)* in this volume and in [Wenzl et al. \(2007\)](#). 1,3-Dichloro-2-propanol (1,3-DCP) cannot be analysed by phenylboronic acid derivatization, which is the most commonly applied procedure for the analysis of 3-MCPD, because phenylboronic acid only reacts with diols.

Similarly to that of 3-MCPD, trace analysis of 1,3-DCP is difficult, especially because its volatility hampers the concentration of solvent extracts without loss of analyte. The solvent extracts frequently include several compounds that potentially co-elute with 1,3-DCP on gas

chromatography (GC), and might not be identified correctly when using electron capture detection (ECD). The major problem of these approaches is that they are time-consuming and require a considerable degree of skill and experience in laboratory manipulations ([Crews et al., 2002](#)). Steam distillation with extraction into co-distilled petroleum ether:ethyl acetate was therefore proposed to determine 1,3-DCP with subsequent GC/ECD of the underivatized analyte ([Van Rillaer & Beernaert, 1989](#)), and an automated headspace (HS) sampling procedure for the analysis of 1,3-DCP was developed ([Crews et al., 2002](#)). The advantages of this method are its rapidity, sensitivity and the need for little sample preparation. It provides accurate identification of 1,3-DCP using mass spectrometry (MS), and precise quantification using a deuterium-labelled internal standard. It requires almost no sample preparation or reagents and a large batch of samples can be processed unattended overnight ([Crews et al., 2002](#)). [Nyman et al. \(2003\)](#) judged this HS-GC-MS method to be very fast and simple but with the disadvantage that simultaneous analysis of 3-MCPD and 1,3-DCP is not possible because the analysis of the underivatized compounds requires different GC columns. In addition, the low-molecular-weight ion fragments of the underivatized compounds render this method susceptible to interference and less reliable for confirmation of the identity of the analyte.

Analysis of heptafluorobutyrate derivatives was found to be more labour-intensive but had the advantage of analysing both 1,3-DCP and 3-MCPD during the same GC-MS run ([Hamlet & Sutton, 1997](#)). Moreover, the heptafluorobutyrate derivative produced higher-molecular-weight ion fragments that were less susceptible to interference.

Methods for the analysis of 1,3-DCP in different matrices are summarized in [Table 1.1](#).

1.2 Production and use

1.2.1 Production

1,3-DCP can be synthesized in a continuous process by the reaction of hydrochloric acid with epichlorohydrin ([Richey, 2000](#)). The hypochlorination of allyl chloride generates a mixture of the glycerol dichlorohydrins, 2,3- and 1,3-DCP, at a ratio of approximately 7:3 ([Richey, 2000](#); [Liu et al., 2005](#)).

1,3-DCP is listed in the CHEMCATS database ([SciFinder, 2010](#)) as being available from 88 suppliers worldwide in amounts up to bulk quantities. Data summarized by the National Toxicology Program ([NTP, 2005](#)) of the United States of America showed that the production volume in 1998 was reported to be between more than 453 600 kg and 4.5 million kg. Unconfirmed information stated that, from the point of view of volume, almost all of the chlorohydrins produced are immediately converted into epoxides, such as epichlorohydrin, and the small quantities sold on the commercial market are used in specialty applications. It was reported that the compound is not produced for the commercial market in the USA ([Richey, 2000](#)).

1.2.2 Use

1,3-DCP is used in large quantities as an intermediate in epichlorohydrin production ([NTP, 2005](#)). Dehydration of 1,3-DCP with phosphoryl chloride forms 1,3-dichloropropene, a soil fumigant. Chlorination of 1,3-DCP (or 2,3-DCP) with phosphorous pentachloride gives 1,2,3-trichloropropane. Hydrolysis of dichlorohydrins has been used in the production of synthetic glycerol ([NTP, 2005](#)). 1,3-DCP has been used as solvent for hard resins and nitrocellulose, in the manufacture of photographic and Zapon lacquer, as a cement for celluloid and as a binder for water colours ([Merck Index, 2010](#)). Its use as a dye fixative/anti-fading agent in detergent formulations appears to be historical, based on a limited patent survey ([NTP, 2005](#)).

Table 1.1 Selected methods for the analysis of 1,3-dichloro-2-propanol in various matrices

Matrix	Analytes	Pre-treatment	Clean up	Derivatization	Detection	LOD for 1,3-DCP (µg/kg)	Reference
HVP	1,3-DCP	Micro-steam distillation, solvent extraction	-	None	GC-ECD	10	Van Rillaer & Beernaert (1989)
Seasonings	2-MCPD, 3-MCPD, 1,3-DCP, 2,3-DCP	Water, pH adjustment	Extrelut	None	GC-MS SIM	50	Wittmann (1991)
Paper	3-MCPD, 1,3-DCP	Acetonitrile extraction	-	BSTFA	GC-MS SIM	40	Bodén et al. (1997)
Soya sauce	1,3-DCP, 2,3-DCP	Ammonium sulfate	HS Extraction	None	GC-MS	3	Crews et al. (2002)
HVP	2-MCPD, 3-MCPD, 1,3-DCP, 2,3-DCP	5M NaCl solution	Extrelut, two-stage extraction	HFBI	GC-ECD, GC-MS	10	van Bergen et al. (1992)
Water	3-MCPD, 1,3-DCP (and bromo-propanediols)	Ethyl acetate extraction	-	HFBA	GC-ECD	1.7	Matthew & Anastasio (2000)
Soya sauce	1,3-DCP, 3-MCPD	5M NaCl solution	Silica gel (60 mesh)	HFBA	GC-MS SIM	5	Chung et al. (2002)
Soya sauce, flavouring	2-MCPD, 3-MCPD, 1,3-DCP, 2,3-DCP	5M NaCl solution	Extrelut	HFBA-Et ₃ N	GC-MS EI SIM or NCI SIM	3 (EI), 0.6 (NCI)	Xu et al. (2006)
Various foods	1,3-DCP, 3-MCPD	Saturated NaCl solution	Aluminium oxide	HFBA	GC-MS SIM	1	Abu-El-Haj et al. (2007)
Water	1,3-DCP	Adjustment to pH 4, addition of NaCl for salting out	HS-SPME	BSTFA	GC-MS/MS	0.4	Carro et al. (2009)
Water	1,3-DCP	(NH ₄) ₂ SO ₄ addition	LLE with ethyl acetate	None	GC-MS SIM	0.1	Schuhmacher et al. (2005)
Seasoning	3-MCPD, 1,3-DCP, 2,3-DCP	No data	No data	TSIM	GC-MS SIM	0.20	Cao et al. (2009)
Soya sauce	1,3-DCP, 3-MCPD	NaCl addition	HS-SPME	MSTFA	GC/MS SIM	0.41	Lee et al. (2007)
Soya and related sauces	1,3-DCP	5M NaCl solution	Extrelut	HFBI	GC/MS SIM	0.06	Nyman et al. (2003)

BSTFA, bis(trimethylsilyl)trifluoroacetamide; DCP, dichloropropanol; 1,3-DCP, 1,3-dichloro-2-propanol; 2,3-DCP, 2,3-dichloro-1-propanol; EI, electron-impact ionization; GC-ECD, gas chromatography with electron capture detection; GC-MS, gas chromatography-mass spectrometry; GC-MS/MS, gas chromatography-tandem mass spectrometry; HFBA, heptafluorobutyric anhydride; HFBI, heptafluorobutyrylimidazole; HS, headspace; HS-SPME, headspace solid phase microextraction; HVP, acid-hydrolysed vegetable protein; LLE, liquid liquid extraction; LOD, limit of detection; MCPD, monochloropropanediol; 2-MSTFA, *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide; NaCl, sodium chloride; NCI, negative chemical ionization; SIM, selected ion monitoring; TSIM, 1-trimethylsilylimidazole

Updated from [Wenzl et al. \(2007\)](#)

1.3 Occurrence

1.3.1 Natural occurrence

1,3-DCP is not known to occur as a natural product.

1.3.2 Occupational exposure

1,3-DCP may occur as a hydrolysis product of epichlorohydrin, which is a major raw material in the chemical and paper industry (see [IARC, 1999](#)). Concerns have therefore been raised that 1,3-DCP may be present in products made with epichlorohydrin as well as in workplace air. However, it was reported that 1,3-DCP is not usually detected, except in the headspace of improperly vented storage tanks ([Dulany et al., 2000](#)). Industrial accidents may result in fatal intoxications (see Section 4.1.1; [Iwasa et al., 1992](#); [Haratake et al., 1993](#); [Shiozaki et al., 1994](#)).

Workers using acrylic paint in spray-painting operations may be exposed to low concentrations of 1,3-DCP present as an impurity in the paint ([NTP, 2005](#)). 1,3-DCP may also be present as an impurity in bis(2-chloro-1-methylethyl)ether and the quaternary ammonium compound, *N*-(3-chloro-2-hydroxypropyl) trimethylammonium chloride (Dextrosil, Dowquat 188). Workers may be exposed indirectly to 1,3-DCP, which is a metabolite of 1,2,3-trichloropropane and tris(1,3-dichloro-2-propyl)phosphate ([NTP, 2005](#)).

1.3.3 Occurrence in food

1,3-DCP is a foodborne contaminant that can be formed during the processing of different foodstuffs ([Wenzl et al., 2007](#)). It was first recognized in 1978 at the Institute of Chemical Technology in Prague ([Velíšek et al., 1978](#)) in acid-hydrolysed vegetable protein, a seasoning ingredient that is widely used in a variety of processed and prepared foods. It generally occurs together with 3-MCPD, which is regarded as the most

abundant chloropropanol found in foodstuff ([Wenzl et al., 2007](#)) (see the *IARC Monograph* on 3-MCPD in this volume for details on the mechanisms of their formation in food). Limited data have shown a linear relationship between the concentrations of 1,3-DCP and 3-MCPD in food ([JECFA, 2007](#)).

In general, 1,3-DCP occurs at lower concentrations than 3-MCPD, except in meat products. Due to the analytical problems described above, and especially because 1,3-DCP cannot be detected by many of the methods developed for the analysis of 3-MCPD, data on the occurrence of 1,3-DCP worldwide are more sparse than those for 3-MDPC ([Table 1.2](#)). Similarly to 3-MCPD, 1,3-DCP occurs most abundantly in soya sauce and soya sauce-based products.

The international, representative average dietary exposure of the general population was estimated to be 0.051 µg/kg body weight (bw) per day, while an exposure of 0.136 µg/kg bw per day was estimated for high consumers (including children). Intakes were calculated by linking data on individual consumption with those on mean occurrence, using the actual body weight of consumers reported in consumption surveys ([JECFA, 2007](#)).

For secondary school students in China, Hong Kong Special Administrative Region, the average exposure was estimated to be 0.003–0.019 µg/kg bw per day, while that for high consumers was 0.009–0.040 µg/kg bw per day ([Yau et al., 2008](#)).

Further exposure may occur when paper treated with epichlorohydrin-based wet resins are used in contact with food, such as tea bag paper, coffee filters, absorbent paper packaged with meats and cellulose casings (for ground meat products such as sausages) ([NTP, 2005](#)). Similar to bound 3-MCPD, bound 1,3-DCP may also be present in foods in the form of esters ([Seefelder et al., 2010](#)).

Table 1.2 Summary of the distribution-weighted concentration of 1,3-dichloro-2-propanol in soya sauce and soya sauce-based products, in other foods and in food ingredients from various countries, 2001–06^a

Product	LOQ (mg/kg)	No.	<i>n</i> < LOQ	Mean ^b (mg/kg)	Maximum (mg/kg)
Soya sauce and soya sauce-based products	0.002–0.15	484	371	0.110	9.84
Meat and meat products	0.005	99	51	0.019	0.11
Fish and sea food	0.005	29	26	0.0025	0.024
Food ingredients (including HVPs and malt extracts)	0.010	56	13	0.008	0.070

^a Includes data of surveys before intervention to reduce occurrence had been undertaken by government or industry.

^b Data below the level of detection or LOQ have been assumed to be half of those limits and the mean was weighted according to the number of samples per country.

HVP, acid-hydrolysed vegetable protein; LOQ, limit of quantification
Data summarized from [JECFA \(2007\)](#)

1.3.4 Environmental occurrence

1,3-DCP and related contaminants can be found in epichlorohydrin polyamine polyelectrolytes used in drinking-water treatment chemicals (coagulation and flocculation products) ([NTP, 2005](#)).

Similar to occupational exposure, environmental exposure to 1,3-DCP predominantly occurs from wastes containing epichlorohydrin. Single studies reported that 1,3-DCP was present in pulp mill effluents and spent kraft paper bleaching liquors, as well as in a municipal waste landfill leachate ([NTP, 2005](#)). Each of more than 300 river water samples from 32 sites in Austria that were analysed contained 1,3-DCP at concentrations of less than 1.0 µg/L, which was the quantification limit of the study ([Schuhmacher et al., 2005](#)).

1.4 Regulations and guidelines

The current regulation of the US Food and Drug Administration for the use of dimethylamine epichlorohydrin copolymer resin establishes a limit for residues of 1,3-DCP in the resin of 1000 ppm ([Code of Federal Regulations, 2010](#)).

Fewer limits have been set for the levels of 1,3-DCP in food than for those of 3-MCPD (see the *Monograph* in this volume), because

its concentration is generally lower than that of 3-MCPD ([NTP, 2005](#)). Hence, the regulatory control of 3-MCPD decreases the need for specific limits on 1,3-DCP, although some countries have imposed maximum limits (Australia/New Zealand, 0.005 mg/kg in soya/oyster sauces; Switzerland, 0.05 mg/kg in savoury sauces; USA, 0.05 mg/kg in acid-hydrolysed vegetable protein) ([Hamlet & Sadd, 2009](#)).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Oral administration

See [Table 3.1](#)

3.1.1 Rat

Groups of 80 male and 80 female Wistar KFM/Han rats were administered 0 (control), 27 (low dose), 80 (mid dose) or 240 (high dose) mg/L [0, 0.21, 0.62 or 1.86 mmol/L] 1,3-DCP in the drinking-water for up to 104 weeks. These

Table 3.1 Carcinogenicity study of 1,3-dichloro-2-propanol administered in the drinking-water to rats

Strain (sex) Duration	Dosing regimen Animals/group at start	Incidence and/or multiplicity of tumours	Significance (Peto trend test)	Comments
Wistar (M) up to 104 wk	0, 27, 80 and 240 mg/L (0, 2.1, 6.3 and 19 mg/kg bw per d) 80/group	Liver (hepatocellular adenoma): 1/80, 0/80, 1/80, 0/80	*** $P < 0.001$	Ten rats per group were killed after 26, 52 and 78 wk of treatment
		Liver (hepatocellular carcinoma): 0/80, 0/80, 2/80, 11/80***		
		Kidney (renal tubule adenoma): 0/80, 0/80, 3/80, 10/80***	*** $P < 0.001$	
		Kidney (renal tubule carcinoma): 0/80, 0/80, 0/80, 1/80		
		Kidney (renal tubule adenoma or carcinoma): 0/80, 0/80, 3/80, 10/80***	*** $P < 0.001$	
		Tongue/oral cavity (papilloma): 0/80, 1/80, 0/79, 6/80***	*** $P < 0.001$	
		Tongue/oral cavity (squamous-cell carcinoma): 0/80, 0/80, 1/79, 6/80***	*** $P < 0.001$	
		Thyroid (follicular-cell adenoma): 0/80, 0/80, 3/80*, 3/78*	* $P < 0.05$	
		Thyroid (follicular-cell carcinoma): 0/80, 0/80, 2/80, 1/78		
		Thyroid (follicular-cell adenoma or carcinoma): 0/80, 0/80, 5/80*, 4/78*		
Wistar (F) up to 104 wk	0, 27, 80 and 240 mg/L (0, 3.4, 9.6 and 30 mg/kg bw per d) 80/group	Liver (hepatocellular adenoma): 1/80, 1/80, 1/80, 6/80**	** $P < 0.01$	Ten rats per group were killed after 26, 52, and 78 wk of treatment
		Liver (hepatocellular carcinoma): 0/80, 0/80, 1/80, 44/80***	*** $P < 0.001$	
		Kidney (renal tubule adenoma): 0/80, 0/80, 0/80, 1/79		
		Kidney (renal tubule carcinoma): 0/80, 0/80, 0/80, 0/79		
		Tongue/oral cavity (papilloma): 0/80, 0/80, 0/80, 7/79***	*** $P < 0.001$	
		Tongue/oral cavity (squamous-cell carcinoma): 0/80, 1/80, 1/80, 4/79**	** $P < 0.01$	
		Thyroid (follicular-cell adenoma): 1/79, 0/80, 3/80, 4/79		
		Thyroid (follicular-cell carcinoma): 0/79, 0/80, 0/80, 2/79*	* $P < 0.05$	

bw, body weight; d, day or days; F, female; M, male; wk, week or weeks

From [Research & Consulting Co. \(1986\)](#), [IECFA \(2002\)](#), and [Williams et al. \(2010\)](#)

doses were reported to provide exposures equal to 0, 2.1, 6.3 or 19 and 0, 3.4, 9.6 or 30 mg/kg body weight (bw) per day for males and females, respectively. Ten rats of each sex per group were killed after 26, 52 and 78 weeks of treatment. The mortality rates of the 50 animals per group that were exposed for 104 weeks were higher in males (32/50, $P < 0.05$) and females (27/50, $P < 0.05$) in the high-dose groups than in controls (males, 18/50; females, 13/50). Those in the low- and mid-dose groups were 11/50 males and 9/50 females and 16/50 males and 14/50 females, respectively. Statistically significant increases in the incidence of the following tumours were observed: in the liver, hepatocellular carcinoma in males and hepatocellular carcinoma and adenoma in females; in the tongue/oral cavity, squamous-cell carcinoma and papilloma in males and females; in the kidney, renal tubule adenoma in males; and in the thyroid, follicular-cell carcinoma in females and follicular-cell adenoma or carcinoma combined in males. With the exception of follicular-cell adenoma of the thyroid in the mid-dose males, the increases in tumour incidence were only statistically significant in the high-dose groups ([Research & Consulting Co., 1986](#); [JECFA, 2002](#); [Williams et al., 2010](#)).

4. Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

The study of toxicity in humans has been restricted to industrial accidents, in which workers were exposed by inhalation to 1,3-DCP. A consistent finding was acute hepatitis, which was fatal in several cases ([Iwasa et al., 1992](#); [Haratake et al., 1993](#); [Shiozaki et al., 1994](#)). [Confounding by co-exposure to other

compounds, including epichlorohydrin, could not be excluded.]

4.1.2 Experimental systems

The limited available data on absorption, distribution, excretion and metabolism of 1,3-DCP in experimental systems have been reviewed previously ([JECFA, 2002](#); [NTP, 2005](#)).

(a) Degradation in bacteria

Two pathways for the degradation of 1,3-DCP have been found in *Corynebacterium* sp. strain N-1074 ([Natarajan et al., 2008](#)), which are catalysed by two groups of two isoenzymes ([Nakamura et al., 1992](#)). One group of two enzymes catalyses the non-stereospecific dechlorination and subsequent hydrolyzation of 1,3-DCP. Both enzymes accept (R)- and (S)-enantiomers as substrates and convert them to racemic mixtures ([Yu et al., 1994](#)). The second group of enzymes also accepts (R)- and (S)-enantiomers, but converts them to (R)-rich products ([Nakamura et al., 1992](#)).

Although *Arthrobacter* sp. strain AD2 can dechlorinate 1,3-DCP and 3-chloro-1,2-propanediol, it has no epoxide hydrolase activity and therefore cannot use either compound as a sole source of carbon ([Nagasawa et al., 1992](#)).

Another species, *Agrobacterium radiobacter* strain AD1, can use 1,3-DCP or epichlorohydrin as a sole source of carbon. The pathway of degradation is non-enantioselective and similar to that of the *Corynebacterium* strain ([Rink et al., 1997](#)).

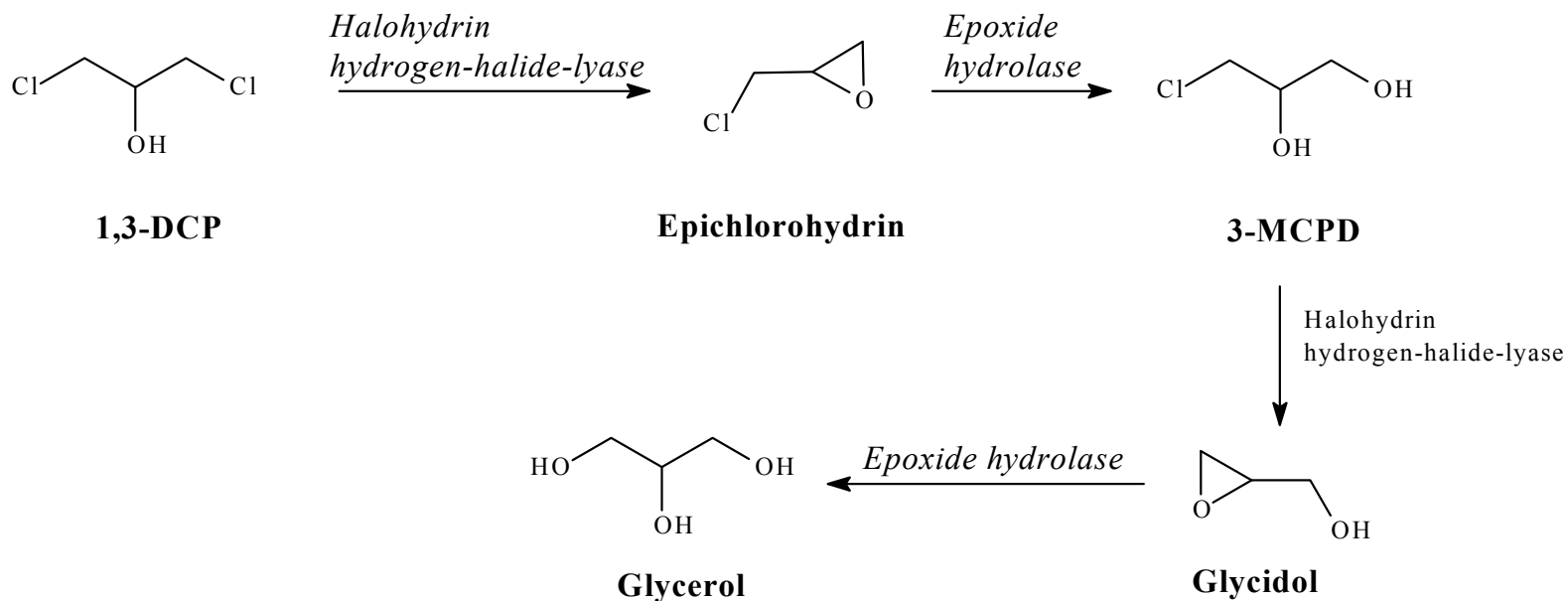
Epichlorohydrin was formed in media used for Ames and SOS chromotest assays with 1,3-DCP ([Hahn et al., 1991](#)).

The proposed bacterial metabolism of 1,3-DCP is summarized in Fig. 4.1.

(b) Metabolism in mammalian systems

Few studies have investigated the metabolism of 1,3-DCP in mammalian systems, although it has been reported to induce and/or

Fig. 4.1 Proposed microbial metabolism of 1,3-dichloro-2-propanol



1,3-DCP, 1,3-dichloro-2-propanol; 3-MCPD, 3-monochloro-1,2-propanediol
 Adapted from [Natarajan et al. \(2008\)](#)

be metabolized by the cytochrome P450 (CYP) enzyme isoform CYP2E1 (Garle *et al.*, 1997; Hammond & Fry, 1997; Fry *et al.*, 1999). Studies in rat hepatocytes in culture (Hammond & Fry, 1999) and in rat liver *in vivo* (Fry *et al.*, 1999) have indicated that 1,3-DCP is metabolized by CYP2E1 to an aldehyde intermediate that depletes glutathione (GSH). Under basal conditions, this metabolite appears to be effectively detoxified, but increased CYP2E1 activity and/or decreased aldehyde dehydrogenase activity promotes accumulation of the metabolite and thus GSH depletion and toxicity. Other factors, such as nutrition status (Fouin-Fortunet *et al.*, 1990), that modify GSH levels in humans may alter susceptibility to 1,3-DCP toxicity.

The metabolites identified in the urine of rats treated orally with 50 mg/kg bw 1,3-DCP per day for 5 days were β -chlorolactate (approximately 5% of the dose), *N,N'*-bis-acetyl-*S,S'*-(1,3-bis-cysteinyl)propan-2-ol (1%) and *N*-acetyl-*S*-(2,3-dihydroxypropyl)cysteine (Jones & Fakhouri, 1979). It was proposed that epoxychloropropane (epichlorohydrin, IARC Group 2A, IARC, 1999) is formed as an intermediate, and may either undergo conjugation with GSH to form mercapturic acid or be hydrolysed to 3-MCPD. The latter undergoes oxidation to β -chlorolactate, which is further oxidized to oxalic acid (see also the *Monograph* on 3-MCPD in this volume). The formation of other epoxides from α -chlorohydrins has been postulated but only at high pH (Jones & Fakhouri, 1979; JECFA, 2002).

Ethyl acetate-extractable metabolites were found in the 24-hour urine of male Wistar rats given a single subcutaneous injection of about 62 mg/kg bw 1,3-DCP. The parent compound accounted for 2.4% of the dose, 3-MCPD for 0.35% and 1,2-propanediol for 0.43%. 2,3-DCP was also found (0.16% of the dose), but the authors attributed this to its presence as an impurity (1.7%) in the 1,3-DCP administered to the rats. Metabolites that were not extractable in

ethyl acetate were not analysed (Koga *et al.*, 1992; JECFA, 2002).

Alcohol dehydrogenase might be responsible for the oxidation of 1,3-DCP to dichloroacetone, a DNA-reactive metabolite, that can also be formed by rearrangement of the epichlorohydrin intermediate (Eder & Dornbusch, 1988; Weber & Sipes, 1992; JECFA, 2002). 1,3-Dichloroacetone is known to deplete GSH (Garle *et al.*, 1999), and may also be produced by CYP2E1-mediated metabolism (Hammond & Fry, 1997).

Because of selective extraction procedures and limited attempts at their identification, only a small percentage of administered doses have been accounted for as metabolites (JECFA, 2002).

1,3-DCP has been reported to deplete GSH both *in vitro* and *in vivo* (Hammond *et al.*, 1996; Garle *et al.*, 1997; Fry *et al.*, 1999; Garle *et al.*, 1999; Hammond & Fry, 1999). 1,3-DCP (up to 1000 μ M [129 μ g/mL]) depleted GSH dose-dependently when incubated with co-factors (i.e. a nicotinamide adenine dinucleotide phosphate-generating system) and liver microsomes from untreated rats. Inclusion of pyridine or omission of the co-factor, however, inhibited the depletion (Garle *et al.*, 1999). In rat hepatocyte cultures, isoniazid (an inducer of CYP) was found to increase the rate and extent of GSH depletion by 1,3-DCP, as well as its toxicity, whereas cyanamide (an aldehyde dehydrogenase inhibitor) did neither. Pretreatment of cultures with 1-aminobenzotriazole (an inhibitor of CYP) prevented the toxicity of 1,3-DCP, while pretreatment with diethyl maleate or buthionine sulfoximine (GSH inhibitors) increased its toxicity (Hammond & Fry, 1996, 1997, 1999).

A dose of 5 mg/kg bw diethyldithiocarbamate significantly protected against the hepatotoxicity induced in rats by intraperitoneal injection of 70 mg/kg bw 1,3-DCP, and also inhibited enzyme markers for CYP2E1 activity. At a dose of 25 mg/kg bw, diethyldithiocarbamate afforded complete protection. It was therefore concluded that the hepatotoxicity of 1,3-DCP was mediated

principally through its metabolism by CYP2E1 ([Stott et al., 1997](#)).

In rats treated with 0.3 mg/kg bw 1,3-DCP, significantly increased hepatic levels of malondialdehyde were associated with decreases in liver GSH S-transferase activity and GSH content. Lipid peroxidation was suggested as a mechanism of the reported hepatotoxicity [diffuse massive necrosis] ([Katoh et al., 1998](#); [Kuroda et al., 2002](#)).

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

Genotoxicity studies of 1,3-DCP *in vitro* and *in vivo* have recently been reviewed ([IECFA, 2002](#)), and are summarized in [Table 4.1](#).

In vitro, 1,3-DCP induced reverse mutation in various strains of *Salmonella typhimurium*. It induced mutations and influenced DNA repair in *Escherichia coli*. 1,3-DCP induced sister chromatid exchange in Chinese hamster V79 cells. It was also mutagenic in HeLa cells and induced malignant transformation of mouse fibroblasts.

In the only available study *in vivo*, 1,3-DCP had no effect on the induction of wing spots in *Drosophila melanogaster* ([Frei & Würigler, 1997](#)).

4.3 Mechanistic data

4.3.1 Effects on cell physiology

Data *in vitro* suggested that 1,3-DCP-induced apoptosis was dependent on Ca²⁺ and that reactive oxygen species were also induced by exposure of B16F10 murine melanoma cells to 1,3-DCP ([Park et al., 2010](#)).

Exposure of A549 lung adenocarcinoma cells to 1,3-DCP was reported to inhibit cell growth, generate reactive oxygen species and to activate p53 and p21^{CIP1/WAF1} ([Jeong et al., 2007](#)).

Six groups of rats received a single intraperitoneal injection of 0.2 mL 20% ethanol (control), or 1/8, 1/4, or 1/2 of the dose that was lethal in 50% of animals (LD₅₀), the LD₅₀ or double the LD₅₀ (LD₅₀ = 149 µg/kg bw) of 1,3-DCP diluted in 20% ethanol. Rats administered ethanol only or 1/8 (18.6 µg/kg bw) and 1/4 (37 µg/kg/bw) of the LD₅₀ showed no serological or histopathological abnormalities. Marked elevation of serum glutamate pyruvate transaminase and diffuse massive necrosis of the liver cells were noted in all rats treated with both the LD₅₀ (149 µg/kg bw) and double the LD₅₀ (298 µg/kg bw), and irregular zonal necroses were found in three of four rats injected with 1/2 the LD₅₀ (74.5 µg/kg bw). No serious toxic changes occurred in other organs. In a second experiment in which rats were exposed to ethanol alone or the LD₅₀, hepatic malondialdehyde levels were significantly increased, associated with decreases in liver GSH S-transferase activity and reduced GSH content in the LD₅₀-treated group. The authors concluded that the hepatotoxicity was dose-dependent and that one of its mechanisms might be lipid peroxidation ([Katoh et al., 1998](#)). [Lipid peroxidation was not shown to be dose-dependent.]

4.3.2 Structure-activity relationships relevant to an evaluation of carcinogenicity and structural analogies with known carcinogens

Carcinogenicity, genotoxicity and toxic effects on reproduction and development were compiled for a limited group of C3-compounds and their derivatives related to 1,3-DCP ([NTP, 2005](#)). Oxygen-containing compounds that induced malignancies in rodents included epichlorohydrin [106-89-8] (Group 2A, [IARC, 1999](#)), 2,3-dibromo-1-propanol [96-13-9] and tris(2,3-dibromopropyl) phosphate [126-72-7] (Group 2A, [IARC, 1999](#)). Oxygen-containing compounds that induced only benign tumours were 3-MCPD [96-24-2] and 1,3-dichloro-2-propanol

Table 4.1 Genetic and related effects of 1,3-dichloro-2-propanol

Test system	Results		Dose (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Salmonella typhimurium</i> TA100, reverse mutation	-	+	0.05 mg/plate	Gold et al. (1978)
<i>Salmonella typhimurium</i> TA100, TA1535, reverse mutation	+	+	0.39 mg/plate	Nakamura et al. (1979)
<i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	0.13 mg/plate	Stolzenberg & Hine (1980)
<i>Salmonella typhimurium</i> TA100, reverse mutation	+	-	0.1 mg/plate	Lynn et al. (1981)
<i>Salmonella typhimurium</i> TA100, TA1537, TA1538, reverse mutation	-	-	26 mg/plate	Silhánková et al. (1982)
<i>Salmonella typhimurium</i> TA100, reverse mutation	NT	+	≤ 0.5 mg/plate	Majeska & Matheson (1983)
<i>Salmonella typhimurium</i> TA100, TA1535, reverse mutation	+	+	0.3–3.33 mg/plate	Zeiger et al. (1988)
<i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	3.4 mg/plate	Hahn et al. (1991)
<i>Salmonella typhimurium</i> TA100, TA1535, reverse mutation	+	+	≤ 1.2 mg/plate	Ohkubo et al. (1995)
<i>Salmonella typhimurium</i> TA1535, reverse mutation	+	+	0.26 mg/plate	Silhánková et al. (1982)
<i>Salmonella typhimurium</i> TA1535, reverse mutation	+	+	0.72 mg/plate	Hahn et al. (1991)
<i>Salmonella typhimurium</i> TA97, reverse mutation	-	+	3.33 mg/plate	Zeiger et al. (1988)
<i>Salmonella typhimurium</i> TA98, reverse mutation	-	+	6.7 mg/plate	Zeiger et al. (1988)
<i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	1.2 mg/plate	Ohkubo et al. (1995)
<i>Salmonella typhimurium</i> TM677, forward mutation	-	+	≤ 0.1 mg/plate	Ohkubo et al. (1995)
<i>Escherichia coli</i> WP2, TM930, TM1080, reverse mutation	-	+	0.26 mg/plate	Silhánková et al. (1982)
Prophage induction, SOS repair, DNA strand breaks or cross-links (<i>Escherichia coli</i> PM21, GC4798)	-	+	1.3–3.9 mg/ sample	Hahn et al. (1991)
Sister chromatid exchange, Chinese hamster lung V79 cells <i>in vitro</i>	+	+	0.032–0.13 mg/ mL	von der Hude et al. (1987)
Mutation, inhibition of DNA synthesis, HeLa S3 cells <i>in vitro</i>	NT	+	0.32 mg/mL	Painter & Howard (1982)
Transformation assay, mouse fibroblasts, M2 clone <i>in vitro</i>	+	NT	0.1 mg/mL	Piasecki et al. (1990)
<i>Drosophila melanogaster</i> , somatic mutation, wing-spot test	-		1.3 mg/mL	Frei & Würigler (1997)

+, positive; -, negative; HID, highest ineffective dose; LED, lowest effective dose; NT, not tested

[13674-87-8]. Two related chlorinated hydrocarbons, 1,3-dichloropropene [542-75-6] (Group 2B, [IARC, 1999](#)) and 1,2,3-trichloropropane [96-18-4] (Group 2A, [IARC, 1995](#)), were also rodent carcinogens.

No long-term study was available for 2,3-dichloropropanol [616-23-9]. The compounds that caused tumours, including 1,3-DCP, were genotoxic in at least some mammalian systems *in vitro*. The metabolism of all of these compounds has not been explored, but their conversion to epichlorohydrin or epibromohydrin [3132-64-7] might be involved in their mode of action of tumour induction.

Brominated analogues evaluated by IARC include 1,2-dibromo-3-chloropropane [96-12-8] (Group 2B, [IARC, 1999](#)) and 2,3-dibromo-1-propanol [96-13-9] (Group 2B, [IARC, 2000](#)).

4.4 Mechanisms of carcinogenesis

While no studies have evaluated the genotoxicity of 1,3-DCP in intact mammalian organisms or humans, the results of *in-vitro* studies demonstrated that 1,3-DCP can readily interact with chromosomal material in cells. Therefore, 1,3-DCP or its metabolites can be expected to have genotoxic activity in target tissues *in vivo* ([JECFA, 2002](#)). Nevertheless, no clear mode of action was established for tumours observed in experimental animals (i.e. of the liver, kidney and tongue).

5. Summary of Data Reported

5.1 Exposure data

1,3-Dichloro-2-propanol is used as an intermediate in the production of epichlorohydrin. Hydrolysis of epichlorohydrin, which is a major raw material in industry, may contribute to occupational exposure to 1,3-dichloro-2-propanol.

1,3-Dichloro-2-propanol may be formed as a heat-induced contaminant during food processing. The levels in food are usually below 100 µg/kg with the exception of soya sauce and soya sauce-based products, which may contain levels up to the milligram per kilogram range. Levels in food have been regulated in some jurisdictions, and indirect regulation also occurs in jurisdictions where 3-monochloro-1,2-propanediol is regulated, because both compounds are formed by similar mechanisms and their concentrations were correlated.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

In a 2-year study in rats, administration of 1,3-dichloro-2-propanol in the drinking-water increased the incidence of tongue carcinoma, tongue papilloma and hepatocellular carcinoma in males and females. The incidence of renal tubule adenoma in males, thyroid follicular-cell carcinoma in females and thyroid follicular-cell adenoma or carcinoma (combined) in males was also increased.

Tumours of the tongue and thyroid are rare spontaneous neoplasms in experimental animals.

5.4 Other relevant data

1,3-Dichloro-2-propanol may be metabolized in bacteria by two consecutive steps of halohydrin hydrogen-halide-lyase followed by epoxide hydrolase, which generates the metabolites epichlorohydrin and glycidol, both of which are classified by IARC as *probably carcinogenic to humans* (Group 2A). The metabolism in mammals is not fully elucidated but may be similar.

β-Chlorolactate was detected in the urine of rats treated orally with 1,3-dichloro-2-propanol.

The compound is assumed to be formed by oxidation of 3-monochloro-1,2-propanediol, which may arise as a hydrolysis product of the epichlorohydrin metabolite.

1,3-Dichloro-2-propanol is mutagenic *in vitro*, but the limited data available from in-vivo assays were negative. At high doses, it exhibits hepatotoxicity in experimental animals and evidence for acute hepatitis was also detected in cases of human intoxication. A possible mechanism for the carcinogenicity of 1,3-dichloro-2-propanol is the induction of DNA damage by the agent itself or its metabolites, and the production of reactive oxygen species.

Overall, the available mechanistic data are considered to be weak. However, the relevance of the tumour response in experimental animals to humans cannot be excluded.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,3-dichloro-2-propanol.

6.3 Overall evaluation

1,3-Dichloro-2-propanol is *possibly carcinogenic to humans (Group 2B)*.

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2,4-HEXADIENAL

1. Exposure Data

1.1 Chemical and physical data

From [Ford et al. \(1988\)](#), [NTP \(2003\)](#) and [HSDB \(2010\)](#)

1.1.1 Nomenclature

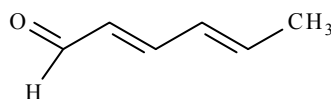
(a) *E,E*- isomer

Chem. Abstr. Services Reg. No.: 142-83-6
Chem. Abstr. Name: 2,4-Hexadienal, (*E,E*)-
IUPAC Name: (2*E*,4*E*)-Hexa-2,4-dienal
EINECS No.: 205-564-3
JECFA: 1175
Flavouring No.: 05.057
Synonyms: Hexa-2,4-dienal; 2,4-hexadienal, (2*E*,4*E*)-; *trans,trans*-2,4-hexadienal; 2,4-hexadien-1-al; (*E,E*)-2,4-hexadien-1-al; *trans,trans*-2,4-hexadien-1-al; 1,3,-pentadiene-1-carboxaldehyde; 2-propyleneacrolein; 2-propylene acrolein; 3-propyleneacrolein; sorbaldehyde; sorbic aldehyde

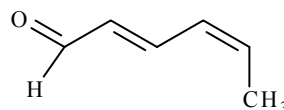
(b) *E,Z* isomer

Chem. Abstr. Services Reg. No.: 53398-76-8
Chem. Abstr. Name:
(2*E*,4*Z*)-2,4-Hexadienal
Synonym: *trans,cis*-2,4-Hexadienal

1.1.2 Structural and molecular formulae and relative molecular mass



(*E,E*)-2,4-Hexadienal



(2*E*,4*Z*)-2,4-Hexadienal

C_6H_8O

Relative molecular mass: 96.13

1.1.3 Chemical and physical properties of the pure substance

Throughout the *Monograph*, when not otherwise specified, 2,4-hexadienal refers to the compound for which the stereoisomeric status has not been indicated.

Description: Colourless liquid with a sweet, green, spicy, floral or citrus odour

Boiling-point: 174 °C at 15 mm Hg; 76 °C at 30 mm Hg

Specific gravity: 0.898 at 25 °C

Vapour pressure: 4.8 mm Hg at 25 °C (estimated)

Refractive index: 1.5384 at 20 °C

Solubility: Insoluble in water; soluble in ethanol

Flash-point: 54.4 °C

Odour threshold: 0.0018 g/m³

Octanol/water partition coefficient: log K_{ow},
1.37 (estimated)

Henry's law constant:

9.78 × 10⁻⁶ atm.m³/mol at 25 °C

1.1.4 Technical products and impurities

2,4-Hexadienal is commercially available as a > 95% pure mixture of *trans,trans* (≈80%) and *cis,trans* (10–16%) isomers with the Chemical Abstract Services Registry Number 80466-34-8.

α-Tocopherol (at 0.50%) is present as an additive in 2,4-hexadienal ([Bedoukian Research Inc., 2010](#)).

1.1.5 Analysis

No data were available to the Working Group.

1.2 Production and use

1.2.1 Production

2,4-Hexadienal is prepared by the condensation of acetaldehyde ([Ford et al., 1988](#)). Current production levels are not available.

1.2.2 Use

2,4-Hexadienal is used as a flavouring agent in the manufacture of the aromatic chemical 3,5,7-nonatrien-2-one, as a chemical intermediate in various organic synthetic reactions and as a raw material in the manufacture of sorbic acid (a widely used food preservative). It is also used as a chemical intermediate in the manufacture of polymethine dyes, as a pharmaceutical intermediate in the manufacture of mitomycins and antihypercholesterolemics, as an inhibitor of corrosion for steel used in oil field operations, as a monomer in reactions with silane comonomers for the manufacture of polyalkenyloxysilane

polymer and as a fumigant against larvae of the Caribbean fruit fly ([NTP, 2003](#)).

1.3 Occurrence

1.3.1 Natural occurrence

2,4-Hexadienal is one of the unsaturated aldehydes that are naturally present as auto-oxidation products of polyunsaturated fatty acids (PUFAs) of plant and animal origin ([NTP, 2003](#)).

1.3.2 Occupational exposure

Exposure to 2,4-hexadienal may occur from its inhalation and through dermal contact at workplaces during its production or use.

1.3.3 Dietary exposure

(a) Natural occurrence in food or development during storage or processing

According to [Burdock \(2005\)](#), 2,4-hexadienal, (*E,E*)- has been detected in olives, tomatoes, caviar, fish, auto-oxidized salmon oil, tea, apricots, strawberries, wheaten bread, Russian cheeses, cooked chicken and beef, boiled mutton, hop oil, raw and roasted peanuts, soya beans, rice, buckwheat, malt, kiwi fruit and scallops. The [Council of Europe \(2000\)](#) reported concentrations of 0.001–0.003 mg/kg 2,4-hexadienal, (*E,E*)- in roasted peanuts and 0.0004 mg/kg in kiwi fruit. Quantitative data in the Netherlands database of volatile compounds in food reported highest concentrations of 2,4-hexadienal in apricots (0–0.2 mg/kg), tomatoes (0.186–0.282 mg/kg) and chicken (0.2 mg/kg); lower concentrations were reported in fish (0.03 mg/kg) and malt (< 0.01–0.02 mg/kg). Much lower concentrations were reported in peanuts (0.0005–0.003 mg/kg) and kiwi fruit (0.0004 mg/kg). In addition, qualitative data are available for beef, buckwheat, caviar, various cheeses, guava and feyoa, hop oil, lamb and mutton, olives, rice, scallops, soya

beans, strawberries, thyme and wheaten bread ([TNO, 2010](#)).

According to a review ([NTP, 2003](#)), 2,4-hexadienal, (*E,E*)- occurs naturally in tomatoes, mangoes, kiwi fruit and Chinese quince. It has been detected in cow's milk fat, potato chips, bread crusts, tropical fruit, herbs and spices, in the essential oils of lovage, thyme leaf and dill and in solid alfalfa extract.

2,4-Hexadienal was identified as one of the volatile flavour components of *Callicarpajaponica* Thunb., which belongs to the Verbenaceae family and is indigenous to Japan, the Republic of Korea, the People's Republic of China, and Taiwan, China ([Kim & Shin, 2004](#)).

During cooking, the auto-oxidation process in oil and fat is enhanced. The concentration of PUFA-derived auto-oxidation products generated depends on the PUFA content of the oil, the nature and capacity of the heating vessel used (surface area), and the durations and conditions of heating and storage ([Haywood et al., 1995](#)). The oxidative deterioration of glycerol-bound PUFAs in culinary oils and fats during episodes of heating associated with normal usage (30–90 minutes at 180 °C) has been studied ([Claxson et al., 1994](#)). Thermal stressing of PUFA-rich culinary oils was found to generate high levels of aldehydes, whereas only low concentrations were produced in oils with a low PUFA content, lard and dripping subjected to the above-mentioned heating episodes. Samples of repeatedly used, PUFA-rich culinary oils obtained from restaurants also contained high levels of each class of aldehyde. Auto-oxidation occurs in particular in repeatedly used frying oils in fast-food and take-away establishments ([Claxson et al., 1994](#)). 2,4-Hexadienal has been identified in numerous oxidized glyceridic oils, including canola (low-erucic acid rapeseed), soya bean, cottonseed, sunflower, sesame and palm oils ([NTP, 2003](#)).

(i) Fish, seafood and their products

2,4-Hexadienal may occur in seafood, and was detected in biota collected in the United States of America at Lake Pontchartrain (New Orleans, LA) between May and June 1980: in oysters from the mouth of the Inner Harbor Navigation Canal (35 ng/g wet weight) and in clams from the Chef Menteur Pass (7.5 ng/g wet weight). It was not detected in clams from the Rigolets Pass ([Ferrario et al., 1985](#)).

In the Republic of Korea, 2,4-hexadienal, (*E,E*)- has been identified as a volatile compound in salt-fermented fish paste at concentrations of 491 and 1570 ng/g in anchovy and big-eyed herring pastes, respectively; it was not found in hair tail viscera or shrimp pastes ([Cha & Cadwallader, 1995](#)).

Vacuum steam-deodorized fish oils oxidized under fluorescent light (950 lux) at 21 °C were shown to contain 2,4-hexadienal at a concentration of 60 ng/g ([Karahadian & Lindsay, 1989](#)).

[Venkateshwarlu et al. \(2004\)](#) examined the profiles of volatile compounds in fish oil-enriched milk during cold storage (2 °C) for 14 days, because the development of objectionable, fishy off-flavours is an obstacle in the development of fish oil-enriched foods. 2,4-Hexadienal, (*E,E*)- was detected only on day 14 of storage but not on day 1, 4, 8 or 11.

(ii) Cheese

2,4-Hexadienal was identified as a contributor to the odour of parmesan cheese ([Qian & Reineccius, 2002](#)).

(iii) Fruit and vegetables

[Takeoka et al. \(1986\)](#) identified 48 volatile compounds in kiwi fruit (*Actinidia chinensis* Planch.); 2,4-hexadienal was identified but represented only 0.01% of total volatile compounds, which constituted 2–10 mg/kg of the fruit pulp.

2,4-Hexadienal was identified as one of the volatile components of tomatoes that increases with high nitrogen and potassium fertilization

([Wright & Harris, 1985](#)), and, according to the authors, may contribute to the undesirable flavour of highly fertilized tomatoes.

(iv) *Animal fats*

[Suzuki & Bailey \(1985\)](#) quantitated 52 volatile compounds from lamb fat. The average concentration of 2,4-hexadienal was 1.16 mg/kg in clover-fed lamb fat but was less than 0.1 mg/kg in corn-fed lamb fat.

(v) *Others*

2,4-Hexadienal, (*E,E*)- has been detected as a volatile component of piled Toyama Kurocha tea processed in Japan. It was not found in fresh, steamed or fermented tea leaves, but was reported at a concentration of 4 mg/kg in the solar-dried product and at 2 mg/kg in this product after storage for 1 year ([Kawakami & Shibamoto, 1991](#)).

Overall, the concentration of 2,4-hexadienal that occurs naturally in fresh vegetal and animal products is generally below 0.3 mg/kg. Higher concentrations (above 1 mg/kg) were observed in lamb fat, in some fish products and in tea. Only qualitative data are available on the concentration of 2,4-hexadienal, (*E,E*)- in foods cooked in PUFA-rich culinary oils.

(b) *Food additives*

In the USA, reported levels of use of 2,4-hexadienal, (*E,E*)- range from 0.073 mg/kg in gravies to 2 mg/kg in condiments, relishes and frozen dairy products. Maximum levels of use of up to 6 mg/kg were reported in non-alcoholic beverages. Other reported uses are in alcoholic beverages, soft candy, gelatins and puddings, hard candy, jam and jellies, seasonings and flavourings, snack foods and soups ([Burdock, 2005](#)).

Some essential oils that are added to food contain 2,4-hexadienal. One of these is peppermint oil which is used to flavour candies, chewing gums and liqueurs ([Mookherjee & Wilson, 2008](#)). In a study conducted in 1988, 2,4-hexadienal was

reported to represent 0.1% of the total volatile compounds in the headspace of partially dried, picked peppermint ([Mookherjee *et al.*, 1989](#), cited by [Mookherjee & Wilson, 2008](#)).

[Kim & Shin \(2004\)](#) suggested that the essential oil of the beautyberry *Callicarpa japonica* Thunb. could potentially be useful as a modified-atmosphere packing agent to extend the shelf-lives of instant food, because its volatile flavour components, including 2,4-hexadienal, have antibacterial activity against foodborne microorganisms.

Overall, the presence of 2,4-hexadienal in a large number of foods and food products — either naturally or as an additive — suggests that the majority of the population is exposed to this compound. Consumers are also exposed to 2,4-hexadienal in oxidized oils and fats in the diet.

1.3.4 Environmental occurrence

The production and use of 2,4-hexadienal as a food additive and chemical intermediate may result in its release into the environment through various waste streams.

(a) *Release*

Aldehydes are emitted directly into the atmosphere from a variety of natural and anthropogenic sources and are also formed in situ from the atmospheric degradation of volatile organic compounds ([O'Connor *et al.*, 2006](#)). 2,4-Hexadienal was identified in polluted urban air as a product of photo-oxidation of toluene ([Dumdei *et al.*, 1988](#)). Unsaturated C6 aldehydes, including 2,4-hexadienal, (*E,E*)-, are emitted into the atmosphere from vegetation as a result of leaf wounding ([de Gouw *et al.*, 1999](#); [Fall *et al.*, 1999](#)), and have been detected in numerous field studies ([König *et al.*, 1995](#); [Helmig *et al.*, 1999](#); [Fukui & Doskey, 2000](#); [O'Connor *et al.*, 2006](#)). 2,4-Hexadienal was tentatively identified as a biogenic volatile organic compound in experiments on 63 vegetal species conducted in 1993

in three specific sites in the USA: Atlanta (GA), near Rhinelander (WI) and near Hayden (CO) ([Helmig et al., 1999](#)).

(b) *Terrestrial fate*

Based on a classification scheme ([Swann et al., 1983](#)), an estimated octanol partition coefficient (K_{oc}) value of 17, determined from a structure estimation method ([Meylan et al., 1992](#)), indicates that 2,4-hexadienal is expected to be highly mobile in soil. Its potential volatilization from dry soil surfaces may be expected based upon an estimated vapour pressure of 4.8 mm Hg at 25 °C, determined from a fragment constant method ([HSDB, 2010](#)).

(c) *Aquatic fate*

Based on an estimated K_{oc} value of 17, if released into water, 2,4-hexadienal is not expected to adsorb to suspended solids and sediment. Volatilization from water surfaces may be expected based upon a Henry's Law constant of 9.78×10^{-6} atm.m³/mol ([HSDB, 2010](#)).

(d) *Atmospheric fate*

According to a model of gas/particle partitioning of semi-volatile organic compounds in the atmosphere, 2,4-hexadienal, which has an estimated vapour pressure of 4.8 mm Hg at 25 °C, is expected to exist solely as a vapour. Vapour-phase 2,4-hexadienal is degraded in the atmosphere by a reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 5.9 hours ([HSDB, 2010](#)).

2,4-Hexadienal, (*E,E*)- was found to undergo rapid isomerization to produce a ketene-type compound (probably *E*-hexa-1,3-dien-1-one). The isomerization process was reversible and formation of the reactant was slightly favoured. Although photolysis appears to be an important atmospheric degradation pathway for 2,4-hexadienal, (*E,E*)-, the reversible nature of the

photolytic process means that gas-phase reactions with hydroxyl and nitrate radicals are ultimately responsible for the atmospheric removal of these compounds ([O'Connor et al., 2006](#)).

(e) *Environmental abiotic degradation*

2,4-Hexadienal is not expected to undergo hydrolysis in the environment, due to its lack of hydrolysable functional groups, or to photolyse directly due to its lack of absorption in the environmental ultraviolet spectrum (> 290 nm) ([HSDB, 2010](#)).

1.3.5 Other occurrence

(a) *Indoor air*

In the USA, indoor and outdoor air concentrations of 2,4-hexadienal were measured in three houses per county (10 samples of indoor and outdoor air in each). The minimum level of detection was 0.01 µg/m³. Average indoor and outdoor values, respectively, were: 8.8 µg/m³ and not detected in Yolo County, 1.1 µg/m³ and 0.4 µg/m³ in Los Angeles county, 1.4 µg/m³ and not detected in Placer County, 1.4 µg/m³ and 0.4 µg/m³ in model homes, and 0.5 µg/m³ and 0.4 µg/m³ in new homes. 2,4-Hexadienal was not detected in nylon carpet, wood adhesive, latex paint or drywall but was present in particle board (1 ng/g). In five species of lumber used as building material, it was detected at concentrations of 9–22 ng/g of material in 'yellow poplar', Douglas fir and pine, while emissions of 1400 ng/g of material were observed in red oak ([Seaman et al., 2007](#)).

(b) *Consumer products*

Some essential oils that are added to consumer products contain 2,4-hexadienal, in particular peppermint oils used in oral hygiene products ([Mookherjee & Wilson, 2008](#)).

(c) *Tobacco smoke*

2,4-Hexadienal is a component of tobacco leaf and tobacco-smoke volatiles ([Florin et al., 1980](#); [Weeks et al., 1989](#)).

(d) *Drugs*

Extracts of *Callicarpa japonica Thunb.*, which contain 2,4-hexadienal, are reportedly used in traditional medicine ([Kim & Shin, 2004](#)).

1.4 Regulations and guidelines

In 1974, 2,4-hexadienal,(E,E)- was given ‘Generally Recognized as Safe’ status (No. 3429) by the Flavor and Extract Manufacturers’ Association (FEMA) ([Ford et al., 1988](#)).

2,4-Hexadienal, (E,E)- is listed in the Toxic Substances Control Act Chemical Substance Inventory of the USA ([EPA, 2010](#)). The American Conference of Governmental Industrial Hygienists has not adopted a time-weighted average threshold limit value for this compound ([NTP, 2003](#)).

2,4-Hexadienal, (E,E)- is listed in the register of chemically defined flavourings authorised at national level in the European Union ([European Commission, 2011](#)), where its safety evaluation is on-going ([EFSA, 2009](#)).

2,4-Hexadienal, (E,E)- has been listed by the Council of Europe in category B (flavouring substances for which further information is required before the Committee of Experts is able to offer a firm opinion on their safety in use; these substances can be used provisionally in foodstuff) ([Council of Europe, 2000](#)). Upper levels of use reported are 0.02 mg/kg in beverages and foods in general, with an exception of 1 mg/kg for candy confectionery ([Council of Europe, 2000](#)).

The International Fragrance Association ([IFRA, 2009](#)) has recommended that 2,4-hexadienal (CAS No. 80466-34-8 including all

geometric isomers) not be used as a fragrance ingredient.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Oral administration

See [Table 3.1](#)

3.1.1 Mouse

In a 2-year study, groups of 50 male and 50 female B6C3F₁ mice were administered 2,4-hexadienal (89% *trans,trans* isomer, 11% *cis,trans* isomer) in corn oil by gavage at doses of 0 (controls), 30, 60 or 120 mg/kg body weight (bw) on 5 days a week for up to 105 weeks ([Chan et al., 2003](#); [NTP, 2003](#)). The incidence of squamous-cell papilloma and squamous-cell papilloma or carcinoma (combined) of the forestomach showed a positive trend in male and female mice and was significantly increased in high-dose males and mid- and high-dose females. Forestomach squamous-cell carcinomas were observed in high-dose males and females, and the incidence was significantly increased in females; the incidence of squamous-cell carcinoma in the high-dose male and female groups exceeded historical control ranges. Two high-dose males developed a squamous-cell carcinoma of the tongue. Although not significantly increased relative to controls, the incidence exceeded historical control range.

[Tumours of the forestomach and the tongue are rare spontaneous neoplasms in experimental animals.]

Table 3.1 Carcinogenicity studies of oral administration of 2,4-hexadienal by gavage to experimental animals

Species, strain (sex) Duration	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F ₁ (M, F) up to 105 wk	0, 30, 60 or 120 mg/kg bw in corn oil 5 d/wk for 104–105 wk 50/group	Forestomach (squamous-cell papilloma): M ^a –2/50, 4/50, 5/50, 8/50 F ^b –2/50, 2/49, 11/50, 13/50	<i>P</i> = 0.035 (high-dose M) <i>P</i> = 0.022 (trend M) <i>P</i> = 0.006 (mid-dose F) <i>P</i> < 0.001 (high-dose F) <i>P</i> < 0.001 (trend F)	89% <i>trans,trans</i> isomer 11% <i>cis,trans</i> isomer
		Forestomach (squamous-cell carcinoma): M ^c –0/50, 1/50, 0/50, 2/50 F ^d –0/50, 0/49, 0/50, 7/50	<i>P</i> = 0.007 (high-dose F) <i>P</i> < 0.001 (trend F)	
		Forestomach (squamous-cell papilloma or carcinoma combined): M ^e –2/50, 4/50, 5/50, 10/50 F ^f –2/50, 2/49, 11/50, 18/50	<i>P</i> = 0.009 (high-dose M) <i>P</i> = 0.004 (trend M) <i>P</i> = 0.006 (mid-dose F) <i>P</i> < 0.001 (high-dose F) <i>P</i> < 0.001 (trend F)	
		Tongue (squamous-cell carcinoma): M ^g –0/50, 0/50, 0/50, 2/50	NS	

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M, F) up to 105 wk	0, 22.5, 45, 90 mg/kg bw in corn oil 5 d/wk for 104–105 wk 50/group	Forestomach (squamous-cell papilloma): M ^b –0/50, 3/50, 10/50, 29/50 F ⁱ –0/50, 1/50, 5/50, 17/50	<i>P</i> < 0.001 (mid- and high-dose M, high-dose F) <i>P</i> = 0.031 (mid-dose F) <i>P</i> < 0.001 (trend M, F)	89% <i>trans,trans</i> isomer 11% <i>cis,trans</i> isomer
		Forestomach (squamous-cell carcinoma): M–0/50, 0/50, 1/50, 2/50	NS	
		Forestomach (squamous-cell papilloma or carcinoma combined): M ^b –0/50, 3/50, 11/50, 29/50	<i>P</i> < 0.001 (mid- and high-dose M) <i>P</i> < 0.001 (trend M)	
		Adrenal gland (malignant pheochromocytoma): M ⁱ –0/50, 1/49, 1/50, 4/49	<i>P</i> = 0.050 (high-dose M) <i>P</i> = 0.014 (trend M)	

From [Chan et al. \(2003\)](#); [NTP \(2003\)](#)

^a Historical incidence (mean ± SD) for 2-year feed studies in mice: 10/659 (1.8% ± 1.9%), range, 0–6%

^b Historical incidence (mean ± SD) for 2-year feed studies in mice: 9/659 (1.4% ± 2.0%), range 0–6%

^c Historical incidence (mean ± SD) for 2-year feed studies in mice: 1/659 (0.2% ± 0.6%), range 0–2%

^d Historical incidence (mean ± SD) for 2-year feed studies in mice: 1/659 (0.2% ± 0.6%), range 0–2%

^e Historical incidence (mean ± SD) for 2-year feed studies in mice: 11/659 (2.0% ± 2.0%), range 0–6%

^f Historical incidence (mean ± SD) for 2-year feed studies in mice: 10/659 (1.6% ± 1.9%), range 0–6%

^g Historical incidence for 2-year feed studies in mice: 0/659

^h Historical incidence (mean ± SD) for 2-year feed studies in rats: 2/609 (0.3% ± 0.7%), range 0–2%

ⁱ Historical incidence for 2-year feed studies in rats: 0/659

^j Historical incidence (mean ± SD) for 2-year feed studies in rats: 10/607 (1.7% ± 1.4%), range 0–4%

bw, body weight; d, day or days; F, female; M, male; NS, not significant; SD, standard deviation; wk, week or weeks

3.1.2 Rat

In a 2-year study, groups of 50 male and 50 female rats F344/N were administered 2,4-hexadienal (89% *trans,trans* isomer, 11% *cis,trans* isomer) in corn oil by gavage at doses of 0 (controls), 22.5, 45 or 90 mg/kg bw on 5 days a week for up to 105 weeks ([Chan et al., 2003](#); [NTP, 2003](#)). The incidence of squamous-cell papilloma of the forestomach showed a positive trend in males and females, and was significantly increased in the mid- and high-dose groups. The incidence of squamous-cell papilloma in the mid- and high-dose males and females exceeded the historical control range. Squamous-cell carcinomas of the forestomach occurred in one mid- and two high-dose males. Although not significantly increased, the incidence of squamous-cell carcinoma in these groups exceeded the historical control range. The incidence of malignant pheochromocytoma of the adrenal gland was also increased in males.

[Tumours of the forestomach are rare spontaneous neoplasms in experimental animals.]

4. Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

No studies on the absorption, distribution, metabolism or excretion of 2,4-hexadienal were found in the literature ([NTP, 2003](#)). This may be due to the poor stability of the radiolabelled compound and to its rapid reactivity with nucleophiles in blood components. Since aldehyde dehydrogenases (ALDHs) are key enzymes in the metabolism of aldehydes, [Picchiottino](#)

[& Lee \(2002\)](#) studied their distribution along the gastrointestinal tract of adolescent rats using 2,4-hexadienal as substrate to measure compound-related enzymatic activity. High levels of 2,4-hexadienal-metabolizing ALDH activity were found in the rat forestomach ([Picchiottino & Lee, 2002](#)). Administration of 2,4-hexadienal (12.5–200 mg/kg bw) by gavage to young Sprague-Dawley rats for 5 days produced a dose-dependent increase in ALDH activity in the forestomach and oesophagus, but not in the glandular stomach, liver, small intestine or kidney ([Lee & Picchiottino, 2003](#)). [This activity may provide partial protection from the direct cytotoxic and genotoxic effects of 2,4-hexadienal after oral administration.]

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

(a) *Salmonella reverse mutation assay*

2,4-Hexadienal initially gave negative results in a 'spot-test' mutation assay using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 regardless of the presence of metabolic activation ([Florin et al., 1980](#)). However, a clear mutagenic effect of 2,4-hexadienal (in a dose-response manner over 0.1–1.0 μmol) was noted in *S. typhimurium* strain TA104 in a liquid preincubation assay in the absence of metabolic activation ([Marnett et al., 1985](#)). Subsequent reports indicated the clear mutagenic activity of 2,4-hexadienal (at 0.01–0.75 $\mu\text{L}/\text{plate}$) in strain TA100 in the presence and absence of metabolic activation using a 30-minute preincubation period ([Eder et al., 1992, 1993](#)). As part of a 2-year gavage study, 2,4-hexadienal was tested for the induction of mutations in strains TA98, TA100 and TA1535 strains, and was mutagenic only in

TA100 in the absence of metabolic activation, indicating its role as a direct-acting alkylating agent ([Marnett et al., 1985](#); [Eder et al., 1992](#); [NTP, 2003](#)).

(b) *Mouse lymphoma cell mutation assay*

2,4-Hexadienal induced gene mutations in mouse lymphoma L5178Y cells in the absence of metabolic activation ([Seifried et al., 2006](#)).

(c) *Micronucleus test*

Results on the induction of micronucleated erythrocytes in the bone marrow of male rats and male mice given intraperitoneal injections of acute doses of 2,4-hexadienal were judged to be inconclusive. No increases in micronucleated normochromic erythrocytes were noted in the peripheral blood of male and female mice administered 2,4-hexadienal by gavage for 14 weeks ([NTP, 2003](#)).

(d) *DNA adducts*

Electrophilic α,β -unsaturated aldehydes are considered to be strong direct-acting alkylating agents and are capable of reacting readily with nucleophilic groups of macromolecules such as DNA ([Esterbauer, 1985](#); [Eder & Hoffman, 1993](#); [Eder et al., 1993](#)). 2,4-Hexadienal interacted with calf thymus DNA *in vitro* ([Frankel et al., 1987](#)) and formed 1, N^2 -cyclic-deoxyguanosine and 7,8-cyclic-guanosine adducts in a cell-free system ([Eder et al., 1993](#)). After exposure to 2,4-hexadienal, a significant increase in the level of crotonaldehyde–deoxyguanosine-2 adducts in rat forestomach, but not in rat liver or mouse forestomach, was observed by ^{32}P -postlabelling ([NTP, 2003](#)). Increased levels of DNA single-strand breaks were noted using the alkaline elution technique in L1210 mouse leukaemia cells treated with subtoxic or slightly toxic doses of 2,4-hexadienal ([Eder & Hoffman, 1993](#)). Induction of oxidative DNA damage was investigated in Chinese hamster lung fibroblasts as a

consequence of glutathione depletion induced 2,4-hexadienal ([Glaab et al., 2001](#); [Janowski et al., 2003](#)). After 1 hour of incubation in the Comet assay, 100 and 300 μM 2,4-hexadienal caused a 20% depletion in glutathione levels in V79 cells, 300 μM caused extensive oxidative DNA breakage (> 20%) and 100 μM caused weak DNA damage (< 5%) ([Janowski et al., 2003](#)). 2,4-Hexadienal also induced DNA damage in human epithelial colorectal adenocarcinoma cells ([Glaab, et al., 2001](#)).

4.3 Mechanistic data

4.3.1 Cytotoxicity

2,4-Hexadienal induced 100% cytotoxicity in murine ascites sarcoma BP8 cells at concentrations of 0.1 and 1.0 mM, and nearly 50% cytotoxicity at 0.01 mM ([Pilotti et al., 1975](#)). The cytotoxicity of 2,4-hexadienal has been attributed to a decrease in membrane lipid fluidity ([Thelestam et al., 1980](#); [Witz, 1989](#)). A 20% increase in membrane permeability was observed in human lung fibroblasts incubated with 25 mM 2,4-hexadienal for 30 minutes ([Thelestam et al., 1980](#)). 2,4-Hexadienal caused a 100% inhibition of non-adrenaline-induced oxidative metabolism in isolated hamster brown fat cells at 1 mM and 20% inhibition at 0.1 mM ([Pettersson et al., 1980](#)).

Glutathione S-transferases and antioxidant enzymes, such as glutathione, catalase and superoxide dismutase, function as inducible systems against electrophiles to modulate xenobiotic toxicity ([Enomoto et al., 2001](#); [Nyska et al., 2001](#)). [Nyska et al. \(2001\)](#) found reduced expression of glutathione S-transferase-Pi in the foci of basal-cell hyperplasia and in tumour cells in the rat forestomach epithelium after gavage with 2,4-hexadienal, suggesting changes in cellular protection against oxidative or electrophilic DNA damage. Furthermore, the ability of α,β -unsaturated aldehydes to

function as electrophilic DNA-alkylating agents is also enhanced by their inhibitory effect on the DNA repair enzyme, *O*⁶-methylguanine–DNA methyltransferase, which has a cysteine residue in its active site (Krokan *et al.*, 1985; Witz, 1989). Other enzymes inhibited by 2,4-hexadienal include microsomal glucose-6-phosphatase, cytochrome P450 (not specified), aminopyrine demethylase and adenylate cyclase (Jørgensen *et al.*, 1992; NTP, 2003).

Aldo-keto reductase family 1 B10 protein is specifically expressed in the small intestine and colon and may be important in protecting the intestinal epithelium by detoxifying cytotoxic dietary and lipid derived α,β -unsaturated carbonyls, such as 2,4-hexadienal. The reduction of 2,4-hexadienal to its corresponding alcohol by this enzyme had a Michaelis-Menten kinetics value of 96 μ M (Zhong *et al.*, 2009).

4.3.2 Cell proliferation

2,4-Hexadienal is cytotoxic and, similarly to other aldehydes, inhibits cell proliferation (Pilotti *et al.*, 1975). In contrast, it also increases the incidence of hyperplasia, squamous-cell papilloma and squamous-cell carcinoma in the forestomach of rats and mice (NTP, 2003).

4.4 Mechanisms of carcinogenesis

Forestomach squamous-cell neoplasms and squamous epithelial hyperplasia (generally recognized as a precursor lesion of neoplasia) developed in rodents administered 2,4-hexadienal by gavage, probably because α,β -unsaturated carbonyls are highly reactive mutagens and carcinogens and tend to be unstable in feed (NTP, 2003). In addition, squamous-cell carcinoma of the oral cavity (tongue) occurred in some male mice, raising the possibility that these rare tumours may be related to exposure to 2,4-hexadienal.

The carcinogenic mechanism is still not clear, but there is convincing evidence that 2,4-hexadienal is a direct-alkylating mutagen in *S. typhimurium* strains TA104 and TA100 (Marnett *et al.*, 1985; NTP, 2003). In addition, 2,4-hexadienal has been shown to interact with calf thymus DNA *in vitro* and to induce DNA strand breaks in mouse leukaemia cells and Chinese hamster lung fibroblasts (Frankel *et al.*, 1987; Eder *et al.*, 1993; Janzowski *et al.*, 2003). These findings imply that highly reactive carbonyl compounds such as 2,4-hexadienal may induce genotoxicity in target tissues by interacting with proteins and enzymes (Esterbauer, 1985; Krokan *et al.*, 1985; Eder *et al.*, 1992). After 14 weeks of exposure to 90 mg/kg 2,4-hexadienal (a dose as high as the high dose used in the 2-year cancer bioassay), crotonaldehyde–deoxyguanosine-2 adduct levels were increased in the rat forestomach (NTP, 2003). Reactive oxygen species, a result of lipid peroxidation during the inflammatory response, can cause accumulation of oxidative DNA damage in forestomach in the form of 8-hydroxydeoxyguanosine. The small increase in chronic inflammation of the forestomach and forestomach ulcers observed in the high-dose group of male rodents only in the 2-year study does not fully support the hypothesis that the cytotoxicity of 2,4-hexadienal was the mechanism that induced dose-related increases in forestomach neoplasms in male and female rats and mice.

There is moderate evidence that tumour induction occurs via a genotoxic mechanism.

5. Summary of Data Reported

5.1 Exposure data

Technical-grade 2,4-hexadienal (80% *trans,trans* isomer, 10–16% *cis,trans* isomer) is prepared by the condensation of acetaldehyde.

2,4-Hexadienal occurs in many foods, and levels increase during cooking as a result of

auto-oxidation of polyunsaturated fatty acids of plant and animal origin. 2,4-Hexadienal is also added to food as a flavouring ingredient. It is used as an intermediate in the production of 3,5,7-nonatrien-2-one, sorbic acid, polymethine dyes, mitomycins and antihypercholesteremics. Occupational exposure to 2,4-hexadienal may occur during its production and use.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

2,4-Hexadienal was tested for carcinogenicity by oral administration by gavage to mice and rats. In mice, it increased the incidence of forestomach squamous-cell papilloma and carcinoma in females, squamous-cell papilloma or carcinoma (combined) in males and females, and squamous-cell carcinoma of the tongue in males. In rats, oral administration of 2,4-hexadienal caused an increase in the incidence of forestomach squamous-cell papilloma in males and females, forestomach squamous-cell papilloma or carcinoma (combined) in males, and malignant pheochromocytoma of the adrenal gland in males.

Tumours of the forestomach and the tongue are rare spontaneous neoplasms in experimental animals.

5.4 Other relevant data

No toxicokinetic data were available to the Working Group.

2,4-Hexadienal is a direct-acting alkylating agent that forms DNA adducts both *in vivo* and *in vitro*, causes glutathione depletion and DNA strand breaks, and is mutagenic in bacteria.

A small increase in chronic inflammation of the forestomach in male rats and in forestomach

ulcers in male mice was observed only in the high-dose groups in the cancer bioassay. This does not fully support the hypothesis that cytotoxicity was the mechanism that induced the dose-related increases in forestomach neoplasms in male and female rats and mice. The relevance of the tumour response in experimental animals to humans cannot be excluded.

There is moderate evidence that tumour induction occurs via a genotoxic mechanism.

The mechanistic data provide some additional support for the relevance of the animal carcinogenicity data to humans.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2,4-hexadienal.

6.3 Overall evaluation

2,4-Hexadienal is *possibly carcinogenic to humans* (Group 2B).

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METHYLEUGENOL

1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 93-15-2

Chem. Abstr. Name: 1,2-Dimethoxy-4-(2-propenyl)benzene

IUPAC Systematic Name:

1,2-Dimethoxy-4-prop-2-en-1-yl-benzene

Synonyms: 1-Allyl-3,4-dimethoxybenzene;

4-allyl-1,2-dimethoxybenzene; 4-allyl-

veratrole; benzene, 4-allyl-1,2-dimethoxy-;

benzene, 1,2-dimethoxy-4-(2-propenyl)-;

1,2-dimethoxy-4-allylbenzene; 3,4-dimethoxyallylbenzene;

1-(3,4-dimethoxyphenyl)-

2-propene; 1,2-dimethoxy-4-(2-propen-1-yl)

benzene; 1,3,4-eugenol methyl ether;

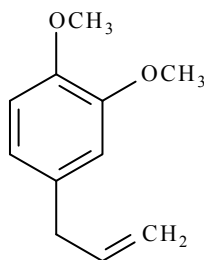
eugenyl methyl ether; methyleugenol;

methyl eugenol; O-methyl eugenol;

veratrole methyl ether

EINECS No.: 202-223-0

1.1.2 Structural and molecular formulae and relative molecular mass



C₁₁H₁₄O₂

Relative molecular mass: 178.23

1.1.3 Chemical and physical properties of the pure substance

Description: Colourless to pale yellow liquid with a clove-carnation odour and a bitter taste ([NTP, 2000](#))

Boiling-point: bp₃₀, 146–147 °C;

bp₇₆₀, 244 °C ([O'Neil et al., 2006](#))

Melting-point: –2 °C ([Lide, 2010](#))

Density: 1.0396 at 20 °C ([Lide, 2010](#))

Solubility: Soluble in ethanol, ethyl ether, chloroform and most other organic solvents; insoluble in water, glycol and propylene glycol ([NTP, 2000](#))

Volatility: Evaporates readily at room temperature ([NTP, 2000](#))

Stability: Darkens and slowly thickens when exposed to air ([NTP, 2000](#))

Octanol/water partition coefficient (P): log K_{ow}, 3.45 ([Sangster, 2010](#))

1.1.4 Technical products and impurities

Methyleugenol is commercially available with the following specifications: purity, 98.0% min.; eugenol, 1.0% max. ([Elan Chemical Company, 2007](#)).

1.1.5 Analysis

The presence of methyleugenol in essential oils and aromatic plants can be determined by gas chromatography (GC)–mass spectrometry (MS) ([Stanfill & Ashley, 1999](#); [Miele *et al.*, 2001](#); [Kothari *et al.*, 2004](#); [Hamm *et al.*, 2005](#); [Boussaada *et al.*, 2008](#); [Verdian-rizi & Hadjiakhoondi, 2008](#); [Zheljazkov *et al.*, 2008](#); [Pino Benitez *et al.*, 2009](#); [Lamas *et al.*, 2010](#)) and high-performance liquid chromatography with ultraviolet (UV) detection ([Chen *et al.*, 2009](#); [Gursale *et al.*, 2010](#)).

Methyleugenol has been determined in cosmetic creams applied to the skin by direct contact sorptive tape extraction–GC–MS ([Sgorbini *et al.*, 2010](#)).

Methyleugenol can be measured in human serum through solid-phase extraction followed by isotope dilution GC–high resolution MS, with a limit of detection of 3.1 pg/g ([Barr *et al.*, 2000](#)).

1.2 Production and use

1.2.1 Production

Methyleugenol is produced by the methylation of eugenol ([Burdock, 2005](#)).

The annual production of methyleugenol in the United States of America in 1990 was estimated at 11.4 tonnes ([NTP, 2000](#)).

Information available in 2010 indicated that methyleugenol was manufactured by 19 companies in the USA, four companies in the People's Republic of China, two companies each in Germany and China (Hong Kong SAR), and one company each in France, India, Indonesia, Japan and the United Kingdom ([Chemical Sources International, 2010](#)). [HSDB \(2010\)](#) reported three additional companies in the USA that produced methyleugenol.

1.2.2 Use

Methyleugenol is used as a flavouring agent in jellies, baked goods, non-alcoholic beverages, chewing gum, candy, puddings, relishes and ice cream. It is also widely used as a fragrance ingredient in perfumes, toiletries and detergents. Methyleugenol has been used as an anaesthetic in rodents. It also is used as an insect attractant in combination with insecticides ([NTP, 2000](#); [HSDB, 2010](#)).

Methyleugenol is a component of several essential oils that are sold for use in aromatherapy, massage oils and alternative medicines ([Government of Canada, 2010](#)).

For centuries, fennel fruits have been used as a traditional herbal medicine in Europe and China. It is administered as a carminative to infants in private homes and in maternity clinics and is highly appreciated for its mild flavour and good tolerance. In several European Union (EU) countries, sweet fennel herbal tea is traditionally used for the treatment of symptoms in digestive upsets. In Germany, bitter fennel herbal tea is used by most of the population as a remedy for colds ([European Medicines Agency, 2008](#)).

Some essential oils, including citronella (*Cymbopogon* spp.), basil (*Ocimum* spp.), bay (*Laurus nobilis*) and tea tree (*Melaleuca* spp.), that may contain a high percentage of methyleugenol are used as fragrances in consumer products, such as personal care products and household cleaners ([Environment Canada, 2010](#)).

Citronella oil, which may contain methyleugenol, is an active ingredient in some commercially available personal insect repellent lotions and sprays that are applied to the skin. It is also used in outdoor candles and torches as an ambient insect repellent ([Environment Canada, 2010](#)).

Methyleugenol is used as a fragrance in perfumes (0.3–0.8%), creams and lotions (0.01–0.05%), and soaps and detergents (0.02–0.2%) ([NTP, 2000](#)).

1.3 Occurrence

1.3.1 Natural occurrence

Methyleugenol is a natural constituent of a large number of essential oils of plant origin and, in some cases, may be the major constituent.

A comprehensive review of the methyleugenol content of essential oils from different botanical sources has been published ([Burfield, 2004a](#)). The data reported in this review were derived from a variety of sources, including unpublished data distributed to its members by the US Flavor and Extract Manufacturers' Association, those related to commercial oils analysed by the British Essential Oil Organization in 2001, those available on the International Fragrance Association web site (www.ifraorg.org), and those reported in the Agricultural Research Services database (www.ars-grin.gov).

Overall, 118 analytical determinations of methyleugenol in essential oils were considered in the review by [Burfield \(2004a\)](#). In four cases, for example, in *Cinnamomum camphora* (camphor oil, white from China), the substance was not detected. In 73 cases, the reported methyleugenol content was below 2%, for example, in *Artemisia dracunculus* French type (tarragon), *Syzygium aromaticum* (clove), *Daucus carota* (carrot), *Myristica fragrans* (nutmeg) and *Rosmarinus officinalis* (rosemary). In 10 cases, high methyleugenol contents were reported: *Anasarum canadense* (snakeroot), 36–45%; *Artemisia dracunculus* (tarragon oil Russian type), 5–29%; *Dacrydium franklinii* (Huon pine oil), up to 98%; *Echinophora tenuifolia* from Turkey, 17.5–50%; *Melaleuca bracteata*, up to 50%; *Melaleuca leucadendron*, up to 97%; *Ocotea pretiosa* (Brazilian sassafras), up to 50%; and *Pimenta racemosa* var. *racemosa* (bay leaf), up to 48.1%. The methyleugenol content of *Ocimum basilicum* (basil) varies considerably according to the reported chemotype, from 1.6% in some products up to 55–65% in the case of var. 'grand

vert' and var. *minimum* (known as small basil) ([Burfield, 2004a](#)). Methyleugenol is also the main constituent of the essential oil of *Melaleuca bracteata* F.v.M. leaves (90–95%) and *Cinnamomum oliveri* Bail. leaves (90–95%) ([Burdock, 2005](#)). Other data on the content of methyleugenol in aromatic plants have been reported ([De Vincenzi et al., 2000](#)).

The amount of methyleugenol in an essential oil extracted from a given type of plant differs according to the variety, plant maturity at the time of harvesting, the method of harvesting, storage conditions and the method of extraction ([Smith et al., 2002](#)).

The [European Medicines Agency \(2005\)](#) has reviewed the methyleugenol content of the parts of plants that are generally used. Thyme species are widely used as medicinal herbs ([ElHadj Ali et al., 2010](#)), and are increasingly used in perfumery, cosmetic and medicinal applications. The mean content of methyleugenol in the essential oil of *Thymus algeriensis* leaves ranged between < 0.01 and 6.9%.

1.3.2 Occupational exposure

The National Institute for Occupational Safety and Health, in its National Occupational Exposure Survey conducted in 1981–83, estimated that, among the 4490 establishments surveyed (522 industry types, employing approximately 1 800 000 workers), 2824 workers (including 877 women) were potentially exposed to methyleugenol in the USA ([NIOSH, 1990](#)).

Methyleugenol was registered as an active pesticide ingredient in the USA in 2006 ([EPA, 2006](#)). It is an insect parapheromone, which is attractive to male fruit flies ([Australian Government, 2005](#)), and is used in insect traps to attract certain species such as the oriental fruit fly. According to the US Environmental Protection Agency (EPA), because methyleugenol is used for the control of fruit flies in traps, no contact is expected by workers using the traps, but may

occur for workers who prepare the pesticide and methyleugenol mixture or fill the traps with the mixture.

Moreover, aromatherapists are liable to be exposed repeatedly to methyleugenol-containing oils through dermal contact ([Burfield, 2004b](#)).

1.3.3 Dietary exposure

(a) Occurrence in food

Some examples of common culinary herbs and spices that contain methyleugenol are basil, tarragon, lemon grass, bay leaf, nutmeg, allspice, cloves and mace ([Environment Canada, 2010](#)).

Methyleugenol is also contained in edible fruit such as grapefruit, bananas and some forest fruit at a level of less than 0.1 mg/kg ([TNO, 2010](#)). Methyleugenol was identified as a volatile flavour compound in the juice of Kogyoku apples ([Yagima et al., 1984](#)).

The methyleugenol content of basil has been studied very extensively ([Tsai & Sheen, 1987](#); [Green & Espinosa, 1988](#), cited by [Smith et al., 2002](#); [Lawrence et al., 1988](#); [Bobin et al., 1991](#); [Sheen et al., 1991](#); [Lawrence & Shu, 1993](#))

(b) Occurrence in food as a flavouring substance or as constituent of essential oils

Processed foodstuffs can be flavoured with essential oils or extracts of specific plants that contain methyleugenol such as sassafras (*Sassafras albidum*) leaf extracts, tarragon (*Artemisia dracuncululus* L.), laurel (*Laurus nobilis* L.) and Ceylon citronella (*Cymbopogon nardus*) ([Burdock, 2005](#)).

It has been reported to be added as a flavouring agent to baked goods (27–40 mg/kg), chewing gum (10–45 mg/kg), condiments and relishes (3–7 mg/kg), frozen dairy products (15–17 mg/kg), gelatins and puddings (15–17 mg/kg), hard candy (0.6 mg/kg), non-alcoholic beverages (9–12 mg/kg) and soft candy (19–24 mg/kg) ([Burdock, 2005](#)).

Some brands of cookies available in the USA were found to contain approximately 3.3 mg/kg methyleugenol as an added flavouring, i.e. 18 µg/cookie. Lower concentrations were found (in decreasing order) in other brands of gingersnaps, cinnamon-flavoured oatmeal, vinaigrette salad dressing, cinnamon-flavoured mints, chewing gum, cake doughnuts and cola beverages. In 20 other brands of gingersnaps and other cookies, doughnuts, colas and foods flavoured with cinnamon, nutmeg or ginger, methyleugenol was either not detected or was found at concentrations < 0.05 mg/kg ([Schechter et al., 2004](#)).

Methyleugenol was measured in a limited number of well defined food products purchased on the Italian national market, and was found to be present in tomato sauce containing basil (0.01–0.33 mg/kg) and in Vienna sausage (0.10–0.14 mg/kg), probably due to the addition of nutmeg ([Siano et al., 2003](#)).

(c) Estimates of dietary exposure

Ocimum basilicum cv. Genovese Gigante is by far the most popular basil cultivar used in the production of a typical Italian sauce called pesto. Pesto is traditionally prepared with basil that is 10–12 cm in height, when the percentage of methyleugenol in the essential oil is generally more than 40%. Considering that, at this stage of growth, the amount of essential oil in *O. basilicum* cv. Genovese Gigante corresponds to ~0.5% and that one portion of pesto contains ~10 g of basil, the resulting dietary exposure to methyleugenol could reach 250 µg/kg body weight (bw) per meal in adults and 500 µg/kg bw per meal in children ([Miele et al., 2001](#)).

[Smith et al. \(2002\)](#) estimated dietary exposure to methyleugenol using data on the annual volumes of plant materials with methyleugenol-containing essential oils (principally spices), methyleugenol-containing essential oils used as flavour ingredients and neat methyleugenol used as a flavouring substance imported and

consumed in the USA in 1999. The dietary intake of methyleugenol was estimated for a 60-kg bw adult and the assumption that methyleugenol would be consumed by 10% of the population of the USA. The plant materials considered were bananas and spices (anise, basil (dried and fresh), mace, nutmeg, pimento berry (allspice) and tarragon) and lead to an estimated dietary exposure of 0.50 µg/kg bw per day, mainly from basil (assuming an average concentration of methyleugenol of 2.6% in dried basil and 0.11% in fresh basil), nutmeg and allspice. The essential oils considered were basil, bay (leaves and sweet oil), citronella, clove (bud), nutmeg, pimento berry and pimento leaf and lead to an estimated dietary exposure of 0.16 µg/kg bw per day, mainly from nutmeg oil. The estimated dietary exposure from methyleugenol used as an added flavouring substance was 0.11 µg/kg bw per day. Thus, the overall dietary exposure was estimated to be 0.77 µg/kg bw per day ([Smith et al., 2002](#)).

The Joint FAO/WHO Expert Committee on Food Additives published an evaluation of a group of alkoxy-substituted allylbenzenes, including methyleugenol ([JECFA, 2009](#)). Per-capita dietary exposure was assessed by dividing the volumes of spices, herbs and oils in the USA and Europe, as reported by industry, by the total population (320 million in Europe and 280 million in the USA), and considering the range of concentrations of methyleugenol in oil samples. The lower/upper limits and mean values for dietary exposure to methyleugenol were: 2.5/424 and 80.5 µg per day (USA) and 0.6/39 and 9.6 µg per day (Europe) ([Williams & Mattia, 2009](#)).

Based on annual production volumes of 77 kg methyleugenol in the USA ([Gavin et al., 2007](#)), per-capita intake as flavouring agents for the whole population was estimated to be 0.8 µg per day in the USA. Estimated total dietary exposure in the USA would therefore amount to 81.3 µg per day versus 9.6 µg per day in Europe ([Smith et al., 2010](#)).

[The per-capita intake assessed by [Williams & Mattia \(2009\)](#) considered the whole population as consumers. Estimates obtained by assuming that 10% of the population were consumers would lead to a total dietary exposure of 813 µg per day in the USA and 96 µg per day in Europe, i.e. 13.5 and 1.6 µg/kg bw per day, respectively, for a 60-kg adult.]

The intake of methyleugenol estimated by the United Kingdom delegation to the Council of Europe was considered by the Scientific Committee on Food ([European Commission, 2001](#)). The average intake (for consumers only) amounted to 13 mg/person per day and the 97.5th percentile was 36 mg/person per day. If expressed on the basis of adult size, these values correspond to 0.19 mg/kg bw per day and 0.53 mg/kg bw per day, respectively. These estimates were based on maximum used levels of methyleugenol provided by the European Flavour and Fragrance Association from a global industry survey. [It should be noted that this estimate pre-dated the decision to remove the use of methyleugenol as a flavouring additive from the EU register. In this study, it was assumed that methyleugenol was maximally added to the following food categories: non-alcoholic beverages (including all soft drinks and fruit juices), alcoholic beverages (liqueurs only), ices (including ice cream and ice lollies), candy (excluding chocolate), baked goods, gelatin-based desserts, meat products and condiments and relishes (including sauces and spreads). A United Kingdom survey performed through 7-day dietary records on 2197 adults (16–65 years old) was used as the basis for consumption estimates.]

[Smith et al. \(2010\)](#) assessed dietary exposure to methyleugenol using the theoretical added maximum daily intake technique, assuming that maximum levels of methyleugenol in food were those regulated by the EU ([European Commission, 2008a](#)). The calculation was made assuming a concomitant daily consumption of 324 g of beverages in general (containing

1 mg/kg methyleugenol), 133.4 g of food in general (20 mg/kg methyleugenol), 20 g of sauces and condiments (60 mg/kg methyleugenol) and 20 g of ready-to-eat savouries (20 mg/kg methyleugenol). A maximum intake of 4.6 mg methyleugenol per day was calculated for the European adult population which, assuming an average body weight of 70 kg, would be equivalent to 66 µg/kg bw per day.

In conclusion, dietary exposure to methyleugenol may arise from: (i) the ingestion of fruits and vegetables containing methyleugenol (minor source), (ii) the ingestion of herbs and spices containing methyleugenol (primary source), (iii) the ingestion of essential oils and extracts of herbs and spices used to flavour food and beverages and (iv) the ingestion of methyleugenol added directly as a flavouring to food and beverages (outside Europe). Average total dietary exposures are in the range of 8–81.3 µg per day, i.e. 0.13–1.35 µg/kg bw per day for a 60-kg bw adult. Total dietary exposures assessed for regular consumers of food containing methyleugenol are in the range of 66–514 µg/kg bw per day (Smith *et al.*, 2010).

1.3.4 Environmental occurrence

(a) Releases

Methyleugenol was detected at a concentration of 5 ppb [0.005 mg/L] in wastewater from a New Jersey (USA) publicly owned treatment works facility located at an industrial site (industrial contribution to the influent is 18%) (Clark *et al.*, 1991), and in the raw and partially treated effluent of one unbleached kraft paper mill at concentrations of 0.001–0.002 mg/L, but not in the final effluent (Keith, 1976).

Shaver & Bull (1980) described the fate of methyleugenol in the environment after its use in a fruit fly control programme; the study was carried out on soil, water and tomatoes, a representative crop that might be affected by the use of methyleugenol. Moreover, the proposed use of

methyleugenol in a male annihilation programme that involved the aerial distribution of cigarette filters saturated with the lure and malathion over fruit fly-infested areas was investigated. Methyleugenol was shown to dissipate rapidly from both soil and water. At 32 °C, 98% of the material was lost within 96 hours, and 77% and 81% were lost from water and soil, respectively, after 96 hours at 22 °C. Methyleugenol had a half-life of approximately 6 hours in soil and water at 32 °C and 16 hours and 34 hours in soil and water at 22 °C, respectively. The persistence of methyleugenol in water was very similar for treatment rates of 1 and 10 mg/100 mL water. Methyleugenol disappeared rapidly from the surface of field-grown tomatoes treated topically with 1 mg/fruit of the fruit fly control product. Only 3.8% of the dose was recovered in the external wash after 24 hours, and none was detected 3, 7 and 14 days after treatment. No methyleugenol was detected in tomatoes from plants that had been exposed to a cigarette filter containing 0.5 mL methyleugenol placed at the base of the plant (Shaver & Bull, 1980).

The California Department of Food and Agriculture began an oriental fruit fly (*Dacus dorsalis*) eradication programme in California (USA) in 1988 (Turner *et al.*, 1989) using methyleugenol (Dorsalure ME) and the pesticide Naled (Dibrom 14 Concentrate). Methyleugenol is used to attract male oriental fruit flies to bait stations that are set up during eradication programmes and to traps placed in fruit trees to detect new infestations.

In October 1988, the Environmental Hazards Assessment Program of the California Department of Food and Agriculture determined the concentrations of methyleugenol in ambient air and fruit during oriental fruit fly trapping for this programme. In Los Angeles county, the air in the vicinity of insect traps baited with methyleugenol was analysed for the presence of the substance 0–5 days after the bait stations were set up. During the first application,

methyleugenol was detected in samples on days 0 (353–1050 ng/m³) and 1, but not on day 5, at a distance of 5 m from the traps. During the fourth application, concentrations did not decrease significantly over time. The variability in methyleugenol concentrations found during the fourth application is believed to be due to microclimate variations within each site and variable bait application. Whole citrus fruit samples were collected from a detection area in September 1988 in Sacramento County. Methyleugenol was detected in several fruit from two of the four sites sampled at concentrations ranging from 70 to 210 ppb [$\mu\text{g}/\text{kg}$] ([Turner et al., 1989](#)).

(b) Terrestrial fate

Based on its physical properties (see Section 1.1.3), methyleugenol is expected to be highly mobile in soil. However, the compound was immobile in a silty loam, Lufkin fine sandy loam, Houston clay and Brazos river bottom sand from Texas (USA) using soil thin-layer chromatography ([Shaver, 1984](#)).

The volatilization of methyleugenol from moist soil surfaces is expected to be an important process ([HSDB, 2010](#)). Dissipation half-lives of 6 and 16 hours in soil and water at 32 and 22 °C, respectively, have been measured ([Shaver & Bull, 1980](#)). Methyleugenol is not expected to volatilize from dry soil surfaces based upon its vapour pressure ([Perry & Green, 1984](#), cited by [HSDB, 2010](#)). Biodegradation may be an important environmental process in soil ([HSDB, 2010](#)).

(c) Aquatic fate

Based on its physical and chemical properties, methyleugenol is not expected to adsorb to suspended solids and sediment. Volatilization from water surfaces is expected ([Lyman, 1990](#), cited by [HSDB, 2010](#)), with half-lives for a model river and model lake of 9 and 69 days, respectively. Dissipation half-lives of 6 and 34 hours in water at 32 and 22 °C, respectively, have been measured ([Shaver & Bull, 1980](#)). Its potential for

bioconcentration in aquatic organisms is low, but biodegradation may be an important environmental process in water ([HSDB, 2010](#)).

(d) Atmospheric fate

Methyleugenol is expected to exist almost entirely as a vapour in the ambient atmosphere ([HSDB, 2010](#)). Vapour-phase methyleugenol is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 5 hours at an atmospheric concentration of 5×10^5 hydroxyl radicals per cm³ ([Meylan & Howard, 1993](#)). The rate constant for the vapour-phase reaction of methyleugenol with photochemically produced hydroxyl radicals has been estimated to be 7.5×10^{-11} cm³/molecule.s at 25 °C using a structure estimation method, which corresponds to an atmospheric half-life of approximately 5 hours ([Meylan & Howard, 1993](#)). The rate constant for the vapour-phase reaction of methyleugenol with ozone has been estimated to be 1.2×10^{-17} cm³/molecule.s at 25 °C using a structure estimation method ([Meylan & Howard, 1993](#)), which corresponds to an atmospheric half-life of about 1 day at an atmospheric concentration of 7×10^{11} ozone molecules per cm³ ([Atkinson & Carter, 1984](#)). Methyleugenol is not expected to undergo hydrolysis in the environment due to its lack of hydrolysable functional groups ([Lyman, 1990](#)) nor to photolyse directly due to its lack of absorption in the environmental UV spectrum (> 290 nm) (cited by [HSDB, 2010](#)).

(e) Environmental exposure models

Based on its physical and chemical properties and taking into consideration its estimated half-lives in air (5 hours), water (measured as 8 days), soil (8 days, estimated to be the same as that in water) and sediments (32 days, estimated at four times that in water), methyleugenol is expected to reside mainly in the environmental compartment into which it is released ([Environment Canada, 2010](#)).

1.3.5 Exposure of the general population

The general population may be exposed to methyleugenol via inhalation and by dermal contact with consumer products that contain methyleugenol.

(a) Indoor exposure

Fragrances are present in household products, air fresheners, insecticides and cosmetics, and, because of their nature, inhalation exposure should be considered as an important pathway, especially in indoor environments ([Lamas et al., 2010](#)). Because people in developed countries spend up to 90% of their time indoors, inhalation of indoor air is potentially the most important exposure pathway to many pollutants ([Brown et al., 1994](#); [Molhave et al., 1997](#)).

(b) Cosmetic ingredients

Potential exposure to methyleugenol from the use of personal care products made with essential oils that contain methyleugenol was assessed by [Environment Canada \(2010\)](#) using consumer exposure modelling software. For adult women, estimated daily systemic exposure to methyleugenol as a result of dermal exposure only through the aggregate use of four types of personal care products (body lotion, face moisturizer, skin cleanser and fragrance) formulated with various essential oils that contain methyleugenol was 1.5 µg/kg bw per day (1 µg/kg bw per day from fragrance, 0.2 µg/kg bw per day from body lotion, 0 µg/kg bw per day from face cream and 0.3 µg/kg bw per day from skin cleanser). These estimates were based on the following assumptions: (i) methyleugenol was present at the upper level authorized in the EU ([SCCNFP, 2000](#)) and Canada ([Health Canada, 2010](#)); (ii) the dermal absorption of methyleugenol was 40% for products applied to the skin; and (iii) the permeability coefficient was 0.0221 cm/h for skin cleanser that was washed off. The estimates of exposure from the use of personal care products

are not expected to differ appreciably across age groups. The concentration of methyleugenol in plant-derived material is quite variable, and there is significant uncertainty associated with these estimates ([Environment Canada, 2010](#)).

These estimates do not include exposure arising from the use of dental or oral hygiene products. For example, clove flower oil is licensed for sale in Canada as a non-prescription dental analgesic ([Environment Canada, 2010](#)).

In a study in which the skin (stratum corneum) of volunteers was treated with a cream of known composition, approximate permeation of methyleugenol through skin was reported to be 14.5% ([Sgorbini et al., 2010](#)). Within a survey of hand soaps performed in Denmark, three products that contained scent of roses were analysed for methyleugenol (methyleugenol is a natural component of rose oil). All were below the limit of detection (10 mg/kg) ([Danish EPA, 2006](#)).

(c) Insect repellent

[Health Canada \(2004\)](#) assessed the exposure to methyleugenol due to its presence in citronella oil that is used as a personal insect repellent. Assuming that 1 mg/cm² citronella oil is applied, that 25% of the body surface is treated (i.e. 4610 cm² in a 70-kg adult and 1641 cm² in a 15-kg child) [based on this estimation, the Working Group calculated that each application would result in 66 and 109 mg/kg bw citronella oil for adults and children, respectively] and that the concentration of methyleugenol in the product would be 0.0002%, an exposure of 0.13 µg/kg bw for adults and 0.21 µg/kg bw for children would occur.

(d) Tobacco smoke

In a study of eight commercial brands of cigarettes in the USA, methyleugenol was identified in the smoke particulate of unblocked cigarettes at a level above the limit of detection (1.1 ng/cigarette) in only one brand (average of three measurements: 46.5 ng in the smoke

particulate of one cigarette) ([Stanfill & Ashley, 2000](#)). The effect of blocking ventilation holes in the cigarette filter was assessed in another brand (containing 81 ng/cigarette). Methyleugenol was not detected in the unblocked cigarette smoke, whereas it was detected in the smoke when the holes were partially or fully blocked (6.4 ng and 10.8 ng in the smoke particulate of one cigarette, respectively).

Bidi cigarettes (small hand-rolled cigarettes produced primarily in India) are sold in the USA in a wide variety of exotic (e.g. clove and mango) and candy-like flavours (e.g. raspberry, dewberry, chocolate and clove) and are popular among adolescents. Certain tobacco flavourings contain alkenylbenzenes, including methyleugenol ([Stanfill et al., 2003, 2006](#)).

Methyleugenol was detected in 11/20 *bidi* cigarettes purchased in the USA and in Indian *bidi* cigarettes at levels ranging from 0.49 µg/g to 61 µg/g. The highest levels were found in Kailas Strawberry brand (47–61 µg/g) followed by Darshan Clove brand (5.1–12 µg/g). Lower levels of methyleugenol were observed in US cigarettes, ranging from 0.018 to 0.021 µg/g ([Stanfill et al., 2003](#)).

In a study by [Stanfill et al. \(2006\)](#), compounds in the burnable portions of the filler and wrapper material actually consumed during the smoking of *bidis* and US cigarettes were analysed. Methyleugenol was not detected (< 6.3 µg/cigarette) in the three US cigarettes, and was detected in only two *bidis*: Azad clove brand (not detected–7.52 µg/cigarette) and Azad herbal brand (27.7–36.6 µg/cigarette).

In Canada, exposure to methyleugenol through cigarette smoking is liable to decrease because, in May 2009, the Government of Canada introduced amendments to the Tobacco Act to prohibit the sale of cigarettes, little cigars and blunt wraps (leaf-wrapped tobacco) with flavours and additives that taste like candy ([Government of Canada, 2009](#)).

1.3.6 Total human exposure

According to the [Government of Canada \(2010\)](#), exposure to methyleugenol is dominated by the ingestion of food and beverages, with smaller contributions from the use of personal care products, cosmetics and citronella-based insect repellents.

(a) Biomonitoring data

The Centers for Disease Control and Prevention in the USA measured methyleugenol in a non-representative subset of adult serum samples collected as a part of the Third National Health and Nutrition Examination Survey conducted during 1988–94 ([Barr et al., 2000](#)). The mean methyleugenol concentration in this subset was approximately 24 pg/g serum, and ranged from < 3.1 to 390 pg/g serum. Methyleugenol was detected in 98% of the 206 adult human serum samples analysed, indicating that human exposure in the USA is ubiquitous. Only four individuals had methyleugenol concentrations below the limit of detection (< 3.1 pg/g). The 5–95% distribution was 5–78 pg/g in serum. Bivariate and multivariate analyses using selected demographic variables showed only marginal relationships between race/ethnicity and sex/fasting status and methyleugenol serum concentrations. The data on integrated exposure to methyleugenol derived from biomonitoring indicate that serum levels as high as 390 pg/g have been measured and may be higher in some individuals depending on such factors as diet, genetics and body weight ([Barr et al., 2000](#)).

In a study by [Schechter et al. \(2004\)](#) involving nine volunteers, the highest blood levels after consumption of about 216 µg methyleugenol (contained in 12 gingersnap cookies) corresponding to 3.16 µg/kg bw were about 100 pg/g. About 15 minutes after ingesting the gingersnaps, the median concentration of methyleugenol peaked at 54 pg/g serum (range, 25–100 pg/g), then fell to a mean level of about 25 pg/g serum

(whole weight) after 2 hours. The results of this study suggest that methyleugenol is present in the blood after oral intake and that levels rapidly decline.

A comparison of the results of these two studies ([Barr *et al.*, 2000](#); [Schechter *et al.*, 2004](#)) suggest that levels of exposure observed in the general population are higher than those obtained after the ingestion of about 3.16 µg/kg bw methyleugenol ([Robison & Barr, 2006](#)).

[The mean level of methyleugenol observed in adults in the USA (24 pg/g serum) corresponds to the mean level reached 2 hours after ingestion of 3.16 µg/kg bw methyleugenol, which is 25% of the estimated high exposure level. However, as stated by the authors, the methods used for the analysis of gingersnap cookies have not been validated for accuracy, reproducibility or detection limits ([Schechter *et al.*, 2004](#)).]

1.4 Regulations and guidelines

In the USA, methyleugenol was affirmed as generally recognized as safe by the US Food and Drug Administration as a food additive under 21 CFR §172.515 ([FDA, 2004](#)). It is also permitted for direct addition to food for human consumption as a synthetic flavouring substance in the USA ([FDA, 2010](#)).

In the EU, EC Regulation 1334/2008, which became effective in January 2011, prohibits the addition of methyleugenol to foods and restricts the concentration of methyleugenol in compound foods that have been prepared with flavourings or food ingredients with flavouring properties. The permitted maximum concentrations were: dairy products, 20 mg/kg; meat preparations and meat products (including poultry and game), 15 mg/kg; fish preparations and fish products, 10 mg/kg; soups and sauces, 60 mg/kg; ready-to-eat savouries, 20 mg/kg; and non-alcoholic beverages, 1 mg/kg. However, if the only food ingredients with flavouring properties that have been added are fresh, dried or frozen herbs and

spices, the maximum limits for methyleugenol do not apply. For instance, pesto made with basil is permitted in food preparations, regardless of its methyleugenol content ([European Commission, 2008a](#)).

In the EU, the technical product of citronella used on non-food crops to control for ragwort must contain no more than 0.1% of the manufacturing impurities methyleugenol and its structurally related methyl-isoeugenol ([European Commission, 2008b](#)).

The Government of Canada will propose a phase-out plan for insect repellents that contain citronella oil if further information to support their continued safety is not provided. The re-evaluation of citronella oil-based personal insect repellents by the Pest Management Regulatory Agency is on-going pending additional data to refine the proposed risk assessment published on 17 September 2004. Following the report from an Independent Science Panel on Citronella Oil as an Insect Repellent, the Pest Management Regulatory Agency required producers of skin application products that contain citronella oil to provide confirmatory data that the levels of methyleugenol do not exceed 0.0002% of the product formulation ([Government of Canada, 2010](#)).

In the EU and in Canada, methyleugenol should not be intentionally added as a cosmetic ingredient. When fragrance compounds containing methyleugenol that is naturally present in essential oils are used as components in cosmetic products, the highest concentration of methyleugenol in the finished products must not exceed 0.01% in fine fragrance, 0.004% in eau de toilette, 0.002% in a fragrance cream, 0.0002% in other leave-on products and in oral hygiene products, and 0.001% in rinse-off products [such as skin cleanser] ([SCCNFP, 2000](#); [Health Canada, 2010](#)).

Australia and the USA permit the use of methyleugenol in insect traps and lure products as an insect attractant in eradication

programmes and as an anaesthetic in rodents ([Australian Government, 2005](#); [EPA, 2010](#)). In March 1982, an exemption from the requirement of a tolerance was established by the US EPA for a methyleugenol:malathion (3:1 ratio) combination impregnated on a carrier and used in US Department of Agriculture Oriental Fruit Fly Eradication Programs. This exemption was modified in April 2004 to define the carrier further. In February 2005, the US EPA published a Tolerance Reassessment Eligibility Document for methyleugenol and concluded "... there is a reasonable certainty that no harm to any population or subgroup will result from the dietary and water exposure to methyleugenol from uses specified in the existing exemption for the requirements for tolerance for methyleugenol under 40 CFR §180.1067." ([EPA, 2006, 2010](#)).

The Government of Canada will propose not to authorize the use of pure methyleugenol in natural health products ([Government of Canada, 2010](#)).

According to the [Government of Canada \(2010\)](#), oral use of methyleugenol present as a component of essential oils should not exceed 200 µg/kg bw per day.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Oral administration

See [Table 3.1](#)

3.1.1 Mouse

In a 2-year study, groups of 50 male and 50 female B6C3F₁ mice were administered methyleugenol (99% pure) in 0.5% methylcellulose by

gavage at doses of 0 (control), 37, 75 or 150 mg/kg bw on 5 days a week for 105 weeks ([Johnson et al., 2000](#); [NTP, 2000](#)). Methyleugenol caused significant dose-related increases in the incidence of hepatocellular adenoma, hepatocellular carcinoma and hepatoblastoma in both sexes. The incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) was significantly increased in all treated groups, and that of hepatocellular carcinoma was significantly increased in males administered 37 or 75 mg/kg and in all groups of treated females. In both sexes, the incidence of hepatoblastoma was increased, and that in all groups of treated females was dose-related and significantly increased. The incidence of hepatoblastoma in both sexes exceeded the historical control range for gavage studies. Tumours of the glandular stomach, including one carcinoma, developed in females and two malignant neuroendocrine tumours occurred in males.

[The Working Group noted that hepatoblastomas are rare spontaneous neoplasms, and that neuroendocrine tumours of the glandular stomach are extremely rare spontaneous neoplasms in experimental animals.]

3.1.2 Rat

In a 2-year study, groups of 50 male and 50 female F344/N rats received methyleugenol (99% pure) in 0.5% methylcellulose by gavage at doses of 37, 75 or 150 mg/kg bw on 5 days a week for 105 weeks ([Johnson et al., 2000](#); [NTP, 2000](#)). Groups of 60 males and 60 females that received the 0.5% methylcellulose vehicle alone served as controls. In a stop-exposure study, additional groups of 60 males and 60 females received 300 mg/kg bw methyleugenol in 0.5% methylcellulose by gavage for 52 weeks followed by the vehicle alone for the remaining 53 weeks of the study. Groups of five male and five female vehicle controls, and five males and five females administered 300 mg/kg were killed at 6 and

Table 3.1 Carcinogenicity studies of oral administration by gavage of methyleugenol to experimental animals

Species, strain (sex) Duration	Dosing regimen Animals/group at start	Incidence of tumours	Significance (poly-3 test)	Comments
Mouse, B6C3F ₁ (M, F) 105 wk	0, 37, 75 or 150 mg/kg bw 0.5% methylcellulose, 5 d/wk 50/group	Liver (hepatocellular adenoma): M–26/49, 43/50*, 38/50*, 39/50** F–20/50, 48/50***, 46/49***, 41/50*** Liver (hepatocellular carcinoma): M–10/49, 20/50*, 19/50**, 9/50 F–7/50, 37/50***, 47/49***, 47/50*** Liver (hepatocellular adenoma or carcinoma combined): M–31/49, 47/50**, 46/50**, 40/50* F–25/50, 50/50***, 49/49***, 49/50*** Liver (hepatoblastoma): M–0/49, 0/50, 1/50, 3/50 (M) F–0/50, 6/50*, 11/49**, 15/50** Liver (hepatocholangiocarcinoma): F–0/49, 0/50, 0/50, 2/50 Glandular stomach (carcinoma): M–0/49, 0/48, 0/49, 1/50 (M) Glandular stomach (malignant neuroendocrine tumour): M–0/49, 0/48, 0/49, 2/50	* $P \leq 0.01$, ** $P = 0.003$, *** $P \leq 0.001$ $P = 0.006$ (trend M) $P \leq 0.001$ (trend F) * $P = 0.030$, ** $P = 0.044$, *** $P < 0.001$ $P < 0.001$ (trend F) * $P = 0.02$, ** $P \leq 0.001$, *** $P \leq 0.001$ $P = 0.02$ (trend M) $P \leq 0.001$ (trend F) * $P = 0.009$, ** $P < 0.001$ $P = 0.019$ (trend M) $P < 0.001$ (trend F) NS NS NS	99% pure In all treated groups the incidence of glandular ectasia and neuroendocrine-cell hyperplasia was significantly increased.
Rat, F344 (M, F) 105 wk	0 ^a , 37, 75, 150 or 300 ^{a,b} mg/kg bw in 0.5% methylcellulose, 5 d/wk 50/group ^a 60/group ^b Stop exposure: 5 d/wk for 52 wks followed by vehicle	Liver (hepatocellular adenoma): M–5/50, 12/50*, 23/50**, 38/50**, 32/50** F–1/50, 8/50*, 11/49**, 33/49**, 43/50** Liver (hepatocellular carcinoma): M–2/50, 3/50, 14/50*, 25/50*, 36/50* F–0/50, 0/50, 4/49, 8/49*, 22/50* Liver (hepatocellular adenoma or carcinoma combined): M–7/50, 14/50*, 28/50**, 43/50**, 45/50** F–1/50, 8/50*, 14/49**, 34/49**, 43/50**	* $P \leq 0.05$, ** $P \leq 0.001$ $P \leq 0.001$ (trend M, F) * $P \leq 0.001$ $P \leq 0.001$ (trend M, F) * $P \leq 0.05$, ** $P \leq 0.001$ $P \leq 0.001$ (trend M, F)	99% pure Five M and F controls and five M and F receiving 300 mg/kg were killed at 6 and 12 mo. All M administered 150 or 300 mg/kg died before the end of the study. Mean body weights of all treated M and F were lower than those of the vehicle controls throughout most of the study.

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen Animals/group at start	Incidence of tumours	Significance (poly-3 test)	Comments
Rat, F344 (M, F) 105 wk (Contd.)		Liver (cholangioma): M-0/50, 0/50, 0/50, 0/50, 2/50	NS	
		Liver (hepatocholangioma): M-0/50, 0/50, 0/50, 1/50, 6/50* F-0/50, 0/50, 0/49, 0/49, 8/50*	* $P \leq 0.001$	
		Liver (hepatocholangiocarcinoma): M-0/50, 0/50, 1/50, 1/50, 7/50* F-0/50, 0/50, 0/49, 3/49, 9/50*	* $P \leq 0.001$	
		Liver (hepatocholangioma or hepatocholangiocarcinoma combined): M-0/50, 0/50, 1/50, 2/50, 13/50* F-0/50, 0/50, 0/49, 3/49, 17/50*	* $P \leq 0.001$	
		Glandular stomach (benign neuroendocrine tumour): M-0/50, 0/50, 0/50, 3/50, 2/50 F-0/50, 0/50, 13/50**, 9/50**, 5/50*	* $P \leq 0.05$, ** $P \leq 0.001$ $P < 0.001$ (trend F)	In all treated groups, the incidence of neuroendocrine- cell hyperplasia of the glandular stomach was significantly increased. The incidence of these non-neoplastic lesions was increased in treated M and F at 6 and 12 mo and at 2 yr. In an extended step-section evaluation of the kidney, the incidence of renal tubule hyperplasia was increased in treated M.
		Glandular stomach (malignant neuroendocrine tumour): M-0/50, 0/50, 0/50, 4/50*, 2/50 F-0/50, 1/50, 12/50**, 26/50**, 36/50**	* $P \leq 0.05$, ** $P \leq 0.001$ $P < 0.001$ (trend, F)	
		Glandular stomach (benign or malignant neuroendocrine tumour combined): M-0/50, 0/50, 0/50, 7/50**, 4/50* F-0/50, 1/50, 25/50**, 34/50**, 41/50**	* $P = 0.03$, ** $P \leq 0.002$ $P \leq 0.001$ (trend M, F)	

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen Animals/group at start	Incidence of tumours	Significance (poly-3 test)	Comments
Rat, F344 (M, F) 105 wk (Contd.)		Kidney (renal tubule adenoma, single sections): M–3/50, 2/50, 6/50, 6/50, 8/50*	* <i>P</i> = 0.018	
		Kidney (renal tubule adenoma, step sections): M–2/50, 5/50, 14/50*, 11/50**, 13/50*	* <i>P</i> ≤ 0.001, ** <i>P</i> = 0.002 <i>P</i> < 0.001 (trend)	
		Kidney (renal tubule carcinoma, single sections): M–1/50, 0/50, 0/50, 0/50, 0/50	NS	
		Kidney (renal tubule adenoma or carcinoma, single sections): M–4/50, 2/50, 6/50, 6/50, 8/50*	* <i>P</i> ≤ 0.05	
		Kidney (renal tubule adenoma, single and step sections): M–4/50, 6/50, 17/50**, 13/50*, 20/50**	* <i>P</i> = 0.003, ** <i>P</i> ≤ 0.001	
		Body cavities (malignant mesothelioma): M–1/50, 3/50, 5/50, 12/50*, 5/50**	* <i>P</i> ≤ 0.001, ** <i>P</i> = 0.041 <i>P</i> < 0.001 (trend)	
		Mammary gland (fibroadenoma): M–5/50, 5/50, 15/50*, 13/50*, 6/50	* <i>P</i> ≤ 0.01 <i>P</i> ≤ 0.001 (trend)	
		Skin (subcutaneous fibroma): M–1/50, 9/50**, 8/50*, 5/50, 4/50	* <i>P</i> = 0.011, ** <i>P</i> = 0.006	
		Skin (subcutaneous fibroma or fibrosarcoma combined): M–1/50, 12/50**, 8/50*, 8/50***, 4/50 (M)	* <i>P</i> = 0.011, ** <i>P</i> < 0.001, *** <i>P</i> = 0.005	
		Skin (subcutaneous fibrosarcoma): M–0/50, 3/50, 0/50, 3/50, 0/50	NS	

From [Johnson et al. \(2000\)](#); [NTP \(2000\)](#)

bw, body weight; d, day or days; F, female; M, male; mo, month or months; NS, not significant; wk, week or weeks; yr, year or years

12 months for histopathological evaluation. Methyleugenol induced rare benign and malignant neuroendocrine tumours of the glandular stomach in both sexes. A positive trend in the incidence of these tumours was observed in females, and the incidence in females administered 75 mg/kg and 150 mg/kg in the main study and 300 mg/kg in the stop-exposure study was significantly increased. The incidence of benign or malignant neuroendocrine tumours (combined) was increased in males administered 150 mg/kg in the main study and 300 mg/kg in the stop-exposure study, and that of malignant neuroendocrine tumours in male rats administered 150 mg/kg was significantly increased. Benign or malignant neuroendocrine tumours have not been observed in the glandular stomach of male or female historical controls in gavage studies. Positive trends were observed in the incidence of hepatocellular adenoma, hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined) with significant increases in most of the treated groups, including the stop-exposure groups. The incidence of hepatocholangioma and hepatocholangiocarcinoma was significantly increased in the male and female stop-exposure (300 mg/kg) groups. At the 12-month interim histopathological evaluation, four males had hepatocellular adenomas, one male had a hepatocholangiocarcinoma and one female had a hepatocellular carcinoma. In males, a positive trend was observed in the incidence of renal tubule adenoma, which was significantly increased (single and step sections combined) in the 75-mg/kg and 150-mg/kg main study groups and in the 300-mg/kg (stop-exposure) group; the incidence in all groups exceeded the historical control range. [The Working Group noted the unusual incidence of renal tubule tumours in the controls that was higher than that of historical controls.] In males of the main study, a positive trend was observed in the incidence of malignant mesothelioma, and the incidence was significantly increased in 150-mg/kg males and stop-exposure

males. The incidence in the 75-mg/kg, 150-mg/kg and stop-exposure (300 mg/kg) groups exceeded the historical control range. Mammary gland fibroadenoma occurred with a positive trend in males; the incidence in 75-mg/kg and 150-mg/kg males was significantly increased, and that in all groups of males exceeded the historical control range. The incidence of skin fibroma in 37- and 75-mg/kg males and that of fibroma or fibrosarcoma (combined) in 37-, 75- and 150-mg/kg males were significantly increased but not in a dose-related manner.

[The Working Group noted that tumours of the kidney, fibromas and fibrosarcomas of the skin, mesotheliomas and hepatocholangiocarcinomas are rare spontaneous neoplasms, and that neuroendocrine tumours of the glandular stomach are extremely rare spontaneous neoplasms in experimental animals. In the main and stop-exposure studies, there was consistency in the tumour response for cancer of the liver and of the glandular stomach in male and female rats, and for renal tubule tumours in male rats.]

3.2 Intraperitoneal injection

See [Table 3.2](#)

3.2.1 Mouse

Groups of male B6C3F₁ mice received intraperitoneal injections of 0 (controls) or 4.75 µmol methyleugenol/mouse (dissolved in trico-tanoin) on lactation days 1, 8, 15 and 22, were weaned at 4 weeks and were then maintained on a purified diet for 18 months. Methyleugenol caused an increase in the incidence of hepatoma [hepatocellular adenoma] ([Miller et al., 1983](#)). [The Working Group noted that the distinction between benign and malignant hepatomas had not been clearly defined at the time when the study was conducted.]

Table 3.2 Carcinogenicity studies of intraperitoneal administration of methyleugenol or 1'-hydroxymethyleugenol to experimental animals

Species, strain (sex) Duration	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F ₁ (M) 18 mo	0 (control) or 4.75 µmol methyleugenol/mouse in tricotanoin on lactation d 1, 8, 15 and 22, weaning at 4 wk then purified diet for 18 mo	Liver (hepatoma [hepatocellular adenoma]): 24/58, 56/58	$P \leq 0.01$	Purity \geq 98%
Mouse, B6C3F ₁ (M) 18 mo	0 (control) or 2.85 µmol 1'-hydroxymethyleugenol/mouse in tricotanoin on lactation d 1, 8, 15 and 22, weaning at 4 wk then purified diet for 18 mo	Liver (hepatoma [hepatocellular adenoma]): 24/58, 41/44	$P \leq 0.01$	Purity \geq 98%

From [Miller *et al.* \(1983\)](#)

d, day or days; M, male; mo, month or months; NR, not reported; wk, week or weeks

3.3 Carcinogenicity of metabolites

See [Table 3.2](#)

3.3.1 Mouse

Groups of male B6C3F₁ mice received intraperitoneal injections of 0 (controls) or 2.85 μmol 1'-hydroxymethyleugenol (a metabolite of methyleugenol)/mouse (dissolved in tricotanoin) on lactation days 1, 8, 15 and 22, were weaned at 4 weeks and were then maintained on a purified diet for 18 months. 1'-Hydroxymethyleugenol caused an increase in the incidence of hepatoma [hepatocellular adenoma] ([Miller et al., 1983](#)). [The Working Group noted that the distinction between benign and malignant hepatomas had not been clearly defined at the time when the study was conducted.]

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

(a) Absorption, distribution and excretion

[Schechter et al. \(2004\)](#) studied nine volunteers who had a mean serum concentration of 16.2 pg/g wet weight methyleugenol after overnight fasting. The subjects then consumed gingersnap cookies (3.16 μg/g) that delivered a dose of ~216 μg methyleugenol/subject, which resulted in a peak serum concentration of 53.9 pg/g wet weight methyleugenol 15 minutes after consumption, with an estimated half-life of ~90 minutes. This peak level was within the range of < 3.1–390 pg/g noted in non-fasting subjects in a biomonitoring study ([Barr et al., 2000](#)).

A study showed that methyleugenol had a 14.5% permeation rate 30 minutes after a cosmetic cream containing 50 ppm of the compound

was applied to the skin of a human volunteer ([Sgorbini et al., 2010](#)).

(b) Metabolism

[Jeurissen et al. \(2006\)](#) showed that incubation of methyleugenol with either supersomes that express individual human cytochrome P450 (CYP) or microsomes from pooled human livers resulted in the formation of the mutagenic metabolite 1'-hydroxymethyleugenol and that this reaction was catalysed by a variety of CYPs, including CYP1A2, -2C9, -2C19 and -2D6. However, when microsomes from the livers of 15 individuals were evaluated, only CYP1A2 and CYP2C9 were clearly important in the bioactivation of methyleugenol. Using microsomes from pooled human livers together with enzyme-specific inhibitors, [Jeurissen et al. \(2006\)](#) also showed that CYP1A2 was the most important enzyme for the hydroxylation of methyleugenol at concentrations found in biomonitoring studies. Other isoforms, such as CYP2C9 and CYP2C19, only had an effect at two- to fourfold higher concentrations of methyleugenol. The authors found a fivefold difference in catalytic activity among microsomal preparations from the 15 different donors. [Gardner et al. \(1997\)](#) showed that microsomes from 13 human liver samples exhibited a 37-fold difference in the conversion of methyleugenol to 1'-hydroxymethyleugenol; the highest activities were similar to those of control rat liver microsomes. Collectively, these data suggest that inter-individual differences might be important in the sensitivity of humans to methyleugenol.

4.1.2 Experimental systems

(a) Absorption, distribution and excretion

In the [NTP \(2000\)](#) rodent study, the mean ± standard deviation of methyleugenol plasma concentrations (wet weight) 15 minutes after gavage with the lowest dose (37 mg/kgbw) was 0.574 ± 0.229 μg/mL for male rats and 0.651 μg/mL (two samples; no standard deviation) for female

rats. The data for mice 10 minutes after gavage with the same dose were $0.417 \pm 0.128 \mu\text{g/mL}$ for males and $0.681 \pm 0.050 \mu\text{g/mL}$ for females.

(b) Metabolism

[Gardner et al. \(1997\)](#) found that CYP2E1 was the most important enzyme in the bioactivation of methyleugenol to 1'-hydroxymethyleugenol by microsomes from control F344 rat liver. In contrast, they showed that liver microsomes from methyleugenol-treated rats were more effective at catalysing this reaction, probably due to the induction of CYP2B and CYP1A2.

Fig. 4.1 shows a schema of the metabolism of allylbenzenes (of which methyleugenol is a member), which is complex but involves similar transformations for many chemicals of this class of agents. Three primary steps in the hepatic metabolism of the parent compounds, which are readily absorbed from the gastrointestinal tract, include *O*-demethylation, epoxidation and 1'-hydroxylation ([Smith et al., 2002](#)). [The Working Group noted that formation of the 2'3'-epoxide of methyleugenol has not been firmly established.] The first two pathways account for formation of the majority of downstream metabolites, including numerous methoxy phenolic derivatives and 2'3'-diols that are rapidly and efficiently metabolized ([Luo & Guenther, 1996](#)). Although 1'-hydroxylation is a minor pathway, subsequent sulfation is thought to produce highly reactive electrophiles that can react with cellular proteins and DNA ([Gardner et al., 1996](#)). Glucuronidation of the 1'-hydroxy compounds has been demonstrated ([Iyer et al., 2003](#)). With increasing doses of these compounds, the proportion of reactive 1'-hydroxy metabolites formed increases compared with *O*-demethylation products, presumably due to saturation of the enzyme systems responsible for *O*-dealkylation ([Zangouras et al., 1981](#)).

(c) Models

Using the available experimental, in-silico and published data, [Al-Subeihi et al. \(2011\)](#) developed a physiologically based biokinetic model for methyleugenol in rats. [Auerbach et al. \(2010\)](#) used toxicogenomics and machine-learning to predict the rodent liver carcinogenicity of alkenylbenzene flavouring agents such as methyleugenol. [Smith et al. \(2010\)](#) analysed rodent data using the margin-of-exposure approach, which considers the relationship between a given point on the dose–response curve in animal and human exposure data. [The Working Group considered these to be risk assessment models, and not relevant to hazard identification.]

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group on the ability of methyleugenol to induce DNA adducts, DNA strand breaks, mutations, chromosomal effects, alterations in oncogenes or suppressor genes in tumours, or changes in gene expression in humans. However, methyleugenol induced DNA adducts in cultured human HepG2 hepatoma cells ([Zhou et al., 2007](#)).

4.2.2 Experimental systems

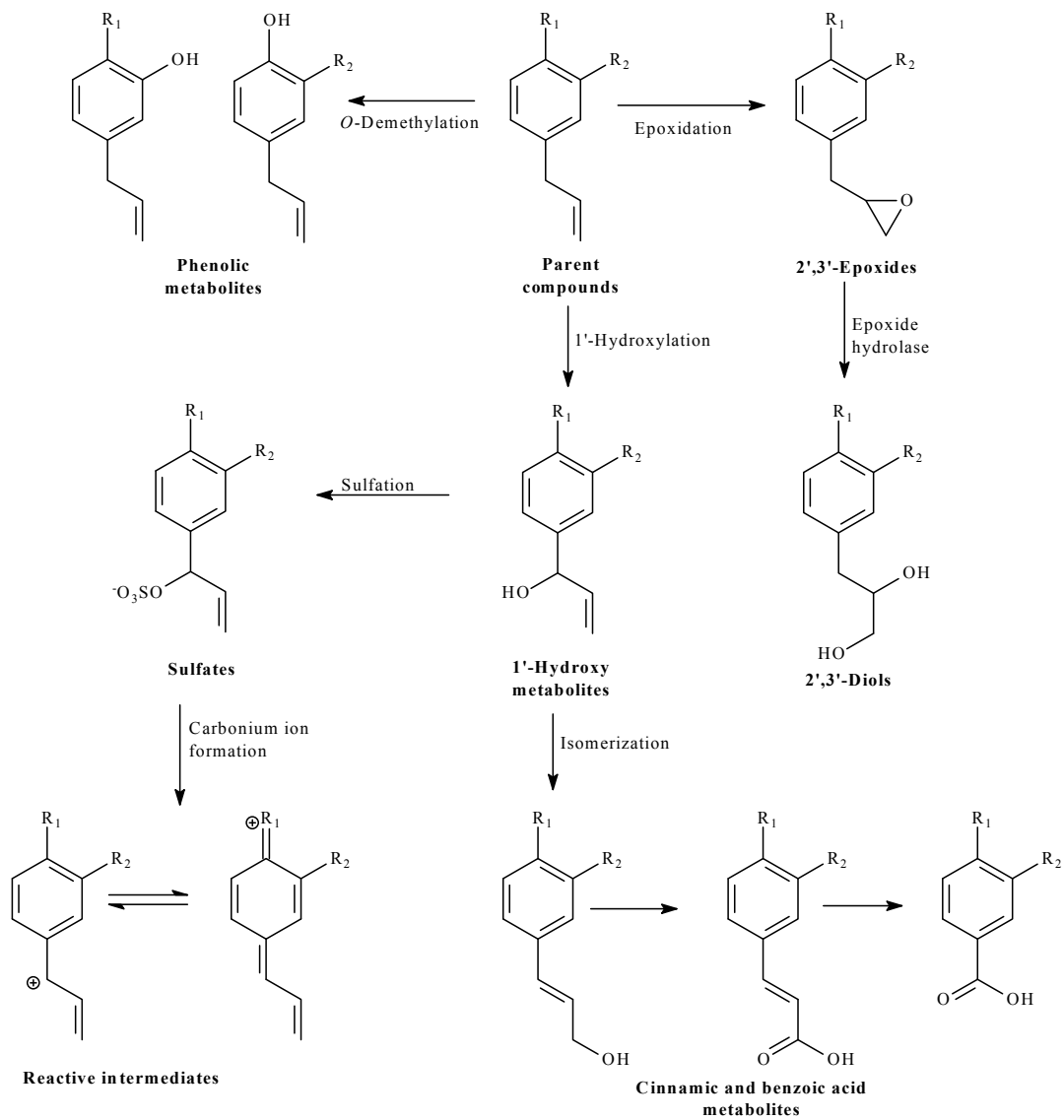
(a) DNA adducts

Intraperitoneal administration of methyleugenol induced DNA adducts in the livers of adult female CD-1 mice ([Randerath et al., 1984](#)) and of newborn male B6C3F₁ mice treated on postnatal days 1, 8, 15 and 22 ([Phillips et al., 1984](#)).

(b) DNA damage

Methyleugenol induced DNA damage in the absence of metabolic activation in the *Bacillus subtilis* rec assay ([Sekizawa & Shibamoto, 1982](#)) and in primary hepatocytes from mice and rats

Fig. 4.1 Schema of different metabolic pathways of allylbenzenes



in the unscheduled DNA synthesis assay ([Howes et al., 1990](#); [Chan & Caldwell, 1992](#); [Burkey et al., 2000](#)). Using various inhibitors, [Burkey et al. \(2000\)](#) showed that the DNA-damaging activity of methyleugenol in the unscheduled DNA synthesis assay was mediated by a sulfotransferase but did not involve epoxide formation.

(c) Mutation

Methyleugenol was not mutagenic in a variety of strains of *Salmonella typhimurium* ([Sekizawa & Shibamoto, 1982](#); [Mortelmans et al., 1986](#)) or in *Escherichia coli* WP2 *uvrA* ([Sekizawa & Shibamoto, 1982](#)) in the presence or absence of metabolic activation.

(d) Chromosomal effects

Methyleugenol induced intra-chromosomal recombination in yeast ([Schiestl et al., 1989](#); [Brennan et al., 1996](#)). In Chinese hamster ovary cells, methyleugenol induced chromosomal aberrations in the presence but not in the absence of metabolic activation and gave weakly positive results for the induction of sister chromatid exchange in both the presence and absence of metabolic activation ([NTP, 2000](#)).

Methyleugenol also induced cell transformation in Syrian hamster embryo cells ([Kerckaert et al., 1996](#); [NTP, 2002](#)).

(e) Alterations in oncogenes and suppressor genes in tumours

Among 29 hepatocellular tumours from methyleugenol-treated mice, 20 (69%) had base-substitutions in the β -catenin gene at codon 32, 33, 34 or 41, all at sites that are also mutated in colon and other cancers in rodents and humans; only two of 22 spontaneous liver tumours (9%) had mutations in β -catenin. The authors also found a relatively high frequency of β -catenin gene mutations in mouse tumours induced by a variety of other chemicals, and noted that this gene was frequently mutated in human liver

tumours ([Devereux et al., 1999](#)). Mutations in this gene cause upregulation of proto-oncogene Wnt-signalling, which results in the stimulation of cell proliferation and the inhibition of apoptosis ([Morin et al., 1997](#)).

(f) Changes in gene expression

[Iida et al. \(2005\)](#) analysed changes in gene expression in the livers of mice after 2 weeks of treatment with methyleugenol, and also the transcriptional profile in methyleugenol-induced mouse liver tumours. They found that methyleugenol upregulated several genes after 2 weeks of treatment during the early carcinogenic process, including p21, early growth response 1, Cyclin G1 and Dnase2a; it downregulated the fragile histidine triad and WW domain-containing oxidoreductase genes. In methyleugenol-induced mouse liver tumours, β -catenin, growth arrest and DNA-damage-inducible (*GADD45*), insulin-like growth factor-binding protein 1, Cyclin D1 and proliferating cell nuclear antigen genes were upregulated, and transcriptional repressor and fragile histidine triad genes were downregulated. These latter two genes, together with the WW domain-containing oxidoreductase gene, are involved in apoptosis, and their downregulation, especially at an early stage, suggests that methyleugenol causes a reduction in apoptosis soon after treatment. [Iida et al., \(2007\)](#) also showed that the transcriptional repressor gene is a suppressor of *GADD45* gene expression.

4.3 Mechanistic data

4.3.1 Structure–activity relationships

Methyleugenol is an alkenylbenzene, as are the structurally related compounds estragole and safrole. [Jeurissen et al. \(2007\)](#) have shown that not all alkenylbenzenes are metabolized by human liver microsomes via the same CYP isoforms. Thus, methyleugenol, and to some extent estragole, are metabolized by CYP1A2,

whereas estragole and safrole are metabolized primarily by CYP2A6. Safrole is not metabolized by CYP1A2, and methyleugenol is not metabolized by CYP2A6. [The Working Group noted the possible importance of polymorphisms of CYP1A2 in the metabolism of methyleugenol by the human liver.]

4.4 Susceptibility

Among the many mutations identified to date in human *CYP1A2*, functional studies showed that three mutations decrease enzyme activity, and one enhances inducibility; no mutation increases enzyme activity ([Karolinska Institutet, 2012](#)). In addition, lifestyle factors, such as exposure to barbiturates, cruciferous vegetables, fried meat or cigarette smoke, can induce *CYP1A2* and may play a critical role in the variation in catalytic ability found among human livers and, ultimately, in potential human susceptibility ([Jiang et al., 2006](#); [Jeurissen et al., 2007](#)). This may also be relevant to susceptibility to the effects of methyleugenol.

4.5 Mechanisms of carcinogenesis

Clear differences in the metabolism of methyleugenol are dose-dependent. Data from studies in both humans and in animals are available for a comparison of plasma concentrations of methyleugenol in different settings ([Barr et al., 2000](#); [NTP, 2000](#); [Schecter et al., 2004](#)). [The Working Group acknowledged the large difference in plasma concentrations measured in experimental animals in the cancer bioassay and those generally observed in humans.]

The data suggest that the doses used in the rodent studies result in the metabolism of methyleugenol by specific CYPs that leads to the formation of high levels of 1'-hydroxymethyleugenol, which can form a reactive carbonium ion. This could then result in DNA damage,

as indicated by the DNA adducts detected in human hepatocytes *in vitro* and in the liver of rats *in vivo*. Mutations have been found in genes such as β -catenin, which alters expression of the Wnt pathway. These effects, together with altered expression of other genes involved in apoptosis and other pathways, could then result in the liver tumours observed in rodent studies. Alterations in these pathways also appear to occur in humans. Thus, there is moderate evidence that a mutational mechanism underlies the formation of methyleugenol-induced tumours in rodents.

5. Summary of Data Reported

5.1 Exposure data

Methyleugenol occurs naturally in a variety of spices and herbs, including basil and tarragon. It is also produced by the methylation of eugenol and is used as a flavouring agent in a variety of foods, as a fragrance ingredient in perfumes, toiletries and detergents, and has been used as an insect attractant.

Daily human exposure to methyleugenol has been estimated at the microgram to milligram level through the ingestion of foods that contain the compound. Exposure can also occur via inhalation and dermal contact through the use of personal care products. Widespread exposure to methyleugenol occurs, as indicated by human biomonitoring data which show that methyleugenol is present in the blood serum of nearly all residents in the USA.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Methyleugenol was tested for carcinogenicity by oral administration by gavage in one study in mice and one study in rats and by intraperitoneal administration to mice in one study.

In mice, oral administration of methyleugenol caused a significantly increased incidence of liver tumours (hepatocellular adenoma, hepatocellular carcinoma and hepatoblastoma) in both sexes. In rats, oral administration of methyleugenol caused a significantly increased incidence of liver tumours (hepatocellular adenoma, hepatocellular carcinoma, hepatocholangioma and hepatocholangiocarcinoma) and benign and malignant neuroendocrine tumours of the glandular stomach in males and females, and renal tubule adenoma of the kidney, mammary gland fibroadenoma, skin fibroma, skin fibroma or fibrosarcoma (combined) and mesothelioma in males in the main and stop-exposure experiments. Tumours of the kidney, fibromas and fibrosarcomas of the skin, mesotheliomas, hepatoblastomas and hepatocholangiocarcinomas are rare spontaneous neoplasms, and neuroendocrine tumours of the glandular stomach are extremely rare spontaneous neoplasms in experimental animals. In the main and stop-exposure experiments in rats, there was consistency in the tumour response for cancers of the liver and glandular stomach in males and females, and of the kidney in males.

Intraperitoneal injection of methyleugenol caused a significantly increased incidence of hepatocellular adenoma in male mice.

1'-Hydroxymethyleugenol, a metabolite of methyleugenol, was tested for carcinogenicity by intraperitoneal injection in one study in mice, and caused a significantly increased incidence of hepatocellular adenoma in males.

5.4 Other relevant data

The three primary steps by which methyleugenol is metabolized by the liver are O-demethylation, epoxidation and 1'-hydroxylation. Although 1'-hydroxylation is a minor pathway, a subsequent sulfation reaction is thought to produce highly reactive electrophiles. Methyleugenol is metabolized by cytochrome P450 1A2, which is polymorphic in humans and is also induced by various dietary factors and cigarette smoking. Thus, a combination of phenotypic variation and lifestyle factors may play a role in the potential differential ability of humans to metabolize methyleugenol to reactive intermediates.

Although methyleugenol is not mutagenic in bacteria, it induces chromosomal aberrations *in vitro* and DNA adducts in the liver of rodents *in vivo*.

The enzymatic pathways by which methyleugenol is metabolized are similar in rodents and humans. Thus, there is moderate evidence that a mutational mechanism underlies the induction of tumours by methyleugenol in rodents.

The mechanistic data provide some additional support for the relevance of animal carcinogenicity data to humans.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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2-METHYLIMIDAZOLE

1. Exposure Data

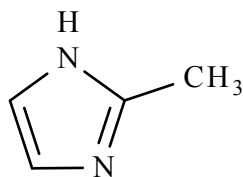
1.1 Chemical and physical data

From [NTP \(2004\)](#), [GESTIS \(2010\)](#) and [HSDB \(2010\)](#)

1.1.1 Nomenclature

Chem. Abstr. Services Reg. No.: 693-98-1
Chem. Abstr. Name: 2-Methylimidazole
Synonyms: 1*H*-Imidazole, 2-methyl-;
imidazole, 2-methyl-; 2 methylglyoxaline
RTECS No.: N17175000
EINECS No.: 211-765-7

1.1.2 Structural and molecular formulae and relative molecular mass



Relative molecular mass: 82.11

1.1.3 Chemical and physical properties of the pure substance

Description: Colourless crystalline solid
Boiling-point: 267 °C
Melting-point: 142–145 °C.

Vapour pressure: 6.9×10^{-4} mm Hg at 25 °C (estimated)

Solubility: Soluble in water (8.09×10^4 mg/L at 25 °C, estimated); very soluble in ethanol

Flash-point: 155 °C

Autoignition: > 600 °C

Octanol/water partition coefficient: $\log K_{ow}$, 0.24

pH value: 10.5–11.5 at 20 °C

Henry's law constant:

4.14×10^{-6} atm.m³/mol at 25 °C

1.1.4 Technical products and impurities

No data were available to the Working Group.

1.1.5 Analysis

Ten alkylated imidazoles, including 2-methylimidazole, can be determined by high-performance liquid chromatography on LiChrosorb Si 60 after chemical derivatization with 4-chloro-7-nitro-benzo-2-oxa-1,3-diazole. This method is very selective because no sample clean-up procedure is necessary, and has been used to identify these chemical agents in cigarette smoke ([Moree-Testa et al., 1984](#)).

1.2 Production and use

1.2.1 Production

A 1,2-dicarbonyl compound is condensed with an aldehyde and ammonia (R1 = H) in a molar ratio of 1:1:2, respectively (Radziszewski reaction). Replacement of a molar equivalent of ammonia with a primary amine (R1 = alkyl or aryl) leads to the corresponding 1-substituted imidazoles. The reaction is usually carried out in water or a water–alcohol mixture at 50–100 °C, and may involve such processes as distillation, extraction and crystallization. Distillation leads to imidazole with a purity > 99% ([Ullmann's Encyclopedia of Industrial Chemistry, 2003](#), cited in [HSDB, 2010](#)).

In the United States of America, production volumes for 2-methylimidazole of < 500 000 pounds [226 800 kg] were reported in 2006 ([HSDB, 2010](#)).

1.2.2 Use

2-Methylimidazole is used as a raw material, a chemical intermediate, and as a component in the manufacture of pharmaceuticals, photographic and photothermographic chemicals, dyes and pigments, agricultural chemicals and rubber. It is also widely used as a polymerization cross-linking accelerator and a hardener for epoxy resin systems for semiconductor potting compounds and soldering masks. It is a component of numerous polymers, including epoxy resin pastes, acrylic rubber-fluororubber laminates, films, adhesives, textile finishes and epoxy silane coatings. It is also used as a dyeing auxiliary for acrylic fibres and plastic foams ([NTP, 2004](#)).

1.3 Occurrence

1.3.1 Natural occurrence

2-Methylimidazole is not known to occur as a natural product.

1.3.2 Occupational exposure

Workers may be potentially exposed to 2-methylimidazole by inhalation or dermal contact during its production and its use in the manufacture of pharmaceuticals, photographic and photothermographic chemicals, dyes, pigments, agricultural chemicals and rubber. It is also widely used in epoxy resin systems for semiconductor potting compounds ([NTP, 2004](#)). No recent information was found on the number of individuals occupationally exposed to 2-methylimidazole. The National Occupational Exposure Survey that was conducted from 1981 to 1983 estimated that 7023 workers were potentially exposed to 2-methylimidazole in the USA, including those employed in the following industries: chemicals and allied products, rubber and miscellaneous plastics products, primary and fabricated metals, machinery, electric and electronic equipment, transportation equipment, and instruments and related products ([NIOSH, 1990](#)).

1.3.3 Environmental occurrence

2-Methylimidazole may be released into the environment (e.g. ambient air, water and soil) via waste streams during its production and use.

[HSDB \(2010\)](#) reviewed information on and calculated parameters related to the environmental fate of 2-methylimidazole in ambient air, water and soil. 2-Methylimidazole is expected to exist only in the vapour phase and be degraded in the atmosphere by a reaction with photochemically produced hydroxyl radicals; its atmospheric half-life has been estimated to be

4.1 hours. It is not expected to undergo photolysis by sunlight.

2-Methylimidazole is not expected to adsorb to sediments and soils in the aquatic environment, but is expected to adsorb more strongly to soils that contain organic carbon and clay than to other types of soil in the terrestrial environment. In the soil, it is predicted to be highly mobile. Volatilization from water surfaces and moist soils is probable but not from dry soil surfaces. The half-lives for volatilization were 190 hours in a model river and 62 days in a model lake. Its potential bioconcentration in the aquatic environment is low, and its estimated bioconcentration factor in fish is 3.2 (reviewed by [HSDB, 2010](#)).

1.3.4 Occurrence in food

Exposure to 2-methylimidazole may occur through the consumption of foods contaminated as a result of the interaction of ammonia with reducing sugars. Forage, typically hay and straw, is sometimes treated with anhydrous ammonia ([Waagepetersen & Vestergaard, 1977](#)). Imidazoles and pyrazines appear to be the dominant groups of toxic by-products formed from the interaction of ammonia and reducing sugars. 2-Methylimidazole was identified in the plasma (0.005 µg/g) and milk (0.13 µg/g) of a sheep fed ammoniated forage (5.5 µg/g) ([Sivertsen et al., 1997](#)), and in the ewe's lamb. It has also been found in the milk of cows fed ammoniated forage ([Müller et al., 1998](#)).

2-Methylimidazole may also be formed during cooking when ammonium hydroxide, glycine and monosodium glutamine are present. In an experimental study that modelled cooking and the reaction of glucose, sodium, hydrogen and different nitrogen sources, large amounts (43.2% relative weight) of 2-methylimidazole were formed when glutamate was the nitrogen source ([Wong & Bernhard, 1988](#)).

1.3.5 Other occurrence

Exposure to 2-methylimidazole may occur from tobacco smoke. It was detected (concentration not reported) in sidestream and mainstream smoke ([Moree-Testa et al., 1984](#); [Sakuma et al., 1984](#)).

1.4 Regulations and guidelines

No data were available to the Working Group.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Oral administration

See [Table 3.1](#)

3.1.1 Mouse

In a 2-year carcinogenicity study, groups of 60 male and 60 female B6C3F₁ mice were fed diets containing 0, 625, 1250 or 2500 ppm 2-methylimidazole (99.5% pure) for 105 weeks (equivalent to average daily doses of approximately 75, 150 or 315 and 80, 150 or 325 mg/kg body weight (bw) for males and females, respectively) ([NTP, 2004](#); [Chan et al., 2008](#)). Ten animals from each group were killed at 6 months for interim evaluation. The food consumption of exposed groups was generally similar to that of controls. 2-Methylimidazole caused increases in the incidence of thyroid follicular-cell adenoma, hepatocellular adenoma and hepatocellular carcinoma in male mice and of hepatocellular adenoma in female mice. The incidence of thyroid follicular-cell adenoma was significantly increased in male mice fed 2500 ppm. The incidence of hepatocellular

Table 3.1 Carcinogenicity studies of 2-methylimidazole administered in the diet to experimental animals

Species, strain (sex) Duration	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F ₁ (M, F) 105 wk	0, 625, 1 250 or 2 500 ppm 7 d/wk 60/group; 10 M and 10 F were necropsied at 6 mo for thyroid hormone analyses and histopathological evaluation.	Thyroid gland (follicular-cell adenoma): M ^a -0/50, 1/50, 0/50, 7/50 F-1/50, 0/50, 0/50, 1/50 Liver (hepatocellular adenoma): M ^b -7/50, 14/50, 13/50, 18/50 F ^c -3/50, 4/49, 6/49, 10/50 Liver (hepatocellular carcinoma): M ^d -4/50, 8/50, 14/50, 6/50 Liver (hepatocellular adenoma and carcinoma): M ^e -10/50, 22/50, 22/50, 22/50	<i>P</i> = 0.006 (high-dose M) <i>P</i> < 0.001 (trend M) <i>P</i> = 0.005 (high-dose M) <i>P</i> = 0.006 (trend M) <i>P</i> = 0.037 (high-dose F) <i>P</i> = 0.015 (trend F) <i>P</i> = 0.003 (mid-dose M) <i>P</i> = 0.09 (low-dose M) <i>P</i> = 0.002 (mid-dose M) <i>P</i> = 0.003 (high-dose M) <i>P</i> = 0.007 (trend M)	99.5% pure The mean body weights of 2 500-ppm males and females were 12% and 11% lower than those of the controls, respectively, at the end of the study.
Rat, F344 (M, F) 106 wk	0, 300, 1 000 or 3 000 ppm (M) 0, 1 000, 2 500 or 5 000 ppm (F) 7 d/wk 60/group; 10 M and 10 F were necropsied at 6 mo for thyroid hormone analyses and histopathological evaluation.	6 mo evaluation Thyroid gland (follicular-cell adenoma): F-0/10, 0/10, 0/10, 2/10 (F) 2-yr evaluation Thyroid (follicular-cell adenoma): M-1/48, 0/46, 1/43, 3/50 F-0/49, 0/48, 0/42, 5/48	<i>P</i> = 0.03 (high-dose F) <i>P</i> < 0.001 (trend F)	99.5% pure Survival of 2 500-ppm females was significantly lower than that of the controls. The mean body weight of 2 500-ppm females was 12% lower than that of the controls at the end of the study; the mean body weights of 5 000-ppm females were significantly lower than those of the controls during most of the study. Serum concentrations of thyroid-stimulating hormone were increased at 6 mo in females. Dose-related increases in relative and absolute thyroid gland weights occurred at 6 mo in both sexes.

Table 3.1 (continued)

Species, strain (sex) Duration	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M, F) 106 wk (contd.)		Thyroid (follicular-cell carcinoma): M–0/48, 2/46, 0/43, 2/50 F–1/49, 1/48, 1/42, 7/48	<i>P</i> = 0.03 (high-dose F) <i>P</i> = 0.003 (trend F)	
		Thyroid (follicular-cell adenoma and carcinoma): M ^f –1/48, 2/46, 1/43, 5/50 F ^g –1/49, 1/48, 1/42, 11/48	<i>P</i> = 0.046 (trend M) <i>P</i> = 0.002 (high-dose F) <i>P</i> < 0.001 (trend F)	
		Liver (hepatocellular adenoma): M–0/50, 1/50, 3/50, 1/50 F ^h –1/50, 0/49, 2/50, 4/50		
		Liver (hepatocellular carcinoma): M–0/50, 0/50, 1/50, 2/50		
		Liver (hepatocellular adenoma and carcinoma): M ⁱ –0/50, 1/50, 3/50, 3/50		

^a Historical incidence (mean ± SD) for 2-yr feed studies in male mice: 3/309 (1.0 ± 1.0%), range 0–2%

^b Historical incidence (mean ± SD) for 2-yr feed studies in male mice: 60/310 (19.0 ± 8.5%), range 10–30%

^c Historical incidence (mean ± SD) for 2-yr feed studies in female mice: 29/309 (9.3 ± 2.4%), range 6–12%

^d Historical incidence (mean ± SD) for 2-yr feed studies in male mice: 43/310 (13.7 ± 5.1%), range 8–20%

^e Historical incidence (mean ± SD) for 2-yr feed studies in male mice: 95/310 (30.2 ± 10.4%), range 20–45%

^f Historical incidence (mean ± SD) for 2-yr feed studies in male rats: 8/307 (2.6 ± 3.0%), range 0–8%

^g Historical incidence (mean ± SD) for 2-yr feed studies in female rats: 3/309 (1.0 ± 1.1%), range 0–2%

^h Historical incidence (mean ± SD) for 2-yr feed studies in female rats: 2/310 (0.6 ± 1.0%), range 0–2%

ⁱ Historical incidence (mean ± SD) for 2-yr feed studies in male rats:^d7/310 (2.2 ± 2.0%), range 0–5%

d, day or days; F, female; M, male; mo, month or months; SD, standard deviation; wk, week or weeks; yr, year or years

From [NTP \(2004\)](#); [Chan et al. \(2008\)](#)

adenoma occurred with positive trends in male and female mice and was significantly increased in the 2500-ppm groups. The incidence of hepatocellular carcinoma was significantly increased in 1250-ppm males. The incidence of hepatocellular adenoma or carcinoma (combined) was significantly increased in all treated males, and that of hepatocellular adenoma in 2500-ppm males and females and hepatocellular carcinoma in 1250-ppm males exceeded the historical control ranges for feed studies.

[Tumours of the thyroid are rare spontaneous neoplasms in experimental animals.]

3.1.2 Rat

In a 2-year carcinogenicity study, groups of 60 male and 60 female F344/N rats were fed diets containing 0, 300, 1000 or 3000 ppm (males) and 0, 1000, 2500 or 5000 ppm (females) 2-methylimidazole (99.5% pure) for 106 weeks (equivalent to average daily doses of approximately 13, 40 or 130 and 50, 120 or 230 mg/kg bw for males and females, respectively) (NTP, 2004; Chan *et al.*, 2008). Ten animals from each group were killed at 6 months for interim evaluation. The food consumption of 3000-ppm males was lower than that of controls from week 4 through to week 28, and that of 5000-ppm females was lower throughout the study. 2-Methylimidazole caused increases in the incidence of thyroid follicular-cell adenoma and follicular-cell carcinoma in females and of thyroid follicular-cell adenoma or carcinoma (combined) in males. At the 6-month interim evaluation, thyroid follicular-cell adenomas occurred in two females exposed to 5000 ppm. At 2 years, increases with a positive trend were observed in the incidence of thyroid follicular-cell adenoma, follicular-cell carcinoma and follicular-cell adenoma or carcinoma (combined) in females, which were significant in the 5000-ppm group. The incidence of thyroid follicular-cell adenoma or carcinoma (combined) in males showed a positive trend

and exceeded the historical control ranges. In exposed males and females, the incidence of some hepatocellular tumours (hepatocellular adenoma in females and hepatocellular adenoma or carcinoma combined in males) exceeded the respective historical control ranges and may have been related to exposure to 2-methylimidazole.

[Tumours of the thyroid are rare spontaneous neoplasms in experimental animals.]

4. Other Relevant Data

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, distribution and excretion

Following intravenous administration of 3 $\mu\text{mol/kg}$ bw [246.5 $\mu\text{g/kg}$ bw] [^{14}C]2-methylimidazole to male Wistar rats, radioactivity associated with 2-methylimidazole and its metabolites was excreted in the urine. About 78% of the injected dose was excreted within 24 hours (Ohta *et al.*, 1998).

In male and female F344 rats, 2-methylimidazole was rapidly absorbed and widely distributed following either intravenous or oral administration by gavage (Johnson *et al.*, 2002). In male F344 rats, 90% of 2-methylimidazole was eliminated in the urine within 24 hours and about 4% in the faeces. The compound was not extensively metabolized and more than half of the dose was found in the urine as the parent compound. Tissue distribution was proportional to dose and independent of the route of administration (oral or intravenous) (Sanders *et al.*, 1998).

(b) Metabolism

Following intravenous administration, approximately 3% of a 3- μ mol/kg bw dose was excreted by Wistar rats as the nucleophilic metabolite, 2-methylimidazolone. Pretreatment with the cytochrome P450 (CYP) inhibitors, SKF-252A or cimetidine, increased the amount of this urinary metabolite and the irreversible binding of 2-methylimidazole equivalents to the aortic connective tissue while both were decreased following pretreatment with triethylenetetramine dihydrochloride (TETA). TETA decreases the activity of copper-containing enzymes such as lysyl oxidase. This suggests that both CYP-dependent and -independent pathways are involved in the formation of this metabolite (Ohta *et al.*, 1998). A chemical model system for CYP has also been shown to oxidize 2-methylimidazole to 2-methylimidazolone efficiently (Miyachi & Nagatsu, 2002).

(c) Toxicokinetics

The toxicokinetics of 2-methylimidazole was characterized in male and female F344 rats (Johnson *et al.*, 2002) following intravenous (10 mg/kg bw) or oral administration by gavage (25, 50 or 100 mg/kg bw). The compound was rapidly absorbed and systemically distributed. Peak plasma concentrations were proportional to dose and were reached within 35–50 minutes. While 2-methylimidazole was quickly eliminated, differences were noted between males and females. In males, clearance of all oral doses was similar to the rate observed following intravenous administration. In contrast, a decreased rate of clearance was observed in females at higher doses. Compared with the 25-mg/kg oral dose, clearance was 36 and 42% lower at 50 and 100 mg/kg, respectively. Nevertheless, the clearance value, although reduced in female rats at doses of 50 mg/kg or higher, was still greater than that obtained for intravenously or orally treated male rats. The data suggest that, at increased

doses, renal clearance is saturated in female but not male rats.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

The genetic effects of 2-methylimidazole have been reviewed (NTP, 2004).

(a) Mutations

2-Methylimidazole was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100 or TA1535 in the presence or absence of a metabolic activation system (detailed protocol presented by Zeiger *et al.*, 1988; NTP, 2004).

Yamaguchi & Nakagawa (1983) reported that 2-methylimidazole did not suppress the mutagenicity of 3-amino-1-methyl-5H-pyrido[2,3-b]indol, 2-acetylaminofluorene or benzo[a]pyrene in *S. typhimurium* strains TA98 and TA100. In contrast, imidazole and 1-methylimidazole decreased the mutagenicity of activation-dependent carcinogens such as benzo[a]pyrene and 2-acetylaminofluorene; the authors suggested that this was a consequence of the inhibition of metabolic activation of the procarcinogens.

(b) Chromosomal effects

The results of the induction of chromosomal damage by 2-methylimidazole *in vivo* were contradictory. When administered as three intraperitoneal injections at 24 hours intervals, 2-methylimidazole did not induce micronucleated polychromatic erythrocytes in the bone marrow of rats or mice 24 hours after the third injection (detailed protocol presented by Shelby *et al.*, 1993; NTP, 2004). However, after male and female mice were fed 2-methylimidazole in the diet for 14 weeks, a significant dose-related

increase in the frequency of micronucleated normochromatic erythrocytes and in the percentage of the micronucleated polychromatic erythrocytes was noted in the peripheral blood (detailed protocol presented by [MacGregor et al., 1990](#); [NTP, 2004](#)).

4.3 Mechanistic data

4.3.1 Effects on cell physiology

2-Methylimidazole induced thyroid lesions in rats and mice in both 15-day and 14-week feeding studies ([NTP, 2004](#); [Chan et al., 2006](#)). Both male and female F344 rats fed 3300 or 10 000 ppm for 15 days exhibited enlarged thyroid glands, hypertrophy of thyroid-stimulating hormone (TSH) cells and thyroid gland follicular-cell hyperplasia. After 14 weeks of exposure to doses greater than 2500 ppm, animals had significant decreases in triiodothyronine (T_3) and thyroxine (T_4) and an increase in TSH. Diffuse follicular hyperplasia was observed in males at 1250 ppm (80 mg/kg bw) and in females at 2500 ppm (160 mg/kg bw). Thyroid follicular adenoma was observed in two males in the 10 000-ppm (560 mg/kg bw) group. Testicular degeneration and a decreased spermatid count were also seen in this group. Females had increased length of the estrous cycle at 10 000 ppm (560 mg/kg bw). Minimal to mild anaemia was present in both sexes at the two highest exposure levels.

In male and female B6C3F₁ mice, 15 days of exposure to 2-methylimidazole resulted in a dose-dependent increase in thyroid follicular-cell hypertrophy and haematopoietic cell proliferation in the spleen ([Chan et al., 2006](#)). The incidence of thyroid, spleen and kidney lesions was dose-dependent in the 14-week study, and doses of 2500 ppm and higher induced thyroid gland follicular-cell hypertrophy in males and females. Haematopoietic cell proliferation in the spleen, anaemia and renal tubule pigmentation

occurred in both males (1250 ppm and higher) and females (2500 ppm and higher).

The proposed mechanism for the hyperthyroid effects of 2-methylimidazole in rats appears to be indirect and results from effects of the compound on hepatic uridine diphosphate glucuronosyltransferase (UDPGT). 2-Methylimidazole induces a significant increase in UDPGT activity in the liver of rats. T_4 is glucuronidated in the liver and then excreted. Low levels of T_4 are consistent with the induction of UDPGT by 2-methylimidazole. Decreased T_4 results in increased TSH secretion that in turn causes thyroid follicular-cell hyperplasia, hypertrophy and ultimately tumours. In mice, the changes in circulating T_4 and TSH levels were less apparent, although hypertrophy was observed, which raises the possibility of another mechanism ([Sanders et al., 1998](#); [NTP, 2004](#); [Chan et al., 2006](#)).

4.3.2 Effects on cell function

Imidazole compounds can inhibit CYPs ([Murray, 1987](#)). Compared with imidazole, 2-methylimidazole was a less potent inhibitor of CYP2E1 ([Hargreaves et al., 1994](#)).

After exposure of male and female rats to 2-methylimidazole in the diet, hepatic UDPGT activity was significantly increased for up to 6 months; relative liver weight was increased in treated males and females and total hepatic CYP level was generally decreased after 6 months ([Chan et al., 2008](#)).

4.4 Mechanisms of carcinogenesis

In both F344/N rats and B6C3F₁ mice, 2-methylimidazole induced a dose-related increase in follicular-cell hyperplasia and hypertrophy in the thyroid. This effect was observed at the 6-month interim analysis, and was increased at the end of the 2-year carcinogenicity study. 2-Methylimidazole induced

thyroid follicular-cell adenoma or carcinoma (combined) and hepatocellular adenoma or carcinoma (combined) in male and female rats and mice ([Chan *et al.*, 2008](#)).

Hepatocellular neoplasms and thyroid follicular-cell neoplasms are often found in association in rodent carcinogenicity studies ([Huff *et al.*, 1991](#); [McConnell, 1992](#); [Haseman & Lockhart, 1993](#)). For chemicals that cause tumours in both the liver and thyroid, hepatic microsomal enzyme induction and thyroid hormone metabolism have been proposed as a possible mechanistic link that connects the pathogenesis of thyroid follicular tumours with that of hepatocellular neoplasms ([McClain, 1989](#); [McClain & Rice, 1999](#)). 2-Methylimidazole induced increases in liver weights in mice and liver microsomal UDPGT activity in rats and mice. The increases, however, were not accompanied by changes in total hepatic CYPs in mice, and microsomal enzyme induction alone appeared to be insufficient to account for the thyroid and liver neoplasms in these studies ([Chan *et al.*, 2008](#)).

2-Methylimidazole in the diet induced micronucleated normochromatic erythrocytes in mice after 14 weeks of treatment, but not in the bone marrow of rats and mice after 3 days of treatment ([NTP, 2004](#)). A mutational mechanism may thus be involved in the carcinogenic effects of 2-methylimidazole observed in the liver and thyroid.

5. Summary of Data Reported

5.1 Exposure data

2-Methylimidazole is used as a raw material, chemical intermediate or component in the manufacture of pharmaceuticals, dyes, pigments and agricultural chemicals. Occupational exposure may occur by inhalation or dermal contact. In a model system, 2-methylimidazole was

formed as a result of the interaction of ammonia with reducing sugars. It has been detected in ammoniated forage and ammoniated molasses used to feed animals, and in the milk from these animals. No data were available on the presence of 2-methylimidazole in commercial supplies of milk. 2-Methylimidazole has been detected in tobacco smoke.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

2-Methylimidazole was tested for carcinogenicity by administration in the diet to mice and rats. Oral administration of 2-methylimidazole caused an increased incidence of thyroid follicular-cell adenoma, hepatocellular adenoma and hepatocellular carcinoma in male mice, and of hepatocellular adenoma in female mice. It increased the incidence of thyroid follicular-cell adenoma and follicular-cell carcinoma in female rats, and of thyroid follicular-cell adenoma or carcinoma (combined) in male rats.

Tumours of the thyroid, which are consistently found in rats and mice, are rare spontaneous neoplasms in experimental animals.

5.4 Other relevant data

No data were available on the toxicokinetics of 2-methylimidazole in humans. After oral administration to rats, 2-methylimidazole was rapidly absorbed and widely distributed, and the parent compound and its metabolites were mainly excreted in urine. 2-Methylimidazole was not extensively metabolized in rats; 3% of the dose was excreted in the urine as 2-methylimidazolone. The formation of this metabolite involves both cytochrome P450-dependent and -independent pathways.

2-Methylimidazole was not mutagenic in bacteria, but induced micronuclei in polychromatic erythrocytes in mice after 14 weeks of administration in the diet, but not in rats or mice given three daily intraperitoneal injections. Thyroid lesions were induced by 2-methylimidazole in rats and mice in both 15-day and 14-week feeding studies. A possible indirect mechanism that causes these changes in the thyroid is the effect of 2-methylimidazole on hepatic uridine diphosphate glucuronosyltransferase.

There is weak evidence that enzyme induction is a mechanism by which thyroid and liver tumours develop in experimental animals. A genotoxic mechanism may also be involved in 2-methylimidazole-induced cancer in animals. The relevance of the tumour response in experimental animals to humans cannot be excluded.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2-methylimidazole.

6.3 Overall evaluation

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

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4-METHYLIMIDAZOLE

1. Exposure Data

1.1 Chemical and physical data

From [NTP \(2007\)](#), [GESTIS \(2010\)](#) and [HSDB \(2010\)](#)

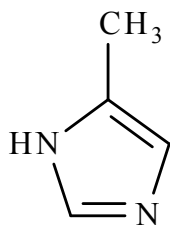
1.1.1 Nomenclature

Chem. Abstr. Services Reg. No.: 822-36-6

Chem. Abstr. Name: 4-Methylimidazole

Synonyms: 1*H*-Imidazole, 4-methyl;
1*H*-imidazole, 5-methyl-; imidazole,
4(or 5)-methyl; 4(5)-methylglyoxaline;
4(5),4(5)-methylimidazole
EINECS No.: 212-497-3

1.1.2 Structural and molecular formulae and relative molecular mass



$C_4H_6N_2$

Relative molecular mass: 82.11

1.1.3 Chemical and physical properties of the pure substance

Description: Light yellow crystalline solid

Boiling-point: 263 °C

Melting-point: 46–48 °C

Vapour pressure: 0.007 mm Hg at 25 °C

Solubility: Very soluble in water and alcohol

Flash-point: 157 °C

Octanol/water partition coefficient: $\log K_{ow}$,
0.23

Henry's law constant: 4.14×10^{-6} atm.m³/
mol at 25 °C (estimated)

1.1.4 Technical products and impurities

No data were available to the Working Group.

1.1.5 Analysis

Ten alkylated imidazoles have been identified in cigarette smoke, of which 4-methylimidazole and imidazole were the most abundant, by high-performance liquid chromatography on LiChrosorb Si 60 after chemical derivatization with 4-chloro-7-nitro-benzo-2-oxa-1,3-diazole. This method is very selective because no clean-up procedure is necessary ([Moree-Testa et al., 1984](#)).

1.2 Production and use

1.2.1 Production

Preparation of 4-methylimidazole involves cyclocondensation of an aldehyde and ammonia with methylglyoxal. Variations include the use of ammonium carbonate or ammonium oxalate as the source of ammonia and cyclocondensation of

ammonia and formamide with hydroxyacetone. Another method to synthesize the compound is by catalytic dehydrogenation of imidazoline derivatives. 4-Methylimidazole may be synthesized from propanol and formamide, by catalytic cyclization of bisformamidopropane or by photolysis of alkenyltetrazole derived from alkenes by sequential epoxidation, ring opening and dehydration ([NTP, 2007](#)).

1.2.2 Use

4-Methylimidazole is used as a chemical intermediate, raw material or component in the manufacture of pharmaceuticals, photographic and photothermographic chemicals, dyes and pigments, agricultural chemicals and rubber. It has also been investigated for use as a raw material in the synthesis of cardiovascular stimulants, epoxy resin anticholesteremics, neurotransmitter antagonists, disinfectants/antiprotozoal antiseptic agents and aromatase inhibitors ([NTP, 2007](#)).

The chemical is also used as a component in imidazolephenoxyalkane oven cleaners, a cross-linking agent for epoxy resin hardeners, a corrosion inhibitor for cooling water in heat exchange apparatuses, a component of absorbents to remove acid gases from hydrocarbon or synthesis gas, and a raw material for inks and paper dyes ([NTP, 2007](#)).

1.3 Occurrence

1.3.1 Natural occurrence

4-Methylimidazole is not known to occur as a natural product.

1.3.2 Occupational exposure

Workers may be exposed to 4-methylimidazole by inhalation or dermal contact during its production, its use as a major pharmaceutical intermediate and from other uses ([NTP, 2007](#)).

1.3.3 Environmental occurrence

4-Methylimidazole may be released into ambient air, water and soil during its production and use.

4-Methylimidazole is expected to exist only in the vapour phase and to be degraded in the ambient atmosphere by a reaction with photochemically produced hydroxyl radicals; its estimated atmospheric half-life is 4.1 hours, and it is not expected to undergo photolysis by sunlight ([HSDB, 2010](#)).

4-Methylimidazole is not expected to adsorb to sediments and soils in the aquatic environment, but is expected to adsorb more strongly to soils that contain organic carbon and clay than to other types of soil in the terrestrial environment. Volatilization from water surfaces and moist soils is probable, but not from dry soil surfaces; the half-lives for volatilization were 194 hours in a model river and 62 days in a model lake. Its potential bioconcentration in the aquatic environment is low, and its estimated bioconcentration factor in fish is 3.2 based on an estimated octanol/water coefficient ($\log K_{ow}$) of 0.23 ([HSDB, 2010](#)).

1.3.4 Occurrence in food

(a) Occurrence in milk through ammoniated forage

Exposure can occur from the consumption of foods contaminated with 4-methylimidazole, which is formed as a result of the interaction of ammonia with reducing sugars. Forage — typically hay and straw — is sometimes treated with anhydrous ammonia to improve its quality (e.g. increase the non-protein nitrogen content) and digestibility ([Waagepetersen & Vestergaard, 1977](#)). Imidazoles (such as 4-methylimidazole) and pyrazines appear to be the dominant groups of toxic by-products formed from the interaction of ammonia with reducing sugars. Experimental studies have shown that higher concentrations of sugar and ammonia, higher temperatures, higher

water activity and longer reaction times increased the amount of 4-methylimidazole (formed at the pH achieved by the addition of ammonia) (Bergström, 1991). Perdok & Leng (1987) reported that 4-methylimidazole was not present in most types of untreated roughage, but was found at concentrations ranging from 8 to 43 mg/kg in thermo-ammoniated roughage. Average concentrations of 0.72 µg/mL 4-methylimidazole were detected in the plasma of sheep fed ammoniated tall fescue that contained an average concentration of 64.36 mg/kg of the chemical (Karangwa *et al.*, 1990a). 4-Methylimidazole has been identified in the plasma, urine and milk of cows and sheep fed ammoniated forage (Müller *et al.*, 1998a, b). Müller *et al.* (1998a) reported that the concentrations of 4-methylimidazole in an ammoniated forage-fed (90 µg/g dry matter) ewe were 0.07 µg/mL in plasma, 0.23–0.31 µg/mL in milk and 21 µg/mL in urine. The plasma concentration in one of the ewes' suckling lambs that developed toxicosis was 0.01 µg/mL. Similar concentrations were found in the plasma and milk of ewes fed ammoniated seed hay (with 4-methylimidazole concentrations greater than 100 µg/g dry matter) for 7 days (Sivertsen *et al.*, 1993). In a dairy cow fed ammoniated forage containing 4-methylimidazole (58 µg/g dry matter), concentrations of the chemical in plasma, milk and urine were 0.28, 2.7 and 5.8 µg/mL, respectively (Müller *et al.*, 1998a).

(b) *Occurrence in food and drinks containing caramel colourings*

4-Methylimidazole is found in ammonia and ammonia-sulfite process caramel colourings. Caramel colourings are produced by heating carbohydrates with specified reagents under defined temperatures and pressures, which results in a dark brown colouring with a characteristic odour of burnt sugar. Their use accounts for 95% by weight of the permitted colour additives used in food. They have been classified by the Joint FAO/WHO Expert Committee on Food

Additives (JECFA), and the European Union Scientific Committee for Food into four classes, two of which are prepared using compounds that contain ammonia (reviewed by Chappel & Howell, 1992; Houben & Penninks, 1994). Class III ammonia caramels are commonly used in various bakery products, soya-bean sauces, brown sauces, gravies, soup aromas, brown (dehydrated) soups, brown malt caramel blends for various applications, vinegars and beers, especially in certain dark-brown beers. Their use accounts for 20–25% of the total use of caramel colourings in the USA and for about 60% in Europe. Class IV ammonia-sulfite caramels are used in soft drinks, pet foods and soups (Houben & Penninks, 1994), and account for approximately 70% of the caramel colourings produced worldwide (Licht *et al.*, 1992a). Reported concentrations of 4-methylimidazole in caramel colourings are provided in Table 1.1. Licht *et al.* (1992b) reported 4-methylimidazole concentrations ranging from < 5 to 184 mg/kg in 40 commercial Class III caramel colourings that met JECFA guidelines for 2-acetyl-4-tetrahydroxybutylimidazole. Other studies have reported higher concentrations in some samples, ranging up to 463 mg/kg. In general, higher 4-methylimidazole levels have been found in Class IV caramel colourings; a study of 90 commercial products found levels ranging from 112 to 1276 mg/kg (see Table 1.1).

Long-term dietary exposure to caramels among children aged 1–10 years has been estimated based on analytical data in 11 European countries (EFSA, 2010). Median exposure ranged from 4.3 to 41 mg/kg body weight (bw) per day for ammonia-sulfite caramels (class IV) and from 32 to 105 mg/kg bw per day for ammonia caramels (class III).

Reported concentrations of 4-methylimidazole ranged from 1.58 to 28.03 mg/kg in dark beer (Klejdus *et al.*, 2006), from 0.3 to 1.45 mg/kg in coffee (Casal *et al.*, 2002; Lojková *et al.*, 2006) and from 0.30 to 0.36 µg/mL in

Table 1.1 Concentrations of 4-methylimidazole in caramel colourings and food and beverages

Product	Number of samples	Concentration (mg/kg)	Reference
Class III	40 commercial colourings ^a	< 5–184	Licht <i>et al.</i> (1992b)
Class III	6 commercial colourings ^b	ND–463	Allen <i>et al.</i> (1992)
Class III	5 colourings	85.6–187.8	Klejdus <i>et al.</i> (2006)
Class III	3 colourings ^b	34–463	Brusick <i>et al.</i> (1992)
Class III	3 commercial colourings		Ciolino (1998)
	Liquid	14–24	
	Powder	50	
Ammonia process caramel colourings	6 colourings	6.6–351 per 20 000 EBC units ^c	Thomsen & Willumsen (1981)
Ammonia caramel colourings	4 samples	7.5–210	Fernandes & Ferreira (1997)
Ammonia caramel colourings	3 samples	122–414	Kvasnička (1989)
Ammonia caramel colourings	5 samples	25–303	Fuschs & Sundell (1975)
Class IV	90 commercial colourings ^d	112–1276	Licht <i>et al.</i> (1992a)
Class IV	2 commercial colourings ^b	146–215	Allen <i>et al.</i> (1992)
Class IV	6 colourings ^b	ND–387	Brusick <i>et al.</i> (1992)
Class IV	3 colourings		Ciolino (1998)
	Liquid	130–300	
	Powder	480	
Ammonia caramel colourings (ammonia-sulfite process)	8 colourings	62–341 per 20 000 EBC units ^c	Thomsen & Willumsen (1981)
Malt extract	2 samples	ND	Fernandes & Ferreira (1997)
Coffee	10 real samples	0.39–2.05	Klejdus <i>et al.</i> (2006)
Coffee	5 types		Lojková <i>et al.</i> (2006)
	Solid	0.77–1.45	
	Liquid	0.35–0.77	
Coffee	7 samples		Casal <i>et al.</i> (2002)
	Roasted	0.307–1.241	
Dark beer	7 real samples	1.58–28.03	Klejdus <i>et al.</i> (2006)
Soda	5 brands	0.30–0.36	Moon & Shibamoto (2010)

Table 1.1 (continued)

Product	Number of samples	Concentration (mg/kg)	Reference
Soft drinks			Yoshikawa & Fujiwara (1981)
Cola type	7 samples	0.17–0.70	
Grape type	2 samples	0.15–0.16	
Alcoholic beverages			
Whisky	5 samples	ND–0.14	
Black beer	2 samples	ND	
Beer, wine brandy	1 sample each	ND	
Milk products	3 samples	Trace	
Seasoning sauces			
Worcestershire sauce	6 samples	1.6–3.4	
Soya sauce	4 samples	0.37–0.55	
Others	5 samples	0.11–1.5	
Foods cooked in soya sauce	5 samples	0.89–3.2	
Confectioneries	6 samples	ND–0.78	

^a Represent full range of commercially available samples, limited to those that meet JECFA specification of 25 mg/kg or less of 2-acetyl-4(5)-tetrahydroxybutylimidazole when expressed per 0.1 colour intensity unit or varied between > 10 and 45 mg/kg on an 'as is' basis.

^b Samples provided by the International Technical Caramel Association

^c EBC units: caramel colour intensity unit of the European Brewery Convention

^d Represent full range of commercially available samples from 11 manufacturers and seven countries.

ND, not detected

soda ([Moon & Shibamoto, 2010](#)). [Yoshikawa & Fujiwara \(1981\)](#) measured 4-methylimidazole in various foods and beverages (see [Table 1.1](#)). The highest levels were found in Worcestershire sauce (up to 3.4 mg/kg) or foods cooked in soya sauce (up to 3.2 mg/kg).

1.3.5 Other occurrence

Exposure to 4-methylimidazole from tobacco smoke may also occur. 4-Methylimidazole has been detected in the condensate of smoke from several brands of cigarettes, ranging from 2.3 (low tar) to 15 (non-filtered) µg/cigarette for dark, air-cured tobacco. Concentrations (µg/cigarette) in other types of tobacco were 2.3 for Virginia and 5.5 for American blend. 4-Methylimidazole is one of the most abundant imidazoles found in cigarette smoke ([Moree-Testa et al., 1984](#)).

1.4 Regulations and guidelines

Specifications issued by the [European Commission \(2008\)](#) and [JECFA \(2006\)](#) stated that the maximum level of 4-methylimidazole in class III ammonia caramel and class IV ammonia-sulfite caramel should be restricted to ≤ 250 mg/kg on a colour intensity basis.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Oral administration

See [Table 3.1](#)

3.1.1 Mouse

In a 2-year carcinogenicity study, groups of 50 male and 50 female B6C3F₁ mice were fed diets containing 0, 312, 625 or 1250 ppm 4-methylimidazole (> 99% pure) for 106 weeks (equivalent to average daily doses of approximately 40, 80 and 170 mg/kg body weight (bw)) ([NTP, 2007](#); [Chan et al., 2008](#)). Food consumption of treated male and female mice was generally similar to that of controls. 4-Methylimidazole significantly increased the incidence of alveolar/bronchiolar adenoma in all treated groups of females, of alveolar/bronchiolar carcinoma in 1250-ppm males and of alveolar/bronchiolar adenoma or carcinoma (combined) in 1250-ppm males and 625- and 1250-ppm females. Although the incidence of alveolar/bronchiolar carcinoma in females was not statistically significant, that in the 1250-ppm exposure group exceeded the range (0–6%) in historical controls.

3.1.2 Rat

In a 2-year carcinogenicity study, groups of 50 male and 50 female F344/N rats were fed diets containing 0, 625, 1250 or 2500 ppm (males) and 0, 1250, 2500 or 5000 ppm (females) 4-methylimidazole (> 99% pure) for 106 weeks (equivalent to average daily doses of approximately 30, 55 or 115 and 60, 120 or 260 mg/kg bw in males and females, respectively) ([NTP, 2007](#); [Chan et al., 2008](#)). The food consumption of 5000-ppm females was lower than that of the controls. The incidence of mononuclear-cell leukaemia in 5000-ppm females was significantly higher than that in the controls, and exceeded the range (12–38%) in historical feed study controls. Mononuclear-cell leukaemia is a common finding with a highly variable incidence in F344/N rats and may have been exacerbated by exposure to 4-methylimidazole, as the onset in 5000-ppm females was earlier (day 368) than that in control females (day 624). [The Working Group also noted the significantly

Table 3.1 Carcinogenicity studies of oral administration of 4-methylimidazole in the diet to experimental animals

Species, strain (sex) Duration	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F ₁ (M, F) 106 wk	0, 312, 625 or 1250 ppm 7 d/wk 50/group	Lung (alveolar/bronchiolar adenoma): M ^a –8/50, 11/50, 13/50, 15/50 F ^b –0/50, 8/50, 16/50, 8/50 Lung (alveolar/bronchiolar carcinoma): M ^c –2/50, 4/50, 4/50, 8/50 F ^d –3/50, 0/50, 2/50, 7/50 Lung (alveolar/bronchiolar adenoma or carcinoma): M ^e –9/50, 13/50, 16/50, 22/50 F ^f –3/50, 8/50, 17/50, 14/50	<i>P</i> = 0.004 (low-dose F) <i>P</i> < 0.001 (mid-dose F) <i>P</i> = 0.003 (high-dose F) <i>P</i> = 0.017 (trend F) <i>P</i> = 0.042 (high-dose M) <i>P</i> = 0.024 (trend M) <i>P</i> = 0.003 (high-dose M) <i>P</i> < 0.001 (trend M) <i>P</i> < 0.001 (mid-dose F) <i>P</i> = 0.002 (high-dose F) <i>P</i> = 0.002 (trend F)	> 99% pure
Rat, F344 (M, F) 106 wk	0, 625, 1250 or 2500 ppm (M) 0, 1250, 2500 or 5000 ppm (F) 7 d/wk 50/group	Haematopoietic system (mononuclear-cell leukaemia): M–15/50, 18/50, 22/50, 20/50 F ^g –9/50, 7/50, 16/50, 20/50	<i>P</i> = 0.013 (high-dose F) <i>P</i> < 0.001 (trend F)	> 99% pure

^a Historical incidence (mean ± SD) for 2-year feed studies in male mice: 75/510 (15.8 ± 6.3%); range, 9–28%

^b Historical incidence (mean ± SD) for 2-year feed studies in female mice: 19/509 (3.7 ± 3.8%); range, 0–10%

^c Historical incidence (mean ± SD) for 2-year feed studies in male mice: 40/510 (7.8 ± 3.8%); range, 4–14%

^d Historical incidence (mean ± SD) for 2-year feed studies in female mice: 16/509 (2.9 ± 2.5%); range, 0–6%

^e Historical incidence (mean ± SD) for 2-year feed studies in male mice: 108/510 (22.2 ± 6.3%); range, 14–32%

^f Historical incidence (mean ± SD) for 2-year feed studies in female mice: 35/509 (6.6 ± 4.2%); range, 0–12%

^g Historical incidence (mean ± SD) for 2-year feed studies in female rats: 121/510 (23.8 ± 9.1%); range, 12–38%

d, day or days; F, female; M, male; SD, standard deviation; wk, week or weeks

From [NTP \(2007\)](#), [Chan et al. \(2008\)](#)

decreased incidence of pituitary (pars distalis) gland adenoma, of benign, complex or malignant pheochromocytoma (combined) of the adrenal gland in males, of pituitary (pars distalis) gland and clitoral gland adenoma, of mammary gland fibroadenoma and of uterine stromal polyps in females. These decreases in incidence could not be attributed to loss of body weight alone. The study in rats is also discussed in [Murray \(2011\)](#).]

4. Other Relevant Data

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, distribution and excretion

Previous studies have shown species differences in the disposition of 4-methylimidazole.

In rats, 5 minutes after a single intraperitoneal injection of 216 mg/kg bw, the uptake of 4-methylimidazole was highest in the intestines, followed by the liver, blood, stomach and kidney ([Hidaka, 1976](#)). The compound was excreted unchanged in the urine, beginning approximately 30 minutes after injection, and reached approximately 90% within 8 hours ([Hidaka, 1976](#)).

In ewes, the absorption and elimination of a single oral dose of 4-methylimidazole followed first-order kinetics. One half of an oral dose (20 mg/kg bw) of 4-methylimidazole was absorbed within about 27 minutes, and the maximum plasma level was reached 5 hours after administration ([Karangwa et al., 1990b](#)). Bioavailability calculated from plasma data from three ewes was 69%, and the biological half-life was 9.37 hours. Only 0.07 mg/kg of the dose was recovered in the urine as the unchanged parent

compound. Metabolites of 4-methylimidazole were not detected by high-performance liquid chromatography. [The Working Group noted that the sensitivity of this assay was difficult to evaluate.]

In goats and heifers, the mean residence time of 4-methylimidazole administered orally or intravenously was about 5 hours, and the volume of distribution was 0.9 L/kg bw. 4-Methylimidazole and its metabolites were excreted mainly in the urine, but also in the milk and faeces, and the administered dose was distributed mainly in the liver, kidney and lung ([Nielsen et al., 1993](#)). 4-Methylimidazole was found in the milk following its oral administration to pregnant and postpartum cows ([Morgan & Edwards, 1986](#)).

Following oral administration by gavage of 5, 50 or 150 mg/kg bw 4-methylimidazole (^{14}C -radiolabelled) to F344/N rats, peak plasma concentrations were reached at 0.5, 1.0 and 3.0 hours, respectively ([Yuan & Burka, 1995](#)). At 150 mg/kg, the plasma concentration of [^{14}C]4-methylimidazole was almost constant during the first 5 hours; at lower doses, the decline was more rapid. The estimated terminal half-life was dose-dependent. The authors suggested that the elimination of parent 4-methylimidazole was saturable. From the total urinary recovery of parent 4-methylimidazole, the estimated bioavailability was approximately 60–70%. Little or no metabolism of 4-methylimidazole was found. Only one minor hydrophilic metabolite was present in the urine and plasma. Faecal, biliary and respiratory elimination of radioactivity were negligible.

(b) Metabolism

Four metabolites were determined in the urine of goats and heifers given 4-methylimidazole ([Nielsen et al., 1993](#)), three of which were identified as 5-methyl-hydantoin, 2-methyl-hydantoic acid and urea. The high polarity of the fourth product prevented further characterization.

(c) Toxicokinetic models

After a single oral administration by gavage of 4-methylimidazole (10, 50 or 100 mg/kg bw) to male and female F344/N rats, the plasma concentration versus time data could be described by a one-compartment model, with no lag phase, and first-order absorption and elimination for both males and females based on the findings of [Yuan & Burka \(1995\)](#) ([NTP, 2007](#)). The absorption half-life ranged from 5 to 23 minutes and decreased with dose. The elimination half-life ranged from 1 to 8 hours and increased with dose. The plasma concentration versus time data following intravenous administration of 10 mg/kg bw 4-methylimidazole was described as a one-compartment model with first-order elimination. From comparisons of the area under the concentration versus time curves for the two routes of administration, bioavailability was determined to be greater than 85%.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

The genetic effects of 4-methylimidazole have recently been reviewed ([NTP, 2007](#)).

(a) Mutations

4-Methylimidazole (up to 10 000 µg/plate) was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100 or TA1535 when tested in the presence or absence of 10% or 30% hamster or rat liver metabolic activation systems (detailed protocol presented by [Zeiger et al., 1988](#); [NTP, 2007](#)). Class III and IV caramel colourings contain various concentrations of 4-methylimidazole, and did not to induce mutagenic activity in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 or TA1538. In these

studies, concentrations of 4-methylimidazole in class III preparations were 9–463 mg/kg, and those in class IV were 146–215 mg/kg ([Allen et al., 1992](#)). Class III and IV caramel colourings were also negative in the *S. typhimurium* Ames test and *Saccharomyces cerevisiae* gene conversion assays. In these studies, concentrations of 4-methylimidazole in class III preparations were 34–463 mg/kg, and those in class IV were 107–387 mg/kg ([Brusick et al., 1992](#)).

(b) Chromosomal effects

No increases in the frequency of micronucleated erythrocytes were observed in the bone marrow of male rats or male mice (detailed protocol presented by [Shelby et al., 1993](#); [NTP, 2007](#)) administered three intraperitoneal injections of 4-methylimidazole at 24-hours intervals or in peripheral blood samples from male and female mice fed the compound in the diet for 14 weeks (detailed protocol presented by [MacGregor et al., 1990](#); [NTP, 2007](#)). No significant alterations in the percentage of polychromatic erythrocytes, an approximate indicator of bone marrow toxicity, were seen in the bone marrow or peripheral blood of mice; however, the percentage declined with increasing dose of 4-methylimidazole and was significantly depressed at the highest dose in the bone marrow of male rats ([NTP, 2007](#)).

Class III caramel colouring did not induce chromosomal damage in Chinese hamster ovary cells ([Allen et al., 1992](#)). In the study of [Brusick et al. \(1992\)](#), class IV caramel colouring gave negative results in the chromosomal aberration assay while class III colouring was weakly clastogenic only in the absence of metabolic activation or in the presence of heat-inactivated metabolic activation. Class IV caramel colouring was not clastogenic in Chinese hamster ovary cells *in vitro* in either the presence or absence of metabolic activation, whereas the weak clastogenic effect of class III caramel colouring was abolished in the presence of metabolic activation. Moreover,

in vivo, class III caramel colouring administered orally to mice did not induce micronuclei in the bone marrow.

4.3 Mechanistic data

4.3.1 Effects on cell physiology

In 15-day feed studies, 4-methylimidazole did not induce any histopathological changes in male or female F344 rats. In a 14-week feed study, some variation in serum thyroxine (males) or triiodothyronine and thyroid-stimulating hormone (females) was observed but the changes were sporadic and independent of dose. No histopathological alterations were observed in B6C3F₁ mice fed 4-methylimidazole for 15 days. However, transient decreases in serum thyroxine and increases in serum tri-iodo-thyronine levels were observed in males and females in a 14-week feed study; levels of thyroid-stimulating hormone were not determined (NTP, 2004; Chan *et al.*, 2006).

No thyroid lesions were observed following 15 days or 14 weeks of exposure to 4-methylimidazole. In contrast, the structural analogue, 2-methylimidazole, induced thyroid lesions in rats and mice in both 15-day and 14-week feed studies (NTP, 2004; Chan *et al.*, 2006).

Class IV caramel colouring was evaluated for toxicity in male and female F344 rats at doses up to 30 g/kg bw for 13 weeks (MacKenzie *et al.*, 1992). Although food and water consumption, body weight and urine volume were decreased, these were considered to be adaptive non-specific changes.

4.3.2 Effects on cell function

4-Methylimidazole forms complexes with haeme-containing enzymes such as cytochrome P450 (CYP) and results in the inhibition of mixed-function oxidase activity (Wilkinson *et al.*, 1983; Karangwa *et al.*, 1990b). It was reported

that 4-methylimidazole significantly inhibited CYP2E1 activity in rat liver (Hargreaves *et al.*, 1994) and tolbutamide hydroxylase (CYP2C9) activity in human and rat microsomes (Back & Tjia, 1985; Back *et al.*, 1988). Moreover, it stimulated the phosphorylation of rabbit kidney (Na⁺ and K⁺)-adenosine triphosphatase (Schuurmans Stekhoven *et al.*, 1988), and exhibited significant antioxidant activity in a lipid peroxyl radical activity trapping assay (Kohen *et al.*, 1988).

4.4 Mechanisms of carcinogenesis

The incidence of hyperplasia of the lung alveolar epithelium was significantly increased in female mice fed 1250 ppm [60 mg/kg bw] 4-methylimidazole for 2 years (NTP, 2007). Hyperplasia of the alveolar epithelium is thought to be a precursor of neoplastic development. [In this study, hyperplasia was analysed only at the end of the 2-year study, which does not ensure that hyperplasia appeared before adenoma.] Interestingly, 4-methylimidazole had no effect on the respiratory epithelium in a 14-week toxicity study at concentrations as high as 10 000 ppm [3180 mg/kg bw] (NTP, 2004). 4-Methylimidazole induced neither mutations nor chromosomal aberrations *in vitro* or *in vivo*. The mechanism of action of 4-methylimidazole in mouse lung tumorigenesis is not clear.

5. Summary of Data Reported

5.1 Exposure Data

4-Methylimidazole is used as a raw material, chemical intermediate or component in the manufacture of pharmaceuticals, dyes, pigments or agricultural chemicals. Occupational exposure may occur by inhalation or dermal contact. 4-Methylimidazole is formed as a result of the interaction of ammonia with reducing sugars.

The general population is exposed to 4-methylimidazole in food through its presence in class III and IV caramels, which are widely used food colourings, especially in beverages. It has been detected in ammoniated forage and ammoniated molasses that were fed to animals, and in the milk from these animals.

4-Methylimidazole has been detected in tobacco smoke.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

4-Methylimidazole was tested for carcinogenicity by oral administration in the diet to mice and rats. It increased the incidence of alveolar/bronchiolar adenoma in female mice, alveolar/bronchiolar carcinoma in male mice and alveolar/bronchiolar adenoma and carcinoma combined in male and female mice. Oral administration of 4-methylimidazole increased the incidence of mononuclear-cell leukaemia in female rats.

5.4 Other relevant data

No data were available on the toxicokinetics of 4-methylimidazole in humans. After oral administration to mammals, 4-methylimidazole was rapidly absorbed and widely distributed. In rats, ewes, goats and heifers, 4-methylimidazole and its metabolites were mainly excreted in the urine. Three urinary metabolites were identified in goats and heifers (5-methyl-hydantoin, 2-methyl-hydantois acid and urea) but none was characterized in rats.

4-Methylimidazole induces neither mutations nor chromosomal aberrations in experimental test systems. It caused no observable histological lesions in rodents following 15 days

or 14 weeks of exposure in the diet. The mechanism of action of 4-methylimidazole that leads to lung tumours in mice is unknown.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 4-methylimidazole.

6.3 Overall evaluation

4-Methylimidazole is *possibly carcinogenic to humans (Group 2B)*.

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INTRODUCTION TO THE *MONOGRAPHS* ON BROMOCHLOROACETIC ACID, DIBROMOACETIC ACID AND DIBROMOACETONITRILE

No epidemiological studies have evaluated exposure specifically to bromochloroacetic acid, dibromoacetic acid or dibromoacetonitrile in humans. Human exposure to these chemicals always occurs in mixtures with other disinfection by-products in chlorinated drinking-water and chlorinated water in swimming pools, which include more than 700 chemicals ([Richardson *et al.*, 2007](#)). The previous *Monograph* on drinking-water disinfectant by-products ([IARC, 2004](#)) reviewed the epidemiological studies published up to 2002. The current Working Group reviewed all of the data detailed below, although a formal evaluation on chlorinated drinking-water and disinfection by-products was not made at this time.

Chlorine is the most common disinfectant used worldwide, although others may also be used (i.e. ozone, chlorine dioxide and bromide in swimming pools). To deal with the complexity of the mixtures of chemicals in disinfected water, epidemiological studies conducted since the 1970s that evaluated the risk of chlorinated water and disinfection by-products for cancer used surrogates of exposure, which have evolved from duration of residence in a household supplied with chlorinated surface water and type and concentration of disinfectant to the quantification of

levels of relevant by-products (e.g. chloroform and total trihalomethanes). However, although the more recent studies measured specific compounds (i.e. trihalomethanes), these are a proxy for a complex mixture in disinfected water, which includes bromochloroacetic acid, dibromoacetic acid and dibromoacetonitrile. The correlation between surrogates of disinfectant by-products and individual constituents in specific samples of treated water is complex and strongly depends on raw water quality and the type of treatment (including the disinfection processes). Brominated disinfection by-products, including bromochloroacetic acid, dibromoacetic acid and dibromoacetonitrile, tend to increase as the bromine content of the raw water increases ([Kampioti & Stephanou, 2002](#)). In general, trihalomethanes and haloacetic acids represent the two major classes of halogenated disinfection by-products on a weight basis, ([Krasner *et al.*, 2006](#)). Levels of total trihalomethanes are correlated with the total organic halide content when chlorine is used as the main disinfectant ([Singer & Chang, 1989](#)).

Several epidemiological studies have evaluated the risk of cancer associated with chlorinated drinking-water and disinfection by-products. They used a variety of methods

to assess exposure and indicators, generally through surrogates of exposure, such as duration of residence in a household supplied with chlorinated surface water, duration of exposure to chlorinated drinking-water, estimated lifetime level of trihalomethanes, amount of tap-water consumed, type of disinfectant or concentration of relevant by-products (e.g. total trihalomethanes).

The risk for cancer of the urinary bladder was reported in nine case-control studies ([Cantor et al., 1987](#); [McGeehin et al., 1993](#); [Vena et al., 1993](#); [King & Marrett, 1996](#); [Freedman et al., 1997](#); [Cantor et al., 1998](#); [Koivusalo et al., 1998](#); [Chevrier et al., 2004](#); [Bove et al., 2007a](#); [Villanueva et al., 2007](#); [Cantor et al., 2010](#)) and three cohort studies ([Wilkins & Comstock, 1981](#); [Doyle et al., 1997](#); [Koivusalo et al., 1997](#)). Positive and duration- or dose-dependent associations were reported in all case-control studies and reached statistical significance in all but three of them. One study ([Cantor et al., 2010](#)) evaluated gene-environment interactions for glutathione S-transferase (GST) and cytochrome P450 (CYP) genes that metabolize disinfection by-products and found them to be statistically significant. A pooled analysis of six case-control studies ([Villanueva et al., 2004](#)) showed that the risk for urinary bladder cancer among men increased with increasing exposure to trihalomethanes. The results from the cohort studies were inconsistent: studies reported elevated risks that were only statistically significant for women in one study ([Koivusalo et al., 1997](#)) and no dose-response relationships were observed.

Other cancer sites have been evaluated in case-control and cohort studies. Increased risks were identified for cancers of the lung ([Doyle et al., 1997](#)), melanoma ([Doyle et al., 1997](#)), oesophagus ([Koivusalo et al., 1997](#)) and breast ([Koivusalo et al., 1997](#)) in cohort studies and cancers of the kidney ([Koivusalo et al., 1998](#)), brain ([Cantor et al., 1999](#)), melanoma ([Nelemans et al., 1994](#)) and non-melanoma skin cancer

([Karagas et al., 2008](#)) in case-control studies. These results either need to be replicated or were not statistically significant (non-melanoma skin cancer); there are also concerns about potential bias (melanoma). Findings were null for childhood leukaemia (one case-control study: [Infante-Rivard et al., 2001, 2002](#)) and were contradictory for colorectal cancer in case-control ([Young et al., 1987](#); [Hildesheim et al., 1998](#); [King et al., 2000](#); [Bove et al., 2007b](#)) and cohort ([Doyle et al., 1997](#); [Koivusalo et al., 1997](#)) studies, pancreatic cancer (three case-control studies: [Ijsselmuiden et al., 1992](#); [Kukkula & Löfroth, 1997](#); [Do et al., 2005](#)) and different types of adult leukaemia (one case-control study: [Kasim et al., 2006](#)).

Cancer in Humans

1. Cohort studies

See [Table 1](#) and [Table 2](#)

[Wilkins & Comstock \(1981\)](#) identified increased risks for urinary bladder cancer in men and women and for liver cancer among women, but the risk estimates were not statistically significant, among users of chlorinated surface water versus those of non-chlorinated deep wells. [Doyle et al. \(1997\)](#) conducted a study among women only and found a significantly increased risk and a dose-response relationship with levels of chloroform for all cancers, melanoma, and cancers of the colon and lung. [Koivusalo et al. \(1997\)](#) identified a significantly increased risk among women for cancers of the urinary bladder, colon, oesophagus and breast with increasing mutagenicity of the water.

Table 1 Studies on the incidence of urinary bladder cancer associated with exposure to chlorinated drinking-water

Study	Population/end-point	Exposure	Exposure categories	Risk estimate (95% CI)	Comments
Cohort					
Wilkins & Comstock (1981)	Residents of Washington County, MD, USA 30 780 persons (14 553 men, 16 227 women), ≥ 25 yr of age, followed up 1963–75; 52 cases	Chlorinated surface water (average chloroform concentration, 107 µg/L) vs non-chlorinated deep wells	Men Women	Relative risk 1.8 (0.8–4.8) 1.6 (0.5–6.3)	Adjusted for differences between cohorts in age, marital status, education, smoking history, church attendance, housing, persons per room
Doyle et al. (1997)	Iowa Women's Health Study (USA): 41 836 women aged 55–69 yr, followed up 1986–93; 42 cases	1108 municipal water supplies (1979 and 1986–87) Chloroform concentration in 1986–87 (µg/L)	< Limit of detection 1–2 3–13 14–287 <i>P</i> for trend	1.0 0.9 (0.4–2.0) 1.2 (0.6–2.7) 0.6 (0.3–1.6) 0.46	Adjusted for age, education, smoking, physical activity, fruit and vegetable intake, energy intake, body mass index, waist-to-hip ratio
Koivusalo et al. (1997)	Finland, incidence 1971–93 56 towns – 32% of country population; 313 464 men, 307 967 women	Estimates of mutagenic potency of drinking-water; 3000 net revertants/L increase in average exposure to mutagenicity	Men Women	1.03 (0.8–1.3) 1.5 (1.01–2.2)	Record-linkage study; adjusted for age, time-period, urbanization and social status; cancers of ureter and urethra included
Case-control					
Cantor et al. (1987)	10 areas in the USA: Atlanta, Connecticut, Detroit, Iowa, New Jersey, New Mexico, New Orleans, Seattle, San Francisco, Utah Incidence, 1-yr period starting December 1997; 2805 cases, 5258 population controls (men and women)	Duration of consumption of chlorinated surface drinking-water in subjects with tap-water consumption above median (1.44 L/d)	Duration (yr) <i>Men</i> 0 1–19 20–39 40–59 ≥ 60 <i>P</i> for trend <i>Women</i> 0 1–19 20–39 40–59 ≥ 60 <i>P</i> for trend	Odds ratio 1.0 1.1 (0.7–1.6) 1.1 (0.7–1.5) 1.2 (0.8–1.7) 1.2 (0.7–2.1) 0.44 1.0 1.8 (0.8–3.7) 1.5 (0.7–3.1) 2.2 (1.0–4.8) 3.2 (1.2–8.7) 0.02	Adjusted for age, smoking habit, high-risk occupation, population size of usual residence, reporting centre

Table 1 (continued)

Study	Population/end-point	Exposure	Exposure categories	Risk estimate (95% CI)	Comments
McGeehin et al. (1993)	Colorado, USA Incidence 1990–91; 327 cases, 261 controls with other cancers excluding lung and colorectal cancer (men and women, all white)	Lifetime exposure to chlorinated water from individual histories of residence and water source	Duration (yr)		Adjusted for coffee consumption, smoking, tap-water intake, family history of bladder cancer, sex, medical history of bladder infection or kidney stone
			0	1.0	
			1–10	0.7 (0.4–1.3)	
			11–20	1.4 (0.8–2.5)	
			21–30	1.5 (0.8–2.9)	
> 30	1.8 (1.1–2.9)				
Vena et al. (1993)	Western New York, USA Incidence, 1979–85; 351 cases, 855 population controls; restricted to white males	Daily intake of tap-water	<i>No. of cups</i>	<i>Age < 65 yr</i>	Adjusted for age, education, cigarette smoking (pack-yr), and coffee, carotene and non-tap-water intake. [Overlaps with Bove et al. (2007a) .]
			0–5	1.00	
			6–7	1.3 (0.7–2.4)	
			8–9	1.6 (0.9–3.0)	
			10–39	2.6 (1.5–4.5)	
			<i>P for trend</i>	< 0.001	
			<i>Age ≥ 65 yr</i>		
			0–5	1.00	
			6–7	1.3 (0.8–2.1)	
			8–9	1.4 (0.8–2.5)	
			10–39	3.0 (1.8–5.0)	
<i>P for trend</i>	< 0.001				
King & Marrett (1996)	Ontario, Canada Incidence, September 1992–May 1994; 696 cases, 1545 population controls (men and women)	Consumption of chlorinated surface drinking-water	Duration (yr)		Adjusted for age, sex, log pack-yr of smoking, current smoking, education, calorie intake
			0–9	1.0	
			10–19	1.04 (0.7–1.5)	
			20–34	1.2 (0.9–1.5)	
			≥ 35	1.4 (1.1–1.8)	
		Trihalomethanes-yr	Quartiles (µg/L-yr)		
			0–583	1.0	
			584–1505	1.2 (0.9–1.6)	
			1506–1956	1.08 (0.8–1.4)	
			1957–6425	1.4 (1.1–1.9)	
		Level of trihalomethanes in water source	Level (µg/L)		
			0–24	1.0	
			25–74	1.4 (1.0–2.0)	
≥ 75	1.7 (1.1–2.5)				
<i>P for trend</i>	0.006				

Table 1 (continued)

Study	Population/end-point	Exposure	Exposure categories	Risk estimate (95% CI)	Comments
Freedman et al. (1997)	Washington County, MD, USA Incidence, 1975–92; 294 cases, 2326 population controls	Duration of residence with municipal water source	Duration (in yr) <i>Men (cases)</i> 0 (54) 1–10 (63) 11–20 (41) 21–30 (31) 31–40 (11) > 40 (9) <i>Women (cases)</i> 0 (25) 1–10 (28) 11–20 (15) 21–30 (7) 31–40 (5) > 40 (4)	1.0 1.1 (0.6–1.9) 1.1 (0.6–1.9) 1.3 (0.7–2.5) 1.5 (0.6–3.3) 2.2 (0.8–5.1) 1.0 0.7 (0.3–1.7) 0.7 (0.3–1.8) 0.6 (0.2–1.6) 0.7 (0.2–2.2) 0.6 (0.2–2.2)	Adjusted for age, sex, smoking, urbanization
Cantor et al. (1998)	Iowa, USA Incidence, 1986–89; 1123 cases, 1983 population controls (men and women)	Total lifetime exposure to trihalomethanes (THM) estimated from lifetime residential histories, water utility survey and measurements of water samples	THM (g) <i>Men</i> ≤ 0.04 0.05–0.12 0.13–0.34 0.35–1.48 1.49–2.41 ≥ 2.42 <i>P</i> for trend <i>Women</i> ≤ 0.04 0.05–0.12 0.13–0.34 0.35–1.48 1.49–2.41 ≥ 2.42 <i>P</i> for trend	1.0 1.3 (1.0–1.7) 1.1 (0.8–1.5) 1.2 (0.9–1.6) 1.3 (0.8–2.0) 1.8 (1.2–2.7) 0.05 1.0 1.2 (0.8–1.8) 0.9 (0.6–1.6) 1.0 (0.6–1.7) 0.9 (0.9–2.0) 0.6 (0.3–1.4) 0.54	Adjusted for age, study period, education, high-risk occupation, cigarette smoking (6 strata)

Table 1 (continued)

Study	Population/end-point	Exposure	Exposure categories	Risk estimate (95% CI)	Comments
Koivusalo et al. (1998)	Finland Incidence, 1991–92; 732 cases (552 men, 180 women), 914 population controls (621 men, 293 women)	Mutagenic potency of drinking-water estimated from historical exposure at past residence, past water source and historical data on water quality and treatment	3000-net revertants/L increase in average exposure to mutagenicity among subjects with ≥ 30 yr of exposure Tertiles of exposure among subjects with ≥ 30 yr of exposure (net revertants/L)	<i>Men</i> 1.2 (0.9–1.7) <i>Women</i> 1.2 (0.7–2.0)	Adjusted for age, smoking, socioeconomic status
			<i>Men</i> Unexposed Low (1–999) Medium (1000–2499) High (≥ 2500)	1.0 1.2 (0.8–1.6) 0.97 (0.7–1.4) 1.4 (0.9–2.0)	
			<i>Women</i> Unexposed Low (1–999) Medium (1000–2499) High (≥ 2500)	1.0 1.2 (0.7–2.0) 1.3 (0.7–2.4) 1.2 (0.6–2.2)	

Table 1 (continued)

Study	Population/end-point	Exposure	Exposure categories	Risk estimate (95% CI)	Comments			
Chevrier et al. (2004)	France Incidence, 1985–87; 281 cases (240 men, 41 women), 272 controls (233 men, 39 women)	Duration of exposure to ozonated water	<i>Men (231 cases)</i>			Adjusted for hospital, age, socioeconomic status, smoking status, coffee consumption, high-risk occupations, tap water consumption, average THM level		
			0 yr	1.00				
			1–9 yr	0.58 (0.3–1.3)				
			10–30 yr	0.27 (0.1–0.6)				
			<i>Women (38 cases)</i>					
			0 yr	1.00				
		1–9 yr	0.40 (0.0–7.3)					
		10–30 yr	0.15 (0.0–2.7)					
		Average level of THM in a 30-yr exposure window from 5 to 35 yr before the interview; analysis restricted to subjects with known exposure of at least 70% of the exposure period.	<i>Men (231 cases)</i>					Adjusted for hospital, age, socioeconomic status, smoking status, coffee consumption, high-risk occupations, tap water consumption, duration of exposure to ozonated water
			< 1 µg/L	1.00				
			1–5 µg/L	1.32 (0.7–2.6)				
			6–50 µg/L	1.97 (0.8–5.2)				
> 50 µg/L	3.73 (1.2–11)							
<i>Women (38 cases)</i>								
< 1 µg/L	-							
1–5 µg/L	1.00							
6–50 µg/L	1.97 (0.2–18)							
> 50 µg/L	1.55 (0.1–32)							

Table 1 (continued)

Study	Population/end-point	Exposure	Exposure categories	Risk estimate (95% CI)	Comments			
Villanueva et al. (2007)	Spain Incidence 1998–2001; 1219 cases (1067 men, 152 women), 1271 controls (1105 men, 166 women)	Average THM level in the residences from age 16 until the time of interview; analysis restricted to subjects with known exposure of at least 70% of the exposure window.	<i>Men (618 cases)</i>			Adjusted for age, smoking status, education, urbanization of longest residence until age 18 yr, overall quality of the interview, geographical area. [Overlaps with Cantor et al. (2010) .]		
			≤ 8 µg/L	1.00				
			> 8–26 µg/L	1.53 (0.95–2.48)				
			> 26–49 µg/L	2.34 (1.36–4.03)				
			> 49 µg/L	2.53 (1.23–5.20)				
			<i>P</i> for trend	< 0.01				
			<i>Women (89 cases)</i>					
			≤ 8 µg/L	1.00				
			> 8–26 µg/L	0.40 (0.13–1.27)				
			> 26–49 µg/L	1.14 (0.31–4.10)				
			> 49 µg/L	1.50 (0.26–8.61)				
			<i>P</i> for trend	0.61				
			Duration of chlorinated surface water in the residence from age 16 yr until the time of interview; analysis restricted to subjects with known exposure of at least 70% of the exposure window.	<i>Men (618 cases)</i>				
			0–3 yr	1.00				
> 3–25 yr	2.26 (1.19–4.29)							
> 25–30 yr	2.58 (1.33–5.01)							
> 30 yr	2.21 (1.17–4.20)							
<i>P</i> for trend	0.20							
	<i>Women (89 cases)</i>							
0–3 yr	1.00							
> 3–25 yr	2.72 (0.56–13.26)							
> 25–30 yr	2.32 (0.44–12.13)							
> 30 yr	2.33 (0.51–10.55)							
<i>P</i> for trend	0.62							

Table 1 (continued)

Study	Population/end-point	Exposure	Exposure categories	Risk estimate (95% CI)	Comments	
Cantor et al. (2010)	Spain Incidence 1998–2001; 680 cases (595 men, 85 women), 714 controls (622 men, 92 women)	Average THM level in the residences from age 16 yr until the time of interview; analysis restricted to subjects with known exposure of at least 70% of the exposure window.	<i>GSTT1</i> present (542 cases)			Adjusted for age (continuous), sex, smoking status (never/former/ current), size of the municipality of longest residence until 18 yr of age, education (3 strata), geographical area (6 strata), overall quality of interview. [Overlaps with Villanueva et al. (2007) .] <i>P</i> -value for multiplicative interaction between THM level and: <i>GSTT1</i> polymorphism = 0.02; <i>GSTZ1</i> polymorphism = 0.02; <i>CYP2E1</i> polymorphism = 0.04
			≤ 8 µg/L	1.0		
			> 8–26 µg/L	1.2 (0.7–1.9)		
			> 26–49 µg/L	2.0 (1.2–3.4)		
			> 49 µg/L	2.2 (1.1–4.3)		
			<i>P</i> for trend	0.0072		
			<i>GSTT1</i> null (136 cases)			
			≤ 8 µg/L	1.0		
			> 8–26 µg/L	1.2 (0.5–2.5)		
			> 26–49 µg/L	1.2 (0.5–2.5)		
			> 49 µg/L	1.0 (0.4–2.5)		
			<i>P</i> for trend	0.28		
			<i>GSTZ1</i> rs1046428 CT/TT (244 cases)			
			≤ 8 µg/L	1.0		
			> 8–26 µg/L	1.4 (0.7–2.7)		
			> 26–49 µg/L	2.2 (1.1–4.2)		
> 49 µg/L	2.9 (1.3–6.7)					
<i>P</i> for trend	0.0043					
<i>GSTZ1</i> rs1046428 CC (405 cases)						
≤ 8 µg/L	1.00					
> 8–26 µg/L	1.1 (0.7–1.9)					
> 26–49 µg/L	1.5 (0.9–2.7)					
> 49 µg/L	1.3 (0.6–2.8)					
<i>P</i> for trend	0.28					

Table 1 (continued)

Study	Population/end-point	Exposure	Exposure categories	Risk estimate (95% CI)	Comments
Cantor et al. (2010) Contd.			<i>CYP2E1 rs2031920 CC</i> (590 cases)		
			≤ 8 µg/L	1.0	
			> 8–26 µg/L	1.3 (0.8–2.0)	
			> 26–49 µg/L	2.1 (1.2–3.5)	
			> 49 µg/L	2.0 (1.0–4.1)	
			<i>P</i> for trend	0.014	
			<i>CYP2E1 rs2031920 CT/TT</i> (37 cases)		
			≤ 8 µg/L	1.0	
			> 8–26 µg/L	0.98 (0.4–2.5)	
			> 26–49 µg/L	1.1 (0.4–3.1)	
			> 49 µg/L	0.6 (0.1–2.7)	
<i>P</i> for trend	0.33				

Table 1 (continued)

Study	Population/end-point	Exposure	Exposure categories	Risk estimate (95% CI)	Comments	
Bove et al. (2007a)	Western New York, USA Incidence, 1979–85; 129 cases, 256 controls (all men)	THM level at the last residence around 20 yr after recruitment	<i>Total THM</i>			Adjusted for daily tap-water consumption, age, cigarette smoking (pack-yr), carotene, water consumption from foods, dietary fibre, alcohol. [Overlaps with Vena et al. (1993) .]
			1st quartile	≤ 38.04 µg/L	1.00	
			2nd quartile	38.18–52.58 µg/L	1.43 (0.78–2.05)	
			3rd quartile	52.59–73.82 µg/L	1.93 (0.80–2.98)	
			4th quartile	74.10–351.73 µg/L	2.34 (1.01–3.66)	
			<i>Chloroform</i>			
			1st quartile	≤ 17.14 µg/L	1.00	
			2nd quartile	17.42–25.72 µg/L	1.79 (0.81–3.09)	
			3rd quartile	26.15–38.61 µg/L	1.76 (0.91–3.35)	
			4th quartile	38.46–192.52 µg/L	2.55 (1.25–4.66)	
			<i>Bromodichloromethane</i>			
			1st quartile	≤ 9.35 µg/L	1.00	
			2nd quartile	9.40–13.31 µg/L	1.89 (0.95–3.59)	
			3rd quartile	13.35–18.75 µg/L	2.20 (1.12–4.26)	
			4th quartile	18.80–78.93 µg/L	2.49 (1.19–4.48)	
			<i>Dibromochloromethane</i>			
			1st quartile	≤ 4.67 µg/L	1.00	
			2nd quartile	4.68–6.89 µg/L	1.29 (0.77–2.83)	
			3rd quartile	6.90–9.35 µg/L	1.34 (0.78–2.85)	
			4th quartile	9.37–35.62 µg/L	1.17 (0.84–3.03)	
<i>Bromoform</i>						
1st quartile	≤ 0.43 µg/L	1.00				
2nd quartile	0.44–0.73 µg/L	2.12 (1.05–4.17)				
3rd quartile	0.75–1.14 µg/L	2.34 (1.18–4.57)				
4th quartile	1.16–41.88 µg/L	3.05 (1.51–5.69)				

CI, confidence interval; d, day or days; THM, trihalomethanes; vs, versus; yr, year or years

Table 2 Cohort studies of cancer at other sites and exposure to chlorinated drinking-water

Reference	Population/follow-up	Exposure	Site (No. of subjects)	Relative risk (95% CI)	Comments	
Wilkins & Comstock (1981)	Residents of Washington County, MD, USA 30 780 persons (14 553 men, 16 227 women), ≥ 25 yr of age followed up 1963–75	Chlorinated surface water (average chloroform concentration, 107 µg/L) vs non-chlorinated deep wells	<i>Liver</i> (12)		Adjusted for differences between cohorts in age, marital status, education, smoking history, church attendance, housing, persons per room	
			Men	0.7 (0.2–3.5)		
			Women	1.8 (0.6–6.8)		
			<i>Kidney</i> (18)			
			Men	0.8 (0.3–2.7)		
			Women	1.01 (0.3–6.0)		
Doyle et al. (1997)	Iowa Women's Health Study (USA) 41 836 women aged 55–69 yr, followed up 1986–93	1108 municipal water supplies (1979 and 1986–87) Chloroform concentration in 1986–87 (µg/L)	< Limit of detection <i>Kidney</i> (30)		Adjusted for age, education, smoking, physical activity, fruit and vegetable intake, energy intake, body mass index, waist-to-hip ratio	
				1–2		0.5 (0.2–1.6)
				3–13		1.2 (0.5–3.1)
				14–287		0.9 (0.3–2.3)
						<i>P</i> for trend = 0.82
				<i>Colon</i> (178)		
				1–2		1.1 (0.7–1.7)
				3–13		1.4 (0.9–2.2)
				14–287		1.7 (1.1–2.5)
						<i>P</i> for trend < 0.01
				<i>Rectum and anus</i> (78)		
				1–2		0.8 (0.4–1.5)
				3–13		0.8 (0.4–1.5)
14–287	1.1 (0.6–1.9)					
	<i>P</i> for trend < 0.01					
<i>Lung</i> (143)						
1–2	1.2 (0.8–2.1)					
3–13	1.8 (1.1–3.0)					
14–287	1.6 (0.97–2.6)					
	<i>P</i> for trend = 0.025					

Table 2 (continued)

Reference	Population/follow-up	Exposure	Site (No. of subjects)	Relative risk (95% CI)	Comments
Doyle et al. (1997)			<i>Melanoma (44)</i>		
Contd.		1-2		2.6 (0.99-6.6)	
		3-13		1.3 (0.4-4.0)	
		14-287		3.4 (1.3-8.6)	
				<i>P</i> for trend = 0.049	
			<i>All cancers (983)</i>		
		1-2		1.04 (0.9-1.3)	
		3-13		1.2 (1.03-1.5)	
		14-287		1.3 (1.1-1.5)	
				<i>P</i> for trend < 0.01	
Koivusalo et al. (1997)	Finland 56 towns; 32% of Finnish population, 1971-93; 621 431 persons (307 967 women, 313 464 men)	Chlorinated/unchlorinated water supplies; mutagenicity assessment	<i>Both sexes</i> Colon Rectum Oesophagus Pancreas Kidney Brain and nervous system Non-Hodgkin lymphoma Leukaemia	0.9 (0.8-1.04) 1.04 (0.9-1.3) 1.4 (0.9-2.1) 1.01 (0.8-1.2) 1.03 (0.8-1.3) 1.00 (0.9-1.2) 1.2 (0.9-1.5) 1.04 (0.9-1.3)	Adjusted for age, time period, urbanization, social status; cancers of the ureter and urethra included
			<i>Women</i> Colon Rectum Oesophagus Breast Pancreas Kidney Brain and nervous system Non-Hodgkin lymphoma Leukaemia	0.95 (0.8-1.9) 1.4 (1.03-1.9) 1.9 (1.02-3.5) 1.1 (1.01-1.2) 1.1 (0.8-1.5) 1.03 (0.7-1.4) 1.08 (0.9-1.4) 1.4 (0.98-1.98) 1.08 (0.8-1.5)	

Table 2 (continued)

Reference	Population/follow-up	Exposure	Site (No. of subjects)	Relative risk (95% CI)	Comments
Koivusalo <i>et al.</i> (1997)			<i>Men</i>		
Contd.			Colon	0.8 (0.7–1.04)	
			Rectum	0.9 (0.7–1.09)	
			Oesophagus	0.9 (0.5–1.7)	
			Prostate	0.97 (0.8–1.1)	
			Pancreas	0.9 (0.7–1.2)	
			Kidney	1.04 (0.8–1.4)	
			Brain and nervous system	0.9 (0.7–1.2)	
			Non-Hodgkin lymphoma	1.03 (0.8–1.4)	
			Leukaemia	1.02 (0.8–1.3)	

CI, confidence interval; vs, versus; yr, year or years

2. Case-control studies

2.1 Cancer of the urinary bladder

See [Table 1](#)

Seven case-control studies of urinary bladder cancer have been reviewed previously ([IARC, 2004](#)). [Cantor et al. \(1987\)](#) found increased odds ratios for bladder cancer among people with both elevated intakes of drinking-water and long-term consumption of chlorinated surface water. [McGeehin et al. \(1993\)](#) found odds ratios that increased with years of exposure to chlorinated water. In a study by [Vena et al. \(1993\)](#), the odds ratios for bladder cancer increased with increasing numbers of cups of tap-water consumed daily. No excess risk was observed in subjects who had used the public water supply for more than 50 years compared with those who had used it for less than 50 years. [The Working Group noted that the unexposed group included subjects with a long duration of exposure to chlorinated drinking-water. This paper did not analyse associations with the water source or the level of trihalomethanes.] [King & Marrett \(1996\)](#) reported odds ratios that increased with increasing duration of use of a chlorinated surface water source. Results for trihalomethanes-years as the exposure variable showed a similar increase in risk. In addition, among subjects with relatively homogeneous exposures for at least 30 years, a trend in risk with increasing levels of trihalomethanes was observed ($P = 0.006$). [Freedman et al. \(1997\)](#) found that the risk for bladder cancer among men increased with duration of exposure to municipal drinking-water; but the associations were not statistically significant. [Cantor et al. \(1998\)](#) reported that odds ratios increased with increasing total lifetime dose of trihalomethanes for men but not for women. Results for average lifetime dose of trihalomethanes followed similar patterns. [Koivusalo et al. \(1998\)](#) identified a small, non-significant excess risk for bladder cancer for an

increase in mutagenicity of 3000 net revertants/L in men and women. The odds ratio for categories of increasing exposure did not show a consistent exposure-response relationship.

[Chevrier et al. \(2004\)](#) analysed data from a hospital-based case-control study conducted in seven hospitals in France. Information on water source and treatment was collected retrospectively in the study areas and mean levels of trihalomethanes were assigned to the different combinations of water source and treatment as predicted by an experimental model. The risk for bladder cancer decreased as duration of exposure to ozonated water increased with a statistically significant dose-response relationship. [The Working Group noted that, in general, chlorinated by-products decrease as the level of ozonation increased.] The risk for bladder cancer increased with duration of exposure to chlorinated surface water and with the estimated trihalomethanes content of the water, but the dose-response relationship was not statistically significant. Results were similar among men and women.

The hospital-based case-control study conducted in Spain by [Villanueva et al. \(2007\)](#) evaluated lifetime exposure to trihalomethanes through different exposure situations involving ingestion, inhalation and dermal absorption. Study subjects were interviewed and provided individual information on water-related habits and residential history from birth. Levels of trihalomethanes, water source history and year when chlorination started in the study areas were ascertained through measurements from drinking-water samples and questionnaires to water companies and local authorities. Historical annual average level of trihalomethanes was calculated in the study municipalities and was linked to study subjects by year and municipality of residence ([Villanueva et al., 2006](#)). Positive associations between lifetime exposure to trihalomethanes were observed among men and null associations were observed among women.

The combined influence of exposure to disinfectant by-products and genetic variants in the metabolic pathways of trihalomethanes was investigated in the Spanish study by [Cantor *et al.* \(2010\)](#). Polymorphisms in GST (*GSTT1*, *GSTZ1*) and CYP (*CYP2E1*) genes that are metabolizing enzymes of trihalomethanes were considered. Results showed that polymorphisms in these genes modified the disinfectant by-product-related risk for bladder cancer. Associations between trihalomethanes and bladder cancer were stronger among subjects who were *GSTT1*^{+/+} or ^{+/-} versus *GSTT1* null, *GSTZ1* rs1046428 CT/TT versus CC, or *CYP2E1* rs2031920 CC versus CT/TT. Among the 195 cases and 192 controls with high-risk forms of *GSTT1* and *GSTZ1*, the odds ratios for quartiles 2, 3 and 4 of trihalomethanes were 1.5 (95% confidence interval [CI], 0.7–3.5), 3.4 (95% CI: 1.4–8.2) and 5.9 (95% CI: 1.8–19.0), respectively.

[Bove *et al.* \(2007a\)](#) re-analysed the population in the study by [Vena *et al.* \(1993\)](#) by including newly estimated indices of exposure to trihalomethanes. Assessment of exposure to disinfectant by-products was based on measurements of trihalomethanes in 1998–2003, which were assigned to study subjects by residence at time of interview (1979–85). A positive association was observed for total trihalomethanes, chloroform, bromodichloromethane, dibromochloromethane and bromoform. [The Working Group noted that there could be potential exposure misclassification due to the exposure assessment based on measurements of trihalomethanes conducted 20 years after the recruitment of study subjects.]

2.2 Cancer of the colorectum

See [Table 3](#)

Four case-control studies on colorectal cancer were reviewed previously ([IARC, 2004](#)). [Cragle *et al.* \(1985\)](#) reported a statistically significantly positive association with years of

living in a residence with chlorinated versus non-chlorinated water. [Young *et al.* \(1987\)](#) concluded that exposure to trihalomethanes was not associated with colon cancer in the state of Wisconsin, USA. [Hildesheim *et al.* \(1998\)](#) reported an increased risk for rectal cancer with average lifetime dose of trihalomethanes. No such trend was observed for colon cancer. [King *et al.* \(2000\)](#) reported increased risks for colon cancer among men exposed to increasing levels of trihalomethanes and with increasing years of chlorinated drinking-water consumption. This effect was not observed in women. There was no association between the risk for rectal cancer and the number of years of exposure to water containing elevated levels of trihalomethanes in either sex.

[Bove *et al.* \(2007b\)](#) evaluated the risk for rectal cancer associated with exposure to total and specific trihalomethanes in a subset of the Upstate New York Diet Study (USA). Cases were identified from hospital pathology records and controls were identified from control groups of other cancer studies for five other unrelated sites (oral cavity, oesophagus, stomach, larynx, and lung). Measurements of trihalomethanes conducted from 1998 to 2003 for a separate independent study by Monroe County Department of Health were used to assign levels at the taps of study subjects in the last residence. The spatial patterns of trihalomethanes and individual measurements of tap-water consumption provided estimates of ingested trihalomethanes. Results indicated that the risk for rectal cancer did not increase with increasing levels of chloroform. Increasing odds ratios for rectal cancer were associated with increasing levels of bromoform consumed at the residence. Ingestion of chlorodibromomethane and bromodichloromethane was marginally associated with an increase in risk. [The use of cancer controls raises some concerns on potential selection bias. The exposure assessment based on non-contemporaneous measurements raises concerns on potential exposure misclassification.]

Table 3 Case-control studies of colorectal cancer and exposure to chlorinated drinking-water

Study	Population/end-point	Exposure	Odds ratio (95% CI)	Comments	
Young <i>et al.</i> (1987)	Wisconsin, USA Incidence, 1951–81; 347 colon cancer cases; 639 other cancer controls; 611 population controls; age, 35–90 yr (both sexes)	Total concentration of trihalomethanes at place of residence (µg/L) in 1951	< 10	1.0	Odds ratio for colon cancer adjusted for sex, age, population size of place of residence; general population controls
			10–40	1.2 (0.6–2.3)	
			> 40	0.98 (0.4–2.3)	
		Cumulative total exposure to trihalomethanes (mg) over lifetime	< 100	1.0	
			100–300	1.1 (0.7–1.8)	
			> 300	0.7 (0.4–1.2)	
Hildesheim <i>et al.</i> (1998)	Iowa, USA Incidence, 1986–89; 560 colon cancer cases; 537 rectal cancer cases; 1983 population controls; age, 40–85 yr (both sexes)	Total lifetime exposure to trihalomethanes (g)	≤ 0.04	1.0	Rectal cancer; adjusted for age, sex; <i>P</i> for trend = 0.08
			0.05–0.12	1.3 (1.0–1.6)	
			0.13–0.34	1.3 (0.9–1.8)	
			0.35–1.48	1.5 (1.1–2.1)	
			1.49–2.41	1.9 (1.2–3.0)	
			≥ 2.42	1.6 (1.0–2.6)	
		Lifetime average concentration of trihalomethanes (µg/L)	≤ 0.7	1.0	Rectal cancer; adjusted for age, sex; <i>P</i> for trend = 0.01
			0.8–2.2	1.05 (0.8–1.4)	
			2.3–8.0	1.2 (0.9–1.7)	
			8.1–32.5	1.2 (0.9–1.7)	
			32.6–46.3	1.7 (1.1–2.6)	
			≥ 46.4	1.7 (1.1–2.6)	

Table 3 (continued)

Study	Population/end-point	Exposure	Odds ratio (95% CI)	Comments	
King et al. (2000)	Southern Ontario, Canada Incidence, 1992–94; 767 colon cancer cases; 661 rectal cancer cases; 1545 population controls; age, 30–74 yr (both sexes)	Consumption of chlorinated drinking- water (yr)	0–9	Men 1.0	Colon cancer; adjusted for sex, age, education, body mass index, intake of energy, cholesterol, calcium, alcohol, coffee
			10–19	1.7 (1.1–2.7)	
			20–34	1.3 (0.96–1.9)	
			≥ 35	1.5 (1.1–2.1)	
			Women		
			0–9	1.0	
			10–19	0.6 (0.3–0.9)	
			20–34	0.9 (0.6–1.2)	
		≥ 35	0.7 (0.5–1.1)	P for trend = 0.005	
		Level of trihalomethanes (µg/L)			
		0–24	Men 1.0	P for trend = 0.211	
		25–74	1.5 (0.99–2.4)		
		≥ 75	1.9 (1.2–3.1)		
		Women			
		0–24	1.0		
		25–74	0.5 (0.3–0.8)		
≥ 75	0.9 (0.5–1.7)				
Exposure to trihalomethanes ≥ 75 µg/L (yr)		Men			
0–9	1.0				
10–19	1.1 (0.9–1.5)				
20–34	1.5 (0.99–2.3)				
≥ 35	2.1 (1.2–3.7)				
		Women			
0–9	1.0				
10–19	0.9 (0.7–1.3)				
20–34	0.9 (0.5–1.6)				
≥ 35	1.2 (0.6–2.4)				
Bove et al. (2007b)	Western New York, USA Incidence, 1979–85; 128 rectal cancer cases, 253 controls (men)	Level of trihalomethanes at the residence approximately 20 yr after recruitment (1998–2003) weighted by the amount of tap-water consumed; bromoform (µg/d)	0.90–0.64	1.00	Adjusted for alcohol, β-carotene, total calories
			0.65–0.97	1.42 (0.73–2.74)	
			0.98–1.68	1.63 (0.85–2.69)	
			1.69–15.43	2.32 (1.22–4.39)	

CI, confidence interval; d, day or days; yr, year or years

Table 4 Case-control studies of cancer at other sites and exposure to chlorinated drinking-water

Cancer site	Study	Population/end-point	Exposure	Odds ratio (95% CI)	Comments
Kidney	Koivusalo et al. (1998)	Finland Incidence 1991–92; 703 cases (386 men, 317 women), 914 population controls (621 men, 293 women)	Mutagenicity assessment; 3 000 net revertants/L increase	≥ 30 yr of estimable exposure Both sexes, 1.3 (1.0–1.7) Women, 1.1 (0.7–1.7) Men, 1.5 (1.1–2.1)	Calculated for all those with at least 30 yr of known exposure; adjusted for age, smoking, socioeconomic status, sex
			Tertiles of exposure (net revertants/L)		
				<i>Women</i>	
			Unexposed	1.0	
			Low (1–999)	0.9 (0.6–1.5)	
			Medium (1000–2499)	1.3 (0.8–2.1)	
			High (≥ 2500)	1.1 (0.7–1.9)	
				<i>Men</i>	
			Unexposed	1.0	
			Low	1.2 (0.8–1.7)	
Medium	1.3 (0.8–1.8)				
High	1.6 (1.0–2.4)				
Brain	Cantor et al. (1999)	Residents of Iowa, USA Incidence 1984–87; 291 glioma cases (155 men, 136 women); 1983 population controls (1308 men, 675 women); aged 40–85 yr	Chlorinated surface water; water utilities surveyed, measurements of trihalomethanes, personal questionnaire for past exposure; yr of exposure to ≥ 75 µg/L		Adjusted for sex, age, farming occupation, population size; 74.4% of cases had proxy respondents; cases and controls with ≥ 70% of lifetime with known source selected; excluded population better educated and more urban
				<i>Both sexes</i>	
			0	1.0	
			1–19	1.1 (0.8–1.6)	
			20–39	1.6 (1.0–2.6)	
			≥ 40	1.3 (0.8–2.3)	
			<i>P</i> for trend	0.1	
				<i>Women</i>	
			0	1.0	
			1–19	1.0 (0.6–1.6)	
20–39	1.6 (0.8–3.0)				
≥ 40	0.7 (0.3–1.6)				
<i>P</i> for trend	0.4				

Table 4 (continued)

Cancer site	Study	Population/end-point	Exposure	Odds ratio (95% CI)	Comments
	Cantor et al. (1999) Contd.			<i>Men</i>	
			0	1.0	
			1–19	1.3 (0.8–2.1)	
			20–39	1.7 (0.9–3.3)	
			≥ 40	2.5 (1.2–5.0)	
			<i>P</i> for trend	0.04	
			Lifetime average concentration of trihalomethanes (µg/L)		
				<i>Both sexes</i>	
			≤ 0.7	1.0	
			0.8–2.2	0.9 (0.6–1.3)	
			2.3–32.5	0.9 (0.6–1.4)	
			≥ 32.6	1.1 (0.7–1.8)	
			<i>P</i> for trend	0.3	
				<i>Both sexes</i>	
			≤ 0.7	1.0	
			0.8–2.2	0.9 (0.5–1.5)	
			2.3–32.5	0.8 (0.5–1.5)	
			≥ 32.6	0.9 (0.4–1.8)	
			<i>P</i> for trend	0.9	
				<i>Men</i>	
			≤ 0.7	1.0	
			0.8–2.2	0.9 (0.6–1.6)	
			2.3–32.5	1.0 (0.6–1.8)	
			≥ 32.6	1.4 (0.7–2.9)	
			<i>P</i> for trend	0.04	
Pancreas	Ijsselmuiden et al. (1992)	Washington County, MD, USA Incidence 1975–89; 101 cases (47 men, 54 women), 206 population controls (96 men, 110 women); all white	Chlorinated drinking-water, as of 1975 census Non-municipal (chlorinated) Municipal (chlorinated)	1.0 2.2 (1.2–3.95)	Adjusted for age, current cigarette smoking; non-municipal but chlorinated water used as baseline for odds ratios

Table 4 (continued)

Cancer site	Study	Population/end-point	Exposure	Odds ratio (95% CI)	Comments
	Kukkula & Löfroth (1997)	Turku area, Finland (220 000 persons) Incidence 1989–91; 183 cases (71 men, 112 women), 360 matched controls	Residence in an area supplied by chlorinated drinking-water until 1981 <i>Exposure (yr)</i> 0 1 5 10 15 20	0.33 (0.2–0.7) 0.54 (0.3–1.2) 0.66 (0.3–1.3) 0.53 (0.3–1.07) 0.32 (0.1–0.8) 0.20 (0.04–0.9)	No adjustment for confounders; odds ratio calculated from exposure data of the discordant case–control set; total trihalomethanes often > 200 µg/L at end of distribution system
	Do et al. (2005)	Canada (provinces of Nova Scotia, Ontario, Manitoba, Saskatchewan, Alberta and British Columbia) Incidence 1994–1997; 576 cases (324 men, 252 women), 4105 matched controls (2066 men, 2039 women)	Total level of trihalomethanes (µg/L), for an exposure time window of 30 yr, ending 3 yr before the interview < 10 10–20 20–50 > 50 <i>P</i> for trend	<i>Both sexes (476 cases)</i> 1.00 0.88 (0.67–1.17) 1.07 (0.83–1.39) 0.86 (0.58–1.28) 0.61	Adjusted for sex, age, province of recruitment, body mass index, per cent weight change, smoking, coffee, beer, alcohol, total fat intake, total energy intake

Table 4 (continued)

Cancer site	Study	Population/end-point	Exposure	Odds ratio (95% CI)	Comments
Acute lymphocytic leukaemia	Infante-Rivard et al. (2001)	Province of Québec, Canada Incidence 1980–93; 491 cases aged 0–9 yr, 491 population controls (boys and girls)	Trihalomethanes, metals (As, Cd, Cr, Pb, Zn) and nitrates in drinking-water; municipality-exposure matrix based on historical data		Adjusted for maternal age, level of education
			<i>Water chlorination</i>	<i>Prenatal</i>	
			Part of the time	1.6 (0.7–3.7)	
			Always	0.8 (0.5–1.2)	Baseline: never
			<i>Cumulative exposure (total trihalomethanes)</i>		
			> 95th percentile	0.8 (0.4–1.8)	Baseline: ≤ 95th percentile
			25th–75th percentile	1.1 (0.8–1.7)	Baseline: ≤ 24th percentile
			> 75% percentile	1.2 (0.7–1.8)	
			<i>Water chlorination</i>	<i>Postnatal</i>	
			Part of the time	1.4 (0.7–2.5)	Baseline: never
			Always	0.9 (0.6–1.3)	
			<i>Cumulative exposure (total trihalomethanes)</i>		
			> 95th percentile	1.5 (0.8–3.0)	Baseline: ≤ 95th percentile
			25th–75th percentile	1.1 (0.8–1.6)	Baseline: ≤ 24th percentile
> 75% percentile	0.9 (0.6–1.4)				
	Infante-Rivard et al. (2002)	Province of Québec, Canada Incidence 1980–83; 161 cases from earlier study (2001)	<i>GSTT1</i> -null; total trihalomethanes > 95th percentile Average Cumulative <i>CYP2E1</i> *5; total trihalomethanes ≥ 75th percentile Average Cumulative	9.1 (1.4–57.8) 2.5 (0.6–10.5) 4.1 (0.8–21.5) 5.96 (0.7–53.8)	Case-only study, postnatal exposure

Table 4 (continued)

Cancer site	Study	Population/end-point	Exposure	Odds ratio (95% CI)	Comments
Leukaemia (adults); acute (myelocytic and lymphocytic), chronic (myelocytic, lymphocytic, and hairy cell), and non-specified leukaemia	Kasim et al. (2006)	Canada (provinces of Prince Edward Island, Nova Scotia, Ontario, Manitoba, Saskatchewan, Alberta, Newfoundland and British Columbia) Incidence 1994–97; 686 total leukaemia cases (421 men, 265 women), 91 chronic myelocytic leukaemia (48 men, 43 women), 161 acute myelocytic leukaemia (90 men, 71 women), 323 chronic lymphocytic leukaemia (217 men, 106 women), 23 acute lymphocytic leukaemia (13 men, 10 women), 48 hairy cell leukaemia (34 men, 14, women) and 3 240 controls (1 580 men, 1 660 women)	Duration of exposure to chlorinated surface water	<i>All leukaemia (686 cases)</i>	Adjusted alternately for age, gender, occupational exposure to benzene, ionizing radiation, body mass index, passive smoking, pack-yr of smoking, education
			Never exposed	1.00	
			1–28 yr	1.15 (0.88–1.51)	
			29–35 yr	1.02 (0.78–1.34)	
			> 35 yr	0.84 (0.63–1.97)	
			<i>P</i> for trend	0.07	
				<i>Chronic myelocytic leukaemia (91 cases)</i>	
			Never exposed	1.00	
			1–28 yr	1.86 (0.79–4.36)	
			29–35 yr	2.14 (0.92–4.94)	
			> 35 yr	2.20 (0.93–5.23)	
			<i>P</i> for trend	0.09	
				<i>Acute myelocytic leukaemia (161 cases)</i>	
			Never exposed	1.00	
1–28 yr	1.48 (0.85–2.59)				
29–35 yr	1.45 (0.84–2.50)				
> 35 yr	1.09 (0.60–1.97)				
<i>P</i> for trend	0.93				
	<i>Chronic lymphocytic leukaemia (323 cases)</i>				
Never exposed	1.00				
1–28 yr	1.10 (0.77–1.59)				
29–35 yr	0.92 (0.64–1.31)				
> 35 yr	0.69 (0.47–1.02)				
<i>P</i> for trend	0.02				

Table 4 (continued)

Cancer site	Study	Population/end-point	Exposure	Odds ratio (95% CI)	Comments
	Kasim <i>et al.</i> (2006) Contd.		Total concentration of trihalomethanes in a 40-yr exposure window (analysis restricted to subjects with 30 or more yr of known level)	<i>All leukaemia (419 cases)</i>	Adjusted alternately for age, gender, occupational exposure to benzene, ionizing radiation, daily tap-water consumption, body mass index, passive smoking, pack-yr of smoking, education
			≤ 20 µg/L	1.00	
			> 20–40 µg/L	0.80 (0.55–1.17)	
			> 40 µg/L	0.90 (0.70–1.10)	
			<i>P</i> for trend	0.14	
				<i>Chronic myelocytic leukaemia (56 cases)</i>	
			≤ 20 µg/L	1.00	
			> 20–40 µg/L	0.90 (0.32–2.58)	
			> 40 µg/L	1.76 (1.01–3.10)	
			<i>P</i> for trend	0.04	
				<i>Acute myelocytic leukaemia (96 cases)</i>	
			≤ 20 µg/L	1.00	
			> 20–40 µg/L	0.90 (0.42–1.80)	
			> 40 µg/L	1.03 (0.68–1.60)	
			<i>P</i> for trend	0.80	
				<i>Chronic lymphocytic leukaemia (199 cases)</i>	
			≤ 20 µg/L	1.00	
			> 20–40 µg/L	0.63 (0.36–1.10)	
			> 40 µg/L	0.73 (0.51–0.97)	
			<i>P</i> for trend	0.03	

Table 4 (continued)

Cancer site	Study	Population/end-point	Exposure	Odds ratio (95% CI)	Comments
Melanoma	Nelemans et al. (1994)	the Netherlands, mideastern Incidence 1988–90; 128 cases, 168 controls (other types of malignancies); sex unspecified	Swimming in pools (vs not swimming or swimming only in lakes or fens) at different ages < 15 yr (<i>n</i> = 73 cases) 15–25 yr (<i>n</i> = 76 cases) > 25 yr (<i>n</i> = 84 cases) Age at which swimming was learned (127 cases) > 12 yr (or never) 9–12 yr < 9 yr	2.20 (1.05–4.62) 2.46 (1.21–5.00) 1.01 (0.51–2.01) 1.00 1.87 (0.91–3.78) 2.22 (1.16–4.26)	Adjusted for age, gender, educational level, hair colour, freckling, tendency to burn, exposure to sun light
Skin cancer (basal-cell carcinoma and squamous-cell carcinoma)	Karagas et al. (2008)	New Hampshire (USA) Incidence 1993–95; 603 basal-cell carcinoma cases, 293 squamous-cell carcinoma cases, 540 controls	Water source and total level of trihalomethanes Private Public < 1 µg/L ≥ 1–20 µg/L > 20–40 µg/L > 40 µg/L Private Public < 1 µg/L ≥ 1–20 µg/L > 20–40 µg/L > 40 µg/L	<i>Basal-cell carcinoma (545 cases)</i> 1.1 (0.7–1.8) 1.0 (referent) 0.9 (0.6–1.5) 1.1 (0.7–1.8) 2.4 (0.9–6.7) <i>Squamous-cell carcinoma (266 cases)</i> 1.1 (0.6–1.9) 1.0 (referent) 0.9 (0.5–1.6) 1.3 (0.7–2.3) 2.1 (0.7–7.0)	Adjusted for age, gender, skin sensitivity to the sun (i.e. tendency to sunburn); further adjustment for toenail arsenic did not affect the results.

CI, confidence interval; d, day or days; vs, versus; yr, year or years

2.3 Cancer at other sites

See [Table 4](#)

(a) Cancer of the kidney

[Koivusalo et al. \(1998\)](#) identified an exposure-related excess risk among men only for a 3000-net revertants/L increase in average exposure to chlorination by-products. No significant risk was observed when cases were placed in tertiles of exposure, although a weak association was suggested.

(b) Cancer of the brain

[Cantor et al. \(1999\)](#) reported elevated risks among men, but not women, with duration of exposure to chlorinated surface waters with levels of trihalomethanes of about 75 µg/L. For lifetime average exposure to trihalomethanes, the odds ratio among men increased to 1.4 (95% CI: 0.7–2.9) for levels > 32.6 µg/L. There was no association with average levels of trihalomethanes among women.

(c) Cancer of the pancreas

[Ijsselmuiden et al. \(1992\)](#) classified subjects as users of chlorinated non-municipal or chlorinated municipal drinking-water, which yielded an odds ratio of 2.2 (95% CI: 1.2–3.95) for users of municipal water compared with those of non-municipal water. [The Working Group noted that the information collected in the census on residence and source of drinking-water was cross-sectional.] [Kukkula & Löfroth \(1997\)](#) found that exposure to chlorinated drinking-water was not associated with risk for pancreatic cancer, with odds ratios ranging from 0.2 to 0.7 depending on the duration of exposure. [The Working Group noted that the study did not provide information on individual water-drinking habits or on potential confounding factors, and that the exposure time window of 20 years before diagnosis was short.]

[Do et al. \(2005\)](#) reported results from a population-based case-control study derived

from the National Enhanced Cancer Surveillance System of Canada. Study subjects were aged between 30 and 75 years. Cases were histologically confirmed and identified from six provincial cancer registries. Controls were frequency matched to the overall case group on age (5-year groups) and sex. Exposure to chlorination by-products was estimated by linking lifetime residential histories to two different databases containing information on levels of disinfectant by-products in municipal water supplies from routine surveys conducted in the past (back to 1962). A null association with exposure to trihalomethanes, bromodichloromethane or chloroform (all odds ratios, < 1.3) was observed for men and women separately and overall. Null findings were also obtained assuming a latency period for pancreatic cancer induction of 3, 8 or 13 years.

(d) Childhood leukaemia

[Infante-Rivard et al. \(2001\)](#) reported no consistent associations for chlorinated water consumption or cumulative exposure to trihalomethanes during the prenatal period, as well as the postnatal period. Subsequently, [Infante-Rivard et al. \(2002\)](#) conducted a case-case analysis among persons for whom data were available on exposure and genotypes of *GSTT1* and *CYP2E1*, genes that are involved in the metabolism of trihalomethanes. The results identified different risks by genotype.

(e) Adult leukaemia

[Kasim et al. \(2006\)](#) conducted a population-based case-control study in Canada using data available in the Canadian National Enhanced Cancer Surveillance System. Cases were histologically confirmed leukaemia patients aged 20–74 years identified from eight provincial cancer registries. Controls were identified from population-based records and were age-/sex-frequency matched to cases. Eligible subjects were contacted and mailed questionnaires (response rates: 70%

of cases, 63% of controls). Personal residential histories and the main source of drinking-water were ascertained. Data on trihalomethanes were as collected from different routine monitoring surveys. Individual exposures were assigned by linking the subjects' residences to the data on trihalomethanes by time and geographical area. Individual exposures to disinfection by-products were estimated for the 40-year period before the interview. The analysis included subjects for whom water quality information was available for at least 30 years. Results differed among subtypes of leukaemia. Duration of exposure to chlorinated surface water was positively associated with chronic myelocytic leukaemia, negatively associated with acute lymphocytic, chronic lymphocytic and hairy-cell leukaemia, and was not associated with acute myelocytic or all leukaemia. None of the point estimates or *P*-values for linear trend was statistically significant. Risk for chronic myeloid leukaemia increased with concentration of total trihalomethanes. A protective effect was observed for chronic lymphoid leukaemia for the highest category of trihalomethane concentration (> 40 versus \leq 20 $\mu\text{g/L}$).

(f) *Melanoma and non-melanoma skin cancer*

[Nelemans et al. \(1994\)](#) conducted a case-control study of melanoma and enrolled patients aged between 25 and 70 years identified from a population-based cancer registry that covers 95% of tumours in the mid-eastern part of the Netherlands. Controls were patients with urogenital cancers (65%), non-Hodgkin lymphomas (24%) and laryngeal carcinomas (11%). Study subjects were interviewed within 1 year after diagnosis, and the response rate was 80% among cases and 47% among controls. Information on aquatic leisure-time activities (age when they learned to swim, frequency of swimming in different type of pools) was collected. A physical examination was conducted to assess the skin, hair and eye colour, degree of freckling and number of naevi on the back. Swimming in

pools before 15 years or between 15 and 25 years of age was associated with an increased risk for melanoma. Younger age at learning to swim was associated with higher odds ratios for melanoma. [Although the results are suggestive of a potential association, they should be interpreted cautiously because of potential recall bias and potential selection bias due to the low response rates among controls and the use of cancer controls. Although these analyses were adjusted for exposure to sunlight, potential residual confounding by ultraviolet radiation cannot be ruled out.]

[Karagas et al. \(2008\)](#) published a preliminary analysis on the risk for skin cancer associated with exposure to trihalomethanes in a population-based case-control study conducted in New Hampshire (USA). They used data obtained in a previous population-based case-control study of keratinocyte-derived malignancies (basal-cell carcinoma and squamous-cell carcinoma) originally designed to examine the effects of arsenic in drinking-water. The study comprised cases and controls aged 25–74 years (response rates: 83% of cases, 69% of controls). Participants completed a self-administered work and residential history calendar and provided information on water supply at lifetime residences together with other risk factors (e.g. sun exposure, smoking history) in a structured interview. Average levels of trihalomethanes were computed from samples taken from public water systems between 1984 and 1994 that were assigned by subjects' residence at their reference date (date of diagnosis of the cases and a comparable date for controls). Increased odds ratios were identified among those in the highest category of exposure to trihalomethanes, but results were not statistically significant and there was no exposure-response trend. [The Working Group noted that there was potential exposure misclassification from the use of average values for multiple water utilities and applying these values to a specific individual, which would limit the power to detect an effect.]

Table 5 Meta-analyses and pooled analysis of cancer and exposure to chlorinated drinking-water

Study	Population/end-point	No. of subjects	Exposure		Risk estimate	Comments
Meta-analyses						
Multiple cancer sites				Site (No. of studies)		
Morris et al. (1992)	Mortality and morbidity studies; individual-based information on exposure and covariates	10 case-control and 2 cohort studies	Consumers of drinking-water containing chlorination by-products vs non-consumers	Urinary bladder (<i>n</i> = 7) Brain (<i>n</i> = 2) Breast (<i>n</i> = 4) Colon (<i>n</i> = 7) Colorectal (<i>n</i> = 8) Oesophagus (<i>n</i> = 5) Kidney (<i>n</i> = 4) Liver (<i>n</i> = 4) Lung (<i>n</i> = 5) Pancreas (<i>n</i> = 6) Rectum (<i>n</i> = 6) Stomach (<i>n</i> = 6) All sites	1.21 (1.09–1.34) 1.29 (0.53–3.14) 1.18 (0.90–1.54) 1.11 (0.91–1.35) 1.15 (0.97–1.37) 1.11 (0.85–1.45) 1.16 (0.89–1.51) 1.15 (0.94–1.40) 1.01 (0.86–1.18) 1.05 (0.91–1.22) 1.38 (1.01–1.87) 1.14 (0.94–1.38) 1.15 (1.09–1.20)	
Urinary bladder cancer						
Villanueva et al. (2003)	Individual-based studies of incident bladder cancer cases including data on long-term patterns of water consumption; studies conducted in North America and Europe	6 case-control studies (6084 cases, 10 816 controls) and 2 cohort studies (124 cases)	Consumption of chlorinated drinking-water was associated with an increased risk	Ever exposure 20 yr 40 yr 60 yr	<i>Men</i> 1.4 (1.1–1.9) <i>Women</i> 1.2 (0.7–1.8) <i>Both sexes</i> 1.13 (1.08–1.20) 1.27 (1.15–1.43) 1.43 (1.27–1.72)	These estimates are based on 5 studies

Table 5 (continued)

Study	Population/end-point	No. of subjects	Exposure	Risk estimate	Comments	
Colorectal cancer						
Rahman et al. (2010)	Case-control or cohort studies on colorectal cancer with an assessment of exposure to disinfectant by-products reporting relative risks or odds ratios	13 studies (10 case-control, 3 cohort)	Mixed exposure variables: chloroform levels, trihalo-methanes levels, chlorine dose, duration of exposure to chlorinated water	Highest versus lowest exposure category <i>Colon cancer</i> Cohort studies Case-control studies All studies <i>Rectal cancer</i> Cohort studies Case-control studies All studies	1.11 (0.73–1.70) 1.33 (1.12–1.57) 1.27 (1.08–1.50) 0.88 (0.57–1.35) 1.40 (1.15–1.70) 1.30 (1.06–1.59)	
Pooled analysis						
Urinary bladder cancer						
Villanueva et al. (2003)	Case-control studies with incident bladder cancer cases with evaluation of personal long-term exposure to trihalomethanes	6 studies (2 from the USA, and 1 from Canada, Finland, France and Italy); analysis included 2806 cases and 5254 controls	Average trihalo-methanes level in the residences from 45 to 5 yr before the interview, with known data of at least 70% of the exposure window Duration of exposure to chlorinated surface water (yr), with known data of at least 70% of the exposure window	<i>Men (n = 2126)</i> ≤ 1 µg/L > 1–5 µg/L > 5–25 µg/L > 25–50 µg/L > 50 µg/L <i>P for trend</i> <i>Women (n = 603)</i> ≤ 1 µg/L > 1–5 µg/L > 5–25 µg/L > 25–50 µg/L > 50 µg/L <i>P for trend</i>	1.00 1.10 (0.92–1.31) 1.26 (1.05–1.51) 1.25 (1.04–1.50) 1.44 (1.20–1.73) < 0.001 1.00 0.99 (0.72–1.36) 0.86 (0.63–1.18) 1.04 (0.76–1.43) 0.93 (0.67–1.28) 0.753	Adjusted for study, age, smoking status, ever worked in high-risk occupations, heavy coffee consumption (> 5 cups/d), education, total fluid intake Adjusted for study, age, smoking status, ever worked in high-risk occupations, heavy coffee consumption (> 5 cups/d), education

Table 5 (continued)

Study	Population/end-point	No. of subjects	Exposure	Risk estimate	Comments
Villanueva et al. (2003) Contd.			<i>Men (n = 692)</i>		
			0 yr	1.00	
			> 0–7 yr	1.40 (1.02–1.94)	
			> 7–15 yr	1.01 (0.74–1.37)	
			> 15–30 yr	1.67 (1.22–2.29)	
			> 30–40 yr	1.62 (1.21–2.16)	
			<i>P for trend</i>	< 0.001	
			<i>Women (n = 174)</i>		
			0 yr	1.00	
			> 0–7 yr	0.83 (0.47–1.47)	
			> 7–15 yr	1.24 (0.72–2.15)	
			> 15–30 yr	0.60 (0.32–1.12)	
			> 30–40 yr	1.08 (0.62–1.88)	
			<i>P for trend</i>	0.725	

CI, confidence interval; d, day or days; vs, versus; yr, year or years

3. Meta-analyses and pooled analyses

See [Table 5](#)

[Morris et al. \(1992\)](#) reviewed the literature on cancer mortality and morbidity for any cancer site related to exposure to chlorination by-products. Studies that identified morbidity or mortality and provided information on exposure and potential confounders at the individual level (i.e. case-control or cohort studies) were included in a meta-analysis. Two independent readers scored each paper for quality. Studies were scored on the basis of selection of subjects, measurement of and adjustment for confounding variables, exposure assessment and statistical analysis. These quality scores were used to conduct subanalyses using different subsets of studies. The odds ratios or relative risks for cancer among consumers of drinking-water containing chlorination by-products were identified for each of the selected studies. The meta-analysis showed a significant association for all cancers overall and specifically for cancers of the urinary bladder and rectum.

In a meta-analysis, [Villanueva et al. \(2003\)](#) evaluated individual consumption of chlorinated drinking-water and incident cases of urinary bladder cancer. They focused on epidemiological studies including incident cases and individual information on long-term patterns of water consumption. The studies used provided information on residential history obtained from individual interviews linked with water source. Summary risk estimates were provided for intermediate and long-term (> 40 years) consumption of chlorinated water, stratified by sex when possible. Results indicated that long-term consumption of chlorinated drinking-water was associated with an increased risk for bladder cancer, particularly in men. Ever consumption of chlorinated drinking-water was associated with an increased risk of bladder cancer in men and women. An estimate was calculated

that summarized the slopes of dose-response analyses and a positive duration-response relationship was observed.

A pooled analysis by [Villanueva et al. \(2004\)](#) re-evaluated the risk for urinary bladder cancer from six case-control studies with available data [of seven eligible studies] that used trihalomethanes as a marker of exposure to disinfection by-products and included individual data on water consumption. The methodology used to evaluate long-term exposure to trihalomethanes differed among studies and a common 40-year exposure window was created, from 45 to 5 years before the interview. Cumulative exposure to trihalomethanes was estimated by combining individual year-by-year average levels of trihalomethanes and daily tap-water consumption. Among men, risk increased with increasing exposure to trihalomethanes.

[Rahman et al. \(2010\)](#) performed a meta-analysis of colorectal cancer and exposure to disinfection by-products. The authors conducted a literature search to identify case-control or cohort studies that reported relative risks or odds ratios (or data that allowed their estimation) and an assessment of exposure to disinfectant by-products. Relative risks or odds ratios comparing the highest exposure category with the lowest were extracted from studies that met the inclusion criteria and were pooled using random effects methods. The results show an increased risk for colon and rectal cancers. [The Working Group noted that this meta-analysis included studies with poor exposure assessment.]

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BROMOCHLOROACETIC ACID

1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

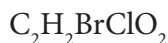
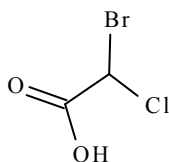
Chem. Abstr. Serv. Reg. No.: 5589-96-8

Chem. Abstr. Name: Acetic acid,
2-bromo-2-chloro-

IUPAC Systematic Name: 2-Bromo-2-chloroacetic acid

Synonyms: Acetic acid, bromochloro- (7CI, 8CI, 9CI); bromochloroacetate; bromochloroacetic acid; chlorobromoacetic acid

1.1.2 Structural and molecular formulae and relative molecular mass



Relative molecular mass: 173.39

1.1.3 Chemical and physical properties of the pure substance

Description: Crystalline compound ([NTP, 2009](#))

Boiling-point: bp₇₆₀ 215 °C ([Weast, 1983](#))

Melting-point: 27.5–31.5 °C ([WHO, 2004](#))

Density: 1.98 at 20 °C ([WHO, 2004](#))

Spectroscopy data: Infrared and magnetic resonance spectra (proton and C-13) have been reported ([NTP, 2009](#)).

Solubility: Soluble in water and methanol ([Xie et al., 1993](#)); in dilute solutions at pH > 6, more than 99.99% of the chemical exists as the dissociated carboxylate anion, bromochloroacetate. Bromochloroacetate contains an asymmetric carbon atom and, therefore, can exist in two non-superimposable forms, the (+)- and (-)-bromochloroacetate stereoisomers ([NTP, 2009](#)).

Octanol/water partition coefficient (P): log P, 1.08 ([WHO, 2004](#))

Conversion factor in air:

1 ppm = 7.09 mg/m³ ([WHO, 2004](#))

1.1.4 Technical products and impurities

Dibromoacetic acid was found to be the major impurity at concentrations of 2.35% and 0.83% in two lots of bromochloroacetic acid used in toxicology studies in rodents ([NTP, 2009](#)).

1.1.5 Analysis

Bromochloroacetic acid can be determined in the drinking-water by gas chromatography with electron capture detection following extraction by anion exchange column and conversion to its methyl ester. The limit of detection is 0.016 µg/L ([EPA, 2003](#)). It can also be determined in drinking-water following ion chromatography by electrospray ionization tandem mass

spectrometry, for which the limit of detection is 0.11 µg/L ([EPA, 2009](#)).

1.2 Production and use

1.2.1 Production

Bromochloroacetic acid can be produced by bromination of chloroacetic acid with a 2:1 bromide/bromate mixture under acidic conditions ([Adimurthy et al., 2006](#)).

Bromochloroacetic acid is produced commercially only in small quantities for research purposes.

Information available in 2010 indicated that bromochloroacetic acid was manufactured by five companies in the USA and one company each in Germany and Switzerland ([Chemical Sources International, 2010](#)).

1.2.2 Use

Bromochloroacetic acid is used only in research.

1.3 Occurrence and exposure

1.3.1 Natural occurrence

Bromochloroacetic acid is not known to occur naturally.

1.3.2 Occurrence and exposure in drinking-water

(a) Formation of halogenated disinfection by-products in drinking-water

The drinking-water disinfectant chlorine reacts with natural organic matter to produce halogenated disinfection by-products, and trihalomethanes and haloacetic acids are the two most prevalent groups of known specific by-products formed during disinfection of natural waters with chlorine-containing oxidizing compounds ([Hua & Reckhow, 2007](#)). These compounds are

formed when drinking-water supplies containing natural organic matter (e.g. humic or fulvic acids) are disinfected with compounds such as chlorine gas, hypochlorous acid and hypochlorite ([Huang et al., 2004](#)). When bromide is present in the source water, it may be oxidized to hypobromous acid–hypobromite ion, which can react with organic matter to form brominated organic compounds. The reaction of brominated and/or chlorinated oxidizing agents with natural organic matter produces mixed brominated and chlorinated compounds. The relative amount of brominated haloacetates produced in chlorinated drinking-water is a function of the concentration of bromide in the source water and of the initial bromine/chlorine ratio. The relative amounts of disinfection by-products produced in drinking-water supplies are affected by the nature and concentration of the organic precursor materials, water temperature, pH, the type of disinfectant, the disinfectant dose and contact time ([Liang & Singer, 2003](#); [Huang et al., 2004](#)). Treatment of natural waters with chloramine or chlorine dioxide produces haloacetic acids, but at levels substantially lower than those formed by free chlorine ([Richardson et al., 2000](#); [Hua & Reckhow, 2007](#)). Because commonly used alternative disinfectants (ozone, chloramines and chlorine dioxide) produce lower levels of most haloacetic acids, many water utilities have switched from chlorination to these alternatives to meet the regulation limits in terms of disinfection by-products ([Krasner et al., 2006](#); [Richardson et al., 2007](#)).

Data from the USA revealed that water-treatment systems that used chlorine dioxide had higher levels of nine haloacetic acids than those that used chlorine or chloramine only ([McGuire et al., 2002](#)). This is because the water-treatment systems that used chlorine dioxide also used chlorine or chloramines (mostly as post-disinfectants). Similarly to chloramines and chlorine dioxide, ozone used in water treatment is well known to lower the levels of haloacetic acids

formed relative to chlorination ([Richardson et al., 2007](#)). However, when source waters contain elevated levels of natural bromide, the levels of brominated compounds were shown to increase when pre-ozone treatment was performed before chlorination ([IPCS, 2000](#); [Richardson et al., 2007](#)).

According to [IPCS \(2000\)](#) and [WHO \(2008\)](#), the optimized use of combinations of disinfectants that function as primary and secondary disinfectants should allow further control of disinfection by-products. There is a trend towards combination/sequential use of disinfectants: ozone is used exclusively as a primary disinfectant; chloramines are used exclusively as a secondary disinfectant; and both chlorine and chlorine dioxide are used in either role.

According to [WHO \(2004\)](#), bromide ions occur naturally in surface water and groundwater; their levels exhibit seasonal fluctuations, and can also increase due to saltwater intrusion resulting from drought conditions or pollution ([IPCS, 2000](#)).

(b) Concentrations in drinking-water

A nationwide study of the occurrence of disinfection by-products in different geographical regions of the USA was conducted between October 2000 and April 2002 ([Weinberg et al., 2002](#)), in which samples were taken from 12 water-treatment plants that had different levels of source water quality and bromide and used the major disinfectants (chlorine, chloramines, ozone and chlorine dioxide). Concentrations of bromochloroacetic acid in finished water samples ranged from 1.3 to 18 µg/L.

Data from drinking-water supplies in the USA ([EPA, 2000](#) cited in [WHO 2004](#)) indicated that bromochloroacetic acid was detected in groundwater and surface water distribution systems at mean concentrations of 1.47 and 3.61 µg/L, respectively.

In a survey of 20 drinking-waters prepared from different source waters in the Netherlands

([Peters et al., 1991](#)), haloacetic acids were found in all drinking-waters prepared from surface water, whereas they could not be detected in drinking-waters prepared from groundwater. The total concentrations of haloacetic acids were in the range of 0.5–14.7 µg/L (surface water only) with levels of bromochloroacetic acid ranging from 0.2 to 2.5 µg/L. The limit of detection of this study was 0.1 µg/L, and brominated acetic acids accounted for 65% of the total haloacetic acid concentration.

Bromochloroacetic acid was measured in water samples taken from a water-treatment plant in Barcelona (Spain) between November 1997 and March 1998 ([Cancho et al., 1999](#)). Haloacetic acids were rapidly formed during the pre-chlorination step, but their concentration did not increase during either sand filtration or the ozonation step. At these two stages, the concentration of total haloacetic acids represented 60% of the total trihalomethane levels. A significant decrease in total concentration of haloacetic acids was observed when ozonated water was passed through granular activated carbon filters, but the acids were formed again during post-chlorination, although at concentrations lower than those during the previous stages. The average total level of haloacetic acids was around 22 µg/L in tap-water (range, 11–32 µg/L). Bromochloroacetic acid was not detected in raw water, but was detected in pre-chlorinated water (mean, 8.8 µg/L; range, 6.4–15 µg/L), sand-filtered water (mean, 8.2 µg/L; range, 7.1–11 µg/L), ozonated water (mean, 9.1 µg/L; range, 8–11.4 µg/L), granulated activated carbon-filtered water (mean, 0.8 µg/L; range, not detected–3.2 µg/L) and post-chlorinated water (mean, 2.5 µg/L; range, 1–3.9 µg/L), i.e. water that was ready for consumption. [The limit of detection was not reported.]

Water samples were collected from 35 Finnish waterworks between January and October in 1994 and from three waterworks and distribution systems during different seasons in 1995 ([Nissinen et al., 2002](#)). Bromochloroacetic acid

was detected at 32 of the 35 Finnish waterworks sampled in 1995 with concentrations between 0.3 and 19 µg/L. Levels at the other facilities were below the limit of quantitation of 0.2 µg/L. The concentration of six haloacetic acids, including bromochloroacetic acid, exceeded that of trihalomethanes. Chlorinated drinking-waters originating from surface waters contained the highest concentration of haloacetic acids (108 µg/L). The lowest concentrations of disinfection by-products were measured from ozonated and/or activated carbon-filtered and chloraminated drinking-waters (20 µg/L). Higher concentrations of the six haloacetic acids were measured in summer than in winter [data not reported].

In the USA, finished waters from the Philadelphia (PA) Suburban Water Co., the Metropolitan Water District of Southern California, and utilities at the cities of Houston (TX) and Corpus Christi (TX) were collected at the point of entry to the water distribution system and analysed for the nine haloacetic acids (Cowman & Singer, 1996). These samples included waters with relatively low (Philadelphia), moderate (Houston), and high (Southern California, Corpus Christi) bromide concentrations. Several of the utilities (Houston, Southern California, Corpus Christi) added ammonia to their waters after chlorination to control disinfection by-product formation. Levels of bromochloroacetic acid were below the limit of detection [not reported] in the Philadelphia utility, where the bromide ion concentration was 50.6 µg/L. For the others utilities, where levels of bromide ion ranged from 72 to 412 µg/L, those of bromochloroacetic acid ranged from 4.68 to 10.8 µg/L.

Drinking-water was studied in Israel because its source water (the Sea of Galilee, a freshwater lake, also called Lake Kinnereth) has among the highest natural levels of bromide in the world for surface water (2000 µg/L) and chlorine dioxide is used for disinfection at full-scale treatment plants (Richardson *et al.*, 2003). Chlorine-containing

disinfection by-products that are usually dominant under conditions of low levels of bromide (for chlorination and chloramine disinfection) — chloroform and dichloroacetic acid — were found at very low concentrations or not at all in these samples, with a shift to bromoform and dibromoacetic acid occurring under these conditions of high levels of bromide. Thus, the high bromide content in the source water had a major impact on the speciation of the disinfection by-products. Bromochloroacetic acid was detected at levels between 1 and 3.9 µg/L.

Between October 1994 and April 1996, a mean concentration of 0.6 µg/L bromochloroacetic acid was measured in the Santa Ana River (USA) downstream of a discharge point for highly treated municipal wastewater effluent (Ding *et al.*, 1999).

A study was conducted in nine distribution systems of the greater area of Québec City (Province of Québec, Canada) (Legay *et al.*, 2010). Nine individual haloacetic acids, including bromochloroacetic acid, were analysed during 2006–08, and concentrations were: mean, 25.3–115.2 µg/L; 25th percentile, 16.7–73.5 µg/L; 50th percentile, 23.0–113.5 µg/L; and 75th percentile, 31.6–145.1 µg/L.

In a study based on data from several European countries (Belgium, France, Germany, Italy, the Netherlands and Spain) and covering two decades (from 1980 to 2000; Palacios *et al.*, 2000), the levels of organohalogenated compounds in surface and groundwaters after chlorination were evaluated. A mean concentration of 3.53 µg/L bromochloroacetic acid was measured in post-treatment surface water (range, not detected–13.7 µg/L), but was not detected in post-treatment groundwater from disinfection utilities [limit of detection not reported].

(c) Dietary exposure from drinking-water

To assess exposure to disinfection by-products through drinking-water, WHO uses a default consumption value of 2 L drinking-water

per capita per day and a typical body weight (bw) of 60 kg (WHO, 2008). The underlying assumption is that of a total water consumption of 3 L per capita per day, including water present in food (WHO, 2003).

The mean concentrations and ranges of bromochloroacetic acid from all references available were used by the Working Group to assess dietary exposure in adults and infants (weighing 60 kg and 5 kg, respectively), assuming a consumption of 2 L and 0.75 L drinking-water, respectively, i.e. 33 mL/kg bw and 150 mL/kg bw, respectively (Table 1.1). The infant scenario (expressed in mL/kg bw) would correspond to the consumption of 9 L drinking-water per day in a 60-kg adult and therefore cover any possible scenario of physically active persons and increased temperature.

Based on the available data on average concentrations of bromochloroacetic acid, dietary exposure through drinking-water in a standard 60-kg adult ranges from 0.02 to 0.08 µg/kg bw per day, and high observed concentration values would lead to a dietary exposure of 0.1–0.6 µg/kg bw per day. Similarly, dietary exposure through drinking-water in a 5-kg infant ranges from 0.1 to 0.4 µg/kg bw per day, and high observed concentration values would lead to a dietary exposure of 0.4–2.8 µg/kg bw per day (Table 1.1).

(d) Other dietary sources

No data on the levels of haloacetic acids in foods (other than drinking-water) could be identified. Extrapolations from values in drinking-water to values in food are difficult to achieve because the conditions of the chemical interactions, dosages, temperatures, contact times and especially the precursors differ considerably (FAO/WHO, 2009).

1.3.3 Exposure through inhalation or dermal contact

Bromochloroacetic acid occurs in water used for showering and bathing due to its presence in household water distribution systems (see Section 1.3.2). Bromochloroacetic acid was also detected in the water of two large public swimming pools disinfected with either chlorine or bromine in Barcelona (Spain) (Richardson *et al.*, 2010).

Exposure to bromochloroacetic acid through dermal contact and inhalation was not measured. Based on the low dermal absorption observed for other haloacetic acids (Kim & Weisel, 1998), dermal exposure to bromochloroacetic acid is not liable to be significant. In contrast, inhalation of the substance in vapour/mist might occur during showering, bathing or swimming, as is anticipated for other disinfection by-products (Richardson *et al.*, 2007).

1.3.4 Environmental occurrence

Many haloacetates are distributed ubiquitously in the biosphere, including lakes and groundwater. The formation of bromochloroacetic acid as a chemical by-product of chlorination and chloramination of drinking-water may result in its release into the environment through various waste streams (Cowman & Singer, 1996).

When released into the air, an estimated vapour pressure of 0.14 mm Hg at 25 °C indicates that bromochloroacetic acid exists solely as a vapour in the atmosphere. Vapour-phase bromochloroacetic acid is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals. Bromochloroacetic acid does not contain chromophores that absorb at wavelengths > 290 nm and is therefore not expected to be susceptible to direct photolysis by sunlight (HSDB, 2010).

When released into water, bromochloroacetic acid is not expected to adsorb to suspended solids or sediment based upon the estimated soil

Table 1.1 Dietary exposure to bromochloroacetic acid from drinking-water^a

Reference	Country	Concentration (µg/L)			Estimated exposure in adults (µg/kg bw per day)			Estimated exposure in children (µg/kg bw per day)		
		Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Weinberg <i>et al.</i> (2002)	USA		1.3	18	0.05		0.60	0.21		2.70
Cancho <i>et al.</i> (1999)	Spain ^b	2.5	1.0	3.9	0.08	0.03	0.13	0.38	0.15	0.59
Nissinen <i>et al.</i> (2002)	Finland		0.3	19		0.01	0.63		0.05	2.85
Richardson <i>et al.</i> (2003)	Israel		1.0	3.9		0.03	0.13		0.15	0.59
Ding <i>et al.</i> (1999)	USA	0.6			0.02			0.09		
Peters <i>et al.</i> (1991)	Netherlands		0.2	2.5		0.006	0.08		0.03	0.38
Palacios <i>et al.</i> (2000)	European Union ^c	3.53	ND	13.7	0.12		0.46	0.53		2.06
Cowman & Singer (1996)	USA		4.68	10.8		0.16	0.36		0.70	1.62

^a Calculated by the Working Group, assuming a daily intake and a body weight for adults of 2 L and 60 kg, and for children of 0.75 L and 5 kg, respectively.

^b The study reported concentrations of bromochloroacetic acid according to different water treatments (e.g. chlorinated water, sand-filtered water, ozonated water, granulated activated carbon-filtered water); for the dietary exposure assessment, the chlorinated water values were used because it was considered as finished water.

^c [Limit of detection not reported]

bw, body weight; max., maximum; min., minimum; ND, not detected

organic carbon–water partitioning coefficient of 1.9 (Swann *et al.*, 1983).

Data on biodegradation were not available to the Working Group, but the dissociation constant of 1.40 indicates that bromochloroacetic acid exists almost entirely in the anion form at pH values of 5–9 and therefore volatilization from water surfaces is not expected to be an important fate process (HSDB, 2010). An estimated bioconcentration factor of 3.2 suggests that the potential for bioconcentration in aquatic organisms is low. Hydrolysis is not expected to be an important environmental fate process because this compound lacks functional groups that hydrolyse under environmental conditions (HSDB, 2010).

1.3.5 Occupational exposure

No data were available to the Working Group.

1.4 Regulations and guidelines

No occupational exposure limits or recommended guidelines for maximum safe levels in drinking-water have been established for bromochloroacetic acid.

Levels of haloacetic acids in drinking-water in the USA are regulated by the Environmental Protection Agency (EPA, 2010). Under the disinfection by-products rule, the sum of the concentrations of monochloroacetic, dichloroacetic, trichloroacetic, monobromoacetic and dibromoacetic acids is limited to 60 µg/L (60 ppb). Bromochloroacetic acid is not included among the five haloacetic acids regulated by the Environmental Protection Agency under this current rule.

2. Cancer in Humans

See Introduction to the *Monographs* on Bromochloroacetic Acid, Dibromoacetic Acid and Dibromoacetonitrile.

3. Cancer in Experimental Animals

3.1 Oral administration

See [Table 3.1](#)

3.1.1 Mouse

In a 2-year carcinogenicity study, groups of 50 male and 50 female B6C3F₁ mice were given drinking-water containing 0 (controls), 250, 500 or 1000 mg/L bromochloroacetic acid (equivalent to average daily doses of approximately 0, 25, 50 or 90 and 0, 15, 30 or 60 mg/kg bw in males and females, respectively). Bromochloroacetic acid caused a significantly increased incidence of benign and malignant liver tumours: hepatocellular adenoma in males of the low- and mid-dose groups and in all exposed groups of females; hepatocellular carcinoma in males of the mid- and high-dose groups and females of the mid-dose group; hepatocellular adenoma or carcinoma (combined) in all exposed groups of males and females; and hepatoblastoma in all exposed groups of males (NTP, 2009).

3.1.2 Rat

In a 2-year carcinogenicity study, groups of 50 male and 50 female F344/N rats were given drinking-water containing 0 (controls), 250, 500 or 1000 mg/L bromochloroacetic acid (equivalent to average daily doses of approximately 0, 10, 20 or 40 and 0, 13, 25 or 50 mg/kg bw in males and females, respectively). Bromochloroacetic acid caused an increased incidence of rare adenomas of the large intestine (colon and rectum) in

Table 3.1 Carcinogenicity studies of exposure to bromochloroacetic acid in the drinking-water in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F ₁ (M, F) 105 wk NTP (2009)	0 (control), 250, 500 or 1000 mg/L 50/group	Liver (hepatocellular adenoma, multiple): M–13/50, 27/50, 25/50, 19/50 (M) F–16/50, 37/50, 34/50, 43/50 (F)	$P \leq 0.01$ (low- and mid-dose, M; all doses, F)	> 95% pure; the incidence of multiple hepatocellular adenoma and multiple hepatocellular carcinoma in exposed males and females and of multiple hepatoblastoma in exposed males was significantly ($P \leq 0.01$) increased.
		Liver (hepatocellular adenoma, including multiple): M ^a –27/50, 40/50, 40/50, 31/50 F ^b –27/50, 48/50, 44/50, 46/50	$P = 0.005$ (low- and mid-dose M) $P < 0.001$ (all doses, F) $P < 0.001$ (trend, F)	
		Liver (hepatocellular carcinoma): M ^c –19/50, 25/50, 36/50, 45/50 F ^d –14/50, 23/50, 26/50, 20/50	$P < 0.001$ (mid- and high-dose M) $P = 0.011$ (mid-dose F) $P < 0.001$ (trend, M)	
		Liver (hepatocellular adenoma or carcinoma, combined): M ^e –34/50, 44/50, 49/50, 49/50 (M) F ^f –31/50, 49/50, 46/50, 46/50 (F)	$P = 0.013$ (low-dose M) $P \leq 0.001$ (mid- and high-dose M) $P < 0.001$ (all doses, F) $P < 0.001$ (trend; M, F)	
		Liver (hepatoblastoma, multiple): M–0/50, 2/50, 12/50, 14/50	$P < 0.01$ (mid- and high-dose M)	
		Liver (hepatoblastoma, including multiple): M ^g –4/50, 11/50, 28/50, 34/50	$P = 0.047$ (low-dose M) $P < 0.001$ (mid- and high-dose M, trend M)	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M, F) 105 wk NTP (2009)	0 (control), 250, 500 or 1000 mg/L 50/group	All organs (malignant mesothelioma): M ^b -1/50, 5/50, 10/50, 6/50 Large intestine, colon and rectum (adenoma): M ⁱ - 0/50, 2/50, 0/50, 4/50 F ^j - 0/50, 0/50, 3/50, 7/50 Mammary gland (fibroadenoma, multiple): F-22/50, 24/50, 43/50, 38/50 Mammary gland (fibroadenoma, including multiple): F ^k -43/50, 43/50, 47/50, 46/50 Pancreatic islets (adenoma): M ^l -3/50, 4/50, 9/50, 3/50 Liver (hepatocellular adenoma): F ^m -0/50, 0/50, 0/50, 3/50	<i>P</i> = 0.003 (mid-dose M) <i>P</i> = 0.009 (high-dose F) <i>P</i> < 0.001 (trend, F) <i>P</i> ≤ 0.01 (mid- and high-dose) <i>P</i> = 0.049 (mid-dose) <i>P</i> = 0.012 (trend, F)	> 95% pure; the number of fibroadenomas/fibroadenoma-bearing rat was significantly (<i>P</i> ≤ 0.01) increased in the 500-mg/L and 1000-mg/L females.

^a Historical incidence for 2-year drinking-water studies in mice: 140/247 (56.7% ± 13.0%); range, 37–72%

^b Historical incidence for 2-year drinking-water studies in mice: 133/297 (44.8 ± 11.9%); range, 29–61%

^c Historical incidence for 2-year drinking-water studies in mice: 91/247 (36.9 ± 8.6%); range, 28–48%

^d Historical incidence for 2-year drinking-water studies in mice: 51/297 (17.1 ± 9.5%); range, 6–28%

^e Historical incidence for 2-year drinking-water studies in mice: 182/247 (73.7 ± 11.7%); range, 57–85%

^f Historical incidence for 2-year drinking-water studies in mice: 158/297 (53.1 ± 11.3%); range, 35–63%

^g Historical incidence for 2-year drinking-water studies in mice: 28/247 (11.3 ± 13.6%); range, 0–34%

^h Historical incidences for 2-year drinking-water studies in rats: 9/300 (3.0 ± 2.8%); range, 0–6%

ⁱ Historical incidences for 2-year drinking-water studies in rats: 0/300

^j Historical incidences for 2-year drinking-water studies in rats: 0/250

^k Historical incidences for 2-year drinking-water studies in rats: 176/250 (70.4 ± 9.8%); range, 62–86%

^l Historical incidences for 2-year drinking-water studies in rats: 23/296 (8 ± 2%); range, 6–10%

^m Historical incidences for 2-year drinking-water studies in rats: 3/250 (1.2 ± 1.8%); range, 0–4%

bw, body weight; d, day or days; F, female; M, male; wk, week or weeks

male and female rats, with a significant increase in high-dose females. The incidence of rare malignant mesotheliomas at multiple sites was increased in all exposed groups of males and was significantly increased in the mid-dose group. Although the incidence of fibroadenoma of the mammary gland in females was not statistically significantly increased, the number of animals with multiple mammary gland fibroadenomas was increased in the mid-dose and high-dose groups. The incidence of pancreatic islet-cell adenoma was significantly increased in mid-dose males. The incidence of hepatocellular adenoma in high-dose females exceeded the historical control range ([NTP, 2009](#)).

4. Other Relevant Data

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, distribution and excretion

Dihaloacetates are rapidly absorbed from the gastrointestinal tract of rats after oral exposure ([James et al., 1998](#); [Schultz et al., 1999](#)). The maximum blood concentration of bromochloroacetate in F344/N rats was reached 1.5 h after administration by gavage ([Schultz et al., 1999](#)).

Dihaloacetates exhibit low binding to rat plasma proteins: in the plasma of treated F344 rats, 93% of the measured bromochloroacetate was in the unbound fraction ([Schultz et al., 1999](#)).

The oral bioavailability of bromochloroacetate was reported to be 47% in male F344/N rats ([Schultz et al., 1999](#)). The lower bioavailability of bromochloroacetate compared with

other dihaloacetates is due to a greater first-pass metabolism in the liver.

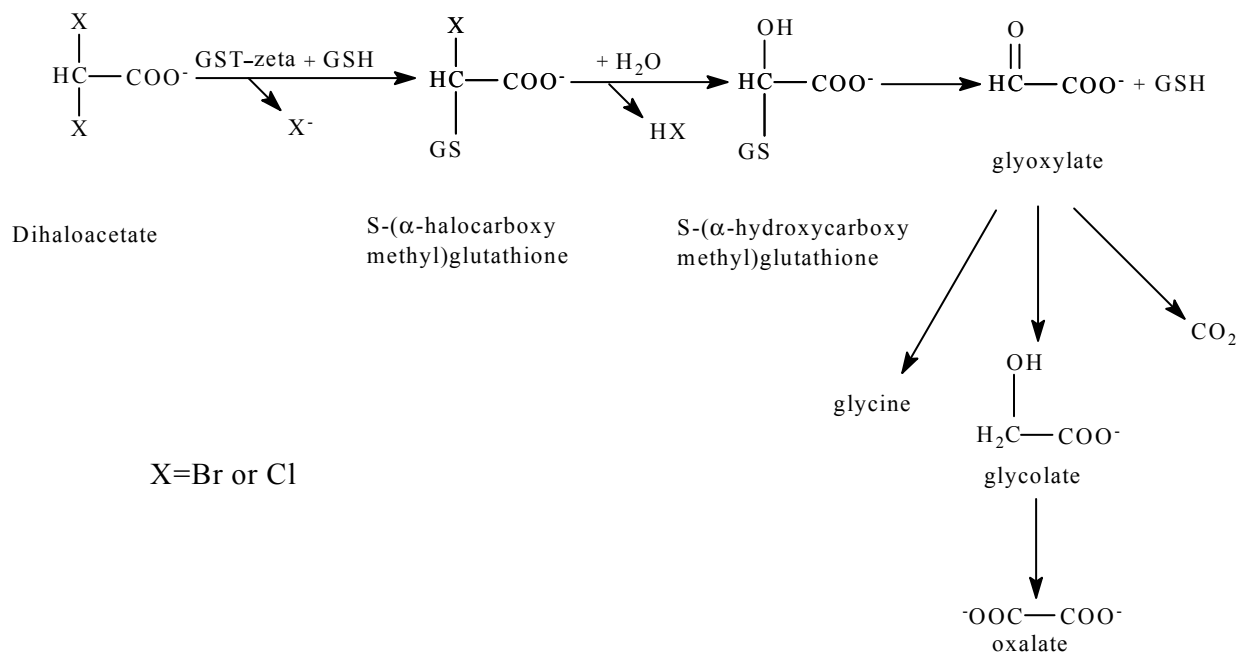
Elimination half-lives of dihaloacetates in the blood of male F344/N rats are less than 4 hours; the plasma half-life of bromochloroacetate after intravenous injection is approximately 45 minutes. Elimination of dihaloacetates occurs primarily by metabolism; after an intravenous dose of 500 $\mu\text{mol/kg bw}$ [86.7 mg/kg bw] bromochloroacetate, excretion as the parent compound was less than 3% in the urine and less than 0.1% in the faeces. Bromine substitution of dihaloacetates increases the rate of metabolic clearance ([Schultz et al., 1999](#)).

(b) Metabolism

The metabolism of bromochloroacetic acid has been reviewed ([NTP, 2009](#)). Biotransformation of dihaloacetates to glyoxylate occurs primarily in the liver cytosol of rats and humans by a glutathione-dependent process ([James et al., 1997](#)) catalysed by glutathione S-transferase-zeta (GST-zeta) ([Tong et al., 1998a](#)).

During GST-zeta-mediated oxygenation of dihaloacetates to glyoxylate, glutathione is required but not consumed. GST-zeta-mediated biotransformation of dihaloacetates (Fig. 4.1) involves displacement of a halide by glutathione to form S-(α -halo-carboxymethyl)glutathione, hydrolysis of this intermediate to form S-(α -hydroxy-carboxymethyl)glutathione and elimination of glutathione to produce glyoxylate ([Tong et al., 1998b](#)). Among the brominated/chlorinated dihaloacetates, the relative rates of glyoxylate formation catalysed by purified GST-zeta are: bromochloroacetate > dichloroacetate > dibromoacetate ([Austin et al., 1996](#)). Glyoxylate can undergo transamination to glycine, decarboxylation to form carbon dioxide and oxidation to oxalate. Glyoxylate may induce toxicity by reacting covalently with proteins, e.g. N-terminal amino groups or lysine ϵ -amino groups ([Anderson et al., 2004](#)).

Fig. 4.1 Biotransformation of dihaloacetates



Adapted from [Tong et al. \(1998a\)](#)

Bromochloroacetic acid is a suicide substrate for GST-zeta; 12 hours after a single injection (0.30 mmol/kg bw), GST-zeta activity in the rat liver is reduced to 19% of that in controls ([Anderson et al., 1999](#)). Hydrolysis of S-(α -halocarboxymethyl)glutathione forms a hemithioacetal that eliminates glutathione and yields glyoxylate. Because this intermediate may inactivate GST-zeta by covalently binding to a nucleophilic site on the enzyme ([Anderson et al., 1999](#); [Wempe et al., 1999](#)), its hydrolysis and GST-zeta inactivation are competing reactions. Recovery of GST-zeta activity occurs via de-novo synthesis of the protein. Because GST-zeta is identical to maleylacetoacetate isomerase, the enzyme that catalyses the penultimate step of the tyrosine degradation pathway, its loss by exposure to dihaloacetates leads to the accumulation of maleylacetoacetate and maleylacetone which may cause tissue damage by reacting with cellular nucleophiles ([Ammini et al., 2003](#)).

The elimination half-life of (-)-bromochloroacetic acid in male F344 rats is approximately sixfold shorter than that of (+)-bromochloroacetic acid, indicating that the rate of GST-zeta-catalysed metabolism of bromochloroacetic acid is much faster for the (-)-stereoisomer ([Schultz & Sylvester, 2001](#)). [The Working Group noted that the carcinogenicity studies in animals were performed using a racemic mixture of bromochloroacetic acid.] Because the metabolism of bromochloroacetic acid stereoisomers in naive and GST-zeta-depleted cytosol of rats is dependent on the presence of glutathione, [Schultz and Sylvester \(2001\)](#) suggested that an additional GST isoenzyme that is not inactivated by dihaloacetates might provide a minor contribution to the formation of glyoxylate in non-pretreated animals.

(c) Toxicokinetic models

No data were available to the Working Group.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

Several studies have demonstrated the genotoxicity of bromochloroacetic acid (see [Table 4.1](#)).

(a) DNA adducts

Oxidative stress can result in oxidative damage to DNA, most commonly measured as increases in 8-hydroxydeoxyguanosine (8-OHdG) adducts. After acute oral administration of bromochloroacetic acid to male B6C3F₁ mice, a significant increase in 8-OHdG/deoxyguanosine ratios in liver nuclear DNA was observed ([Austin et al., 1996](#)). After administration of bromochloroacetic acid in the drinking-water to male B6C3F₁ mice at concentrations of 0.1, 0.5 or 2.0 g/L for 3–10 weeks, the 8-OHdG content in liver nuclear DNA was increased ([Parrish et al., 1996](#)). These findings demonstrate that bromochloroacetic acid causes oxidative stress/damage.

(b) DNA damage

Bromochloroacetic acid induced DNA damage in Chinese hamster ovary cells, as measured in the Comet assay ([Plewa et al., 2010](#)).

(c) Mutations

In two bacterial mutagenicity assays, bromochloroacetic acid gave positive results in *Salmonella typhimurium* strain TA100 regardless of the presence of a metabolic activation system. It was not mutagenic in strain TA98 or in *Escherichia coli* WP2 *uvrA*/pKM101 regardless of the presence of a metabolic activation system ([NTP, 2009](#)). Glyoxylate (a metabolite of dihaloacetate biotransformation) was mutagenic in *S. typhimurium* strains TA97, TA100 and TA104 in the absence of a metabolic activation

system and in strain TA102 in the presence of a metabolic activation system ([Sayato et al., 1987](#)).

(d) Chromosomal effects

No increase in chromosomal damage (micro-nucleus formation in blood lymphocytes) was reported after administration of bromochloroacetic acid in the drinking-water to mice for 3 months ([NTP, 2009](#)).

4.3 Mechanistic data

4.3.1 Effects on cell physiology

No data were available to the Working Group.

4.3.2 Effects on cell function

After daily administration of 0, 8, 24, 72 or 216 mg/kg bw bromochloroacetic acid by gavage for 14 days, male mice showed altered expression of the genes involved in cell communication and adhesion, cell cycle control, proliferation, metabolism, signal transduction, stress response, spermatogenesis and male fertility ([Tully et al., 2005](#)).

4.4 Susceptibility

No data were available to the Working Group. [However, the Working Group noted that disruption of GST-zeta in type-I hereditary tyrosinaemia has been linked to liver cancer in humans.]

4.5 Mechanisms of carcinogenesis

The mechanism by which bromochloroacetic acid induces neoplasms is not known.

It has been suggested that the reduction of GST-zeta activity by dihaloacetic acids may cause accumulation of toxic intermediates because this enzyme is involved in the tyrosine degradation pathway ([Ammini et al., 2003](#)).

Table 4.1 Genetic and related effects of bromochloroacetic acid and glyoxylate

Test system	Results		Dose ^a (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	33	NTP (2009)
<i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	33	NTP (2009)
<i>Escherichia coli</i> WP2 <i>uvrA</i> /pKM101, mutant colonies	-	-	1000	NTP (2009)
DNA strand breaks (Comet assay), Chinese hamster ovary cells <i>in vitro</i>	+	NT	520	Plewa et al. (2010)
DNA adducts (8-OHdG) in liver nuclear DNA, male B6C3F1 mice <i>in vivo</i>	+		30 po × 1	Austin et al. (1996)
DNA adducts (8-OHdG) in liver nuclear DNA, male B6C3F1 mice <i>in vivo</i>	+		125, dw, 3 wk	Parrish et al. (1996)
Micronucleus formation, B6C3F1 mouse blood lymphocytes <i>in vivo</i>	-		62.5, dw, 3 mo	NTP (2009)
<i>Glyoxylate (metabolite of bromochloroacetic acid)</i>				
<i>Salmonella typhimurium</i> TA100, TA104, TA97, reverse mutation	+	-	400 µg/plate	Sayato et al. (1987)
<i>Salmonella typhimurium</i> TA102, reverse mutation	-	+	1000	Sayato et al. (1987)

^a *in vitro* test, µg/mL; *in vivo* test, mg/kg bw/d

+, positive; -, negative; bw, body weight; d, day or days; dw, drinking-water; HID, highest ineffective dose; LED, lowest effective dose; mo, month or months; NT, not tested; 8-OHdG, 8-hydroxydeoxyguanosine; po, oral; wk, week or weeks

DNA damage due to oxidative stress in the livers of mice exposed to halogenated acetic acids, including bromochloroacetic acid, may contribute to the hepatocarcinogenicity of these chemicals (NTP, 2009).

The carcinogenicity of bromochloroacetic acid may also involve a genotoxic mechanism because it induces DNA damage (Austin *et al.*, 1996; Parrish *et al.*, 1996; Plewa *et al.*, 2010). Glyoxylate, a metabolite of dihaloacetates biotransformation, is mutagenic in *S. typhimurium* (Sayato *et al.*, 1987; NTP, 2009).

5. Summary of Data Reported

5.1 Exposure Data

Bromochloroacetic acid is formed as a by-product during the disinfection of water by chlorination in the presence of organic matter and bromide. The concentration of bromochloroacetic acid measured in drinking-water was up to 19 µg/L. The highest concentrations of bromochloroacetic acid were observed in waters with the highest bromide content. The maximum daily human exposure to bromochloroacetic acid through drinking-water, estimated from such measurements, is at the low microgram per kilogram body weight level.

5.2 Human carcinogenicity data

No epidemiological studies were identified that evaluated exposure specifically to bromochloroacetic acid. This chemical occurs in mixtures in disinfected water, studies on which are reviewed in the Introduction to the *Monographs on Bromochloroacetic Acid, Dibromoacetic Acid and Dibromoacetone*.

5.3 Animal carcinogenicity data

Bromochloroacetic acid was tested for carcinogenicity by administration in the drinking-water in one study in mice and one study in rats. In mice, bromochloroacetic acid caused a significantly increased incidence of hepatocellular adenoma and hepatocellular carcinoma in males and females, and of hepatoblastoma in males. In rats, bromochloroacetic acid caused a significantly increased incidence of mesothelioma in males, of large intestine adenoma in males and females, and of pancreatic islet cell adenoma in males. It also increased the multiplicity of fibroadenomas of the mammary gland in females. Tumours of the large intestine, mesotheliomas and hepatoblastomas are rare spontaneous neoplasms in experimental animals.

5.4 Other relevant data

No data were available to the Working Group on the toxicokinetics of bromochloroacetic acid in humans. In rats, dihaloacetates are rapidly absorbed from the gastrointestinal tract after oral administration.

Bromochloroacetic acid is primarily biotransformed to glyoxylate in the liver cytosol of rats and humans by a glutathione-dependent process catalysed by glutathione *S*-transferase-zeta. Glyoxylate can further undergo transamination to glycine, decarboxylation to carbon dioxide and oxidation to oxalate.

The mechanism by which bromochloroacetic acid induces tumours is not known, but a reduction in glutathione *S*-transferase-zeta activity may be involved. There is moderate evidence that the carcinogenicity of bromochloroacetic acid may involve a genotoxic mechanism because this chemical is a bacterial mutagen, produces 8-hydroxydeoxyguanosine in mouse liver (after acute oral administration or administration for three weeks in the drinking-water) and induces DNA damage in Chinese hamster ovary cells.

Glyoxylate, a metabolite of bromochloroacetic acid, is also mutagenic in bacteria.

The mechanistic data provide some additional support for the relevance of the data on cancer in experimental animals to humans.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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DIBROMOACETIC ACID

1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 631-64-1

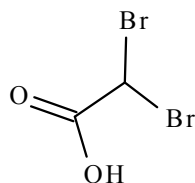
Chem. Abstr. Name: Acetic acid,
2,2-dibromo-

IUPAC Systematic Name:

2,2-Dibromoacetic acid

Synonyms: Acetic acid, dibromo; dibromoacetate; dibromoethanoic acid

1.1.2 Structural and molecular formulae and relative molecular mass



$C_2H_2Br_2O_2$

Relative molecular mass: 217.8

1.1.3 Chemical and physical properties of the pure substance

Description: White deliquescent crystals
([NTP, 2007](#))

Boiling-point: 232–234 °C (decomposition)
(Kirk-Othmer, 1985); 195 °C at 250 mm Hg
([Lide, 2005](#))

Melting-point: 49 °C ([Lide, 2005](#))

Density: 2.3899 at 25 °C ([Yaws & Chen, 2009](#))

Spectroscopy data: Infrared and magnetic resonance spectra (proton and C-13) have been reported ([NTP, 2007](#)).

Solubility: Very soluble in water, ethanol and ether ([Lide, 2005](#))

Octanol/water partition coefficient (P):
log P, 1.22 ([Schultz et al., 1999](#))

Conversion factor in air:

1 ppm = 8.91 mg/m³ ([WHO, 2004](#))

1.1.4 Technical products and impurities

Monobromoacetic acid was found to be an impurity at a concentration of < 1% in a lot of dibromoacetic acid used in toxicology studies in rodents ([NTP, 2007](#)).

1.1.5 Analysis

Dibromoacetic acid can be determined in drinking-water by gas chromatography with electron capture detection following extraction by an anion exchange column and conversion to its methyl ester at a limit of detection of 0.012 µg/L ([EPA, 2003](#)). It can also be determined in drinking-water following ion chromatography by electrospray ionization tandem mass spectrometry, for which the detection limit is 0.015 µg/L ([EPA, 2009](#)).

1.2 Production and use

1.2.1 Production

Dibromoacetic acid can be produced by bromination of bromoacetic acid with a 2:1 bromide/bromate mixture under acidic conditions ([Adimurthy et al., 2006](#)).

Dibromoacetic acid is produced commercially only in small quantities for research purposes.

Information available in 2010 indicated that dibromoacetic acid was manufactured by six companies in the USA and one company each in India and Switzerland ([Chemical Sources International, 2010](#)).

1.2.2 Use

Dibromoacetic acid is used only in research.

1.3 Occurrence

1.3.1 Natural occurrence

Dibromoacetic acid is not known to occur naturally.

1.3.2 Occurrence and exposure in drinking-water

(a) Formation of halogenated disinfection by-products in drinking-water

The drinking-water disinfectant chlorine reacts with natural organic matter to produce halogenated disinfection by-products, and trihalomethanes and haloacetic acids are the two most prevalent groups of known specific by-products formed during disinfection of natural waters with chlorine-containing oxidizing compounds ([Hua & Reckhow, 2007](#)). These compounds are formed when drinking-water supplies containing natural organic matter (e.g. humic or fulvic acids) are disinfected with compounds such as chlorine gas, hypochlorous acid and hypochlorite ([Huang et al., 2004](#)). When

bromide is present in the source water, it may be oxidized to hypobromous acid-hypobromite ion, which can react with organic matter to form brominated organic compounds. The reaction of brominated and/or chlorinated oxidizing agents with natural organic matter produces mixed brominated and chlorinated compounds. The relative amount of brominated haloacetates produced in chlorinated drinking-water is a function of the concentration of bromide in the source water and of the initial bromine/chlorine ratio. The relative amounts of disinfection by-products produced in drinking-water supplies are affected by the nature and concentration of the organic precursor materials, water temperature, pH, the type of disinfectant, the disinfectant dose and contact time ([Liang & Singer, 2003](#); [Huang et al., 2004](#)). Treatment of natural waters with chloramine or chlorine dioxide produces haloacetic acids, but at levels substantially lower than those formed by free chlorine ([Richardson et al., 2000](#); [Hua & Reckhow, 2007](#)). Because commonly used alternative disinfectants (ozone, chloramines and chlorine dioxide) produce lower levels of most of the haloacetic acids, many water utilities have switched from chlorination to these alternatives to meet the regulation limits in terms of disinfection by-products ([Krasner et al., 2006](#); [Richardson et al., 2007](#)).

Data from the USA revealed that water-treatment systems that used chlorine dioxide produced higher levels of nine haloacetic acids than those that used chlorine or chloramine only ([McGuire et al., 2002](#)). This is because the water-treatment systems that used chlorine dioxide also used chlorine or chloramines (mostly as post-disinfectants). Similarly to chloramines and chlorine dioxide, ozone used in water treatment is well known for lowering the levels of haloacetic acids formed relative to chlorination ([Richardson et al., 2007](#)). However, when source waters contain elevated levels of natural bromide, the levels of brominated compounds were shown to increase when pre-ozone treatment was performed before

chlorination ([IPCS, 2000](#); [Richardson et al., 2007](#)).

According to [IPCS \(2000\)](#) and [WHO \(2008\)](#), the optimized use of combinations of disinfectants that function as primary and secondary disinfectants, should allow further control of disinfection by-products. There is a trend towards combination/sequential use of disinfectants: ozone is used exclusively as a primary disinfectant; chloramines are used exclusively as a secondary disinfectant; and both chlorine and chlorine dioxide are used in either role ([IPCS, 2000](#); [WHO, 2008](#)).

According to [WHO \(2004\)](#), bromide ions occur naturally in surface water and groundwater; their levels exhibit seasonal fluctuations, and can also increase due to saltwater intrusion resulting from drought conditions or pollution ([IPCS, 2000](#)).

(b) Concentrations in drinking-water

A nationwide study of the occurrence of disinfection by-products in different geographical regions of the USA was conducted between October 2000 and April 2002 ([Weinberg et al., 2002](#)), in which samples were taken from 12 water-treatment plants that had different source water quality and bromide levels and used the major disinfectants (chlorine, chloramines, ozone and chlorine dioxide). Concentrations of dibromoacetate in the finished water ready for distribution ranged from 2.1 to 18 µg/L.

The occurrence of disinfection by-products in drinking-water in the USA was evaluated at 35 water-treatment facilities in 1988–89 that used a broad range of source water qualities and treatment processes ([Krasner et al., 1989](#)). Median total concentrations of haloacetic acids ranged from 13 to 21 µg/L, with those of dibromoacetic acid ranging from 0.9 to 1.5 µg/L. At a drinking-water utility with high levels of bromide, clearwell effluent contained dibromoacetic acid at concentrations ranging from 7.8 to 19 µg/L. At a utility where levels of bromide varied according

to the season, levels of dibromoacetic acid ranged from 13 to 17 µg/L.

Data for drinking-water supplies in the USA ([EPA, 2005](#)) indicated that dibromoacetic acid is present in groundwater and surface water distribution systems at mean concentrations of 0.91 µg/L (range, < 1.0–12.85 µg/L; 90th percentile, 3.03 µg/L) and 0.96 µg/L (range, < 1.0–11.77 µg/L; 90th percentile, 2.80 µg/L), respectively. For all types of distribution system (groundwater and surface), the mean concentration of dibromoacetic acid was 0.97 µg/L (range, < 1.0–12.85 µg/L; 90th percentile, 2.96 µg/L). The minimum level reported for dibromoacetic acid was 1.0 µg/L. All observations below this level for individual species were considered to be zero for the purposes of calculations.

In a survey of 20 drinking-waters prepared from different source waters in the Netherlands ([Peters et al., 1991](#)), haloacetic acids were found in all those prepared from surface water, whereas they could not be detected in those prepared from groundwater. Total haloacetic acids concentrations were in the range of 0.5–14.7 µg/L (surface water only), with levels of dibromoacetic acid ranging from not detected to 6.5 µg/L. The limit of detection of this study was 0.1 µg/L, and brominated acetic acids accounted for 65% of the total haloacetic acid concentration.

Drinking-water was studied in Israel because its source water (the Sea of Galilee, a freshwater lake, also called Lake Kinereth) has among the highest natural levels of bromide in the world for surface water (2000 µg/L) and chlorine dioxide is used for disinfection at full-scale treatment plants ([Richardson et al., 2003](#)). Chlorine-containing disinfection by-products that are usually dominant under conditions of low levels of bromide (for chlorine and chloramine disinfection) — chloroform and dichloroacetic acid — were found at very low concentrations or not at all in these samples, with a shift to bromoform and dibromoacetic acid occurring under these conditions of high levels of bromide. Thus, the bromide

content in the source water had a major impact on the speciation of the disinfection by-products. The concentration of dibromoacetic acid was 12.5 µg/L (for chloramine plus chlorine dioxide disinfection), between 12 and 38.7 µg/L (for chlorination) and between 14.1 and 23.3 µg/L (for chlorination plus chlorine dioxide disinfection).

Water collected from 53 Canadian drinking-water treatment facilities in the winter of 1993 contained dibromoacetic acid ([Williams et al., 1997](#)). When bromide concentrations were very low (< 0.01 mg/L), the water contained < 0.01 µg/L dibromoacetic acid; when they were low (0.06 mg/L), the water contained 0.9 µg/L dibromoacetic acid; and when they were moderate (0.5 mg/L), the water contained 0.8 µg/L dibromoacetic acid.

In a study based on data from several European countries (Belgium, France, Germany, Italy, the Netherlands and Spain) and covering two decades (from 1980 to 2000; [Palacios et al., 2000](#)), levels of organohalogenated compounds were evaluated in surface and groundwaters after chlorination. A mean concentration of 6.95 µg/L dibromoacetic acid was measured in post-treatment surface water (range, not detected–29.6 µg/L), whereas a mean concentration of 3.0 µg/L dibromoacetic acid was measured in post-treatment groundwater (range, not detected–7 µg/L) [limit of detection not reported].

Dibromoacetic acid was measured in water samples taken from a water-treatment plant in Barcelona (Spain) between November 1997 and March 1998 ([Cancho et al., 1999](#)). Haloacetic acids were rapidly formed during the pre-chlorination step, but their concentration did not increase during either sand filtration or ozonation. At these two stages, the concentration of total haloacetic acids represented 60% of the level of total trihalomethanes. A significant decrease in total haloacetic acids concentration was observed when ozonated water was passed through granular activated carbon filters, but the acids were formed again during post-chlorination,

although at lower concentrations than during the previous stages. The average concentration of total haloacetic acids was around 22 µg/L in tap-water (range, 11–32 µg/L). Dibromoacetic acid was detected in pre-chlorinated water (mean, 5.6 µg/L; range, 3.1–10 µg/L), sand-filtered water (mean, 6.7 µg/L; range, 5–8.4 µg/L), ozonated water (mean, 7.7 µg/L; range, 5.2–10 µg/L), granulated activated carbon-filtered water (mean, 0.6 µg/L; range, not detected–3.1 µg/L) and post-chlorinated water (mean, 3.7 µg/L; range, 2.1–5.7 µg/L).

Water samples were collected from 35 Finnish waterworks between January and October in 1994 and from three waterworks and distribution systems during different seasons in 1995 ([Nissinen et al., 2002](#)). Dibromoacetic acid was detected in six of the 35 Finnish waterworks between January and October 1994 with concentrations between 1.3 and 27 µg/L. Levels at the other facilities were below the limit of quantitation (0.8 µg/L). The concentration of six haloacetic acids, including dibromoacetic acid, exceeded that of trihalomethanes. Chlorinated drinking-waters originating from surface waters contained the highest concentration of haloacetic acids (108 µg/L). The lowest concentrations of disinfection by-products (20 µg/L) were measured in ozonated and/or activated carbon-filtered and chloraminated drinking-waters. Higher concentrations were measured in summer than in winter [data not reported].

Between October 1994 and April 1996, a mean concentration of 0.4 µg/L dibromoacetic acid was measured in the Santa Ana River (USA) downstream from a discharge point for highly treated municipal wastewater effluent ([Ding et al., 1999](#)).

In the USA, finished waters from the Philadelphia (PA) Suburban Water Co., the Metropolitan Water District of Southern California, and utilities at the cities of Houston (TX) and Corpus Christi (TX) were collected at the point of entry to the water distribution system and analysed for the nine haloacetic acids ([Cowman &](#)

[Singer, 1996](#)). These samples included waters with relatively low (Philadelphia), moderate (Houston) and high (Southern California, Corpus Christi) concentrations of bromide. Several of the utilities (Houston, Southern California, Corpus Christi) were reported to add ammonia to their waters after chlorination to control the formation of disinfection by-products. Dibromoacetic acid was found at levels below the limit of detection [not reported] in the Philadelphia and Houston utilities where bromide ion concentration ranged from 50.6 to 134 µg/L. For the others utilities, where bromide ion levels ranged from 220 to 412 µg/L, the concentration of dibromoacetic acid was 8.39–9.18 µg/L.

(c) Dietary exposure from drinking-water

To assess exposure to disinfection by-products through drinking-water, a default consumption value of 2 L drinking-water per capita per day and a typical body weight (bw) of 60 kg is generally used ([WHO, 2008](#)). The underlying assumption is that of a total water consumption of 3 L per capita per day, including food consumption, which usually represents a conservative value ([WHO, 2003](#)).

The mean concentrations and ranges of dibromoacetic acid from all references available were used by the Working Group to assess dietary exposure in adults and infants (weighing 60 kg and 5 kg, respectively) assuming a consumption of 2 L and 0.75 L drinking-water, respectively, i.e. 33 and 150 mL/kg bw, respectively ([Table 1.1](#)). The infant scenario (expressed in mL/kg bw) would correspond to the consumption of 9 L drinking-water per day in a 60-kg adult and therefore cover any possible scenario of physically active persons and increased temperature.

Based on concentrations of dibromoacetic acid reported in the literature, average dietary exposure through drinking-water in a standard 60-kg adult ranges from 0.013 to 0.42 µg/kg bw per day; high observed concentration values would

lead to a dietary exposure of 0.05–1.29 µg/kg bw per day. Similarly, average dietary exposure through drinking-water in a 5-kg infant ranges from 0.06 to 1.88 µg/kg bw per day; and high observed concentration values would lead to a dietary exposure of 0.16–5.81 µg/kg bw per day ([Table 1.1](#)).

An estimate of dietary exposure to dibromoacetic acid arising from the consumption of drinking-water was performed by the Joint FAO/WHO expert meeting for Europe, the USA and Australia ([FAO/WHO, 2009](#)). The mean concentration of dibromoacetic acid from the 12 drinking-water utilities in the USA and Canada (3.4 µg/L) reported by [FAO/WHO \(2009\)](#) was used to estimate of dietary exposure. For Europe, the estimate was based on the mean consumption of ‘tap-water’ observed in adults in the 15 countries for which these data were available in the Concise European Food Consumption Database developed by the European Food Safety Authority ([EFSA, 2008](#)). The highest observed mean consumption of tap-water was 11 mL/kg bw per day (average consumption of 0.84 and 0.886 L per day for an average body weight of 74 and 77 kg, respectively, in Denmark and Finland). Estimated mean dietary exposure to dibromoacetic acid in Europe was therefore up to 0.039 µg/kg bw per day.

For the USA and Australia, mean dietary exposure to dibromoacetic acid was estimated to be 0.048 µg/kg bw per day (assuming a mean body weight of 65 and 68 kg and a mean consumption of drinking-water of 0.926 and 0.969 L per day, respectively, in the USA and Australia).

(d) Other dietary sources

No data on the levels of haloacetic acids in foods (other than drinking-water) were identified. Extrapolations from concentrations of disinfection by-products in drinking-water to those in food are difficult to achieve because the conditions of the chemical interactions, dosages,

Table 1.1 Dietary exposure to dibromoacetic acid from drinking-water^a

Reference (country) Source	Concentration (µg/L)			Estimated exposure in adults (µg/kg bw per day)			Estimated exposure in children (µg/kg bw per day)		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Weinberg et al. (2002) (USA)		2.1	18		0.08	0.603		0.32	2.7
Krasner et al. (1989) ; IPCS (2000) (USA)									
<i>Distribution systems</i>		0.9	1.5		0.03	0.05		0.14	0.23
<i>Clearwell effluent with high bromide levels</i>		7.8	19		0.26	0.63		1.17	2.85
<i>Utility with seasonally variable bromide levels</i>		13	17		0.43	0.57		1.95	2.55
EPA (2005) (USA)									
<i>Distribution systems</i>	0.97	0.00	12.85	0.03	–	0.43	0.15	–	1.93
<i>Groundwater^b</i>	0.91	0.00	12.85	0.03	–	0.43	0.14	–	1.93
<i>Surface water^b</i>	0.96	0.00	11.77	0.03	–	0.39	0.14	–	1.77
Peters et al. (1991) (Netherlands)		0.1	6.5		0.00	0.22		0.02	0.98
Palacios et al. (2000) (European Union)									
<i>Post-treatment surface water</i>	6.95	ND ^d	29.6	0.32	–	0.99	1.04	–	4.44
<i>Post-treatment groundwater</i>	3.0	ND ^d	7	0.10	–	0.23	0.45	–	1.05
Cancho et al. (1999) (Spain) ^c									
<i>Post-chlorinated water (considered as finished water)</i>	3.7	2.1	5.7	0.12	0.07	0.19	0.56	0.32	0.86
Williams et al. (1997) (Canada)									
<i>Distribution systems</i>		< 0.01	1.9			0.04			0.16
<i>Very low bromide concentrations (< 0.01 mg/L)</i>	< 0.01								
<i>Low bromide concentrations (0.06 mg/L)</i>	0.9			0.03			0.14		
<i>Moderate bromide concentrations (0.5 mg/L)</i>	0.8			0.03			0.12		
Richardson et al. (2003) (Israel)									
<i>Chloramine plus chlorine dioxide disinfection</i>	12.5			0.42			1.88		
<i>Chlorine disinfection</i>		12	38.7		0.40	1.29		1.80	5.81
<i>Chlorine plus chlorine dioxide disinfection</i>		14.1	23.3		0.47	0.78		2.12	3.50
Nissinen et al. (2002) (Finland)		< 0.8	27		–	0.90		0.20	4.05
Ding et al. (1999) (USA)	0.4			0.01			0.06		
Cowman & Singer. (1996) (USA)		ND ^d	9.18		–	0.31		1.26	1.38

^a Calculated by the Working Group, assuming a daily intake and a body weight for adults of 2 L and 60 kg, and for children of 0.75 L and 5 kg, respectively.

^b [From the paper, it is not clear if it is considered as water that is ready to drink.]

^c The study reported the levels of dibromoacetic acid according to different water treatments (e.g. chlorinated water, sand-filtered water, ozonated water, granulated activated carbon-filtered water). For the dietary exposure assessment, the chlorinated water values were used because this was considered as finished water.

^d [Limit of detection not reported]

ND, not detected

temperatures, contact times and especially the precursors differ considerably ([FAO/WHO, 2009](#)).

1.3.3 Exposure through inhalation or dermal contact

Dibromoacetic acid occurs in water used for showering and bathing due to its presence in household water distribution systems (see Section 1.3.2). Dibromoacetic acid was also detected in the water of two large public swimming pools disinfected with either chlorine or bromine in Barcelona (Spain) ([Richardson et al., 2010](#)).

Exposure to dibromoacetic acid through dermal contact and inhalation has not been measured. Based on low dermal absorption observed for other haloacetic acids ([Kim & Weisel, 1998](#)), dermal exposure to dibromoacetic acid is not liable to be significant. In contrast, inhalation of the substance in vapour/mist might occur during showering, bathing or swimming, as is anticipated for other disinfection by-products ([Richardson et al., 2007](#)).

1.3.4 Environmental occurrence

Many haloacetates are distributed ubiquitously in the biosphere, including in lakes and groundwater ([Guo et al., 2006](#)). Dibromoacetic acid has been identified in the environment only as a by-product of the treatment of ground- and surface waters with chlorine-containing oxidizing compounds in the presence of bromide. The formation of dibromoacetic acid as a chemical by-product of chlorination and chloramination of drinking-water ([Cowman & Singer, 1996](#)) may result in its release into the environment through various waste streams.

Dibromoacetic acid is not expected to volatilize from dry or moist soil surfaces. In the atmosphere, it is expected to exist solely as a vapour ([HSDB, 2010](#)). Vapour-phase dibromoacetic acid is degraded by reaction with photochemically produced hydroxyl radicals, with a half-life of 25.3 days.

1.3.5 Occupational exposure

No data were available to the Working Group.

1.4 Regulations and guidelines

No occupational exposure limits have been established for dibromoacetic acid. Levels of haloacetic acids in drinking-water are regulated in the USA by the Environmental Protection Agency ([EPA, 2010](#)). Under the disinfection by-products rule, the sum of the concentrations of monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid and dibromoacetic acid is limited to 60 µg/L (60 ppb).

2. Cancer in Humans

See the Introduction to the *Monographs on Bromochloroacetic Acid, Dibromoacetic Acid and Dibromoacetonitrile*.

3. Cancer in Experimental Animals

Carcinogenicity studies of dibromoacetic acid in mice and rats are limited to those of oral administration in the drinking-water conducted by the [NTP \(2007\)](#), which are summarized in [Table 3.1](#) (see also [Melnick et al., 2007](#)).

3.1 Oral administration

3.1.1 Mouse

In a 2-year study, groups of 50 male and 50 female B6C3F₁ mice were administered dibromoacetic acid in the drinking-water at doses of 0 (controls), 50, 500 or 1000 mg/L (corresponding to average daily doses of approximately 0, 4, 45 or 87 and 0, 4, 35 or 65 mg/kg bw in male and female mice, respectively). Significant increases in the incidence of hepatocellular adenoma and

Table 3.1 Carcinogenicity studies of exposure to dibromoacetic acid in the drinking-water in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F ₁ (M) 105–106 wk Melnick et al. (2007) ; NTP (2007)	0 (control), 50, 500, 1000 mg/L (daily dose of 0, 4, 45, 87 mg/ kg bw) 50/group	Liver (hepatocellular adenoma): 18/49, 37/50, 37/50, 42/50 Liver (hepatocellular carcinoma): 14/49, 9/50, 19/50, 26/50 Liver (hepatocellular adenoma or carcinoma, combined): 28/49, 41/50, 42/50, 47/50 Liver (hepatoblastoma) ^a : 0/49, 4/50, 6/50, 18/50 Lung (alveolar/bronchiolar adenoma): 7/49, 5/50, 17/50, 12/50 Lung (alveolar/bronchiolar adenoma or carcinoma, combined): 12/49, 12/50, 22/50, 17/50	$P < 0.001$ (all doses), $P < 0.001$ (trend) $P = 0.016$ (high dose), $P < 0.001$ (trend) $P < 0.001$ (high dose), $P < 0.001$ (mid-dose), $P = 0.004$ (low dose), $P < 0.001$ (trend) $P < 0.001$ (high dose), $P = 0.019$ (mid-dose), $P < 0.001$ (trend) $P = 0.016$ (mid-dose), $P = 0.019$ (trend) $P = 0.027$ (mid-dose)	> 99% pure
Mouse, B6C3F ₁ (F) 105–106 wk Melnick et al. (2007) ; NTP (2007)	0 (control), 50, 500, 1000 mg/L (daily dose of 0, 4, 35, 65 mg/ kg bw) 50/group	Liver (hepatocellular adenoma): 19/49, 26/50, 32/50, 35/49 Liver (hepatocellular carcinoma): 3/49, 3/50, 12/50, 8/49 Liver (hepatocellular adenoma or carcinoma, combined): 22/49, 28/50, 37/50, 37/49 Lung (alveolar/bronchiolar adenoma): 1/50, 3/50, 3/50, 6/50	$P < 0.001$ (high dose), $P = 0.004$ (mid-dose), $P < 0.001$ (trend) $P = 0.009$ (mid-dose), $P = 0.019$ (trend) $P < 0.001$ (high dose), $P < 0.001$ (mid-dose), $P < 0.001$ (trend) $P = 0.044$ (trend)	> 99% pure
Rat, F344/N (M) 105–106 wk Melnick et al. (2007) ; NTP (2007)	0 (control), 50, 500, 1 000 mg/L (daily dose of 0, 2, 20, 40 mg/ kg bw) 50/group	All organs (malignant mesothelioma) ^b : 3/50, 1/50, 0/50, 10/50 Blood (mononuclear-cell leukaemia) ^c : 17/50, 31/50, 24/50, 13/50	$P = 0.035$ (high dose), $P < 0.001$ (trend) $P = 0.003$ (low dose), $P = 0.026$ (negative trend)	> 99% pure
Rat, F344/N (F) 105–106 wk Melnick et al. (2007) ; NTP (2007)	0 (control), 50, 500, 1 000 mg/L (daily dose of 0, 2, 25, 45 mg/ kg bw) 50/group	Blood (mononuclear-cell leukaemia) ^d : 11/50, 13/50, 16/50, 22/50	$P = 0.016$ (high dose), $P = 0.006$ (trend)	> 99% pure

^a Historical control incidence for 2-year drinking-water studies (mean \pm standard deviation): 11/197 (4.5 \pm 6.2%); range, 0–13%

^b Historical control incidence for 2-year drinking-water studies (mean \pm standard deviation): 15/250 (6.0 \pm 4.2%); range, 0–12%

^c Historical control incidence for 2-year drinking-water studies (mean \pm standard deviation): 79/250 (31.6 \pm 3.3%); range, 26–34%

^d Historical control incidence for 2-year drinking-water studies (mean \pm standard deviation): 47/200 (23.5 \pm 4.4%); range, 20–30%

bw, body weight; F, female; M, male; wk, week or weeks

hepatocellular carcinoma in both males and females and of hepatoblastoma in males were observed. A significant increase in the incidence of alveolar/bronchiolar adenoma also occurred in males and females (NTP, 2007). [The Working Group noted that hepatoblastomas are rare spontaneous tumours in experimental animals.]

3.1.2 Rat

In a 2-year study, groups of 50 male and 50 female F344/N rats were administered dibromoacetic acid in the drinking-water at doses of 0 (controls), 50, 500 or 1000 mg/L (corresponding to average daily doses of approximately 0, 2, 20 or 40 and 0, 2, 25 or 45 mg/kg bw in male and female rats, respectively). Significant increases in the incidence of malignant mesothelioma in males and of mononuclear-cell leukaemia in females were observed. A significant increase in the incidence of mononuclear-cell leukaemia in low-dose males and a non-significant increase in mid-dose males also occurred, but the trend was negative. [It was therefore unclear whether the increase in low-dose males was treatment-related] (NTP, 2007). [The Working Group noted that malignant mesotheliomas are rare spontaneous tumours in experimental animals.]

4. Other Relevant Data

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, distribution and excretion

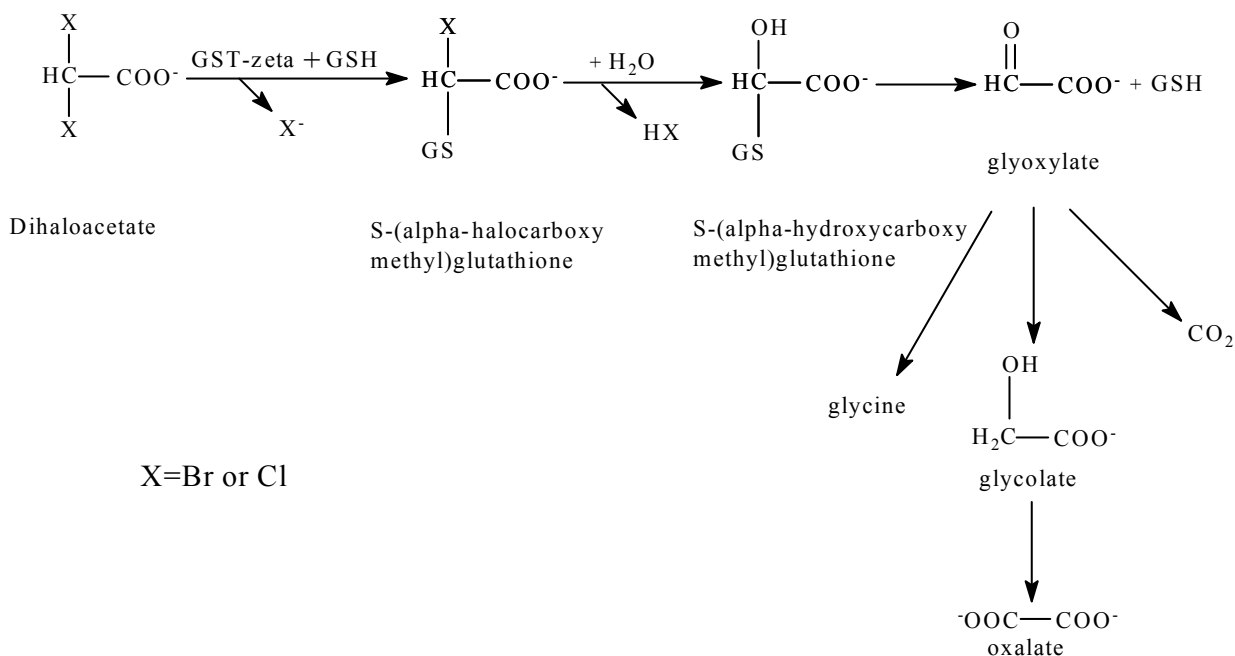
Dihaloacetates are rapidly absorbed from the gastrointestinal tract after oral exposure in rats. The maximum blood concentration of dibromoacetate in F344/N rats was reached one hour after gavage administration (Schultz *et al.*, 1999).

Dihaloacetates exhibit low binding to rat plasma proteins (Schultz *et al.*, 1999). Dibromoacetate was measured in the testicular interstitial fluid of male Sprague-Dawley rats after five daily gavage doses of 250 mg/kg bw. The level in testicular fluid peaked at 79 µg/mL (approximately 370 µM) 30 minutes after the last dose, and the half-life was approximately 1.5 hours (Holmes *et al.*, 2001).

After exposure of Sprague-Dawley rats to 125–1000 mg/L in the drinking-water beginning 14 days before mating and continuing throughout gestation and lactation, dibromoacetate was quantifiable in parental and fetal plasma, placental tissue, amniotic fluid and milk (Christian *et al.*, 2001), showing that dibromoacetate can cross the placenta and be absorbed by fetal tissue.

The oral bioavailability of dibromoacetate was reported to be 30% in male F344/N rats (Schultz *et al.*, 1999). The lower bioavailability compared with that of dichloroacetate is due to a greater first-pass metabolism in the liver (Bull *et al.*, 1985).

Elimination half-lives of dihaloacetates in the blood of male F344/N rats are less than 4 hours; the plasma half-life of dibromoacetate after intravenous injection is approximately 30–40 minutes (Schultz *et al.*, 1999). Elimination of dibromoacetate occurs primarily by metabolism; less than 3% of an intravenous dose of 500 µmol/kg bw (109 mg/kg bw) was excreted as the parent compound in urine and less than 0.1% was eliminated in the faeces. Bromine substitution of dihaloacetates increases the rate of metabolic clearance (Xu *et al.*, 1995), because

Fig. 4.1 Biotransformation of dihaloacetates

Adapted from [Tong et al. \(1998a\)](#)

dichloroacetate is cleared at half the rate of dibromoacetate ([Lin et al., 1993](#); [Narayanan et al., 1999](#)).

(b) Metabolism

The metabolism of dibromoacetic acid has been reviewed ([NTP, 2007](#)). Biotransformation of dihaloacetates to glyoxylate occurs primarily in the liver cytosol of rats, by a glutathione-dependent process ([James et al., 1997](#)) that is catalysed by glutathione S-transferase zeta (GST-zeta) ([Tong et al., 1998a](#)). This enzyme also catalyses the penultimate step in the tyrosine degradation pathway.

GST-zeta-mediated biotransformation of dihaloacetates (Fig. 4.1) involves the displacement of a halide by glutathione to form S-(α -halocarboxymethyl)glutathione, hydrolysis of this intermediate to form S-(α -hydroxycarboxymethyl)glutathione and elimination of glutathione to produce glyoxylate ([Tong et al., 1998b](#)). Among the brominated/

chlorinated dihaloacetates, the relative rates of glyoxylate formation catalysed by purified GST-zeta are: bromochloroacetate > dichloroacetate > dibromoacetate ([Austin et al., 1996](#)). Glyoxylate can undergo transamination to glycine, decarboxylation to carbon dioxide and oxidation to oxalate.

Dibromoacetate is a suicide substrate for GST-zeta; 12 hours after a single injection of 0.30 mmol/kg bw, GST-zeta activity in the rat liver was reduced to 17% of that in controls ([Anderson et al., 1999](#)). Hydrolysis of S-(α -halocarboxymethyl)glutathione forms a hemi-thioacetal that eliminates glutathione and yields glyoxylate. Because this intermediate may inactivate GST-zeta by covalently binding to a nucleophilic site on the enzyme ([Wempe et al., 1999](#)), its hydrolysis and GST-zeta inactivation are competing reactions.

4.1.3 Toxicokinetic models

In a recent study, [Matthews et al. \(2010\)](#) developed a novel physiologically-based pharmacokinetic model, which included submodels for the common metabolites glyoxylate and oxalate that may be involved in the toxicity or carcinogenicity of dibromoacetic acid, and took into account hepatic metabolism as the primary mechanism of elimination (see Fig. 4.2 and Fig. 4.3).

Suicide inhibition induced by dibromoacetic acid was modelled by the irreversible covalent binding of the intermediate metabolite, α -halocarboxymethylglutathione, to the GST-zeta enzyme. Moreover, [Matthews et al. \(2010\)](#) introduced a secondary non-GST-zeta-mediated metabolic pathway for dibromoacetate. The model was calibrated using data on plasma and urine concentrations from studies of female F344 rats exposed to dibromoacetate by intravenous injection, oral gavage and administration in the drinking-water and was validated. The authors hypothesized that the model presented for dibromoacetic acid can be extended to structurally similar dihaloacetic acids.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

Studies on the genotoxicity of dibromoacetic acid are summarized in [Table 4.1](#).

(a) DNA adducts

Oxidative stress can result in oxidative DNA damage, which is most commonly measured as increases in 8-hydroxydeoxyguanosine (8-OHdG) adducts. After acute oral administration of dibromoacetate to male B6C3F₁ mice, a significant increase in 8-OHdG/deoxyguanosine

ratios in nuclear DNA isolated from livers was observed ([Austin et al., 1996](#)). After administration of dibromoacetate to male B6C3F₁ mice (0.1, 0.5 or 2.0 g/L in the drinking-water for 3–10 weeks), 8-OHdG content in liver nuclear DNA was increased ([Parrish et al., 1996](#)). These findings demonstrate that dibromoacetate causes oxidative stress/damage.

(b) DNA damage

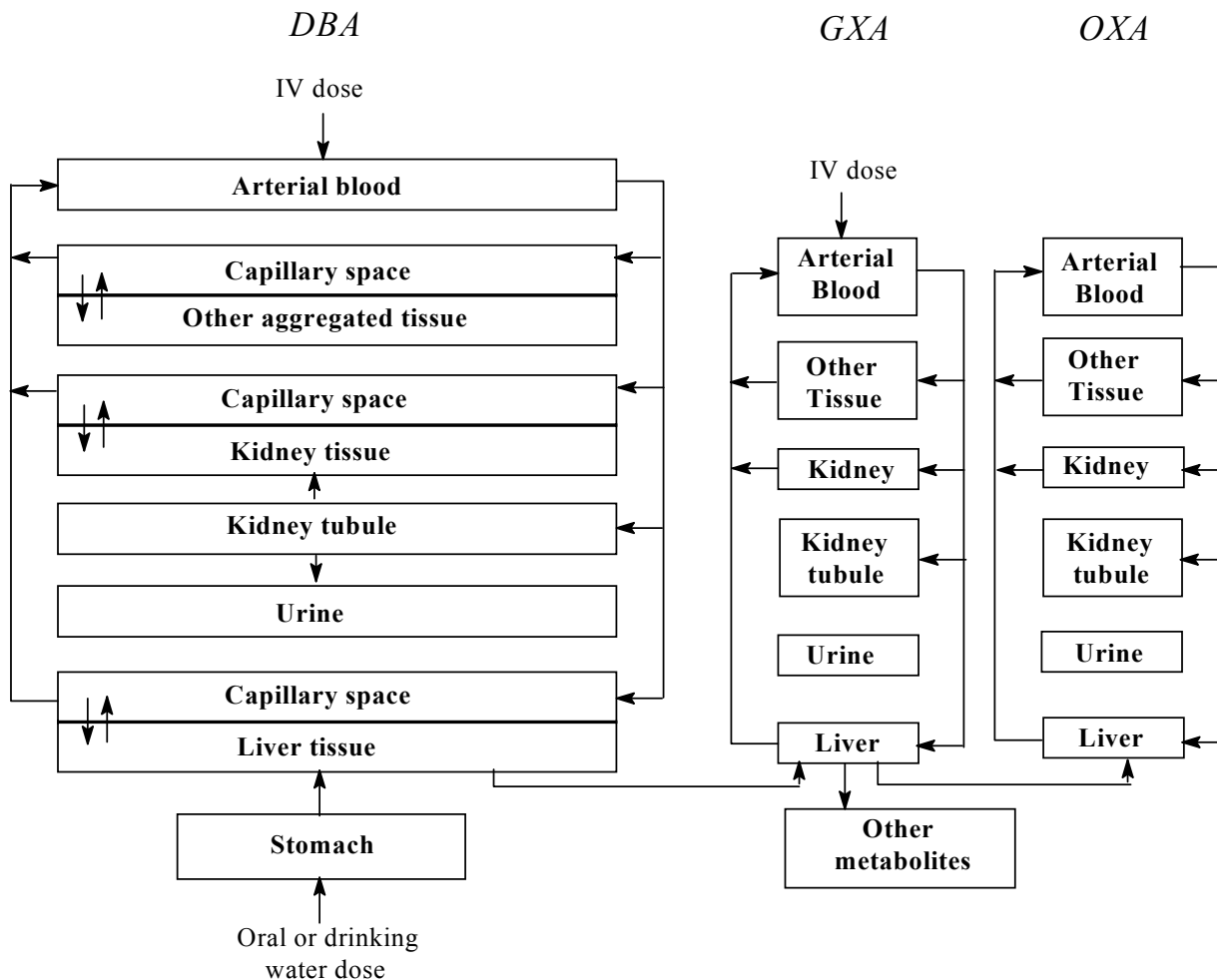
Dibromoacetate induced DNA damage in Chinese hamster ovary cells, as measured in the Comet assay ([Plewa et al., 2002, 2010](#)), and DNA strand breaks in human lymphoblast cell lines ([Daniel et al., 1986](#)). DNA damage was also induced in *Escherichia coli* in the SOS repair assay ([Giller et al., 1997](#)) and in primary rat hepatocytes in the unscheduled DNA synthesis assay ([Fang et al., 2001](#)).

(c) Mutations

Dibromoacetate was mutagenic in *Salmonella typhimurium* strain TA100 in the Ames fluctuation test ([Giller et al., 1997](#)), in TA98 ([Kargalioglu et al., 2002](#)) and in TA100 in the presence and absence of metabolic activation ([Fang et al., 2001](#); [Kargalioglu et al., 2002](#)). It was not mutagenic in strain RSJ100, a derivative of TA1535 that contains a rat *GSTT1-1* gene. In another series of tests, Dibromoacetic acid was mutagenic in TA100, but not TA98, in the presence or absence of metabolic activation ([NTP, 2007](#)). Glyoxylate was mutagenic in *S. typhimurium* strains TA97, TA100 and TA104 in the absence of and in strain TA102 in the presence of metabolic activation ([Sayato et al., 1987](#)).

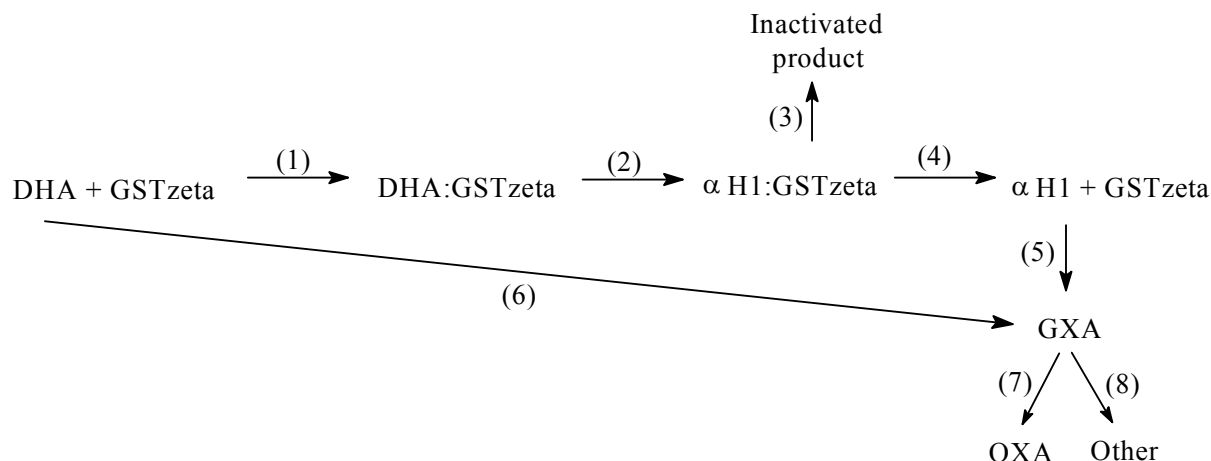
Dibromoacetate was mutagenic in the hypoxanthine-guanine phosphoribosyltransferase gene mutation assay in Chinese hamster ovary cells ([Zhang et al., 2010](#)).

Fig. 4.2 Pharmacokinetic model for dibromoacetate, with glyoxylate and oxalate submodels



DBA, dibromoacetate; GXA, glyoxylate; IV, intravenous; OXA, oxalate
 Reprinted from [Matthews et al. \(2010\)](#) with permission from Elsevier.

Fig. 4.3 Metabolism of dihaloacetates as implemented in the model



DHA, dihaloacetate; GST, glutathione-*S*-transferase; GXA, glyoxylate; αH1, α-halocarboxymethylglutathione; OXA, oxalate
 Reprinted from [Matthews et al. \(2010\)](#) with permission from Elsevier.

(d) Chromosomal effects

Significant increases in micronucleated normochromatic erythrocytes were observed in the peripheral blood of male, but not female, B6C3F₁ mice treated with dibromoacetate in the drinking-water for 3 months ([NTP, 2007](#)). Moreover, dibromoacetic acid induced chromosomal damage *in vivo* in the mouse bone-marrow micronucleus assay and increased the number of micronuclei in NIH3T3 cells *in vitro* ([Fang et al., 2001](#)). It failed to induce micronuclei in the erythrocytes of newt (*Pleurodeles waltl*) larvae ([Giller et al., 1997](#)).

(e) Alterations in oncogenes and suppressor genes in tumours

Dibromoacetic acid (1 or 2 g/L in the drinking-water) induced liver hypomethylation of the proto-oncogene *c-myc* and of the growth factor gene *IGF-II* and increased both mRNA expressions in female B6C3F₁ mice and male F344 rats ([Tao et al., 2004](#)).

(f) Changes in DNA methylation pattern

Dibromoacetic acid (1 or 2 g/L in the drinking-water for 28 days) induced liver hypomethylation of *c-myc* in both female B6C3F₁ mice and male F344 rats ([Tao et al., 2004](#)) and renal hypomethylation of DNA and of *c-myc* in both male B6C3F₁ mice and F344 rats ([Tao et al., 2005](#)).

Table 4.1 Genetic and related effects of dibromoacetic acid (dibromoacetate) and glyoxylate

Test system	Results		Dose ^a (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Salmonella typhimurium</i> TA100, reverse mutation, Ames-fluctuation	+	+	10	Giller et al. (1997)
<i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	500 µg/plate	Fang et al. (2001)
<i>Salmonella typhimurium</i> TA100, reverse mutation	+	+	218 µg/plate	Kargalioglu et al. (2002)
<i>Salmonella typhimurium</i> TA100, reverse mutation	+		1000 µg/plate	NTP (2007)
<i>Salmonella typhimurium</i> TA100, reverse mutation		+	333 µg/plate	NTP (2007)
<i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	5000 µg/plate	Fang et al. (2001)
<i>Salmonella typhimurium</i> TA98, reverse mutation	+	+	610 µg/plate	Kargalioglu et al. (2002)
<i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	10 000 µg/plate	NTP (2007)
<i>Salmonella typhimurium</i> RSJ100, reverse mutation	-	-	0.015	Kargalioglu et al. (2002)
Primary DNA damage, <i>Escherichia coli</i> strain PQ37 (SOS chromotest)	+	+	100	Giller et al. (1997)
Unscheduled DNA synthesis, rat primary hepatocytes <i>in vitro</i>	+	NT	50	Fang et al. (2001)
DNA strand break (Comet assay), Chinese hamster ovary cells <i>in vitro</i>	+	NT	163.3	Plewa et al. (2002)
Gene mutation, <i>Hprt</i> locus, 6-thioguanine resistance, Chinese hamster ovary K1 cells <i>in vitro</i>	+	-	21.8	Zhang et al. (2010)
Micronucleus formation, NIH3T3 cell <i>in vitro</i>	+	NT	100 µg/plate	Fang et al. (2001)
DNA adducts (8-OHdG), liver nuclear DNA, male B6C3F ₁ mice <i>in vivo</i>	+		30 po × 1	Austin et al. (1996)
DNA adducts (8-OHdG), liver nuclear DNA, male B6C3F ₁ mice <i>in vivo</i>	+		100, dw, 3 wk	Parrish et al. (1996)
Micronucleus formation, mouse bone marrow <i>in vivo</i>	+		50 µg/plate	Fang et al. (2001)
Micronucleus formation, male B6C3F ₁ mouse peripheral erythrocytes <i>in vivo</i>	+		250, dw, 3 mo	NTP (2007)
Micronucleus formation, female B6C3F ₁ mouse peripheral erythrocytes <i>in vivo</i>	-		2000, dw, 3 mo	NTP (2007)
Micronucleus formation, <i>Pleurodeles waltl</i> <i>in vivo</i>	-		160	Giller et al. (1997)
Glyoxylate (metabolite of dibromoacetic acid)				
<i>Salmonella typhimurium</i> TA100, TA104, TA97, reverse mutation	+	-	400 µg/plate	Sayato et al. (1987)
<i>Salmonella typhimurium</i> TA100, TA102, TA97, reverse mutation	-	+	1000 µg/plate	Sayato et al. (1987)

^a *in vitro* test, µg/mL; *in vivo* test, mg/kg bw per day

+, positive; -, negative; bw, body weight; d, day or days; dw, drinking-water; HID, highest ineffective dose; *Hprt*, hypoxanthine-guanine phosphoribosyltransferase gene; LED, lowest effective dose; mo, month or months; NT, not tested; 8-OHdG, 8-hydroxydeoxyguanosine; po, oral; wk, week or weeks

4.3 Mechanistic data

4.3.1 Effects on cell physiology

Dibromoacetic acid induced alveolar epithelial hyperplasia in female rats exposed for 2 years via the drinking-water ([Melnick et al., 2007](#)).

Dibromoacetic acid (1 or 2 g/L in the drinking-water for 3 months) caused cytoplasmic vacuolization in hepatocytes and marginal increases in DNA hepatocyte replication in male rats ([NTP, 2007](#)).

4.3.2 Effects on cell function

Treatment of cultured hepatocytes isolated from male Long Evans rats with 1 mM (217 mg/L) dibromoacetate for 72 hours induced peroxisome proliferation ([Walgren et al., 2004](#)). Dibromoacetic acid in the drinking-water caused liver peroxisome proliferation in both female B6C3F₁ mice (4 days at 2 g/L and 7 days at 1 g/L) and male F344 rats (2 days at 2 g/L) ([Tao et al., 2004](#)). [The Working Group noted that it is not known whether peroxisome proliferation occurs at doses of dibromoacetic acid below 1000 mg/L.]

4.3.3 Other relevant data

Several comparative genotoxicity and mutagenicity studies ([Giller et al., 1997](#); [Kargalioglu et al., 2002](#); [Plewa et al., 2010](#); [Zhang et al., 2010](#)) have demonstrated that dibromoacetic acid is more potent than its chlorinated analogue, dichloroacetic acid, and that they have several molecular and biochemical activities in common ([Tao et al., 2004](#)). Dichloroacetic acid is considered as a possible (Group 2B) human carcinogen ([IARC, 2004](#)).

4.4 Susceptibility

No data were available to the Working Group. [However, the Working Group noted that disruption of GST-zeta in type-I hereditary

tyrosinaemia has been linked to liver cancer in humans.]

4.5 Mechanisms of carcinogenesis

The mechanism by which dibromoacetic acid causes tumours is not known.

It has been suggested that the reduction of GST-zeta activity by dibromoacetic acid may cause accumulation of toxic intermediates because this enzyme is involved in the tyrosine degradation pathway ([Ammini et al., 2003](#)).

DNA hypomethylation and increased expression of *c-myc* and *IGF-II* genes were suggested to be possible early events in the hepatocarcinogenicity of dihaloacetic acids in mice. An early increase in hepatocyte proliferation is probably not involved in the mechanism because no increases in the DNA labelling index were observed in mice exposed for 26 days, and the slight increase that occurred in male F344/N rats was not accompanied by an increase in liver tumour response ([Tao et al., 2004](#)).

DNA damage due to oxidative stress in the livers of mice exposed to dibromoacetic acid may contribute to the hepatocarcinogenicity of this chemical ([Austin et al., 1996](#); [Parrish et al., 1996](#)).

The carcinogenicity of dibromoacetic acid may also involve a genotoxic mechanism because it induces DNA damage in bacteria, and rodent and human cell lines, as well as mutations in bacteria and a rodent cell line ([Daniel et al., 1986](#); [Giller et al., 1997](#); [Fang et al., 2001](#); [Kargalioglu et al., 2002](#); [Plewa et al., 2002](#); [NTP, 2007](#); [Plewa et al., 2010](#); [Zhang et al., 2010](#)). In addition, glyoxylate, a metabolite of dihaloacetates biotransformation, is mutagenic in bacteria ([Sayato et al., 1987](#)).

5. Summary of Data Reported

5.1 Exposure data

Dibromoacetic acid is formed as a by-product during the disinfection of water by chlorination in the presence of organic matter and bromide. The concentration of dibromoacetic acid measured in drinking-water was up to 39 µg/L. The highest concentrations are observed in waters with the highest bromide content. The maximum daily human exposure to dibromoacetic acid through drinking-water, estimated from such measurements, is at the low microgram per kilogram of body weight level.

5.2 Human carcinogenicity data

No epidemiological studies were identified that evaluated exposure specifically to dibromoacetic acid. This chemical occurs in mixtures in disinfected water. Studies on disinfected water are reviewed in the Introduction to the *Monographs* on Bromochloroacetic Acid, Dibromoacetic Acid and Dibromoacetonitrile.

5.3 Animal carcinogenicity data

Dibromoacetic acid was tested for carcinogenicity by administration in the drinking-water in one study in mice and one study in rats. In mice, dibromoacetic acid increased the incidence of hepatocellular adenoma and hepatocellular carcinoma in males and females, of hepatoblastoma in males, and of alveolar/bronchiolar adenoma in males and females. In rats, dibromoacetic acid increased the incidence of mesothelioma in males and of mononuclear-cell leukaemia in females. Mesotheliomas and hepatoblastomas are rare spontaneous neoplasms in experimental animals.

5.4 Other relevant data

No data were available to the Working Group on the toxicokinetics of dibromoacetic acid in humans. In rats, dibromoacetate is rapidly absorbed from the gastrointestinal tract after oral exposure.

Dibromoacetic acid is primarily biotransformed to glyoxylate in the liver cytosol of rats and humans by a glutathione-dependent process that is catalysed by glutathione *S*-transferase-zeta. Glyoxylate can further undergo transamination to glycine, decarboxylation to carbon dioxide and oxidation to oxalate.

Dibromoacetic acid induces DNA adducts in mouse liver (after acute oral administration or administration in the drinking-water for three weeks) and causes DNA damage in bacteria, and rodent and human cell lines. In addition, it caused mutations in bacteria and a rodent cell line, and micronucleus formation in male mice *in vivo*. Glyoxylate, a metabolite of dibromoacetate, is also mutagenic in bacteria.

The mechanism of tumour induction by dibromoacetic acid has not been clearly identified. The reduction of glutathione *S*-transferase-zeta activity may be involved. DNA hypomethylation and increased expression of a proto-oncogene and a growth factor gene were also suggested as possible early events. There is moderate evidence that the carcinogenicity of dibromoacetic acid involves a genotoxic mechanism. Moreover, glyoxylate, a metabolite of dibromoacetic acid, is mutagenic in bacteria.

The mechanistic data provide some additional support for the relevance of data on cancer in experimental animals to humans.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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DIBROMOACETONITRILE

Dibromoacetonitrile was considered by previous IARC Working Groups in June 1990 and February 1998 ([IARC, 1991, 1999](#)). Since that time, new data have become available and have been incorporated into this *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.: 3252-43-5

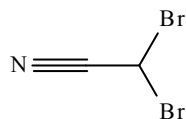
EC Number: 221-843-2

Chem. Abstr. Name: Acetonitrile,
2,2-dibromo-

IUPAC Systematic Name:
2,2-Dibromoacetonitrile

Synonyms: Acetonitrile, dibromo-;
dibromocyanomethane

1.1.2 Structural and molecular formulae and relative molecular mass



C_2HBr_2N

Relative molecular mass: 198.84

1.1.3 Chemical and physical properties of the pure substance

Description: Colourless to pale-yellow liquid with an organohalide odour ([NTP, 2010](#))

Boiling-point: bp₇₆₀ 169 °C; bp₂₄ 68 °C ([Lide, 2005](#))

Density: 2.369 at 20 °C ([Lide, 2005](#))

Spectroscopy data: Infrared and magnetic resonance spectra (proton and C-13) have been reported ([IARC, 1991; NTP, 2010](#)).

Solubility: Slightly soluble in water

Vapour pressure: 0.3 mm Hg at 25 °C ([HSDB, 2010](#))

Octanol/water partition coefficient (P): log P, 0.420 ([IARC, 1991](#))

Conversion factor: mg/m³ = 8.13 × ppm, calculated from: mg/m³ = (relative molecular mass/24.45) ppm, assuming a temperature of 25 °C and a pressure of 101 kPa

1.1.4 Technical products and impurities

No information on technical products and impurities was available to the Working Group.

1.1.5 Analysis

Dibromoacetonitrile can be determined in drinking-water by gas chromatography with electron capture detection following liquid-liquid extraction, with a limit of detection of 0.034 µg/L ([EPA, 1990](#)).

1.2 Production and use

1.2.1 Production

Dibromoacetonitrile can be produced by treatment of cyanoacetic acid with *N*-bromosuccinimide ([Wilt, 1956](#)).

Information available in 2010 indicated that dibromoacetonitrile was manufactured by eight companies in the USA, two companies in the United Kingdom and one company in Germany ([Chemical Sources International, 2010](#)).

1.2.2 Use

Dibromoacetonitrile has been used as an anti-microbial component (≤ 3%) of a metalworking fluid ([DOW, 2006](#)).

1.3 Occurrence and exposure

1.3.1 Natural occurrence

Dibromoacetonitrile is not known to occur naturally.

1.3.2 Occurrence and exposure in drinking-water

(a) Formation of halogenated acetonitriles disinfection by-products

Halogenated acetonitriles are formed during water disinfection as a result of the reaction of chlorinated oxidizing compounds (e.g. chlorine gas, hypochlorous acid and hypochlorite) with natural organic matter, such as algae, humic substances and proteinaceous material, present

in water ([IARC, 1991](#)), and particularly nitrogen-containing organic compounds in water that contains bromide; it is also a by-product of disinfection by ozonation ([Huang *et al.*, 2003, 2004](#)).

Halogenated acetonitriles form rapidly, but then decay in the distribution system as a result of hydrolysis ([IPCS, 2000](#)); they have not been detected in raw (untreated) water sources ([Trehy & Bieber, 1981](#)).

Plants that used chloramines (with or without chlorination) had the highest levels of halogenated acetonitriles in their finished drinking-water. Higher levels were also observed in distribution-system waters treated by chloramination compared with free chlorine. However, the increased levels following chloramination may be the result of the higher levels of total organic carbon in the source waters ([McGuire *et al.*, 2002](#)).

Factors that affect the formation of halogenated acetonitriles in drinking-water supplies include water temperature, pH, the dose and type of disinfectant and contact time ([IPCS, 2000](#); [Huang *et al.*, 2003](#); [Liang & Singer, 2003](#); [Huang *et al.*, 2004](#); [WHO, 2004](#)).

(b) Concentrations in drinking-water

Haloacetonitriles have been measured in several studies of occurrence ([Richardson *et al.*, 2007](#)).

Chloro-, bromochloro-, dibromo- and trichloroacetonitrile are the most commonly measured halogenated acetonitrile species and have been included in a survey of 35 water utilities in the USA conducted in 1988–89 with a broad range of source water qualities and treatment processes ([Krasner *et al.*, 1989](#); [IPCS 2000](#)). Median concentrations of total halogenated acetonitrile over the four seasons ranged from 2.5 to 4 µg/L, with median concentrations of dibromoacetonitrile ranging from 0.46 to 0.54 µg/L. At a drinking-water utility with high levels of bromide, clearwell effluent contained concentrations of dibromoacetonitrile ranging from

5.9 µg/L to 6.7 µg/L according to the season. At a utility with seasonally variable levels of bromide, concentrations of dibromoacetonitrile ranged from 4.6 µg/L to 11 µg/L.

Water collected from 53 Canadian drinking-water treatment facilities in the winter of 1993 was found to contain dibromoacetonitrile (Williams *et al.*, 1997). When bromide concentrations were very low (< 0.01 mg/L), the water contained < 0.1 µg/L dibromoacetonitrile; when they were low (0.06 mg/L), the water contained 0.6 µg/L dibromoacetonitrile; and when they were moderate (0.5 mg/L), the water contained 1.2 µg/L dibromoacetonitrile.

A nationwide study of the occurrence of disinfection by-products in diverse geographical regions of the USA was conducted between October 2000 and April 2002 (Weinberg *et al.*, 2002). Twelve water-treatment plants that had different source water quality and levels of bromide and used the major disinfectants chlorine, chloramines, ozone and chlorine dioxide were sampled. Concentrations of dibromoacetonitrile in the finished water ranged from 0.6 to 2.0 µg/L.

Dibromoacetonitrile was identified in stored, chlorinated Rhine water in the Netherlands. The concentration of dibromoacetonitrile was less than 0.1 µg/L before chlorination and 1 µg/L after chlorination (Zoeteman *et al.*, 1982).

Treatment plant samples collected in 1984 and 1985 from 29 community water systems in the USA (that used free chlorine disinfection) contained dibromoacetonitrile at levels of < 0.2–11 µg/L (14 of 29 sites). Samples from the distribution system contained dibromoacetonitrile at < 0.2–2.5 µg/L (11/26 sites) (Reding *et al.*, 1989).

Water samples were collected in 1985 from 10 utilities in the USA that used free chlorine disinfection (one of which also added ammonia before distribution). Dibromoacetonitrile was detected at concentrations of < 10 µg/L at three of

the seven sites and was not detected in the others (Stevens *et al.*, 1989).

Groundwater samples were collected from utilities in Taiwan, China, which are subject to saltwater intrusion and, therefore, have high levels of bromide (up to 1.5 mg/L) (Huang *et al.*, 2003). Concentrations of dibromoacetonitrile resulting from ozonation of such groundwater — when detected — ranged from 3.1 to 18.1 µg/L (eight of 28 samples).

Seasonal variation in concentrations of haloacetonitriles was investigated in tap-water samples collected from five sampling points (one groundwater and four surface water sources) in İzmir, Turkey, between July 2006 and April 2007 (Baytak *et al.*, 2008). Dibromoacetonitrile was detected in 95% of samples ($n = 217$) with a mean concentration of 4.23 µg/L (median, 2.77 µg/L; range, not detected–16.4 µg/L; 90th percentile, 9.72 µg/L; 95th percentile, 11.4 µg/L). The limit of detection for dibromoacetonitrile was 0.073 µg/L. The highest concentrations of total haloacetonitriles were detected in spring and the lowest in summer and autumn at all locations. The highest levels of dibromoacetonitrile were detected at the groundwater sampling point, most probably due to bromide ion intrusion from seawater.

In a national survey in Canada, concentrations of dibromoacetonitrile ranged from < 0.1 µg/L (minimum quantifiable limit) to 1.2 µg/L in groundwater and surface water distribution systems (Health Canada, 1995). Samples were collected in 1993 during the winter (February–March) and summer (August–September), when levels of disinfection by-products were expected to be lowest and highest, respectively.

Dibromoacetonitrile was measured in water samples taken from a water-treatment plant in Barcelona between November 1997 and March 1998 (Cancho *et al.*, 1999). Dibromoacetonitrile was detected in pre-chlorinated water (mean, 2.5 µg/L; range, 0.6–7.6 µg/L), sand-filtered water (mean, 4.6 µg/L; range, 4.6–8.7 µg/L), ozonated

water (mean, 7 µg/L; range, 5.5–9.9 µg/L) and post-chlorinated water (mean, 1.5 µg/L; range, 0.6–3.1 µg/L).

(c) *Dietary exposure from drinking-water*

To assess exposure to disinfection by-products through drinking-water, WHO uses a default consumption value of 2 L drinking-water per capita per day and a typical body weight (bw) of 60 kg (WHO, 2008). The underlying assumption is that of a total water consumption of 3 L per capita per day, including water contained in food, which usually represents a conservative value (WHO, 2003).

The mean concentrations and ranges of dibromoacetonitrile concentrations from all available references were used by the Working Group to assess dietary exposure in adults and infants (60 and 5 kg bw, respectively) assuming a consumption of 2 and 0.75 L drinking-water, respectively, i.e. 33 mL/kg bw and 150 mL/kg bw, respectively (Table 1.1). The infant scenario (expressed in mL/kg bw) would correspond to the consumption of 9 L drinking-water per day in a 60-kg adult and therefore cover any possible scenario of physically active persons and increased temperature.

Based on concentrations of dibromoacetonitrile reported in the literature, average dietary exposure through drinking-water in a standard 60-kg adult ranges from 0.02 to 0.14 µg/kg bw per day; high observed concentration values would lead to a dietary exposure of 0.02–0.60 µg/kg bw per day. Similarly, average dietary exposure through drinking-water in a 5-kg infant ranges from 0.09 to 0.63 µg/kg bw per day; and high observed concentration values would lead to a dietary exposure of 0.08–2.71 µg/kg bw per day (Table 1.1).

An estimate of dietary exposure to dibromoacetonitrile arising from the measured consumption of drinking-water was performed by the Joint FAO/WHO expert meeting for Europe, the USA and Australia (FAO/WHO, 2009). The estimate for Europe was based on

the mean consumption of ‘tap-water’ observed in adults in the 15 countries for which these data were available in the Concise European Food Consumption Database developed by the European Food Safety Authority (EFSA, 2008). The highest observed mean consumption of tap-water was 11 mL/kg bw per day (average consumption of 0.84 and 0.886 L per day for an average body weight of 74 and 77 kg, respectively, in Denmark and Finland). Estimated mean dietary exposure to dibromoacetonitrile was therefore up to 0.007 µg/kg bw per day in Europe. For the USA and Australia, mean dietary exposure to dibromoacetonitrile was estimated to be 0.009 µg/kg bw per day (assuming a mean body weight of 65 and 68 kg and a mean consumption of drinking-water of 0.926 and 0.969 L per day, respectively, in the USA and Australia).

(d) *Other dietary sources*

No data on the levels of dibromoacetonitrile in foods (other than drinking-water) could be identified. Extrapolations from values in drinking-water to values in food are difficult to achieve because the conditions of the chemical interactions, dosages, temperatures, contact times and especially the precursors differ considerably (FAO/WHO, 2009).

1.3.3 *Exposure through inhalation or dermal contact*

Dibromoacetonitrile occurs in water used for showering and bathing due to its presence in household water distribution systems (see Section 1.3.2). Dibromoacetonitrile was also detected in the water of two large public swimming pools disinfected with either chlorine or bromine in Barcelona, Spain (Richardson *et al.*, 2010).

No data were available to the Working Group in relation to dermal absorption of or inhalation exposure to dibromoacetonitrile.

Table 1.1 Dietary exposure to dibromoacetonitrile in drinking-water

Reference (country) Source	Concentration (µg/L)			Estimated exposure in adults (µg/kg bw per day)			Estimated exposure in children (µg/kg bw per day)		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Weinberg et al. (2002) (USA)		0.60	2.0		0.02	0.07		0.09	0.30
Krasner et al. (1989); IPCS (2000) (USA)									
<i>Distribution systems</i>		0.46	0.54		0.01	0.02		0.07	0.08
<i>Clearwell effluent with high bromide levels</i>		5.90	6.70		0.20	0.22		0.89	1.00
<i>Utility with seasonally-varying bromide levels</i>		4.60	11		0.15	0.37		0.69	1.65
Baytak et al. (2008) (Turkey)									
<i>Groundwater and surface water</i>	4.23	ND ^b	16.4	0.14	-	0.55	0.63	-	2.46
Health Canada (1995) (Canada)									
<i>Groundwater and surface water</i>		< 0.1 ^c	1.2		-	0.04		-	0.18
Cancho et al. (1999) (Spain)									
<i>Post-chlorinated water (considered as finished water)^d</i>	1.5	0.6	3.1	0.05	0.02	0.10	0.23	0.09	0.47
Williams et al. (1997) (Canada)									
<i>Very low bromide concentrations (< 0.01 mg/L)</i>	< 0.1 ^c			-			-		
<i>Low bromide concentrations (0.06 mg/L)</i>	0.6			0.02			0.09		
<i>Moderate bromide concentrations (0.5 mg/L)</i>	1.2			0.04			0.18		
Zoeteman et al. (1982) (Netherlands)									
<i>Before chlorination</i>	< 0.1			-			-		
<i>After chlorination</i>			1			0.03			0.15
Reding et al. (1989) (USA)									
<i>Distribution system</i>		< 0.2	2.5		< 0.01	0.08		0.03	0.37
Stevens et al. (1989) (USA)			< 10			< 0.33	< 1.5		
Huang et al. (2003) (Taiwan, China)									
<i>Groundwater after ozonation (high bromide levels: 1.5 mg/L)</i>		3.1	18.1		0.10	0.60		0.47	2.71

^a Calculated by the Working Group assuming a daily intake and a body weight of 2 L and 60 kg for adults, and 0.75 L and 5 kg for children, respectively.

^b Detection limit, 0.073 µg/L

^c Minimum quantifiable limit, 0.1 µg/L

^d The study reported the levels of dibromoacetonitrile according to different water treatments (e.g. chlorinated water, sand-filtered water, ozonated water, granulated activated carbon-filtered water). For dietary exposure assessment, the chlorinated water values were considered as finished water.

ND, not detected

1.3.4 Environmental occurrence

Halogenated acetonitriles have been identified in the environment only as by-products of the disinfection of ground- and surface waters for drinking-water supplies. Therefore, the only known route of their environmental release is as a constituent of potable water supplies.

Halogenated acetonitriles such as dibromoacetonitrile undergo hydrolysis in water, which occurs at a faster rate in alkaline waters and in the presence of chlorine. Approximately 5 and 20% of dibromoacetonitrile are lost via hydrolysis within 10 days at pH 6 and 8, respectively. Volatilization losses are expected to be minimal, and adsorption to sediment and bioconcentration in aquatic organisms are not expected. In the atmosphere, dibromoacetonitrile reacts extremely slowly with photochemically produced hydroxyl radicals, with a resulting half-life of 696 days ([WHO, 2004](#); [HSDB, 2010](#)).

1.3.5 Occupational exposure

No data were available to the Working Group.

1.4 Regulations and guidelines

[WHO \(2004\)](#) has established a tolerable daily intake of 11 µg/kg bw per day for dibromoacetonitrile. A guideline value of 70 µg/L (rounded figure) was calculated by allocating 20% of the tolerable daily intake to drinking-water and assuming a body weight of 60 kg and a daily drinking-water intake of 2 litres ([WHO, 2004](#); [2008](#)).

The Dow Chemical Company has established an industrial Hygiene Guide value of 0.1 ppm (0.8 mg/m³) (time-weighted average, skin) for dibromoacetonitrile, and has set this value as an occupational exposure ceiling value based on their assessment of its toxicology in the absence of an industry-accepted value or a government-regulated level.

2. Cancer in Humans

See the Introduction to the *Monographs* on Bromochloroacetic Acid, Dibromoacetic Acid and Dibromoacetonitrile.

3. Cancer in Experimental Animals

Studies on the carcinogenicity of dibromoacetonitrile after oral administration and skin application in mice were reviewed by a previous IARC Working Group ([IARC, 1991](#)). The only additional carcinogenicity studies since that time are those conducted by the [NTP \(2010\)](#). Significant results are summarized in [Table 3.1](#).

3.1 Oral administration

3.1.1 Mouse

Groups of 40 female A/J mice were administered dibromoacetonitrile by gavage at a dose of 0 (controls) or 10 mg/kg bw three times a week for 8 weeks and were then held until they reached 9 months of age. No treatment-related increases in lung adenoma incidence or multiplicity were observed ([Bull & Robinson, 1985](#)).

In a 2-year study, groups of 50 male and 50 female B6C3F₁ mice were administered dibromoacetonitrile in the drinking-water at doses of 0 (controls), 50, 100 or 200 mg/L (corresponding to average daily doses of approximately 0, 4, 7 or 13 and 0, 3, 6 or 11 mg/kg bw in males and females, respectively). Dibromoacetonitrile significantly increased the incidence of forestomach squamous-cell papilloma or carcinoma (combined) in males and forestomach squamous-cell papilloma in females ([NTP, 2010](#)). [Squamous-cell tumours of the forestomach are rare spontaneous tumours in experimental animals.]

Table 3.1 Carcinogenicity studies of exposure to dibromoacetonitrile in experimental animals

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F ₁ (M) 105–106 wk NTP (2010)	Drinking-water 0 (control), 50, 100, 200 mg/L (daily dose of ~0, 4, 7, 13 mg/kg bw) 50/group	Forestomach (squamous-cell papilloma): 0/50, 1/50, 0/50, 3/50	$P = 0.042$ (trend)	98.6% pure
		Forestomach (squamous-cell carcinoma): 0/50, 0/50, 0/50, 2/50	NS	
		Forestomach (squamous-cell papilloma or carcinoma, combined) ^a : 0/50, 1/50, 0/50, 5/50	$P = 0.031$ (high dose), $P = 0.003$ (trend)	
Mouse, B6C3F ₁ (F) 105–106 wk NTP (2010)	Drinking-water 0 (control), 50, 100, 200 mg/L (daily dose of ~0, 3, 6, 11 mg/kg bw) 50/group	Forestomach (squamous-cell papilloma) ^b : 1/50, 0/50, 5/50, 14/50	$P < 0.001$ (high dose), $P < 0.001$ (trend)	98.6% pure; survival: 36/50, 36/50, 43/50*, 47/50*
Mouse, Sencar (F) 1 yr Bull (1985) ; Bull et al. (1985) ; IARC (1991)	Skin application (initiation–promotion) 0, 200, 400, 800 mg/kg bw (in 0.2 mL acetone), 3 times/wk for 2 wk followed 2 wk later by 1.0 µg 12- <i>O</i> -tetradecanoylphorbol 13-acetate (in 0.2 mL acetone) 3 times/wk for 20 wk and observed for 1 yr	Skin (squamous-cell papilloma or carcinoma, combined): 9/105, 8/36, 33/70, 10/74 Skin (squamous-cell papilloma): 4/105, 6/36, 16/70, 6/74 Skin (squamous-cell carcinoma): 5/105, 2/36, 17/70, 4/74		96% pure (4% tribromoacetonitrile); survival: 105/120, 36/40, 70/80, 74/80; data compiled from 3 separate experiments; limited reporting of the study

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M) 105–106 wk NTP (2010)	Drinking-water 0 (control), 50, 100, 200 mg/L (daily dose of ~0, 2, 4, 7 mg/kg bw) 50/group	Oral cavity (oral mucosa or tongue) (squamous-cell papilloma or carcinoma, combined) ^c : 0/50, 0/50, 2/50, 5/50	<i>P</i> = 0.035 (high dose), <i>P</i> = 0.003 (trend)	98.6% pure; glandular stomach (gland hyperplasia): 0/50, 0/50, 2/50, 2/50
		Oral cavity (oral mucosa or tongue) (squamous-cell carcinoma) ^d : 0/50, 0/50, 1/50, 3/50	<i>P</i> = 0.021 (trend)	
		Glandular stomach (adenoma) ^e : 0/50, 0/50, 0/50, 2/50	<i>P</i> = 0.046 (trend)	

* $P \leq 0.05$

^a Historical incidence for 2-year drinking-water studies (mean \pm standard deviation): 3/249 (1.2% \pm 1.8%); range, 0–4%

^b Historical incidence for 2-year drinking-water studies (mean \pm standard deviation): 3/300 (1.0% \pm 1.1%); range, 0–2%

^c Historical incidence for 2-year drinking-water studies (mean \pm standard deviation): 1/300 (0.3% \pm 0.8%); range, 0–2%

^d Historical incidence for 2-year drinking-water studies: 0/300

^e Historical incidence for 2-year drinking-water studies: 0/300

bw, body weight; F, female; M, male; NS, not significant; wk, week or weeks; yr, year or years

3.1.2 Rat

In a 2-year study, groups of 50 male and 50 female F344/N rats were administered dibromoacetonitrile in the drinking-water at doses of 0 (controls), 50, 100 or 200 mg/L (corresponding to average daily doses of approximately 0, 2, 4 or 7 and 0, 2, 4 or 8 mg/kg bw in males and females, respectively). A significant increase in the incidence of squamous-cell papilloma or carcinoma (combined) and squamous-cell carcinoma of the oral cavity (oral mucosa or tongue) was observed in males, as well as an increased incidence of adenoma of the glandular stomach (NTP, 2010). [Squamous-cell carcinomas of the oral cavity are rare spontaneous tumours in experimental animals.]

3.2 Skin application

Groups of 40 female Sencar mice received skin applications of 0 (controls) or 400 mg/kg bw dibromoacetonitrile in 0.2 mL acetone three times a week for 24 weeks [total duration of the study not reported]. Dibromoacetonitrile did not induce skin tumours (Bull *et al.*, 1985). [The Working Group noted the limited reporting of the study.]

3.3 Co-exposure with modifying agents

In a series of three initiation–promotion studies, female Sencar mice received skin applications of 0 (controls), 200, 400 or 800 mg/kg bw dibromoacetonitrile three times a week for 2 weeks. Two weeks after the final dose, 1.0 µg 12-*O*-tetradecanoylphorbol 13-acetate was applied three times a week for 20 weeks and the animals were then observed for 1 year. Treatment with 200 and 400 mg/kg bw dibromoacetonitrile plus 12-*O*-tetradecanoylphorbol 13-acetate increased the incidence of squamous-cell papilloma or carcinoma (combined), but not that with

800 mg/kg (Bull, 1985; Bull & Robinson, 1985; Bull *et al.*, 1985). [The Working Group noted the limited reporting of the study and that these data were compiled from three separate, independent studies.]

4. Other Relevant Data

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, distribution and excretion

Disposition studies in F344/N rats and B6C3F₁ mice were conducted after oral (0.2, 2.0 or 20 mg/kg bw) and intravenous (2.0 mg/kg bw) administration of [2-¹⁴C]-dibromoacetonitrile. The compound was well absorbed in both species. Approximately 60% of the oral radiolabelled dose was excreted in the urine (none as the parent compound) within 24 hours in rats and 72 hours in mice; 8–17% was excreted in the faeces and 10–13% was exhaled as carbon dioxide (¹⁴CO₂). At 72 hours after oral administration, 5–6% was recovered in the tissues of rats and approximately 2–3% in the tissues of mice. Most of the radiolabel remained in the stomach and liver and was not extractable with organic solvents, suggesting covalent binding in these tissues. At 72 hours after intravenous administration, 3% was recovered in the faeces of rats, and retention in the tissues was three to four times greater (19% in rats and 10% in mice) than that after oral administration in both species. The parent compound accounted for less than 6% of circulating radiolabel in rats and was not detected in mouse blood; at 24 hours, 50–80%

was not extractable into acetone ([Mathews et al., 2010](#); [NTP, 2010](#)).

Within 24 hours, 8% of a single oral dose (0.75 mmol/kg bw; 149 mg/kg bw) of dibromoacetonitrile administered to male Sprague-Dawley rats was excreted in the urine as thiocyanate ([Pereira et al., 1984](#)).

[Mathews et al. \(2010\)](#) showed that dibromoacetonitrile reacts rapidly with rat blood *in vitro*, and binds covalently. Absorption of oral radiolabelled doses was about 90%. At 72 hours after intravenous administration, the amount of radioactivity recovered in mouse and rat tissues was 10 and 20% of the dose, respectively, while that recovered after oral dosing was three to four times less and was mostly covalently bound in the stomach. Excretion was higher in the urine than in the faeces. Within 72 hours, 9–15% was exhaled as $^{14}\text{CO}_2$ and 1–3% as volatile compounds.

(b) Metabolism

The metabolism of dibromoacetonitrile has been reviewed ([NTP, 2010](#)). The finding by [Pereira et al. \(1984\)](#) that 8% of a single oral dose in rats was excreted as thiocyanate, the product of a reaction of cyanide with thiosulfate that is catalysed by rhodanese, suggests that haloacetonitriles are metabolized to hydroxyacetonitriles by direct displacement of a halide ion by a hydroxyl group or by cytochrome P450-mediated oxidation. Moreover, subsequent release of cyanide or halide ion might result in the formation of formylhalide or cyanoformaldehyde. Dibromoacetonitrile is also transformed to cyanide by the hypoxanthine/xanthine oxidase/iron system *in vitro* ([Mohamadin & Abdel-Naim, 2003](#)).

In-vitro studies have suggested some additional metabolic pathways for haloacetonitriles. For example, dichloroacetonitrile was oxidized with the release of cyanide in a system that generates hydroxyl free radicals (a Fenton-like reaction involving ferrous salts and hydrogen peroxide); the oxidation of dichloroacetonitrile

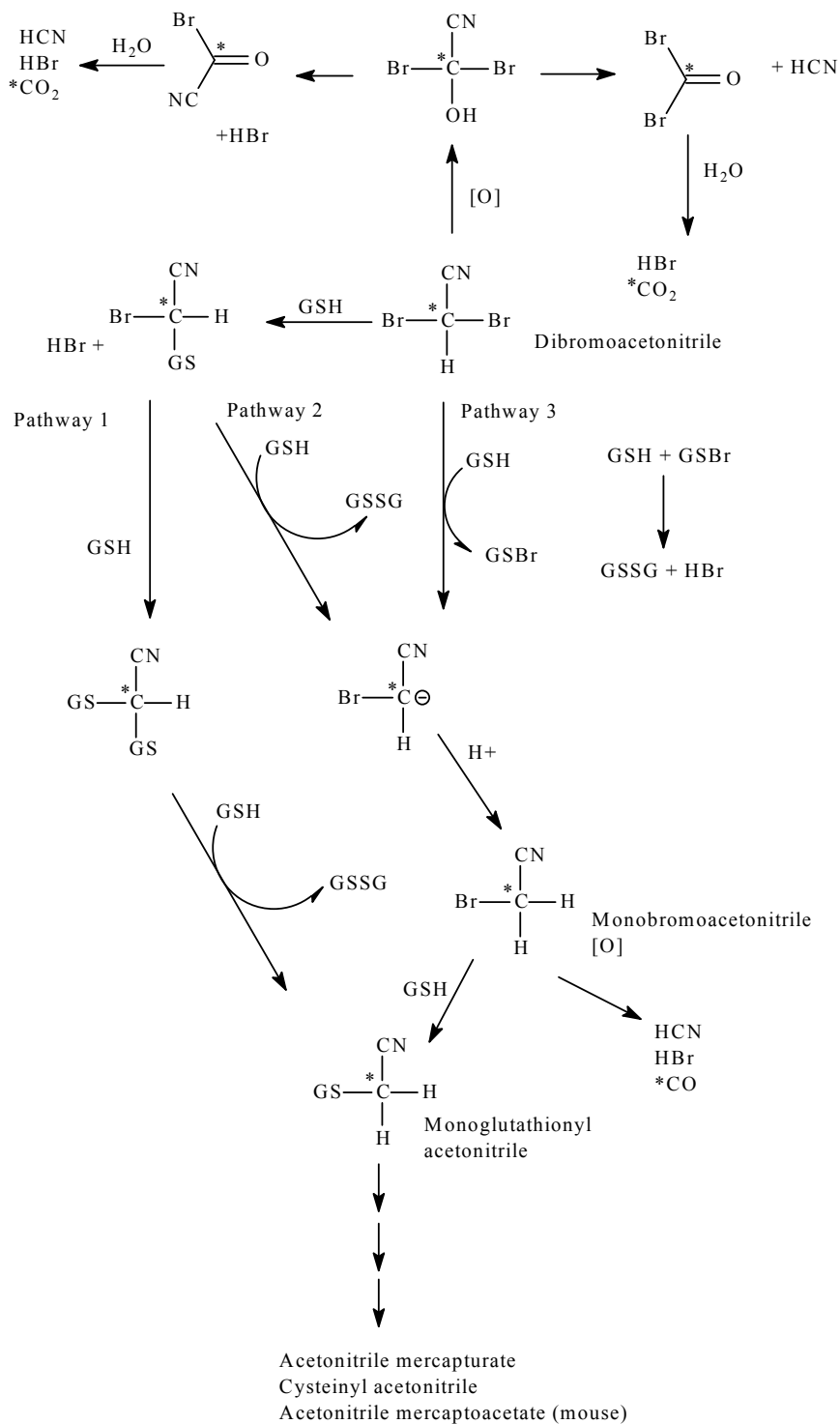
was sensitive to hydrogen peroxide scavengers (e.g. catalase), an iron chelator (desferrioxamine), or free radical scavengers (e.g. mannitol) ([Mohamadin, 2001](#)).

Because dibromoacetonitrile was also oxidized by a hydroxyl radical generated *in vitro* in a hypoxanthine/xanthine oxidase/iron system ([Mohamadin & Abdel-Naim, 2003](#)), oxidative activation of haloacetonitriles may occur via a reactive oxygen-mediated mechanism ([Mohamadin, 2001](#)).

The major metabolite extracted with acetone and methanol from rat stomach or from rat or mouse liver was monogluthionyl acetonitrile. The profiles of rat and mouse urinary metabolites were unaffected by incubation with glucuronidase or sulfatase, but were altered by acylase. The major urinary metabolites identified were acetonitrile mercapturate in rats, and acetonitrile mercaptoacetate, acetonitrile mercapturate and cysteinyl acetonitrile in mice. Because one equivalent of dibromoacetonitrile reacted with 2.5–2.7 equivalents of glutathione (GSH) *in vitro* ([Lin & Guion, 1989](#)) and because bromoacetonitrile was detected in reaction media containing both dibromoacetonitrile and GSH, it was suggested that monogluthionyl conjugate derivatives are formed via a GSH-dependent reduction of dibromoacetonitrile to bromoacetonitrile, followed by the reaction of bromoacetonitrile with another GSH ([NTP, 2010](#)).

Results from *in vitro* studies indicate that dibromoacetonitrile: (1) reacts directly with glutathione, but not with lysine, to form an intermediate that can alkylate histidine; (2) reacts with rat caecal contents to form polar products; and (3) reacts rapidly with rat blood to form polar metabolites and a large non-extractable fraction that may represent covalent protein adducts ([NTP, 2010](#)).

[Mathews et al. \(2010\)](#) studied the metabolism of radiolabelled dibromoacetonitrile in male rats and mice after oral and intravenous administration. It was noted that the prior depletion of

Fig. 4.1 Proposed pathways for the metabolism of [¹⁴C]dibromoacetonitrile in mice and rats

* Denotes position of [¹⁴C] label.

GSSG, glutathione disulfide; GSH, glutathione

Adapted from [Mathews et al. \(2010\)](#)

GSH markedly diminished the loss of dibromoacetonitrile and that the chemical reaction with GSH led immediately to glutathionyl acetonitrile. Thus, the main pathway of dibromoacetonitrile metabolism is via GSH, and covalent binding may be due to a reaction with tissue sulfhydryls ([Mathews et al., 2010](#); see Fig. 4.1).

(c) Toxicokinetic models

No data were available to the Working Group.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

The genetic and related effects of dibromoacetonitrile are summarized in [Table 4.1](#).

(a) DNA damage

Dibromoacetonitrile induced sister chromatid exchange in cultured Chinese hamster ovary cells ([Bull et al., 1985](#)), DNA strand breaks in human lymphoblast cell lines ([Daniel et al., 1986](#)), a dose-related increase in DNA damage in HeLa S3 cells ([Muller-Pillet et al., 2000](#)) and primary DNA damage in *Escherichia coli* strain PQ37 ([Le Curieux et al., 1995](#)).

Two studies found that the potency to induce DNA damage was directly related to the number of halogen atoms present, and that bromine-substituted compounds produced stronger responses than chlorinated compounds ([Daniel et al., 1986](#); [Muller-Pillet et al., 2000](#)).

(b) Mutations

Dibromoacetonitrile was shown to be a weak mutagen in *Salmonella typhimurium* strains TA100 and TA1535 and in the presence of induced hamster liver metabolic activation enzymes; equivocal responses were observed in

these strains in the presence of rat liver metabolic activation ([Mortelmans et al., 1986](#); [NTP, 2010](#)). Another study showed no increase in gene mutations in TA100, TA1535, TA1537, TA1538 or TA98 exposed to dibromoacetonitrile in the presence and absence of metabolic activation ([Bull et al., 1985](#)). The responses for these end-points were found to be weakly positive in a review of these studies ([NTP, 2010](#)). Mutagenic activity was observed in *Escherichia coli* strain WP2 *uvrA*/pKM101 in the presence of metabolic activation, particularly hamster liver metabolic activation ([NTP, 2010](#)), but not in *S. typhimurium* TA100 in the Ames-fluctuation assay ([Le Curieux et al., 1995](#); [Muller-Pillet et al., 2000](#)). No induction of sex-linked recessive lethal mutations was observed in germ cells of male *Drosophila melanogaster* after feeding or injection of dibromoacetonitrile ([Valencia et al., 1985](#)).

(c) Chromosomal effects

Dibromoacetonitrile induced mitotic recombination in *Saccharomyces cerevisiae* ([Zimmermann & Mohr, 1992](#)), did not induce aneuploidy in the oocytes of female *Drosophila melanogaster* after inhalation ([Osgood & Sterling, 1991](#)) and increased the frequencies of micronucleated erythrocytes in newt (*Pleurodeles waltl*) larvae after 12 days of exposure in water ([Le Curieux et al., 1995](#)). It did not induce micronuclei in the bone marrow of mice ([Bull et al., 1985](#)).

4.3 Mechanistic data

Several studies have demonstrated that dibromoacetonitrile induces oxidative stress both *in vitro* and *in vivo*. In cultured glioma cells, it induced the generation of reactive oxygen species, lipid peroxidation and the accumulation of oxidized proteins ([Ahmed et al., 2008](#)). In male mice, dibromoacetonitrile caused GSH depletion and inhibition of GSH S-transferase (GST), superoxide dismutase and catalase activity in

Table 4.1 Genetic and related effects of dibromoacetonitrile

Test system	Results		Dose ^a (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Primary DNA damage, <i>Escherichia coli</i> strain PQ37 (SOS chromotest)	+	–	10	Le Curieux et al. (1995)
<i>Salmonella typhimurium</i> TA100, TA1535, TA1538, TA98 reverse mutation	–	–	0.58	Bull et al. (1985)
<i>Salmonella typhimurium</i> TA100, TA1535 reverse mutation	–	(+)	10 000	Mortelmans et al. (1986)
<i>Salmonella typhimurium</i> TA100, reverse mutation, Ames-fluctuation	–	–	30	Le Curieux et al. (1995)
<i>Salmonella typhimurium</i> TA100, reverse mutation, Ames-fluctuation	–	NT	5.00	Muller-Pillet et al. (2000)
<i>Salmonella typhimurium</i> TA100, reverse mutation	(+)	(+)	67 ^c	NTP (2010)
<i>Salmonella typhimurium</i> TA1535, reverse mutation	–		200	NTP (2010)
<i>Salmonella typhimurium</i> TA1535, reverse mutation		(+)	67 ^b	NTP (2010)
<i>Salmonella typhimurium</i> TA1537, reverse mutation	–		166	NTP (2010)
<i>Salmonella typhimurium</i> TA1537, reverse mutation		–	333	NTP (2010)
<i>Salmonella typhimurium</i> TA98, reverse mutation	–		200	NTP (2010)
<i>Salmonella typhimurium</i> TA98, reverse mutation		–	750	NTP (2010)
<i>Salmonella typhimurium</i> TA97, reverse mutation	(+)		33	NTP (2010)
<i>Salmonella typhimurium</i> TA97, reverse mutation		(+)	100 ^c	NTP (2010)
<i>Escherichia coli</i> WP2 <i>uvrA</i> /pKM101, reverse mutation	NT	+	100 ^b	NTP (2010)
<i>Saccharomyces cerevisiae</i> , mitotic recombination	+	NT	11.42	Zimmermann & Mohr (1992)
<i>Drosophila melanogaster</i> , sex-linked recessive lethal mutation <i>in vivo</i>	–		200 ppm	Valencia et al. (1985)
<i>Drosophila melanogaster</i> , aneuploidy in oocytes <i>in vivo</i>	–		0.30 ppm	Osgood & Sterling (1991)
DNA strand breaks (Comet assay), HeLa S3 cells <i>in vitro</i>	+		0.02	Muller-Pillet et al. (2000)
Sister chromatid exchange, Chinese hamster ovary cells <i>in vitro</i>	+	+	17.33	Bull et al. (1985)
DNA strand breaks, human lymphoblast cell line <i>in vitro</i>	+	NT	3	Daniel et al. (1986)
Micronucleus test, CD-1 mouse erythrocytes <i>in vivo</i>	–		50	Bull et al. (1985)
Micronucleus test, male and female B6C3F1 mouse peripheral erythrocytes, <i>in vivo</i>	–		200, dw, 3 mo	NTP (2010)
Micronucleus test, <i>Pleurodeles waltl</i> erythrocytes <i>in vivo</i>	+		0.12	Le Curieux et al. (1995)
Sperm morphology, B6C3F1 mice <i>in vivo</i>	–		50	Meier et al. (1985)

^a *in vitro* test, µg/mL; *in vivo* test, mg/kg bw per day

^b Active with 10% hamster liver metabolic activation, not with 10% rat liver metabolic activation

^c Active with 10% hamster liver metabolic activation, usually not with 10% rat liver metabolic activation

+, positive; (+), weakly positive; –, negative; bw, body weight; d, day or days; dw, drinking-water; HID, highest ineffective dose; LED, lowest effective dose; mo, month or months; NT, not tested

stomach tissues after a single oral dose ([Abdel-Wahab *et al.*, 2002](#)), and GSH depletion in the testis after a single intraperitoneal dose ([Abdel-Wahab, 2003](#)). In rat primary hepatocytes, it inhibited aldehyde dehydrogenase and GST activity ([Poon *et al.*, 2003](#)). *In vivo*, no significant change in the hepatic activity of these enzymes or in the level of hepatic GSH occurred after 13 weeks of exposure to dibromoacetonitrile via the drinking-water, while it increased the levels of peroxisomal enzymes in both sexes and lipid peroxidation in males ([Poon *et al.*, 2003](#)).

4.4 Susceptibility

No data were available to the Working Group.

4.5 Mechanisms of carcinogenesis

The mechanisms that lead to the carcinogenicity of dibromoacetonitrile are not known.

Some findings suggest that covalent binding occurs in tissues such as the stomach and liver, possibly following GSH-mediated activation. Oxidative stress associated with reduced GSH levels and deficiency in GST activity and/or binding to protein may also be involved. Dibromoacetonitrile may also act via a genotoxic mechanism.

5. Summary of Data Reported

5.1 Exposure data

Dibromoacetonitrile is formed as a by-product during the disinfection of water by chlorination in the presence of natural organic matter (e.g. algae) and bromide. Concentrations of dibromoacetonitrile up to 18 µg/L were measured in drinking-water. Maximum daily human exposure to dibromoacetonitrile through drinking-water, estimated from such measurements, is at

the low microgram per kilogram of body weight level.

Dibromoacetonitrile is also produced for use as an antimicrobial component in metalworking fluids. Occupational exposure may occur during its production or use.

5.2 Human carcinogenicity data

No epidemiological studies were identified that evaluated exposure specifically to dibromoacetonitrile. This chemical occurs in mixtures in disinfected water. Studies on disinfected water are reviewed in the Introduction to the *Monographs* on Bromochloroacetic Acid, Dibromoacetic Acid and Dibromoacetonitrile.

5.3 Animal carcinogenicity data

In one study in mice, administration of dibromoacetonitrile in the drinking-water increased the incidence of squamous-cell papilloma or carcinoma (combined) of the forestomach in males and of squamous-cell papilloma of the forestomach in females. In one study in rats, administration of dibromoacetonitrile in the drinking-water increased the incidence of glandular stomach adenoma, and of squamous-cell papilloma or carcinoma (combined) and squamous-cell carcinoma of the oral cavity (oral mucosa or tongue) in males. Squamous-cell tumours of the forestomach and squamous-cell carcinomas of the oral cavity are rare spontaneous neoplasms in experimental animals.

5.4 Other relevant data

No data were available to the Working Group on the toxicokinetics of dibromoacetonitrile in humans. Dibromoacetonitrile was well absorbed (almost 90%) after oral administration in mice and rats, and there is evidence that covalent binding occurs in the stomach and liver. It can

be metabolized via several pathways, including direct displacement of bromide by a hydroxyl group, and cytochrome P450-mediated or -independent oxidation. The main metabolite identified in rat stomach and rat or mouse livers was monogluthathionyl acetonitrile. The major urinary metabolites identified were acetonitrile mercapturate in rats and acetonitrile mercaptoacetate, acetonitrile mercapturate and cysteinyl acetonitrile in mice. Thiocyanate was also identified in rats.

Dibromoacetonitrile induced DNA damage in bacteria and in human cell lines, mutations in bacteria and micronuclei in newt larvae, but not in mice. No mutations were found in *Drosophila*.

The mechanisms that lead to the carcinogenicity of dibromoacetonitrile are not known, but there is weak evidence that oxidative stress and/or genotoxicity may lead to cancer in rodents exposed to dibromoacetonitrile.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

Dibromoacetonitrile is *possibly carcinogenic to humans (Group 2B)*

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LIST OF ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
ACO	acyl-coenzyme A oxidase
ADH	alcohol dehydrogenase
AhR	aryl hydrocarbon receptor
ALDH	aldehyde dehydrogenase
AQ	anthraquinone
AR	androgen receptor
ATPase	adenine triphosphatase
AUC	area under the curve
BaX	Bcl-2-associated X
Bcl-2	B-cell lymphoma-2
bw	body weight
CALUX	chemically activated luciferase gene expression
CAPD	continuous ambulatory peritoneal dialysis
CAR	constitutive androstane receptor
CAT	carnitine acetyl transferase
CI	confidence interval
CYP	cytochrome P450
DBP	dibutyl phthalate
DCP	1,3-dichloropropanol
DEHP	di(2-ethylhexyl) phthalate
DEP	diethyl phthalate
DINP	di-isononyl phthalate
E2	17 β -estradiol
EC50	half the minimum effect concentration
ECD	electron capture detection
ECH	epichlorohydrin
EFSA	European Food Safety Authority
2-EH	2-ethylhexanol
2-EHA	2-ethylhexanoic acid
EPA	Environmental Protection Agency
ER	estrogen receptor
EU	European Union
FID	flame ionization detection
GC	gas chromatography
GSH	glutathione

GST	glutathione <i>S</i> -transferase
HFBI	heptafluorobutyrylimidazole
hPXR	human pregnane X receptor
HVP	acid-hydrolysed vegetable protein
IC50	half maximal inhibitory concentration
Ig	immunoglobulin
IL	interleukin
K _{ow}	octanol/water constant
KZG	Kurz Zeit Gedächtnis
LBW	low birth weight
MAK	Maximale Arbeitsplatz-Konzentration
MBzP	monobenzyl phthalate
MCMHP	mono(2-carboxymethyl)hexyl phthalate
3-MCPD	3-monochloro-1,2-propanediol
MCPP	mono(3-carboxypropyl) phthalate
MDR	multidrug response
MECPP	mono(2-ethyl-5-carboxypentyl) phthalate
MEHHP	mono(2-ethyl-5-hydroxyhexyl) phthalate
MEHP	mono(2-ethylhexyl) phthalate
MEOHP	mono(2-ethyl-5-oxohexyl) phthalate
MEP	monoethyl phthalate
MiBP	monoisobutyl phthalate
MMP	monomethyl phthalate
MnBP	mono- <i>n</i> -butyl phthalate
mPXR	mouse pregnane X receptor
mRNA	messenger RNA
MS	mass spectrometry
MSHA	Mine Safety and Health Administration
NADPH	nicotinamide adenine dinucleotide phosphate
NAT	<i>N</i> -acetyl transferase
ND	not detected
NDEA	<i>N</i> -nitrosodiethylamine
NDMA	<i>N</i> -nitrosodimethylamine
NIOSH	National Institute of Occupational Safety and Health
NO _x	nitrogen oxides
NPRI	National Pollution Release Inventory
NR	not reported
NS	not significant
NTP	National Toxicology Program
OEL	occupational exposure limit
8-OHdG	8-hydroxydeoxyguanosine
OSHA	Occupational Safety and Health Administration
P450R	nicotinamide adenine dinucleotide phosphate-cytochrome P450 oxidoreductase
PAH	polycyclic aromatic hydrocarbon
PBA	phenylboronic acid
PBT	persistent, bioaccumulative and toxic
PEL	permissible exposure limit
PID	photoionization detection

PLD	phospholipidase D
POBN	α -(4-pyridyl-1-oxide)- <i>N</i> - <i>tert</i> -butylnitrone
PPAR	peroxisome proliferator-activated receptor
PPRE	peroxisome proliferator response element
PUFA	polyunsaturated fatty acid
PVC	polyvinyl chloride
REL	recommended exposure limit
RT-PCR	reverse transcriptase-polymerase chain reaction
SAM	S-adenosyl methionine
SD	standard deviation
SHE	Syrian hamster embryo
SIR	standardized incidence ratio
sk	skin notation
SMR	standardized mortality ratio
STEL	short-term exposure limit
SULT	sulfotransferase
T4	thyroxine
TDI	tolerable daily intake
TGF	transforming growth factor
TLV	threshold limit value
TNF α / α Ama	tumour necrosis factor α / α -amanitine
TPA	12- <i>O</i> -tetradecanoyl phorbol-13-acetate
TSH	thyroid-stimulating hormone
TWA	time-weighted average
UDGPT	uridine diphosphate glucuronosyl transferase
UGT	5'-diphospho-glucuronosyl transferase
UV	ultraviolet
VME	valeur moyenne d'exposition
WY-14 643	4-chloro-6-(2,3-xylidino)-2-pyrimidinylthioacetic acid
XRE	xenobiotic response element

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2012; 586 pages

Supplement No. 1

Chemicals and Industrial Processes Associated with Cancer in Humans (IARC Monographs, Volumes 1 to 20)
1979; 71 pages (out-of-print)

Supplement No. 2

Long-term and Short-term Screening Assays for Carcinogens: A Critical Appraisal
1980; 426 pages (out-of-print)
(updated as IARC Scientific Publications No. 83, 1986)

Supplement No. 3

Cross Index of Synonyms and Trade Names in Volumes 1 to 26 of the IARC Monographs
1982; 199 pages (out-of-print)

Supplement No. 4

Chemicals, Industrial Processes and Industries Associated with Cancer in Humans (IARC Monographs, Volumes 1 to 29)
1982; 292 pages (out-of-print)

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1985; 259 pages (out-of-print)

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Genetic and Related Effects: An Updating of Selected IARC Monographs from Volumes 1 to 42
1987; 729 pages (out-of-print)

Supplement No. 7

Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1–42
1987; 440 pages (out-of-print)

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1990; 346 pages (out-of-print)

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Calcium chromate (see Chromium and chromium compounds)	
Calcium cyclamate (see Cyclamates)	
Calcium saccharin (see Saccharin)	
Cantharidin	10 (1976); Suppl. 7 (1987)
Caprolactam	19 (1979) (corr. 42); 39 (1986) (corr. 42); Suppl. 7 (1987); 71 (1999)
Captafol	53 (1991)
Captan	30 (1983); Suppl. 7 (1987)
Carbaryl	12 (1976); Suppl. 7 (1987)
Carbazole	32 (1983); Suppl. 7 (1987); 71 (1999)
3-Carboxypsoralen	40, 317 (1986); Suppl. 7, 59 (1987)
Carbon black	3 (1973); 33 (1984); Suppl. 7 (1987); 65 (1996); 93, (2010)
Carbon electrode manufacture	92 (2010)
Carbon tetrachloride	1 (1972); 20 (1979); Suppl. 7 (1987); 71 (1999)
Carmoisine	8 (1975); Suppl. 7 (1987)
Carpentry and joinery	25 (1981); Suppl. 7 (1987)
Carrageenan	10 (1976) (corr. 42); 31 (1983); Suppl. 7 (1987)
Cassia occidentalis (see Traditional herbal medicines)	
Catechol	15 (1977); Suppl. 7 (1987); 71 (1999)
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- Chloral (see also Chloral hydrate) 63 (1995); 84 (2004)
- Chloral hydrate 63 (1995); 84 (2004)
- Chlorambucil 9 (1975); 26 (1981); Suppl. 7 (1987); 100A (2012)
- Chloramine 84 (2004)
- Chloramphenicol 10 (1976); Suppl. 7 (1987); 50 (1990)
- Chlordane (see also Chlordane and Heptachlor) 20 (1979) (corr. 42)
- Chlordane and Heptachlor Suppl. 7 (1987); 53 (1991); 79 (2001)
- Chlordecone 20 (1979); Suppl. 7 (1987)
- Chlordimeform 30 (1983); Suppl. 7 (1987)
- Chlorendic acid 48 (1990)
- Chlorinated dibenzodioxins (other than TCDD) 15 (1977); Suppl. 7 (1987)
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- Chlorinated drinking-water 52 (1991)
- Chlorinated paraffins 48 (1990)
- α -Chlorinated toluenes and benzoyl chloride Suppl. 7 (1987); 71 (1999)
- Chlormadinone acetate 6 (1974); 21 (1979); Suppl. 7 (1987); 72 (1999)
- Chlornaphazine (see *N,N*-Bis(2-chloroethyl)-2-naphthylamine)
- Chloroacetonitrile (see also Halogenated acetonitriles) 71 (1999)
- para*-Chloroaniline 57 (1993)
- Chlorobenzilate 5 (1974); 30 (1983); Suppl. 7 (1987)
- Chlorodibromomethane 52 (1991); 71 (1999)
- 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone 84 (2004)
- Chlorodifluoromethane 41 (1986) (corr. 51); Suppl. 7 (1987); 71 (1999)
- Chloroethane 52 (1991); 71 (1999)
- 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea 26 (1981) (corr. 42); Suppl. 7 (1987)
(see also Chloroethyl nitrosoureas)
- 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea Suppl. 7 (1987); 100A (2012)
(see also Chloroethyl nitrosoureas)
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- Chlorofluoromethane 41 (1986); Suppl. 7 (1987); 71 (1999)
- Chloroform 1 (1972); 20 (1979); Suppl. 7 (1987); 73 (1999)
- Chloromethyl methyl ether (technical-grade) 4 (1974); Suppl. 7 (1987); 100F (2012)
(see also Bis(chloromethyl)ether)
- (4-Chloro-2-methylphenoxy)acetic acid (see MCPA)
- 1-Chloro-2-methylpropene 63 (1995)
- 3-Chloro-2-methylpropene 63 (1995)
- 2-Chloronitrobenzene 65 (1996)
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<i>para</i> -Chloro- <i>ortho</i> -toluidine and its strong acid salts.....	16 (1978); 30 (1983); Suppl. 7 (1987); (see also Chlordimeform)	48 (1990); 77 (2000)
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5-Chloro- <i>ortho</i> -toluidine	77 (2000)	
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Ciclosporin.....	50 (1990); 100A (2012)	
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Cinnamyl anthranilate	16 (1978); 31 (1983); Suppl. 7 (1987); 77 (2000)	
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Cisplatin (see also Etoposide)	26 (1981); Suppl. 7 (1987)	
Citrinin.....	40 (1986); Suppl. 7 (1987)	
Citrus Red No. 2	8 (1975) (corr. 42); Suppl. 7 (1987)	
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Clofibrate	24 (1980); Suppl. 7 (1987); 66 (1996)	
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Clonorchis sinensis, infection with	61 (1994); 100B (2012)	
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- Coal-tar distillation 92 (2010); 100F (2012)
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- Cobalt[III] acetate (see Cobalt and cobalt compounds)
- Cobalt-aluminium-chromium spinel (see Cobalt and cobalt compounds)
- Cobalt and cobalt compounds (see also Implants, surgical) 52 (1991)
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- Cobalt metal with tungsten carbide..... 86 (2006)
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- Coconut oil diethanolamine condensate 101 (2012)
- Coffee 51 (1991) (corr. 52)
- Coke production 34 (1984); Suppl. 7 (1987); 92 (2010); 100F (2012)
- Combined estrogen–progestogen contraceptives ... Suppl. 7 (1987); 72 (1999); 91 (2007); 100A (2012)
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- Conjugated estrogens (see also Steroidal estrogens)..... 21 (1979); Suppl. 7 (1987)
- Continuous glass filament (see Man-made vitreous fibres)
- Copper 8-hydroxyquinoline 15 (1977); Suppl. 7 (1987)
- Coronene 32 (1983); Suppl. 7 (1987); 92 (2010)
- Coumarin 10 (1976); Suppl. 7 (1987); 77 (2000)
- Creosotes (see also Coal-tars) 35 (1985); Suppl. 7 (1987); 92 (2010)
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- Cycasin (see also Methylazoxymethanol)..... 1 (1972) (corr. 42); 10 (1976); Suppl. 7 (1987)
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- Cyclochlorotine 10 (1976); Suppl. 7 (1987)
- Cyclohexanone..... 47 (1989); 71 (1999)
- Cyclohexylamine (see Cyclamates)
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5,6-Cyclopenteno-1,2-benzanthracene	92 (2010)
Cyclopropane (see Anaesthetics, volatile)	
Cyclophosphamide	9 (1975); 26 (1981); Suppl. 7 (1987); 100A (2012)
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Dantron	50 (1990) (corr. 59)
D&C Red No. 9	8 (1975); Suppl. 7 (1987); 57 (1993)
Dapsone	24 (1980); Suppl. 7 (1987)
Daunomycin	10 (1976); Suppl. 7 (1987)
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DDE (see DDT)	
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Decabromodiphenyl oxide	48 (1990); 71 (1999)
Deltamethrin	53 (1991)
Deoxynivalenol (see Toxins derived from <i>Fusarium graminearum</i> , <i>F. culmorum</i> and <i>F. crookwellense</i>)	
Diacetylaminoazotoluene	8 (1975); Suppl. 7 (1987)
<i>N,N'</i> -Diacetylbenzidine	16 (1978); Suppl. 7 (1987)
Diallate	12 (1976); 30 (1983); Suppl. 7 (1987)
2,4-Diaminoanisole and its salts	16 (1978); 27 (1982); Suppl. 7 (1987); 79 (2001)
4,4'-Diaminodiphenyl ether	16 (1978); 29 (1982); Suppl. 7 (1987)
1,2-Diamino-4-nitrobenzene	16 (1978); Suppl. 7 (1987)
1,4-Diamino-2-nitrobenzene	16 (1978); Suppl. 7 (1987); 57 (1993)
2,6-Diamino-3-(phenylazo)pyridine (see Phenazopyridine hydrochloride)	
2,4-Diaminotoluene (see also Toluene diisocyanates)	16 (1978); Suppl. 7 (1987)
2,5-Diaminotoluene (see also Toluene diisocyanates)	16 (1978); Suppl. 7 (1987)
<i>ortho</i> -Dianisidine (see 3,3'-Dimethoxybenzidine)	
Diatomaceous earth, uncalcined (see Amorphous silica)	
Diazepam	13 (1977); Suppl. 7 (1987); 66 (1996)
Diazomethane	7 (1974); Suppl. 7 (1987)
Dibenz[<i>a,h</i>]acridine	3 (1973); 32 (1983); Suppl. 7 (1987)
Dibenz[<i>a,j</i>]acridine	3 (1973); 32 (1983); Suppl. 7 (1987)
Dibenz[<i>a,c</i>]anthracene	32 (1983) (corr. 42); Suppl. 7 (1987); 92 (2010)
Dibenz[<i>a,h</i>]anthracene	3 (1973) (corr. 43); 32 (1983); Suppl. 7 (1987); 92 (2010)
Dibenz[<i>a,j</i>]anthracene	32 (1983); Suppl. 7 (1987); 92 (2010)
7 <i>H</i> -Dibenzo[<i>c,g</i>]carbazole	3 (1973); 32 (1983); Suppl. 7 (1987)
Dibenzodioxins, chlorinated, other than TCDD (see Chlorinated dibenzodioxins, other than TCDD)	
Dibenzo[<i>a,e</i>]fluoranthene	32 (1983); Suppl. 7 (1987); 92 (2010)
13 <i>H</i> -Dibenzo[<i>a,g</i>]fluorene	92 (2010)
Dibenzo[<i>h,rst</i>]pentaphene	3 (1973); Suppl. 7 (1987); 92 (2010)

- Dibenzo[*a,e*]pyrene 3 (1973); 32 (1983); Suppl. 7 (1987); 92 (2010)
- Dibenzo[*a,h*]pyrene 3 (1973); 32 (1983); Suppl. 7 (1987); 92 (2010)
- Dibenzo[*a,i*]pyrene 3 (1973); 32 (1983); Suppl. 7 (1987); 92 (2010)
- Dibenzo[*a,l*]pyrene 3 (1973); 32 (1983); Suppl. 7 (1987); 92 (2010)
- Dibenzo[*e,l*]pyrene 92 (2010)
- Dibenzo-*para*-dioxin 69 (1997)
- Dibromoacetic acid 101 (2012)
- Dibromoacetonitrile (see also Halogenated acetonitriles) 71 (1999); 101 (2012)
- 1,2-Dibromo-3-chloropropane 15 (1977); 20 (1979); Suppl. 7 (1987); 71 (1999)
- 1,2-Dibromoethane (see Ethylene dibromide)
- 2,3-Dibromopropan-1-ol 77 (2000)
- Dichloroacetic acid 63 (1995); 84 (2004)
- Dichloroacetonitrile (see also Halogenated acetonitriles) 71 (1999)
- Dichloroacetylene 39 (1986); Suppl. 7 (1987); 71 (1999)
- ortho*-Dichlorobenzene 7 (1974); 29 (1982); Suppl. 7 (1987); 73 (1999)
- meta*-Dichlorobenzene 73 (1999)
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- 3,3'-Dichlorobenzidine 4 (1974); 29 (1982); Suppl. 7 (1987)
- trans*-1,4-Dichlorobutene 15 (1977); Suppl. 7 (1987); 71 (1999)
- 3,3'-Dichloro-4,4'-diaminodiphenyl ether 16 (1978); Suppl. 7 (1987)
- 1,2-Dichloroethane 20 (1979); Suppl. 7 (1987); 71 (1999)
- Dichloromethane 20 (1979); 41 (1986); Suppl. 7 (1987); 71 (1999)
- 2,4-Dichlorophenol (see Chlorophenols; Chlorophenols, occupational exposures to; Polychlorophenols and their sodium salts)
- (2,4-Dichlorophenoxy)acetic acid (see 2,4-D)
- 2,6-Dichloro-*para*-phenylenediamine 39 (1986); Suppl. 7 (1987)
- 1,2-Dichloropropane 41 (1986); Suppl. 7 (1987); 71 (1999)
- 1,3-Dichloro-2-propanol 101 (2012)
- 1,3-Dichloropropene, technical-grade 41 (1986); Suppl. 7 (1987); 71 (1999)
- Dichlorvos 20 (1979); Suppl. 7 (1987); 53 (1991)
- Dicofol 30 (1983); Suppl. 7 (1987)
- Dicyclohexylamine (see Cyclamates)
- Didanosine 76 (2000)
- Dieldrin 5 (1974); Suppl. 7 (1987)
- Dienoestrol (see also Nonsteroidal estrogens) 21 (1979); Suppl. 7 (1987)
- Diepoxybutane (see also 1,3-Butadiene) 11 (1976) (corr. 42); Suppl. 7 (1987); 71 (1999)
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- Diesel fuels 45 (1989) (corr. 47)
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- 1,2-Diethylhydrazine 4 (1974); Suppl. 7 (1987); 71 (1999)
- Diethylstilbestrol 6 (1974); 21 (1979) (corr. 42); Suppl. 7 (1987); 100A (2012)
- Diethylstilbestrol dipropionate (see Diethylstilbestrol)
- Diethyl sulfate 4 (1974); Suppl. 7 (1987); 54 (1992); 71 (1999)

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Diglycidyl resorcinol ether	11 (1976); 36 (1985); Suppl. 7 (1987); 71 (1999)
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1,2-Dihydroaceanthrylene	92 (2010)
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Dihydroxybenzenes (see Catechol; Hydroquinone; Resorcinol)	
1,3-Dihydroxy-2-hydroxymethylantraquinone	82 (2002)
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Dimethoxane	15 (1977); Suppl. 7 (1987)
3,3'-Dimethoxybenzidine	4 (1974); Suppl. 7 (1987)
3,3'-Dimethoxybenzidine-4,4'-diisocyanate	39 (1986); Suppl. 7 (1987)
<i>para</i> -Dimethylaminoazobenzene	8 (1975); Suppl. 7 (1987)
<i>para</i> -Dimethylaminoazobenzenediazo sodium sulfonate	8 (1975); Suppl. 7 (1987)
<i>trans</i> -2-[(Dimethylamino)methylimino]-5-[2-(5-nitro-2-furyl)-vinyl]-1,3,4-oxadiazole (corr. 42); Suppl. 7 (1987)	7 (1974)
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4,5'-Dimethylangelicin plus ultraviolet radiation (see also Angelicin and some synthetic derivatives)	Suppl. 7 (1987)
2,6-Dimethylaniline	57 (1993)
<i>N,N</i> -Dimethylaniline	57 (1993)
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3,3'-Dimethylbenzidine	1 (1972); Suppl. 7 (1987); 100F (2012)
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Dimethylformamide	47 (1989); 71 (1999)
1,1-Dimethylhydrazine	4 (1974); Suppl. 7 (1987); 71 (1999)
1,2-Dimethylhydrazine	4 (1974) (corr. 42); Suppl. 7 (1987); 71 (1999)
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1,4-Dimethylphenanthrene	32 (1983); Suppl. 7 (1987); 92 (2010)
Dimethyl sulfate	41 (1974); Suppl. 7 (1987); 71 (1999)
3,7-Dinitrofluoranthene	46 (1989); 65 (1996)
3,9-Dinitrofluoranthene	46 (1989); 65 (1996)
1,3-Dinitropyrene	46 (1989)
1,6-Dinitropyrene	46 (1989)
1,8-Dinitropyrene	33 (1984); Suppl. 7 (1987); 46 (1989)
Dinitrosopentamethylenetetramine	11 (1976); Suppl. 7 (1987)
2,4-Dinitrotoluene	65 (1996) (corr. 66)
2,6-Dinitrotoluene	65 (1996) (corr. 66)
3,5-Dinitrotoluene	65 (1996)
1,4-Dioxane	11 (1976); Suppl. 7 (1987); 71 (1999)
2,4'-Diphenyldiamine	16 (1978); Suppl. 7 (1987)
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Direct Brown 95 (see also Benzidine-based dyes)	29 (1982)

Disperse Blue 1	48 (1990)
Disperse Yellow 3	8 (1975); Suppl. 7 (1987); 48 (1990)
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Dithranol	13 (1977); Suppl. 7 (1987)
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Doxefazepam	66 (1996)
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Endrin	5 (1974); Suppl. 7 (1987)
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1,2-Epoxybutane	47 (1989); 71 (1999)
1-Epoxyethyl-3,4-epoxycyclohexane (see 4-Vinylcyclohexene diepoxide)	
3,4-Epoxy-6-methylcyclohexylmethyl-3,4-epoxy-6-methyl-cyclohexane carboxylate	11 (1976); Suppl. 7 (1987); 71 (1999)
<i>cis</i> -9,10-Epoxystearic acid	11 (1976); Suppl. 7 (1987); 71 (1999)
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<i>d</i> -Equilenin	72 (1999)
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Estradiol	6 (1974); 21 (1979); Suppl. 7 (1987); 72 (1999)
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Ethinylestradiol	6 (1974); 21 (1979); Suppl. 7 (1987); 72 (1999)

Ethionamide	13 (1977); Suppl. 7 (1987)
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Ethyl carbamate.....	7 (1974); Suppl. 7 (1987); 96 (2010)
Ethylbenzene	77 (2000)
Ethylene	19 (1979); Suppl. 7 (1987); 60 (1994); 71 (1999)
Ethylene dibromide	15 (1977); Suppl. 7 (1987); 71 (1999)
Ethylene oxide	11 (1976); 36 (1985) (corr. 42); Suppl. 7 (1987); 60 (1994); 97 (2008); 100F (2012)
Ethylene sulfide	11, 257 (1976); Suppl. 7, 63 (1987)
Ethylenethiourea	7 (1974); Suppl. 7 (1987); 79 (2001)
2-Ethylhexyl acrylate	60 (1994)
Ethyl methanesulfonate	7 (1974); Suppl. 7 (1987)
<i>N</i> -Ethyl- <i>N</i> -nitrosourea	1 (1972); 17 (1978); Suppl. 7 (1987)
Ethyl selenac (see also Selenium and selenium compounds)	12 (1976); Suppl. 7 (1987)
Ethyl tellurac	12 (1976); Suppl. 7 (1987)
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Fluorspar (see Fluorides)	
Fluosilicic acid (see Fluorides)	
Fluroxene (see Anaesthetics, volatile)	
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Formaldehyde	29 (1982); Suppl. 7 (1987); 62 (1995) (corr. 65; corr. 66); 88 (2006); 100F (2012)
2-(2-Formylhydrazino)-4-(5-nitro-2-furyl)thiazole	7 (1974) (corr. 42); Suppl. 7 (1987)
Frusemide (see Furosemide)	
Frying, emissions from high-temperature	95 (2010)
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Patulin	10 (1976); 40 (1986); Suppl. 7 (1987)
Paving and roofing with coal-tar pitch	92 (2010)
Penicillic acid	10 (1976); Suppl. 7 (1987)
Pentachloroethane	41 (1986); Suppl. 7 (1987); 71 (1999)
Pentachloronitrobenzene (see Quintozene)	
Pentachlorophenol	20 (1979); 53 (1991)
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Permethrin	53 (1991)
Perylene	32 (1983); Suppl. 7 (1987); 92 (2010)
Petasitenine	31 (1983); Suppl. 7 (1987)
Petasites japonicus (see also Pyrrolizidine alkaloids)	10 (1976)
Petroleum refining, occupational exposures in	45 (1989)
Petroleum solvents	47 (1989)
Phenacetin	13 (1977); 24 (1980); Suppl. 7 (1987); 100A (2012)
Phenanthrene	32 (1983); Suppl. 7 (1987); 92 (2010)
Phenazopyridine hydrochloride	8 (1975); 24 (1980) (corr. 42); Suppl. 7 (1987)
Phenelzine sulfate	24 (1980); Suppl. 7 (1987)
Phenicarbazide	12 (1976); Suppl. 7 (1987)
Phenobarbital and its sodium salt	13 (1977); Suppl. 7 (1987); 79 (2001)
Phenol	47 (1989) (corr. 50); 71 (1999)
Phenolphthalein	76 (2000)
Phenoxyacetic acid herbicides (see Chlorophenoxy herbicides)	
Phenoxybenzamine hydrochloride	9 (1975); 24 (1980); Suppl. 7 (1987)
Phenylbutazone	13 (1977); Suppl. 7 (1987)
meta-Phenylenediamine	16 (1978); Suppl. 7 (1987)
para-Phenylenediamine	16 (1978); Suppl. 7 (1987)
Phenyl glycidyl ether (see also Glycidyl ethers)	71 (1999)
N-Phenyl-2-naphthylamine	16 (1978) (corr. 42); Suppl. 7 (1987)
ortho-Phenylphenol	30 (1983); Suppl. 7 (1987); 73 (1999)
Phenytoin	13 (1977); Suppl. 7 (1987); 66 (1996)
Phillipsite (see Zeolites)	
PhIP	56 (1993)
Phosphorus-32 as phosphate	100D (2012)
Picene	92 (2010)
Pickled vegetables	56 (1993)
Picloram	53 (1991)

- Piperazine oestrone sulfate (see Conjugated estrogens)
- Piperonyl butoxide 30 (1983); Suppl. 7 (1987)
- Pitches, coal-tar (see Coal-tar pitches)
- Plutonium-239 100D (2012)
- Polyacrylic acid 19 (1979); Suppl. 7 (1987)
- Polybrominated biphenyls 18 (1978); 41 (1986); Suppl. 7 (1987)
- Polychlorinated biphenyls 7 (1974); 18 (1978) (corr. 42); Suppl. 7 (1987)
- Polychlorinated camphenes (see Toxaphene)
- Polychlorinated dibenzo-*para*-dioxins
(other than 2,3,7,8-tetrachlorodibenzodioxin) 69 (1997)
- Polychlorinated dibenzofurans 69 (1997)
- Polychlorophenols and their sodium salts 71 (1999)
- Polychloroprene 19 (1979); Suppl. 7 (1987)
- Polyestradiol phosphate (see Estradiol-17 β)
- Polyethylene (see also Implants, surgical) 19 (1979); Suppl. 7 (1987)
- Poly(glycolic acid) (see Implants, surgical)
- Polymethylene polyphenyl isocyanate 19 (1979); Suppl. 7 (1987)
(see also 4,4'-Methylenediphenyl diisocyanate)
- Polymethyl methacrylate (see also Implants, surgical) 19 (1979); Suppl. 7 (1987)
- Polypropylene (see also Implants, surgical) 19 (1979); Suppl. 7 (1987)
- Polystyrene (see also Implants, surgical) 19 (1979); Suppl. 7 (1987)
- Polytetrafluoroethylene (see also Implants, surgical) 19 (1979); Suppl. 7 (1987)
- Polyurethane foams (see also Implants, surgical) 19 (1979); Suppl. 7 (1987)
- Polyvinyl acetate (see also Implants, surgical) 19 (1979); Suppl. 7 (1987)
- Polyvinyl alcohol (see also Implants, surgical) 19 (1979); Suppl. 7 (1987)
- Polyvinyl chloride (see also Implants, surgical) 7 (1974); 19 (1979); Suppl. 7 (1987)
- Polyvinyl pyrrolidone 19 (1979); Suppl. 7 (1987); 71 (1999)
- Ponceau MX 8 (1975); Suppl. 7 (1987)
- Ponceau 3R 8 (1975); Suppl. 7 (1987)
- Ponceau SX 8 (1975); Suppl. 7 (1987)
- Post-menopausal estrogen therapy Suppl. 7 (1987); 72 (1999); 100A (2012)
- Potassium arsenate (see Arsenic and arsenic compounds)
- Potassium arsenite (see Arsenic and arsenic compounds)
- Potassium bis(2-hydroxyethyl)dithiocarbamate 12 (1976); Suppl. 7 (1987)
- Potassium bromate 40 (1986); Suppl. 7 (1987); 73 (1999)
- Potassium chromate (see Chromium and chromium compounds)
- Potassium dichromate (see Chromium and chromium compounds)
- Prazepam 66 (1996)
- Prednimustine 50 (1990)
- Prednisone 26 (1981); Suppl. 7 (1987)
- Printing processes and printing inks 65 (1996)
- Procarbazine hydrochloride 26 (1981); Suppl. 7 (1987)
- Proflavine salts 24 (1980); Suppl. 7 (1987)
- Progesterone (see also Progestins; Combined oral contraceptives) 6 (1974); 21 (1979) (corr. 42)
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- Progestogens Suppl. 7 (1987); 72 (1999)

Pronetalol hydrochloride	13 (1977) (corr. 42); Suppl. 7 (1987)
1,3-Propane sultone	4 (1974) (corr. 42); Suppl. 7 (1987); 71 (1999)
Propham	12 (1976); Suppl. 7 (1987)
β -Propiolactone	4 (1974) (corr. 42); Suppl. 7 (1987); 71 (1999)
<i>n</i> -Propyl carbamate	12 (1976); Suppl. 7 (1987)
Propylene	19 (1979); Suppl. 7 (1987); 60 (1994)
Propyleneimine (see 2-Methylaziridine)	
Propylene oxide	11 (1976); 36 (1985) (corr. 42); Suppl. 7 (1987); 60 (1994)
Propylthiouracil	7 (1974); Suppl. 7 (1987); 79 (2001)
Ptaquiloside (see also Bracken fern)	40 (1986); Suppl. 7 (1987)
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Pyrene	32 (1983); Suppl. 7 (1987); 92 (2010)
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Pyrido[3,4- <i>c</i>]psoralen	40 (1986); Suppl. 7 (1987)
Pyrimethamine	13 (1977); Suppl. 7 (1987)
Pyrrolizidine alkaloids (see Hydroxysenkirkine; Isatidine; Jacobine; Lasiocarpine; Monocrotaline; Retrorsine; Riddelliine; Seneciophylline; Senkirkine)	

Q

Quartz (see Crystalline silica)	
Quercetin (see also Bracken fern)	31 (1983); Suppl. 7 (1987); 73 (1999)
<i>para</i> -Quinone	15 (1977); Suppl. 7 (1987); 71 (1999)
Quintozene	5 (1974); Suppl. 7 (1987)

R

Radiation (see gamma-radiation, neutrons, ultraviolet radiation, X-radiation)	
Radionuclides, internalized, that emit α -particles	78 (2001); 100D (2012)
Radionuclides, internalized, that emit β -particles	78 (2001); 100D (2012)
Radioisotopes of iodine, short-lived, including Iodine-131	100D (2012)
Radium-224, radium-226, radium-228	100D (2012)
Radon-222 with its decay products	43 (1988) (corr. 45); 100D (2012)
Refractory ceramic fibres (see Man-made vitreous fibres)	
Reserpine	10 (1976); 24 (1980) (corr. 42); Suppl. 7 (1987)
Resorcinol	15 (1977); Suppl. 7 (1987); 71 (1990)
Retrorsine	10 (1976); Suppl. 7 (1987)
Rhodamine B	16 (1978); Suppl. 7 (1987)
Rhodamine 6G	16 (1978); Suppl. 7 (1987)
Riddelliine	10 (1976); Suppl. 7 (1987); 82 (2002)
Rifampicin	24 (1980); Suppl. 7 (1987)
Ripazepam	66 (1996)
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Rubber industry 28 (1982) (corr. 42); Suppl. 7 (1987) ; 100F (2012)
Rubia tinctorum (see also Madder root; Traditional herbal medicines) 82 (2002)
 Rugulosin 40 (1986); Suppl. 7 (1987)

S

Saccharated iron oxide 2 (1973); Suppl. 7 (1987)
 Saccharin and its salts 22 (1980) (corr. 42); Suppl. 7 (1987); 73 (1999)
 Safrole 1 (1972); 10 (1976); Suppl. 7 (1987)
 Salted fish, Chinese-style 56 (1993); 100E (2012)
 Sawmill industry, including logging
 (see Lumber and sawmill industry, including logging)
 Scarlet Red 8 (1975); Suppl. 7 (1987)
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 Selenium and selenium compounds 9 (1975) (corr. 42); Suppl. 7 (1987)
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Senecio jacobaea L. (see also Pyrrolizidine alkaloids) 10 (1976)
Senecio longilobus 10 (1976); 82 (2002)
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Senecio riddellii (see also Traditional herbal medicines) 82 (1982)
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 Shale-oils 35 (1985); Suppl. 7 (1987); 100F (2012)
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 Shikimic acid (see also Bracken fern) 40 (1986); Suppl. 7 (1987)
 Shoe manufacture and repair (see Boot and shoe manufacture and repair)
 Silica (see also Amorphous silica; Crystalline silica) 42 (1987); 100C (2012)
 Silicone (see Implants, surgical)
 Simazine 53 (1991); 73 (1999)
 Slag wool (see Man-made vitreous fibres)
 Sodium arsenate (see Arsenic and arsenic compounds)
 Sodium arsenite (see Arsenic and arsenic compounds)
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 Sodium chlorite 52 (1991)
 Sodium chromate (see Chromium and chromium compounds)
 Sodium cyclamate (see Cyclamates)
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Sodium fluoride (see Fluorides)	
Sodium monofluorophosphate (see Fluorides)	
Sodium <i>ortho</i> -phenylphenate	30 (1983); Suppl. 7 (1987); 73 (1999)
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Sodium saccharin (see Saccharin)	
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Solar radiation	55 (1992); 100D (2012)
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Spirolactone	24 (1980); Suppl. 7 (1987); 79 (2001)
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Streptozotocin	4 (1974); 17 (1978); Suppl. 7 (1987)
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Strontium chromate (see Chromium and chromium compounds)	
Styrene	19 (1979) (corr. 42); Suppl. 7 (1987); 60 (1994) (corr. 65); 82 (2002)
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Styrene-butadiene copolymers	19 (1979); Suppl. 7 (1987)
Styrene-7,8-oxide	11 (1976); 19 (1979); 36 (1985); Suppl. 7 (1987); 60 (1994)
Succinic anhydride	15 (1977); Suppl. 7 (1987)
Sudan I	8 (1975); Suppl. 7 (1987)
Sudan II	8 (1975); Suppl. 7 (1987)
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T

2,4,5-T 15 (1977)
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 Talc 42 (1987); Suppl. 7 (1987)
 Talc, inhaled, not containing asbestos or asbestiform fibres 93 (2010)
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 Tamoxifen 66 (1996); 100A (2012)
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 Teniposide 76 (2000)
 Terpene polychlorinates 5 (1974); Suppl. 7 (1987)
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 2,2',5,5'-Tetrachlorobenzidine 27 (1982); Suppl. 7 (1987)
 2,3,7,8-Tetrachlorodibenzo-*para*-dioxin 15 (1977); Suppl. 7 (1987); 69 (1997); 100F (2012)
 1,1,1,2-Tetrachloroethane 41 (1986); Suppl. 7 (1987); 71 (1999)
 1,1,2,2-Tetrachloroethane 20 (1979); Suppl. 7 (1987); 71 (1999)
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 2,3,4,6-Tetrachlorophenol (see Chlorophenols; Chlorophenols, occupational exposures to;
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Thorium-232 (as Thorotrast)	100D (2012)
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2,4-Toluene diisocyanate (see also Toluene diisocyanates)	19 (1979); 39 (1986)
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Trichlormethine	9 (1975); Suppl. 7 (1987); 50 (1990)
Trichloroacetic acid	63 (1995) (corr. 65); 84 (2004)
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1,1,2-Trichloroethane	20 (1979); Suppl. 7 (1987); 52 (1991); 71 (1999)
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2,4,6-Trichlorophenol	20 (1979)
(see also Chlorophenols; Chlorophenols, occupational exposures to; Polychlorophenols and their sodium salts)	
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1,2,3-Trichloropropane	63 (1995)
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2,4,6-Trimethylaniline	27 (1982); Suppl. 7 (1987)
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<i>Tussilago farfara</i> L. (see also Pyrrolizidine alkaloids)	10 (1976)

U

Ultraviolet radiation	40 (1986); 55 (1992); 100D (2012)
Underground haematite mining with exposure to radon (see Haematite mining, underground)	
Uracil mustard	9 (1975); Suppl. 7 (1987)
Uranium, depleted (see Implants, surgical)	
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V

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Vinyl bromide	19 (1979); 39 (1986); Suppl. 7 (1987); 71 (1999); 97 (2008)
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Vinyl chloride-vinyl acetate copolymers.....	7 (1976); 19 (1979) (corr. 42); Suppl. 7 (1987)
4-Vinylcyclohexene.....	11 (1976); 39 (1986) Suppl. 7 (1987); 60 (1994)

4-Vinylcyclohexene diepoxide	11 (1976); Suppl. 7 (1987); 60 (1994)
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W

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Y

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Z

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This Volume of the *IARC Monographs* provides an assessment of the carcinogenicity of 18 chemicals present in industrial and consumer products or food (natural constituents, contaminants, or flavourings) or occurring as water-chlorination by-products. The compounds evaluated include the widely used plasticizer di(2-ethylhexyl) phthalate and the food contaminant 4-methylimidazole. In view of the limited agent-specific information available from epidemiological studies, the *IARC Monographs Working Group* relied mainly on carcinogenicity bioassays, and mechanistic and other relevant data to evaluate the carcinogenic hazards to humans exposed to these agents.

