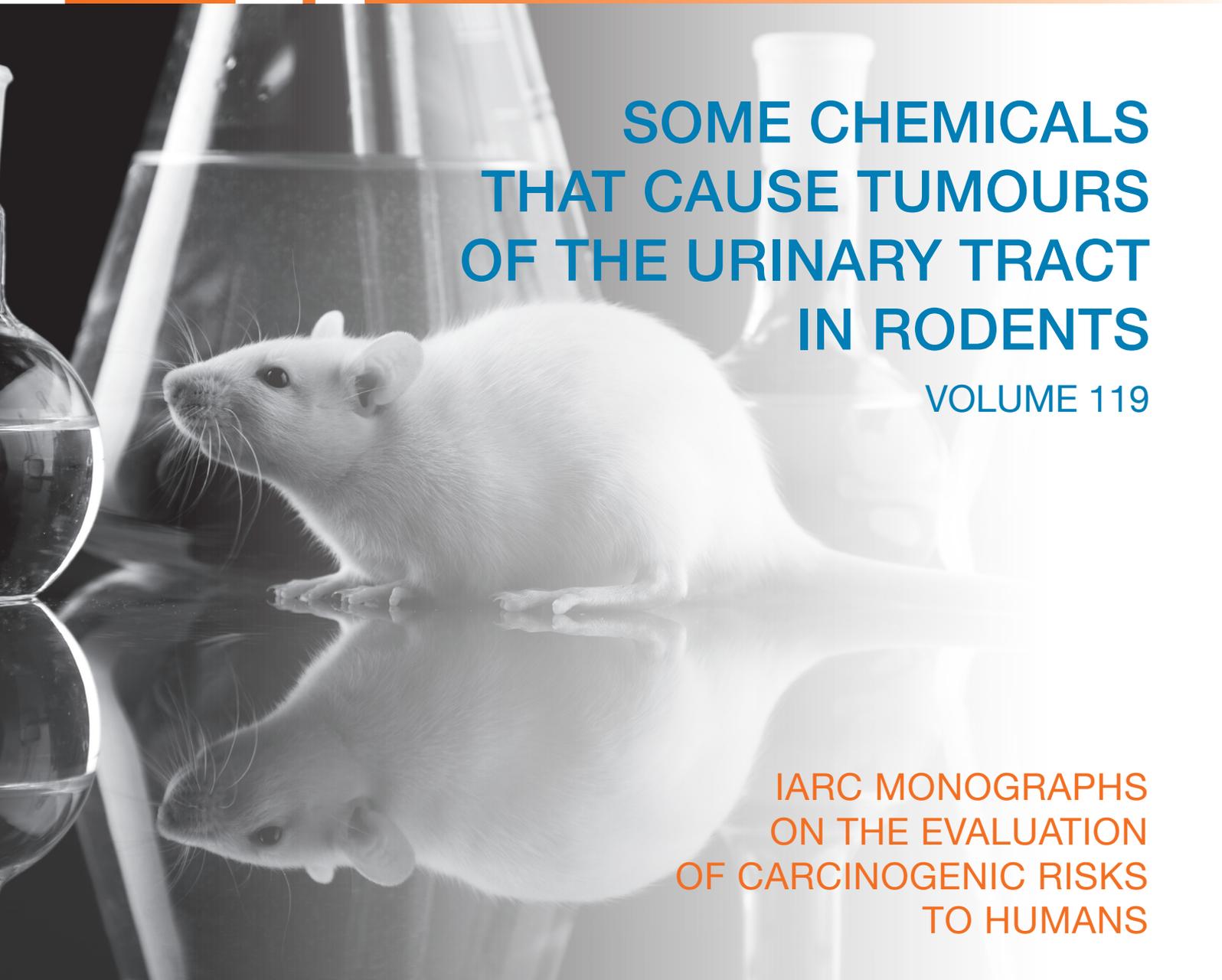


IARC MONOGRAPHS



**SOME CHEMICALS
THAT CAUSE TUMOURS
OF THE URINARY TRACT
IN RODENTS**

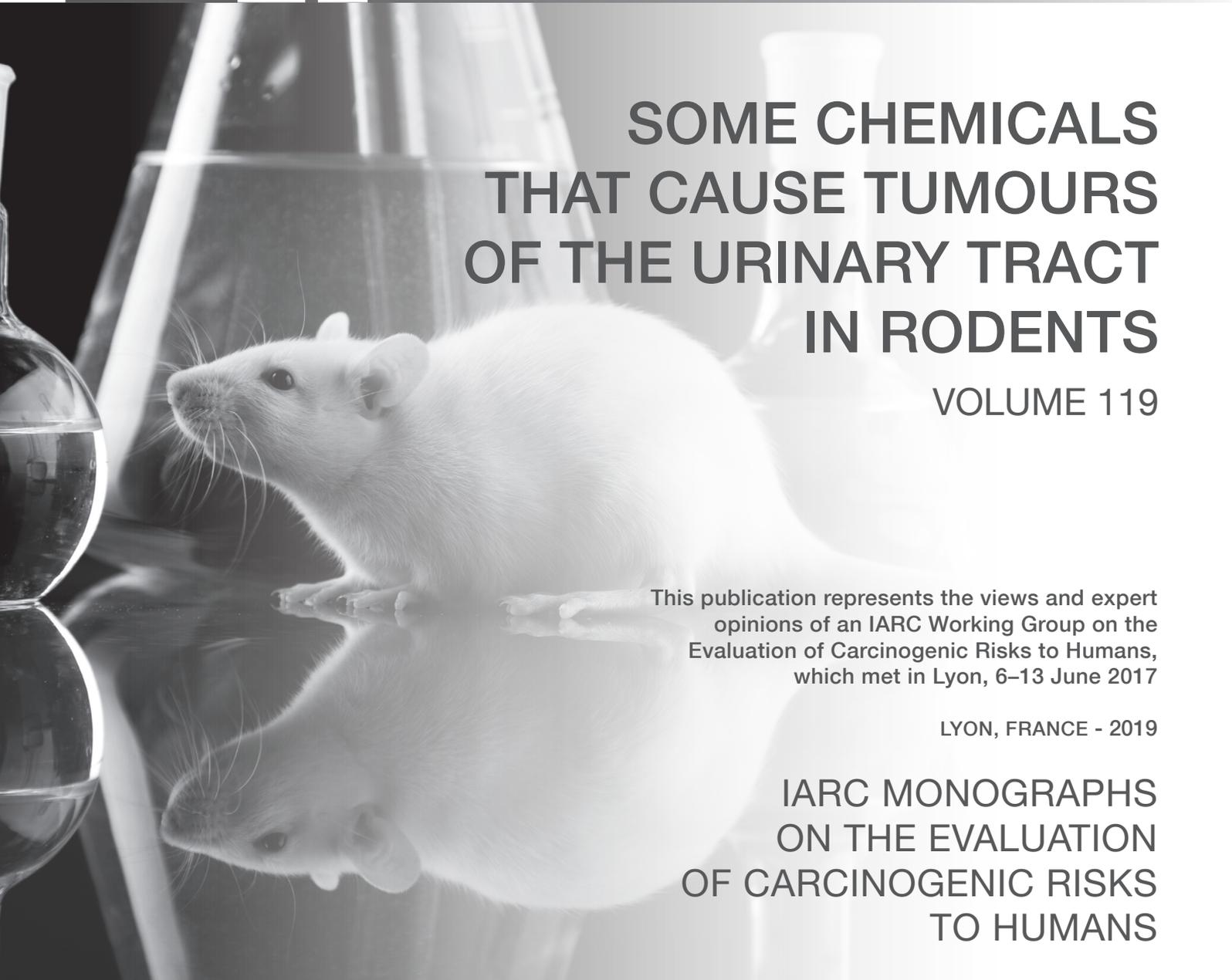
VOLUME 119

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

International Agency for Research on Cancer



World Health
Organization

A white mouse is shown in profile, facing left, on a reflective surface. In the background, there are laboratory glassware items, including a round-bottom flask and a beaker, all rendered in a light, semi-transparent style. The overall image has a scientific and clinical feel.

SOME CHEMICALS THAT CAUSE TUMOURS OF THE URINARY TRACT IN RODENTS

VOLUME 119

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, 6–13 June 2017

LYON, FRANCE - 2019

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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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-variables 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 multistage 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, physico-chemical 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 one-hundred-and-nineteenth volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of seven industrial chemicals (melamine, 1-*tert*-butoxypropan-2-ol, β -myrcene, furfuryl alcohol, pyridine, tetrahydrofuran, and vinylidene chloride) to which workers or the general population are or can potentially be exposed. These chemicals have been recommended for evaluation primarily because of new data on cancer in experimental animals. Epidemiological data for each of the chemicals included in Volume 119 are either lacking or scant.

Vinylidene chloride, melamine, pyridine, and 1-*tert*-butoxypropan-2-ol were evaluated previously in Volumes 71, 73, 77, and 88 of the *IARC Monographs* ([IARC, 1999a](#), [1999b](#), [2000](#), [2006](#)), respectively, as *not classifiable as to their carcinogenicity to humans* (Group 3). Apart from the availability of new data, a separate consideration pertaining to experimental data for chemicals subject to re-evaluation (e.g. 1-*tert*-butoxypropan-2-ol, pyridine) is that certain criteria for specification of *sufficient* and *limited* evidence of carcinogenicity in experimental animals (as detailed in the Preamble to the *IARC Monographs* as amended in January 2006) had changed since the previous evaluation. In relation to experimental data, administration of all seven compounds to rats and mice of both sexes resulted, in at least some instances, in an increase in the incidence of urological tumours, that is tumours of the kidney or urinary bladder. A summary of the findings of this volume appears in *The Lancet Oncology* ([Grosse et al., 2017](#))

High production volume chemicals, and quantification and relative contributions of sources of exposure

Five “high production volume” chemicals (melamine, furfuryl alcohol, pyridine, tetrahydrofuran, and vinylidene chloride) were evaluated. The Working Group noted that most of the agents reviewed in this volume do not have a single source of exposure, and humans may be exposed occupationally or through food and beverages, drinking-water, and the environment. Quantitative determination of the most important sources of human exposure is relevant and was attempted by the Working Group when valid information was available in the scientific literature. However, for many of the agents, quantitative information on many – if not all (e.g. 1-*tert*-butoxypropan-2-ol) – sources of exposure was lacking or inconclusive.

α_{2u} -Globulin-associated kidney tumours in male rats

Of the seven chemicals under review in this volume, four (1-*tert*-butoxypropan-2-ol, β -myrcene, pyridine, and tetrahydrofuran) caused renal tubule tumours in male rats. With each of these four chemicals, the concentration of α_{2u} -globulin was increased in the rat kidneys. The induction of α_{2u} -globulin nephropathy and carcinogenesis is a male-rat-specific disease; thus, if the induction of renal tubule tumours in male rats can be attributed to an α_{2u} -globulin-associated mechanism, the induction of these kidney tumours may not be relevant for humans. IARC established seven criteria that need to be met in order to conclude that renal tubule tumours arising in male rats are due to an α_{2u} -globulin-associated mechanism (Capen *et al.*, 1999). Although each of the four chemicals caused an accumulation of α_{2u} -globulin and most caused a characteristic sequence of histopathological changes associated with α_{2u} -globulin accumulation, many of the other five criteria were not determined or were not met, or the experimental data were inconsistent; thus, the available evidence did not fully satisfy an α_{2u} -globulin-associated mechanism for the induction by these four chemicals of renal tubule tumours in male rats.

Transgenic mouse models

The Working Group noted the difficulty in evaluating a short-term study in transgenic mice (Spalding *et al.*, 2000; see also Tennant *et al.*, 1996) in light of the article by Pritchard *et al.* (2003) on “The role of transgenic mouse models in carcinogen identification”, which states that although they have great promise, transgenic models also have actual or potential limitations for use in a carcinogen identification effort.

Many transgenic models have mutations in only one pathway that may or may not be relevant to human cancer processes for a given chemical. In addition, the specific gene defect may influence tumour development and type, increasing the difficulty of modelling the human response. It is also of concern that the genetic background can influence tumour type, incidence, and location. Thus, short-term, gene-specific transgenic assays may not be able to assess critical information that can be obtained in longer-term bioassays (e.g. multiple target organ effects, interactions of time and age). These issues do not preclude the use of transgenic models, but they must be considered in the selection and interpretation of data obtained using such models.

Evaluation of data on the mechanisms of cancer

In its evaluation of data on mechanisms of carcinogenesis, the Working Group used the procedures first introduced in Volume 112 of the *IARC Monographs* for assessing the strength of evidence with respect to 10 key characteristics of carcinogens (Smith *et al.*, 2016) and for reviewing data from large-scale toxicity testing programmes (IARC, 2017).

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1-TERT-BUTOXYPROPAN-2-OL

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 57018-52-7

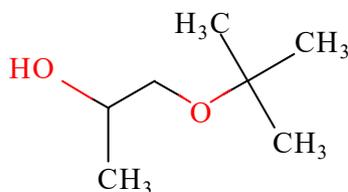
Chem. Abstr. Serv. name: 1-(1,1-Dimethylethoxy)-2-propanol

IUPAC systematic name: 1-(*tert*-Butoxy)propan-2-ol

Synonyms: 1-*tert*-Butoxy-2-propanol; 1-*tert*-butoxypropan-2-ol; 1-methyl-2-*tert*-butoxyethanol; propyleneglycol 1-(*tert*-butyl ether); propylene glycol mono-*tert*-butyl ether; PGMBE; PGTBE; propylene glycol *t*-butyl ether; *tert*-butoxypropanol

From [ChemIDplus \(2018\)](#); [ECHA \(2018\)](#); [NTP \(2018\)](#).

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: C₇H₁₆O₂

Relative molecular mass: 132.201

1.1.3 Chemical and physical properties of the pure substance

Description: Clear colourless liquid with an ethereal odour ([PubChem, 2018](#))

Boiling point: 151 °C

Freezing/melting point: -56 °C

Density: 0.872 g/cm³ at 20 °C

Solubility: ≥ 100 mg/mL at 19 °C, in water

Volatility: Vapour pressure, 0.64 kPa at 20 °C

Relative vapour density: 4.6 (air = 1)

Flash point: 44.4–45.0 °C (open cup)

Auto-ignition temperature: 373 °C

Explosive limits: 1.8–6.8 vol% in air

Octanol/water partition coefficient (*P*): log K_{ow}, 0.87 (estimated)

Conversion factor: 1 ppm = 5.41 mg/m³, at normal temperature (25 °C) and pressure (103.5 kPa)

From [NTP \(2004\)](#); [IPCS \(2006\)](#); [HSDB, \(2017\)](#); [PubChem \(2018\)](#).

1.2 Production and use

1.2.1 Production process

1-*tert*-Butoxypropan-2-ol is manufactured by reacting isobutylene with excess propylene glycol in the presence of a solid resin etherification catalyst. It is then distilled to produce the *a* isomer with a purity of ≥ 99% ([Boatman, 2001](#)).

1.2.2 Production volume

No data were available to the Working Group.

1.2.3 Use

1-*tert*-Butoxypropan-2-ol is used as a solvent (as a substitute for ethylene glycol mono alkyl ethers) and in all-purpose cleaners, coatings, inks, nail polish, lacquers, latex paints, and adhesives. Because of widespread use, there is potential for human exposure via inhalation or dermal routes ([Boatman, 2001](#); [NTP, 2004](#)).

In investigations carried out during 2000–2006 in France, no information on the use of 1-*tert*-butoxypropan-2-ol was recorded ([AFSSET, 2008](#)). The European Chemicals Agency in its inventory indicated only that data on the tonnage of the substance were confidential, which implies that there are some uses in Europe ([ECHA, 2018](#)).

1.3 Analytical methods

No officially validated methods exist specifically for the detection and measurement of 1-*tert*-butoxypropan-2-ol.

Some methods using gas chromatography-mass spectrometry (GC-MS) are available to measure glycol ethers as a group or as individual chemical entities, for example, the Environment Canada reference method ([Environment Canada, 2010](#)).

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

1-*tert*-Butoxypropan-2-ol does not occur naturally.

No data on environmental exposure to 1-*tert*-butoxypropan-2-ol were available to the Working Group.

The use of 1-*tert*-butoxypropan-2-ol as a solvent may result in its release to the environment through various waste streams. As

modelled ([HSDB, 2017](#)), if 1-*tert*-butoxypropan-2-ol is released to air, its vapour pressure indicates that it will exist solely as a vapour in the ambient atmosphere. The vapour phase of 1-*tert*-butoxypropan-2-ol will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 23 hours. If released to soil, 1-*tert*-butoxypropan-2-ol is expected to have very high mobility based upon an estimated K_{oc} of 5. Volatilization from moist soil surfaces is expected to be an important fate process based upon an estimated Henry's law constant of 4.73×10^{-6} atm·m³/mole. The structurally similar compound 2-tertiary butoxy ethanol was not degraded in a 16-day test for biological oxygen demand. Tertiary and ether structures are non-biodegradable. Based on the test result for biological oxygen demand for 2-tertiary butoxy ethanol, 1-*tert*-butoxypropan-2-ol is not expected to biodegrade in soil. If released into water, 1-*tert*-butoxypropan-2-ol is not expected to adsorb to suspended solids and sediment based upon the estimated K_{oc} . Based on the biological oxygen demand test result for 2-tertiary butoxy ethanol, 1-*tert*-butoxypropan-2-ol is not expected to biodegrade in water. Volatilization from water surfaces may be an important fate process based upon this compound's estimated Henry's law constant. Estimated volatilization half-lives for a model river and model lake are 6 days and 69 days, respectively. An estimated bioconcentration factor of 0.8 suggests that the potential for bioconcentration in aquatic organisms is low. Hydrolysis is not expected to be an important environmental fate process since this compound lacks functional groups that hydrolyse under environmental conditions ([HSDB, 2017](#)).

1.4.2 Occupational exposure

Occupational exposure to 1-*tert*-butoxypropan-2-ol may occur through inhalation at workplaces where this compound is produced or used. The general population may be exposed to this compound through contact with consumer products ([HSDB, 2017](#)). A review of investigations of glycol ethers carried out during 2000–2006 in France did not identify any usage for 1-*tert*-butoxypropan-2-ol, or any data on exposure of the working population or general population ([AFSSET, 2008](#)). No qualitative or quantitative data on occupational exposure were available to the Working Group.

1.4.3 Exposure of the general population

No qualitative or quantitative data on exposure of the general population were available to the Working Group.

1.5 Regulations and guidelines

The Committee of Experts on Transport of Dangerous Goods (TDG) and Globally Harmonized System of Classification and Labelling of Chemicals (GHS) of the United Nations Economic Commission for Europe (UNECE) identified 1-*tert*-butoxypropan-2-ol as: United Nations Hazard Class 3; United Nations Pack Group III ([UNECE, 2016](#)).

The WHO International Chemical Safety Card ICSC 1615 ([IPCS, 2006](#)), and the French National Institute for Industrial Environment and Risks (INERIS) ([INERIS, 2015](#)) have labelled this chemical as: “Warning”, “Flammable liquid and vapour”, “Causes serious eye irritation”.

In the workplace, no occupational exposure limits have been established for 1-*tert*-butoxypropan-2-ol ([GESTIS, 2017](#)).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

In 2004, the Working Group evaluated the carcinogenicity of 1-*tert*-butoxypropan-2-ol in experimental animals and concluded that there was *limited evidence* for the carcinogenicity of 1-*tert*-butoxypropan-2-ol ([IARC, 2006](#)). No new studies of carcinogenicity in experimental animals have since become available.

See [Table 3.1](#).

3.1 Mouse

Inhalation

Groups of 50 male and 50 female B6C3F₁ mice (age, 6 weeks) were exposed to propylene glycol mono-*t*-butyl ether [1-*tert*-butoxypropan-2-ol] vapour (purity, ≥ 99%) by whole-body inhalation. The exposure dosage was 0 (control), 75, 300, or 1200 ppm for 6 hours plus T₉₀ (12 minutes) per day on 5 days per week for 104 weeks. Necropsies including gross and microscopic examination of all major tissues were carried out on all mice. No effect on survival was observed. Mean body weights of male mice were generally similar to those of the controls throughout the study; those of females at the highest dose (1200 ppm) were slightly less at the end of the study. The incidence of hepatocellular adenoma (males: 18/50, 23/49, 26/50, 36/50; females: 14/49, 8/50, 10/50, 37/49) and of hepatocellular adenoma or carcinoma combined (males: 25/50, 26/49, 33/50, 41/50; females: 18/49, 14/50, 16/50, 41/49) increased with a significant positive trend in males and females ($P < 0.001$, poly-3 test); the incidence in the groups at the highest dose was also significantly increased ($P < 0.001$, poly-3 test).

Table 3.1 Studies of carcinogenicity in experimental animals exposed to 1-tert-butoxypropan-2-ol by inhalation (whole-body exposure)

Species, strain (sex) Age at start Duration Reference	Purity Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Mouse, B6C3F ₁ (M) 6 wk 104 wk NTP (2004)	Purity, ≥ 99% 0, 75, 300, 1200 ppm 6 h + T ₉₀ (12 min)/d, 5 d/wk 50, 50, 50, 50 35, 40, 40, 37	<i>Liver</i> Hepatocellular adenoma: 18/50*, 23/49, 26/50, 36/50** Hepatocellular carcinoma: 9/50, 8/49, 13/50, 11/50 Hepatocellular adenoma or carcinoma (combined): 25/50*, 26/49, 33/50, 41/50** Hepatoblastoma: 0/50*, 0/49, 1/50, 5/50**	 * <i>P</i> < 0.001 (poly-3 trend test) ** <i>P</i> < 0.001 (poly-3 test) NS * <i>P</i> < 0.001 (poly-3 trend test) ** <i>P</i> < 0.001 (poly-3 test) * <i>P</i> < 0.001 (poly-3 trend test) ** <i>P</i> = 0.028 (poly-3 test)	Principal strengths: GLP study; study in males and females Significant increase in the incidence of multinucleated hepatocyte, eosinophilic foci, and basophilic foci Incidence in historical controls, range: hepatocellular adenoma or carcinoma (combined), 50–68%; hepatocellular carcinoma, 18–32% Incidence of hepatoblastoma in historical controls for inhalation studies: 0/250; all routes, 16/1159 (1.4%)
Mouse, B6C3F ₁ (F) 6 wk 104 wk NTP (2004)	Purity, ≥ 99% 0, 75, 300, 1200 ppm 6 h + T ₉₀ (12 min)/d, 5 d/wk 50, 50, 50, 50 39, 36, 42, 39	<i>Liver</i> Hepatocellular adenoma: 14/49*, 8/50, 10/50, 37/49** Hepatocellular carcinoma: 4/49, 8/50, 7/50, 10/49 Hepatocellular adenoma or carcinoma (combined): 18/49*, 14/50, 16/50, 41/49** Hepatoblastoma: 0/49, 0/50, 0/50, 2/49	 * <i>P</i> < 0.001 (poly-3 trend test) ** <i>P</i> < 0.001 (poly-3 test) NS * <i>P</i> < 0.001 (poly-3 trend test) ** <i>P</i> < 0.001 (poly-3 test) NS	Principal strengths: GLP study; study in males and females Significant increase in the incidence of eosinophilic foci Incidence of hepatocellular adenoma or carcinoma (combined) in high-dose animals exceeded historical control range (22–37%) Incidence of hepatocellular carcinoma in all groups of treated animals (but not in controls) exceeded historical control range (8–12%) Historical control incidence of hepatoblastoma for inhalation studies: 0/248; all routes, 0/1152

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Rat, F344/N (M) 6 wk 104 wk NTP (2004)	Purity, ≥ 99% 0, 75, 300, 1200 ppm 6 h + T ₉₀ (12 min)/d, 5 d/wk 50, 50, 50, 50 27, 29, 16, 22	<i>Kidney, standard (single section) evaluation</i>		Principal strengths: GLP study; study in males and females Significant increase in the incidence of renal tubule hyperplasia, renal tubule hyaline droplet accumulation, and liver basophilic foci Historical control incidence of renal tubule adenoma (standard evaluation): 3/299 (1.0 ± 1.1%); range, 0–2% Historical control incidence of renal tubule carcinoma (standard evaluation): 1/299 Historical control incidence of renal tubule adenoma or carcinoma (standard evaluation): 4/299 (1.3 ± 1.0%); range, 0–2% Historical control incidence of hepatocellular adenoma for inhalation studies: 4/299; range, 0–6%
		Renal tubule adenoma (includes multiple):	NS	
		1/50, 1/50, 3/49, 2/50		
		Renal tubule carcinoma:	NS	
		0/50, 0/50, 0/49, 1/50		
		Renal tubule adenoma or carcinoma (combined):	NS	
		1/50, 1/50, 3/49, 3/50		
<i>Kidney, extended evaluation (step sections)</i>				
Renal tubule adenoma (includes multiple):	NS			
0/50, 1/50, 3/49, 2/50				

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Rat, F344/N (M) 6 wk 104 wk NTP (2004) (cont.)		<i>Kidney, standard (single section) evaluation and extended evaluation (step sections) (combined)</i> Renal tubule adenoma (includes multiple): 1/50, 2/50, 5/49, 4/50 Renal tubule carcinoma: 0/50, 0/50, 0/49, 1/50 Renal tubule adenoma or carcinoma (combined): 1/50, 2/50, 5/49, 5/50 <i>Liver</i> Hepatocellular adenoma: 3/50*, 0/50, 2/49, 6/50 Cholangiocarcinoma: 0/50, 0/50, 0/49, 1/50	NS NS NS * <i>P</i> = 0.022 (poly-3 trend test) NS	
Rat, F344/N (F) 6 wk 104 wk NTP (2004)	Purity, ≥ 99% 0, 75, 300, 1200 ppm 6 h + T ₉₀ (12 min)/d, 5 d/wk 50, 50, 50, 50 33, 34, 28, 36	<i>Kidney</i> Renal tubule adenoma: 0/49, 0/50, 0/50, 1/50 <i>Liver</i> Hepatocellular adenoma: 1/49, 0/50, 0/50, 2/50	NS NS NS	Principal strengths: GLP study; study in males and females Significant increase in the incidence of liver clear cell foci

d, day; F, female; GLP, good laboratory practice; h, hour; M, male; min, minute; NS, not significant; ppm, parts per million; T₉₀, time to achieve 90% of the target concentration after the beginning of vapour generation; wk, week

In males, the incidence of hepatocellular adenoma or carcinoma (combined) in the group at the highest dose (41/50, 82%) exceeded the range for historical controls (range, 50–68%; fed the NTP-2000 diet). However, the incidence of hepatocellular carcinoma in all groups of males (including controls) was below or within the upper bound of the range for historical controls (18–32%). In females at the highest dose, the incidence of hepatocellular adenoma or carcinoma (combined) (41/49, 84%) also exceeded the range observed in historical controls (range, 22–37%). The incidence of hepatocellular carcinoma in all groups of treated females (but not in controls) exceeded the range for historical controls (8–12%). Hepatoblastoma [an embryonal tumour of liver cells,] was also found in treated males and females. The incidence of hepatoblastoma (0/50, 0/49, 1/50, 5/50) increased with a significant positive trend in males ($P < 0.001$, poly-3 test); the incidence in the group at the highest dose was also significantly increased ($P = 0.028$, poly-3 test) compared with controls. At the highest dose, 2 females out of 49 (4.1%) also developed hepatoblastoma compared with none in 49 controls; this incidence was clearly above that for historical controls in inhalation studies (0/248) in females. The incidence of liver eosinophilic foci in males and females at the highest dose, and the incidence of liver basophilic foci in males at the intermediate dose were significantly increased compared with controls. [On the basis of criteria specified in [Thoolen et al. \(2010\)](#), the Working Group noted that the liver basophilic foci (observed in the group at the intermediate dose) may have progressed to hepatoblastoma (observed in the group at the highest dose).] The incidence of multinucleated hepatocytes in males at the highest dose was significantly increased; the severity of this change was generally mild and based on the number of multinucleated hepatocytes ([Doi et al., 2004](#); [NTP, 2004](#)). [The Working Group noted this was a well-conducted

study in males and females and complied with good laboratory practice (GLP).]

3.2 Rat

Inhalation

Groups of 50 male and 50 female Fischer 344/N rats (age, 6 weeks) were exposed to propylene glycol mono-*t*-butyl ether [1-*tert*-butoxy-propan-2-ol] vapour (purity, $\geq 99\%$) at a concentration of 0 (control), 75, 300, or 1200 ppm by whole-body inhalation for 6 hours plus T_{90} (12 minutes) per day on 5 days per week for 104 weeks. For all rats, a complete necropsy was performed, and gross and microscopic examination of all major organs and tissues was carried out. Survival rates in males were 27/50 (control), 29/50, 16/50, and 22/50; those in females were 33/50 (control), 34/50, 28/50 and 36/50. Survival of males in the group receiving the intermediate dose (300 ppm) was less than that of controls. Survival of all exposed groups of females was similar to that of controls. Mean body weights of males and females at the highest dose (1200 ppm) were less than those of controls during the second year of the study. No significant increase in the incidence of tumours was observed in treated females. Some increase in the incidence of tumours of the kidney and liver was observed in exposed males. The incidence of adenoma of the renal tubules in exposed males was non-significantly increased; one renal tubule carcinoma also occurred in the group at the highest dose. The combined incidence of these tumours of the renal tubules (standard and extended evaluation, combined) was 1/50 (control), 2/50, 5/49, and 5/50 (not statistically significant by pair-wise comparison or trend test). Historically, in all six inhalation bioassays carried out by the National Toxicology Program (NTP) and using the NTP-2000 diet, no more than one kidney neoplasm had been observed in the matched chamber-control groups of male

rats fed the NTP-2000 diet; overall, the incidence in historical controls was 4/299. There was a significant increase in the incidence of renal tubule hyperplasia and of hyaline droplet accumulation in male rats at the intermediate and highest dose. Hepatocellular adenoma occurred with a significant positive trend ($P = 0.022$, poly-3 test) in male rats, with the incidence in the group at the highest dose exceeding the range for historical controls. The incidence of hepatocellular adenoma in males was 3/50 (control, 6%), 0/50, 2/49 (4%), and 6/50 (12%); the incidence of hepatocellular adenoma in historical controls for inhalation studies was 4/299 (range, 0–6%). No hepatocellular carcinomas were observed. There was a significant increase in the incidence of liver basophilic foci in all treated groups of males (Doi et al., 2004; NTP, 2004). [The Working Group noted that this was a well-conducted GLP study in males and females.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

The absorption, distribution, metabolism, and excretion of 1-*tert*-butoxypropan-2-ol were described previously in *IARC Monographs* Volume 88 (IARC, 2006).

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

(a) Absorption

Much of the information available on the toxicokinetics of 1-*tert*-butoxypropan-2-ol was provided in an NTP (1994) report, summarized in NTP (2004). In male Fischer 344 rats,

the [^{14}C -propanol]-labelled chemical (purity, 98%) was rapidly and extensively absorbed when given in water by gavage. Approximately 22–26% was recovered as exhaled radiolabelled carbon dioxide ($^{14}\text{CO}_2$), indicating that further metabolism occurred. Dermal exposure occurred to a limited extent, with about 3% and 7.8% of the applied dose absorbed systemically by rats and mice, respectively (NTP, 1994).

(b) Distribution

In male Fischer 344 rats evaluated 72 hours after oral administration of ^{14}C -labelled 1-*tert*-butoxypropan-2-ol at a dose of 3.8, 37.7, or 377.1 mg/kg body weight (bw), radiolabel was distributed fairly evenly throughout the body (NTP, 1994). Concentrations in skeletal muscle, skin, fat, and liver were somewhat higher than in other organs. Less than 6% of the doses remained in the carcass.

(c) Metabolism

1-*tert*-Butoxypropan-2-ol is metabolized primarily to glucuronide and sulfate conjugates and excreted in the urine. When administered orally to male Fischer 344 rats, 48–76% was eliminated in the urine (NTP, 1994). The major urinary metabolite was 1-*tert*-butoxypropan-2-ol glucuronide. The sulfate conjugate was also identified. The proportion of the sulfate increased from 7% to 13% as the dose increased (NTP, 1994). Much (22–26%) of the remainder of each oral dose was exhaled as CO_2 . The glucuronide conjugate was the only metabolite identified in the bile in a separate experiment in male Fischer 344 rats performed by NTP (1994). The finding of exhaled CO_2 is consistent with the metabolism of 1-*tert*-butoxypropan-2-ol to CO_2 by *O*-dealkylation, but this pathway remains to be demonstrated.

(d) Excretion

Elimination kinetic parameters were calculated from the results of an experiment in which male Fischer 344 rats received 1-*tert*-butoxypropan-2-ol at an intravenous dose of 37.8 mg/kg bw ([NTP, 1994](#)). Elimination of the parent compound was very rapid. Clearance exceeded hepatic blood flow. The plasma elimination half-life was only 16 minutes.

In male and female B6C3F₁ mice and Fischer 344 rats, 1-*tert*-butoxypropan-2-ol (administered intravenously or by inhalation) exhibited concentration-dependent nonlinear kinetics in its elimination from the blood ([Dill et al., 2004](#)). Both mice and rats showed longer half-lives, lower clearance, and disproportionate increases in area under the curve (AUC) when intravenous doses were increased from 15 to 200 mg/kg bw. The mice were more efficient than the rats in elimination of 1-*tert*-butoxypropan-2-ol. After inhalation, mice eliminated 1-*tert*-butoxypropan-2-ol more rapidly (shorter $t_{1/2}$) and had a higher efficiency (lower K_m) and capacity (higher V_{max}). Saturable Michaelis–Menten kinetics was most evident in each species at 1200 ppm. A slow, zero-order decline in blood concentrations was manifest for the first several hours at this high exposure level. The most notable sex-specific difference was higher blood concentrations in female rats, ostensibly due to the lower urinary excretion of 1-*tert*-butoxypropan-2-ol conjugates. Total conjugates (glucuronide and sulfate) increased in proportion to exposure level from 75 to 300 ppm, but were less than proportional from 300 to 1200 ppm.

4.2 Mechanisms of carcinogenesis

1-*tert*-Butoxypropan-2-ol has been studied for genotoxic effects in mice and in mammalian and non-mammalian systems in vitro ([Doi et al., 2004](#); [NTP, 2004](#)). Other than studies on altered cell proliferation in experimental animals,

discussed in Section 4.5, no other data relevant to carcinogenic mechanisms were available to the Working Group.

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

A statistically significant positive trend and a marginal increase in the frequency of micronucleated normochromatic erythrocytes were observed in peripheral blood of female, but not male, B6C3F₁ mice exposed to 1-*tert*-butoxypropan-2-ol at concentrations ranging from 75 to 1200 ppm (6 hours per day, 5 days per week) for 3 months. No change was seen in the percentage of immature polychromatic erythrocytes in peripheral blood, suggesting no effect on erythropoiesis.

1-*tert*-Butoxypropan-2-ol (at doses of up to 5000 µg/mL) did not induce sister-chromatid exchange or chromosomal aberrations in the presence or absence of metabolic activation in cultured Chinese hamster ovary cells.

1-*tert*-Butoxypropan-2-ol (100–10 000 µg/plate) induced a dose-related increase in mutagenicity in *Salmonella typhimurium* strain TA97 when tested in the absence, but not the presence, of rat liver S9 supernatant; however, results were negative in other strains (TA98, TA100, TA1535 and TA1537) ([NTP, 2004](#)).

4.3 Data relevant to comparisons across agents and end-points

All seven agents evaluated in the present volume were tested in high-throughput screening assays of the Toxicity Testing in the 21st Century (Tox21) and/or Toxicity Forecaster (ToxCast) research programmes of the government of the USA ([Kavlock et al., 2012](#); [Tice et al., 2013](#); [EPA, 2016a,b](#); [Filer et al., 2016](#)). Four agents were tested

in Tox21 and ToxCast assays, and the other three agents only in Tox21 assays.

One factor of note for the chemicals evaluated in the present volume is that compounds with a very low relative molecular mass (roughly less than 150) generally have only a low affinity for biomolecular interactions due to limited free energy for binding ([Hopkins et al., 2004](#)). All seven of the chemicals evaluated in the present volume have a relative molecular mass of less than 150. Hence screening in vitro at the concentrations used in ToxCast and Tox21 assays may not be able to detect molecular receptor-type interactions for non-reactive effects. These compounds of low relative molecular mass may also have high vapour pressure, which could lead to loss of sample during storage and/or testing and thus failure to reach effective active concentrations. In addition, the Tox21 and ToxCast in vitro assays either fully lacked or had uncharacterized and generally low xenobiotic metabolism capacity, limiting effects generally to parent compounds.

The Tox21 and ToxCast in vitro assays cover a broad range of potential toxicity mechanisms and are not specifically focused on carcinogenesis. The Working Group of *IARC Monograph Volume 112* mapped the 821 assay end-points available at that time to the key characteristics of known human carcinogens ([IARC, 2017a](#)). The consensus assignments resulted in 263 assay end-points mapped to 7 of 10 key characteristics ([IARC, 2017b](#)). Upon review of assay end-points added to Tox21 and ToxCast data since that determination, subsequent Working Groups mapped 28 additional assay end-points to key characteristics, resulting in 291 assays mapped to 7 of 10 key characteristics in total. The 7 chemicals evaluated in the present volume were only tested in 249 of these assays.

The assay end-points used, the bioactivity determination, and the mapping to “key characteristics” are included as supplemental material to the present volume (Annex 1).

The assays mapped to each key characteristic are briefly described below.

1. *Is electrophilic or can be metabolically activated* – 7 assay end-points consisting of cytochrome P450 (CYP) biochemical activity assays including aromatase;
2. *Is genotoxic* – 10 assay end-points consisting of cellular TP53 induction and DNA repair-sensitive cellular assays;
3. *Alters DNA repair or causes genomic instability* – 0 assay end-points;
4. *Induces epigenetic alterations* – 4 assay end-points including biochemical assays targeting histone deacetylases and other enzymes modifying chromatin as well as cellular transcription factor assays involved in epigenetic regulation;
5. *Induces oxidative stress* – 14 assay end-points, all cellular assays, targeting nuclear factor erythroid 2-related factor/antioxidant responsive element (NRF2/ARE), other stress-related transcription factors, and protein upregulation in response to reactive oxygen species;
6. *Induces chronic inflammation* – 47 assay end-points measuring protein expression levels in primary human cells in complex environments;
7. *Is immunosuppressive* – 0 assay end-points;
8. *Modulates receptor-mediated effects* – 83 assay end-points targeting nuclear receptors (including the aryl hydrocarbon receptor) in cellular assays for transactivation, receptor dimerization, and nuclear translocation, as well as biochemical radioligand-binding assays and coregulatory recruitment assays;
9. *Causes immortalization* – 0 assay end-points;
10. *Alters cell proliferation, cell death, or nutrient supply* – 84 assay end-points measuring cytotoxicity by a wide variety of assay formats in cell lines, primary human cells and developing zebrafish larvae.

A summary of results for each agent evaluated in the present volume is given below.

(a) *1-tert-Butoxypropan-2-ol*

1-tert-Butoxypropan-2-ol (CAS No. 57018-52-7) was inactive for 64 Tox21 assay end-points mapped to the key characteristics (it was not in the ToxCast library). The analytical chemistry of the tested sample showed that the intended structure was present and purity was > 90%. [The Working Group noted the low relative molecular mass of the chemical (132), which may limit biomolecular interactions at the concentrations tested.]

(b) *β-Myrcene*

β-Myrcene (CAS No. 123-35-3) was bioactive in 5 of 238 ToxCast and Tox21 assay end-points mapped to the key characteristics. One assay end-point for oxidative stress, (ATG_NRF2) showed bioactivity at 78 μ M. Three other assays demonstrating bioactivity were mapped to key characteristic 8, consisting of PPAR γ activation, PXR activation, and PPAR δ inhibition (ATG_PPAR, ATG_PXRE, and TOX21_PPAR δ). Neither PPAR γ nor PXR activation were supported by orthogonal assays (none existed for PPAR δ antagonist). The other mapped end-point was for key characteristic 10 and was inhibition of cell viability in a single assay for an immortalized cell line (TOX21_VDR_viability). However, *β*-myrcene had no bioactivity in assays of inhibition of viability in many other cell lines. The analytical chemistry determination of the Tox21 sample indicated the expected structure was not present at the time of analysis. [The Working Group noted the low relative molecular mass of the chemical (136), and the low water solubility, factors which may limit biomolecular interactions at the concentrations tested.]

(c) *Furfuryl alcohol*

Furfuryl alcohol (CAS No. 98-00-0) was bioactive in 8 of 217 ToxCast and Tox21 assays mapped to the key characteristics. Furfuryl alcohol activated both a ToxCast NRF2 assay (ATG_NRF2) and a Tox21 NRF2 assay (TOX21_ARE), with AC₅₀ values of 48 and 99 μ M, respectively, supportive of induction of oxidative stress. It showed bioactivity against 3 assay end-points mapped to key characteristic 8, all in the range 40–50 μ M. [The Working Group noted, however, that orthologous ToxCast and Tox21 assays for the activated receptors did not support activation of these assays and the activity may be nonspecific]. Finally, furfuryl alcohol inhibited proliferation in the range of 28–30 μ M of three different primary human cell cultures (BSK_3C_Proliferation, BSK_hDFCGF_Proliferation, BSK_SAg_Proliferation), which included endothelial cells, fibroblasts, and peripheral blood mononuclear cells, mapped to key characteristic 10. Conversely, it did not affect viability of many immortalized cell lines that were also evaluated. The analytical chemistry determination of the Tox21 tested sample showed the appropriate structure was detected with a purity > 90%. [The Working Group noted the low relative molecular mass of the chemical (98), which may limit biomolecular interactions at the concentrations tested.]

(d) *Melamine*

Melamine (CAS No. 108-78-1) had bioactivity in only 1 of 64 Tox21 assay end-points mapped to the key characteristics. The assay end-point in which it showed bioactivity mapped to key characteristic 10; however, activity was only seen at the lowest concentration tested. The analytical chemistry determination of the Tox21-tested sample showed the appropriate structure was detected with a purity > 90%. [The Working Group noted the low relative molecular mass

of the chemical (126), which may limit biomolecular interactions at the concentrations tested.]

(e) *Pyridine*

Pyridine (CAS No. 110-86-1) showed no bioactivity in 64 Tox21 assay end-points mapped to the key characteristics. The analytical chemistry determination of the Tox21-tested sample showed the concentration was < 5% of the expected value. [The Working Group noted the low relative molecular mass of the chemical (79), which may limit biomolecular interactions at the concentrations tested, and that volatility of the chemical may result in limited exposure in vitro.]

(f) *Tetrahydrofuran*

Tetrahydrofuran (CAS No. 109-99-9) showed bioactivity, for one PXR activation assay (ATG_PXRE) mapped to key characteristic 8, out of 118 Tox21 and ToxCast assay end-points mapped to the key characteristics. [The Working Group noted the low relative molecular mass of the chemical (72), which may limit biomolecular interactions at the concentrations tested, and that volatility may result in limited exposure in vitro.]

(g) *Vinylidene chloride*

Vinylidene chloride (CAS No. 75-35-4) was inactive for all except one of 118 ToxCast and the Tox21 programme assay end-points mapped to the key characteristics. The only activity was in a single pregnane X receptor (PXR) transcription factor activation assay (ATG_PXRE) mapped to key characteristic 8. [The Working Group noted the low relative molecular mass of the chemical (97), which may limit biomolecular interactions at the concentrations tested, and that volatility may result in limited exposure in vitro.]

4.4 Susceptibility to cancer

No data were available to the Working Group.

4.5 Other adverse effects

4.5.1 Humans

No data were available to the Working Group.

4.5.2 Experimental systems

IARC has established seven criteria that need to be fully met in order to conclude that an agent induces tumours of the kidney by an α_{2u} -globulin-associated response ([IARC, 1999](#)). Three criteria were met for the present agent, specifically: (1) induction of the characteristic sequence of histopathological changes associated with α_{2u} -globulin accumulation; (2) identification of the accumulating protein as α_{2u} -globulin; and (3) induction of sustained increases in cell proliferation in the renal cortex ([NTP, 2004](#); [Doi et al., 2007](#)). However, four of these criteria were not met, specifically: (1) lack of genotoxic activity of the agent and/or metabolite (1-*tert*-butoxypropan-2-ol was mutagenic in *S. typhimurium* TA97 and increased the frequency of micronucleated erythrocytes in peripheral blood of B6C3F₁ mice (see Section 4.2.2); (2) male rat specificity for nephropathy and renal tumorigenicity (nephropathy was also induced by 1-*tert*-butoxypropan-2-ol in female Fischer 344 rats) ([NTP, 2004](#)); (3) reversible binding of the chemical or metabolite to α_{2u} -globulin (no data were available on the binding of 1-*tert*-butoxypropan-2-ol to α_{2u} -globulin); and (4) similarities in dose-response relationships of the tumour outcome with histopathological end-points associated with α_{2u} -globulin nephropathy ([Doi et al., 2007](#)).

Regarding the issue of dose-response relationships between tumour outcome and α_{2u} -globulin-associated effects in male rats, [Doi et al. \(2007\)](#) compared renal tubule tumour responses in 2-year studies with the extent of α_{2u} -globulin

nephropathy in 3-month studies conducted by the NTP for several chemicals, including decalin, 1-*tert*-butoxypropan-2-ol, Stoddard solvent, and *d*-limonene. While 1-*tert*-butoxypropan-2-ol had the highest 90-day labelling index at doses used in the carcinogenicity studies on these agents, it caused the smallest increase in the number of regenerating renal tubules, the fewest granular cast count, and renal tubule tumour incidence in male Fischer 344 rats that was higher than that observed in the NTP database of historical controls ([NTP, 2004](#)).

In B6C3F₁ mouse liver, 2-year exposure to 1-*tert*-butoxypropan-2-ol by inhalation increased the incidence of eosinophilic foci in exposed male and females, and of basophilic foci and multinucleated hepatocytes in exposed males. In Fischer 344 rat liver, 2-year exposure by inhalation increased the incidence of basophilic foci in males at all exposure concentrations and increased clear cell foci in females at 1200 ppm ([Doi et al., 2004](#); [NTP, 2004](#)).

Liver weights were also increased in male and female Fischer 344 rats and B6C3F₁ mice exposed to 1-*tert*-butoxypropan-2-ol by inhalation for 3 months ([NTP, 2004](#)).

Other target sites of 1-*tert*-butoxypropan-2-ol were the nose and eyes of exposed Fischer 344 rats, and the nose, eyes, and forestomach of exposed B6C3F₁ mice. In the long-term inhalation bioassays, 1-*tert*-butoxypropan-2-ol increased hyaline degeneration of the olfactory epithelium in rats, goblet cell hyperplasia in male rats, and forestomach inflammation and squamous epithelial hyperplasia in male mice ([Doi et al., 2004](#); [NTP, 2004](#)).

5. Summary of Data Reported

5.1 Exposure data

1-*tert*-Butoxypropan-2-ol is a solvent used as a substitute for ethylene glycol mono alkyl ethers and in all-purpose cleaners, coatings, inks, nail polish, lacquers, latex paints, and adhesives. Its use as a solvent may result in its release to the environment through various waste streams. The general population may be exposed to 1-*tert*-butoxypropan-2-ol through contact with consumer products or as a result of environmental contamination. No quantitative information was available on occupational or environmental exposure.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

In one well-conducted study that complied with good laboratory practice (GLP) in male and female mice treated by whole-body inhalation, 1-*tert*-butoxypropan-2-ol significantly increased the incidence (with a significant positive trend) of hepatocellular adenoma, of hepatocellular adenoma or carcinoma (combined), and of hepatoblastoma in males; and significantly increased the incidence (with a significant positive trend) of hepatocellular adenoma, and of hepatocellular adenoma or carcinoma (combined) in females.

In a second well-conducted GLP study in male and female rats treated by whole-body inhalation, 1-*tert*-butoxypropan-2-ol caused a significant positive trend in the incidence of hepatocellular adenoma, and the occurrence of rare neoplasms of the renal tubules in males.

5.4 Mechanistic and other relevant data

No data on the absorption, distribution, metabolism, and excretion of 1-*tert*-butoxypropan-2-ol in humans were available. One study of oral administration and one study of inhalation were available in rats. Orally administered 1-*tert*-butoxypropan-2-ol is rapidly and extensively absorbed. Systemically absorbed 1-*tert*-butoxypropan-2-ol is uniformly distributed to tissues throughout the body. 1-*tert*-Butoxypropan-2-ol is metabolized primarily to glucuronide and sulfate conjugates. These conjugates are excreted in the urine. Much of the remainder is exhaled as CO₂. 1-*tert*-Butoxypropan-2-ol undergoes very rapid metabolic clearance. Its plasma half-life is 16 minutes in rats. Inhalation of high vapour concentrations (e.g. 1200 ppm) exceeds the capacity of conjugation, resulting in supra-proportional increases in 1-*tert*-butoxypropan-2-ol blood concentrations.

With respect to the key characteristics of carcinogens, there is *weak* evidence that 1-*tert*-butoxypropan-2-ol is genotoxic. No data in humans were available. It gave marginally positive results for micronucleus formation in female B6C3F₁ mice, but negative results in males. It gave negative results for induction of sister-chromatid exchange and chromosomal aberration in Chinese hamster ovary cells in the presence or absence of metabolic activation. 1-*tert*-Butoxypropan-2-ol was mutagenic in *Salmonella typhimurium* strain TA97 in the absence, but not the presence, of metabolic activation. It gave negative results in other strains.

Few other data on other key characteristics of carcinogens were available.

Kidney weights, the renal tubule cell labelling index, and kidney α_{2u} -globulin concentrations were increased in male rats exposed by inhalation; renal toxicity, including increased severity of chronic nephropathy, was also evident in female rats. Four of the seven criteria established

by IARC for concluding that an agent induces tumours of the kidney by an α_{2u} -globulin-associated response were not met.

In the long-term inhalation bioassays, 1-*tert*-butoxypropan-2-ol increased hyaline degeneration of the olfactory epithelium in rats, goblet cell hyperplasia in male rats, and forestomach inflammation and squamous epithelial hyperplasia in male mice.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 1-*tert*-butoxypropan-2-ol.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1-*tert*-butoxypropan-2-ol.

6.3 Overall evaluation

1-*tert*-Butoxypropan-2-ol is *possibly carcinogenic to humans* (Group 2B).

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β-MYRCENE

1. Exposure Data

Myrcene exists as two isomers: the naturally occurring β-isomer, containing an isopropylidene group, and the isopropenyl form, often called the α-isomer ([Behr & Johnen, 2009](#)); however, the term “myrcene” in the literature may not exclusively refer to β-myrcene.

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 123-35-3

Chem. Abstr. Serv. name:

7-Methyl-3-methylene-1,6-octadiene

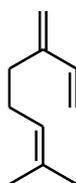
EC/List No.: 204-622-5

IUPAC systematic name: 7-Methyl-3-methylideneocta-1,6-diene

Synonyms: 2-Methyl-6-methylene-2,7-octadiene; 3-methylene-7-methyl-1,6-octadiene; myrcene; NSC No. 406264; β-geraniolene

From [NTP \(2010\)](#); [Merck Index \(2013\)](#); [NCBI \(2018\)](#)

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: C₁₀H₁₆

Relative molecular mass: 136.24

1.1.3 Chemical and physical properties of the pure substance

Description: Yellow oily liquid with a characteristic pleasant terpene odour and citrus-like taste

Boiling point: 167 °C

Melting point: < -10 °C

Density: 0.794 g/cm³ at 20 °C

Octanol/water partition coefficient (P): log K_{ow}, 4.33

Refractive index: 1.4709 at 20 °C

Solubility: Practically insoluble in water; soluble in alcohol, chloroform, ether, and glacial acetic acid

Stability: Polymerizes spontaneously at room temperature, whether air is excluded or not

Conversion factor: 1 ppm = 5.57 mg/m³, at normal temperature (25 °C) and pressure (103.5 kPa)

From [Merck Index \(2013\)](#); [NCBI \(2018\)](#); [Behr & Johnen \(2009\)](#).

1.1.4 Impurities

Technical-grade β -myrcene has a purity of 75%, but rectification can achieve a purity of > 90%. Impurities include limonene, *psi*-limonene, *dl*-limonene, terpenes, β -pinene, dipentene from a cyclization reaction, and isomers and dimers of β -myrcene ([Behr & Johnen, 2009](#); [NTP, 2010](#)). A polymerization inhibitor such as butylhydroxytoluene or tenox propyl gallate is normally added to crude or high-purity β -myrcene during shipment or extended storage ([NTP, 2010](#)). Most commercial preparations contain inhibitors of polymerization, such as tocopherol ([Behr & Johnen, 2009](#)).

1.2 Production and use

1.2.1 Production process

Monoterpenes such as β -myrcene are naturally formed in plants by stereospecific condensation of isopentenyl diphosphate with dimethylallyl diphosphate leading to geranyl diphosphate, which is hydrolysed to the monoterpene alcohol geraniol. β -Myrcene is then formed by dehydration and isomerization of geraniol ([Eggersdorfer, 2012](#)).

β -Myrcene occurs naturally in many organisms. It is a major component of essential oils of plants such as hops, bay leaf, and lemongrass, but since extraction is uneconomical, it is produced industrially by the pyrolysis of β -pinene, which is one of the key components of turpentine ([Behr & Johnen, 2009](#); [NTP, 2010](#); [Eggersdorfer, 2012](#)).

1.2.2 Production volume

β -Myrcene is listed in the USA as a chemical with a high production volume; > 1 million pounds [$> 453\,592$ kg] were produced in or

imported into the USA in 1990–1994 ([NCBI, 2018](#)). Aggregated national production volumes for β -myrcene reported under the inventory update rule show production in the range of > 10 million to < 50 million pounds [> 4536 to < 22680 tonnes] for the years 1986, 1990, 1994, 1998, 2002, and 2006 ([HSDB, 2012](#)).

The Joint Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) reported annual production volumes of β -myrcene used as a flavouring agent of 58 076 kg for Europe and 1188 kg for the USA, while the annual volume of β -myrcene in naturally occurring foods was estimated as 66 842 kg for the USA (based on summarized data published between 1987 and 1999) ([Pronk & Bend, 2006](#)). [Burdock \(2010\)](#) reported an annual volume of 3500 pounds [1588 kg; presumably in the USA, date not specified].

β -Myrcene is listed as a chemical with a high production volume by the Organisation for Economic Co-operation and Development (OECD) ([OECD, 2004](#)). It was produced at a level of greater than 1000 tonnes per year in at least one member country or region of the OECD. β -Myrcene is manufactured and/or imported in the European Economic Area at a level of 0–100 tonnes per year ([ECHA, 2017a](#)).

The database [Chemical Sources International \(2017\)](#) listed 27 manufacturing companies worldwide, of which 10 are located in the USA, 12 in European countries, and 5 in Asia.

1.2.3 Use

One use of technically prepared β -myrcene is as a flavouring agent, for example, in foods and beverages. It is also used widely in cosmetics, soaps, and detergents as well as other fragranced products such as perfumes, air care products, polishes, wax blends, adhesives, disinfectants, biocides, paints, plasters, fuels, inks, and toners

([NTP, 2010](#); [ECHA, 2017b](#)), and electronic cigarette liquids ([Yang et al., 2015](#)). β -Myrcene is a starting material for a range of industrially important products such as menthol, geraniol, nerol, linalool, and isophytol ([Behr & Johnen, 2009](#); [Eggersdorfer, 2012](#)). Besides its main use as an intermediate for the production of terpene alcohols, β -myrcene is also used in the production of terpene polymers, terpene-phenol resins, and terpene-maleate resins ([Eggersdorfer, 2012](#)).

β -Myrcene also occurs naturally in various plants (see Sections 1.4.1 and 1.4.2), and many plants and plant essential oils containing β -myrcene are used in medicinal, food, cosmetic, and other consumer products. For example, β -myrcene is a major constituent of hops used in the manufacture of beer ([Okaru & Lachenmeier, 2017](#)) (see [Table 1.1](#); average, 37% of volatiles; [Salanta et al., 2016](#)).

1.3 Analytical methods

β -Myrcene is typically analysed along with various other compounds in assays for the characterization of terpenes and essential oils, which are predominantly based on hydrodistillation for sample preparation, followed by gas chromatography with flame ionization detection (GC-FID) or with mass spectrometry (GC-MS) ([Okaru & Lachenmeier, 2017](#)). The International Organization for Standardization (ISO) provides an international standard for the GC-FID analysis of essential oils, which includes β -myrcene as analyte ([ISO, 1998](#)). The various ISO standards for essential oils also contain representative gas chromatograms for each matrix (see summary in Section 1.5).

Another means of sample preparation is head-space solid-phase microextraction ([Lachenmeier et al., 2006](#)). For determination of the percentage of β -myrcene in mastic gum oil, a rapid procedure using Fourier transform Raman spectroscopy has been suggested ([Daferera et al., 2002](#)).

Selected methods for the analysis of β -myrcene in various matrices are listed in [Table 1.2](#).

No methods for analysis of β -myrcene in biological matrices in humans were reported.

1.4 Occurrence and exposure

1.4.1 Natural occurrence

β -Myrcene is a compound that occurs naturally in more than 200 plants, including verbena, lemongrass, hops, and bay ([NTP, 2010](#); [Merck Index, 2013](#)). β -Myrcene has been reported qualitatively in more than 200 foods and beverages, including citrus peel oils and juices, apricot, sweet and sour cherry, berries, guava, pineapple, carrot, celery, potato, bell pepper, blackcurrants, anise, anise seed, cardamom, cinnamon, cassia, clove, capsicum varieties, ginger, *Mentha* oils, mace, parsley, thyme, cheeses, cream, pork, hop oil, beer, white wine, rum, cocoa, coffee, tea, mango, tamarind, coriander, gin, sweet bay, prickly pear, calamus, dill, lovage, caraway, buckwheat, corn, basil, fennel, kiwi fruit, rosemary, myrtle berry, turmeric, lemon balm, sage, pimento, angelica oil, Roman and German chamomile oil, eucalyptus and mastic gum oil ([HSDB, 2012](#)).

[Table 1.1](#) and [Table 1.3](#) provide a quantitative overview of the natural occurrence of β -myrcene in essential oils, some natural products, food, medicinal and related products.

While the highest concentrations of β -myrcene in natural materials have been detected in hops (up to 10 g/kg dry weight), the final concentration in beer was very low (0.4–80 μ g/L) due to dilution, low extraction, and potential deterioration during processing ([Kishimoto et al., 2005](#); [Okaru & Lachenmeier, 2017](#)).

β -Myrcene has been measured in air in forests in different parts of the world. Concentrations vary considerably by day and season, and by height of measurement in the forest. Measured concentrations of β -myrcene were often less than

Table 1.1 Relative concentrations of β -myrcene in essential oils and some natural products

Product	Average concentration ^a	Range	Unit	Year ^b	Country or region	Reference
Essential oils from <i>Distichoselinum tenuifolium</i>	67.2	47.7–84.6	%	2010	Portugal	Tavares et al. (2010)
<i>Curcuma mangga</i>	46.50	NR	%	2011	Malaysia	Wahab et al. (2011)
Essential oil of <i>Schinopsis brasiliensis</i>	45.12	NR	%	2011	Brazil	Donati et al. (2015)
Leaf volatiles of <i>Zanthoxylum gillettii</i>	42.87	NR	%	1997	Cameroon	Jirovetz et al. (1999)
Hops (<i>n</i> = 12)	37.90	23.29–52.63	% of volatiles	2011, 2012	Romania	Salanta et al. (2016)
Essential oil of <i>Cannabis sativa</i> (<i>n</i> = 5)	28.41	21.08–35.02	%	2001	Austria	Novak et al. (2001)
Essential oil of lemongrass (<i>Cymbopogon citratus</i>)	27.83	NR	%	2013	Benin	Gbenou et al. (2013)
Peel of pomelo (<i>n</i> = 4)	27.801	22.811–30.928	% of relative content	2014	China	Shao et al. (2014)
Mastic gum oil (<i>n</i> = 10)	24.5	4.5–57.9	% in oil	2002	Greece	Daferera et al. (2002)
Essential oil and the gum of <i>Pistacia lentiscus</i> Var. <i>chia</i> (<i>n</i> = 6)	15.6	7.8–25.0	%	2002	Greece	Koutsoudaki et al. (2005)
Essential oil of <i>Lippia alba</i>	15.0	NR	% of content	2002	Brazil	Oliveira et al. (2006)
Essential oil of <i>Thymus serpyllum</i> ssp. <i>serpyllum</i> (<i>n</i> = 52)	14.30	NR	%	1987	Finland	Stahl-Biskup & Laakso (1990)
Essential oil of <i>Artemisia annua</i>	12.6	0.0–37.7	%	2007	China	Yu et al. (2011)
Essential oil of <i>Santolina rosmarinifolia</i> L. ssp. <i>Rosmarinifolia</i> (<i>n</i> = 13)	11.8	0.3–15.5	% of content	1995–1996	Spain	Palá-Paúl et al. (2001)
Essential oil of <i>Houttuynia</i> Thunb.	11.51	2.58–18.47	%	2004	China	Lu et al. (2006)
Essential oil of Korean endemic citrus species (<i>n</i> = 14)	9.51	2.06–32.10	%	2005	Republic of Korea	Baik et al. (2008)
Essential oil of <i>Thymus serpyllum</i> ssp. <i>tanaensis</i> (<i>n</i> = 133)	9.1	NR	%	1987	Finland	Stahl-Biskup & Laakso (1990)
Essential oils of <i>Juniperus rigida</i> Siebold & Zucc.	9.0	0.0 (stems, needles) –27.00 (berries)	%	2014	China	Liu et al. (2016)
Essential oil of <i>Teucrium stocksianum</i> Bioss.	8.64	NR	%	2012	Pakistan	Shah et al. (2012)
Essential oil of juniper berry (<i>Juniperus communi</i> L.)	8.3	NR	%	2014	Bulgaria	Höferl et al. (2014)
Carrots (<i>Daucus carota</i>) (<i>n</i> = 7)	7.56	0.87–29.90	% of volatiles	2008	NR	Soria et al. (2008)
Essential oil of lemongrass (<i>Cymbopogon citratus</i>)	6.52	NR	%	2015	Cuba	Pinto et al. (2015)
Essential oil of thyme (<i>Thymus kotschyanus</i> and <i>Thymus persicus</i>)	6.46	0.26–12.65	%	2000	Iran (Islamic Republic of)	Rasooli & Mirmostafa (2003)

Table 1.1 (continued)

Product	Average concentration ^a	Range	Unit	Year ^b	Country or region	Reference
Essential oil of sweet fennel (<i>Ocimum gratissimum</i> L.)	6.4	NR	%	2013	Benin	Adjou et al. (2013)
Odorants in frankincense (<i>Boswellia sacra</i>) (n = 6)	6.3	2.8–8.0	% (total peak area)	2014	Oman and Somalia	Niebler & Buettner (2015)
Essential oil of <i>Thymus serpyllum</i> L. (n = 33)	6.2	0.0–20.2	% of content	2001–2004	Estonia	Paaver et al. (2008)
Essential oil of <i>Murraya koenigii</i> L.	6.12	NR	%	2014	India	Rajendran et al. (2014)
<i>Evodia rutaecarpa</i> fruits	5.83	NR	% of volatiles	2005	Japan	Pellati et al. (2005)
Essential oils of wild populations of <i>Stachys lavandulifolia</i> Vahl (Lamiaceae)	5.8	0.0–26.2	%	2011	Iran (Islamic Republic of)	Aghaei et al. (2013)
Essential oil of <i>Thymus serpyllum</i> L. (n = 20)	5.65	0.0–20.2	%	2002, 2003	Estonia	Raal et al. (2004)
Essential oil from <i>Stachys lavandulifolia</i> Vahl (n = 7)	5.49	0.52–15.87	%	2010	Iran (Islamic Republic of)	Pirbalouti & Mohammadi (2013)
Essential oils from <i>Gynura bicolor</i> DC	5.10	NR	%	2012	Japan	Miyazawa et al. (2016)
Oil from <i>Thymus serpylloides</i> ssp. <i>gadorensis</i> (n = 34)	5.0	0.13–30.39	%	1990–1993	Spain	Sáez (2001)
Essential oils of rosemary (<i>Rosmarinus officinalis</i> , Lamiaceae)	4.8	3.4–5.9	%	2008–2009	Serbia	Lakusić et al. (2013)
Japanese pepper (<i>Xanthoxylum piperitum</i> DC.)	4.41	1.75–7.08	%	2001	Japan	Jiang & Kubota (2004)
Essential oils from black pepper ^c (<i>Piper guineense</i>)	4.37	NR	% of content	2013	Nigeria	Obboh et al. (2013)
Essential oil of pineapple weed (<i>Chamomilla suaveolens</i>) (n = 2)	4.2	1.1–7.9	% of content	2007	Estonia	Orav et al. (2010)
Leaves from species of <i>Clausena</i> (Rutaceae)	4.0	0.1–14.3	%	2012	Viet Nam	Trung et al. (2014)
Essential oil from Danggui and Zhiqiao ^d	3.71	NR	%	2016	China	Wang et al. (2016)
Essential oil of carrot seeds (<i>Daucus carota</i>)	3.7	0.5–10.5	%	2014	Italy	Flamini et al. (2014)
Essential oil of wormwood (<i>Artemisia absintium</i>) (n = 15)	3.5	Trace – 9.2	% in oil	1999–2007	Lithuania	Judzentiene et al. (2009)
Essential oil of <i>Lippia alba</i> f. <i>intermedia</i>	3.5	NR	% of content	2002	Brazil	Oliveira et al. (2006)
Cardamom oil (<i>Elettaria cardamomum</i> (L.) Maton)	3.3	2.1–6.6	%	2004	Italy	Marongiu et al. (2004)

Table 1.1 (continued)

Product	Average concentration ^a	Range	Unit	Year ^b	Country or region	Reference
Essential oils of three <i>Thymus</i> species	2.8	0.6–6.8	%	2008	Iran (Islamic Republic of)	Asbaghian et al. (2011)
Essential oil from <i>Satureja intermedia</i> CA Mey	2.5	NR	%	2014	Iran (Islamic Republic of)	Sharifi-Rad et al. (2015)
Essential oils of ripe berries of <i>Juniperus oxycedrus</i> L. ssp. <i>macrocarpa</i> (S.&m) Ball	2.4	1.9–2.8	%	2007	Tunisia	Hanène et al. (2012)
Orange fruit juice (<i>Citrus sinensis</i>) L.	2.38	NR	% of volatiles	2008	China	Qiao et al. (2008)
Essential oils of herbs	2.26	0.11–6.29	%	2006	South-Western Rwanda	Qiao et al. (2008)
Peel oil of <i>Citrus natsudaoidai</i> Hayata (Natsudaoidai)	2.25	NR	% (w/w)	2002	Japan	Mukazayire et al. (2011)
Cardamom essential oil (<i>Elettaria cardamomum</i>)	2.2	NR	%	2016	Iran (Islamic Republic of)	Lan Phi et al. (2006)
Essential oil of <i>Citrus tamurana</i> Hort. ex Tanaka (Hyuganatsu)	2.20	2.11–2.28	% (w/w)	2000	Japan	Masoumi-Ardakani et al. (2016)
Essential oils of fennel fruits ($n = 7$)	2.15	1.48–3.00	%	2010	Romania	Choi & Sawamura (2000)
Orange peel oil (<i>Citrus sinensis</i> L.)	1.88	NR	% of volatiles	2008	China	Aprotosoai et al. (2013)
Peel oil of kumquat (<i>Fortunella japonica</i> Swingle)	1.84	NR	%	2003	Republic of Korea	Choi (2005)
Essential oil of cardamom (<i>Amomum subulatum</i> Roxb.)	1.57	1.16–2.36	%	2013	India	Joshi et al. (2013)
Essential oil from ripe fruits of Jordanian <i>Pistacia palaestina</i> Boiss.	1.2	NR	%	2002	Jordan	Flamini et al. (2004)
Essential oil of <i>Origanum vulgare</i> L. (Lamiaceae) ($n = 12$)	1.1	0.0–3.4	%	2011	Europe	Lukas et al. (2015)
Essential oils of <i>Gynura bicolor</i> DC. Leaves	0.75	NR	%	2012	Japan	Miyazawa et al. (2016)
Essential oil of <i>Thymus serpyllum</i> L. ($n = 7$)	0.6	0.2–1.1	% of content	2001–2004	Armenia, Latvia, the Russian Federation	Paaver et al. (2008)
Essential oil of <i>Eucalyptus citriodora</i>	0.11	NR	%	2013	Benin	Gbenou et al. (2013)
Taperebá fruits	0.1–0.7	NR	% of volatiles	2002	Brazil	Ceva-Antunes et al. (2003)
Cajá fruits	38–41	NR	% of volatiles	2001	Brazil	Ceva-Antunes et al. (2003)

Table 1.1 (continued)

Product	Average concentration ^a	Range	Unit	Year ^b	Country or region	Reference
Essential oil of <i>Lingularia persica</i> Boiss.	0.5 (root) 2.0 (leaf) 2.8 (stem) 4.4 (flower)	NR	%	2012	Iran (Islamic Republic of)	Mohadjerani et al. (2016)
Essential oil of <i>Lavandula</i> L. species	0.3–7.5	NR	%	2012	Tunisia	Messaoud et al. (2012)
Essential oil of <i>Pistacia lentiscus</i> var. <i>chia</i>	8.34 (resin) 20.58 (leaves) 47.92 (twigs)	NR	%	1997	Greece	Magiatis et al. (1999)

^a Calculated by the Working Group if not provided in reference; values below limit of quantification were calculated as zero

^b Year of harvest/sampling; if not provided, year of publication

^c Ashanti black pepper (*Piper guineense*)

^d *Radix Angelica sinensis* and *Fructus aurantii*

NR, not reported; trace, traces below limit of quantification

Table 1.2 Selected methods for the analysis for β -myrcene

Sample matrix	Assay procedure	Limit of detection	Reference
Essential oils	GC-FID	NR	ISO (1998)
Beer	SBSE-GC/MS	0.001 μ g/L	Kishimoto et al. (2005)
Hops and beer	HS trap-GC/MS	NR	Aberl & Coelhan (2012) ; Schmidt & Biendl (2016)
Cheese	HS-SPME-GC/MS	NR	Giuseppe et al. (2005)
Herbs	GC/MS	NR	Gherman et al. (2000)
Liver pâtés	SPME-GC/MS	NR	Estévez et al. (2004)
Tropical fruits	SPME-GC/MS	NR	Ceva-Antunes et al. (2003)
Orange juice	HS-SPME-GC/MS	NR	Lachenmeier et al. (2006)
Mastic gum oil	FT-Raman spectroscopy	NR	Daferera et al. (2002)
Tangerines	GC-O	NR	Miyazaki et al. (2012)
Pomelos	TDS-GC/MS	NR	Shao et al. (2014)

FT, Fourier transform; GC-FID, gas chromatography-flame ionization detection; GC/MS, gas chromatography-mass spectrometry; GC-O, gas chromatography-olfactometry; HS, headspace; NR, not reported; SPME, solid-phase microextraction; SBSE, stir bar-sorptive extraction; TDS, thermal desorption system

Table 1.3 Concentration of β -myrcene in foods, medicinal products, and related products

Product	Average concentration ^a	Range	Unit	Year ^b	Country or region	Reference
Alcoholic beverages	1.12	Max. 5.00	ppm [$\mu\text{g/L}$]	1994	USA	HSDB (2012)
Baked goods	10.05	Max. 14.92	ppm	1994	USA	HSDB (2012)
Beer (bottled and canned, $n = 2$)	25.0	8.9–41.0	$\mu\text{g/L}$	2016	Germany	Wietstock et al. (2016)
Beer ($n = 2$)	62.7	45.6–79.7	$\mu\text{g/L}$	2016	USA and Germany	Schmidt & Biendl (2016)
Beer ($n = 3$)	0.7	0.4–1.1	ppb	2005	Japan	Kishimoto et al. (2005)
Bullock's heart fruit (<i>Annona reticulata</i> L.) ($n = 24$)	16.24	12.62–20.06	mg/kg	2003	Cuba	Pino et al. (2003)
Carrots (<i>Daucus carota</i> L.)	125.25	80.0–219.0	ng/g	1999	Denmark	Kjeldsen et al. (2003)
Chewing gum	116.2	Max. 126.00	ppm	1994	USA	HSDB (2012)
Condiments, relishes	5.00	Max. 10.00	ppm	1994	USA	HSDB (2012)
Dekopon peel (<i>Shiranihi mandarin</i>)	36.54	NR	mg/kg	2002	Japan	Umano et al. (2002)
Fennel fruits	1150	NR	$\mu\text{g/g}$	2006	Hungary	Zeller & Rychlik (2006)
Fennel tea (prepared)	140	NR	$\mu\text{g/L}$	2006	Hungary	Zeller & Rychlik (2006)
Frozen dairy	12.32	Max. 15.68	ppm	1994	USA	HSDB (2012)
Gelatins, puddings	19.96	Max. 22.91	ppm	1994	USA	HSDB (2012)
Hops ($n = 12$)	5489	2330–10 494	$\mu\text{g/g dw}$	2008	Germany	Aberl & Coelhan (2012)
<i>Houttuynia cordata</i> ($n = 13$)	138.0	57.68–271.2	$\mu\text{g/g}$	2010	China	Ji et al. (2011)
Italian lemon liquors (Limoncello) ($n = 12$)	12.2	3.0–31.0	mg/L	2003	Italy	Andrea et al. (2003)
Leaves and stalks of celery	31.5	8.0 (raw stalk)–73.0 (boiled leaves)	$\mu\text{g/kg}$	2006	Japan	Kurobayashi et al. (2006)
Mango	65.9	NR	$\mu\text{g/kg}$	2014	USA	Munafa et al. (2016)
Meat products	5.00	Max. 10.00	ppm	1994	USA	HSDB (2012)
Non-alcoholic beverages	7.72	Max. 11.15	ppm	1994	USA	HSDB (2012)
Soft candy	6.22	Max. 8.07	ppm	1994	USA	HSDB (2012)
Spanish pomegranates (<i>Punica granatum</i> L.), sour cultivars ($n = 2$)	0.01	0.01–0.01	g/kg	2009	Spain	Calín-Sánchez et al. (2011)
Spanish pomegranates (<i>Punica granatum</i> L.), sour-sweet cultivars ($n = 3$)	0.03	0.02–0.04	g/kg	2009	Spain	Calín-Sánchez et al. (2011)
Spanish pomegranates (<i>Punica granatum</i> L.), sweet cultivars ($n = 4$)	0.03	0.01–0.07	g/kg	2009	Spain	Calín-Sánchez et al. (2011)

^a Calculated by the Working Group if not provided in reference; values below limit of quantification were calculated as zero

^b Year of harvest/sampling. If not provided, year of publication.
dw, dry weight; NR, not reported

Table 1.4 Exposure to β-myrcene in the general population

Region, country Year	Exposure (integrated/mixed exposure data)			Reference
	Mean	Range	Comments	
USA Before 2008	3 µg/kg bw per day	NR	Estimated daily per capita intake for eaters only; calculation based on annual volume of 1338 kg	Adams et al. (2011)
Europe Before 1999	138 µg/kg bw per day	NR	Estimation based on data sources 1989–1999 and an annual production of 58076 kg	Pronk & Bend (2006)
USA Before 1999	3 µg/kg bw per day	NR	Estimation based on data sources 1989–1999 and an annual production of 1188 kg	Pronk & Bend (2006)
USA NR	2.966 µg/kg bw per day	NR	Individual consumption based on annual consumption of 3500 lb [1587.5 kg]	Burdock (2010)

bw, body weight; NR, not reported

10 ppt during the day, rising to ppb levels during the night ([Clement et al., 1990](#); [Janson, 1992](#)).

1.4.2 Exposure in the general population

The general population may be exposed to β-myrcene via ingestion of food and medicinal products containing β-myrcene, inhalation of ambient air in natural environments containing plants that emit β-myrcene, and dermal contact with products containing β-myrcene ([HSDB, 2012](#)). Inhalation exposure may also occur due to the emission of β-myrcene from various household products such as detergent, fabric deodorizer, or general purpose cleaner ([Kwon et al., 2007](#)). The United States consumer product information database lists five air freshener products containing β-myrcene ([Household Products Database, 2017](#)). [The Working Group noted that quantitative data for inhalation exposure, e.g. in households, were not available.]

Since β-myrcene is an approved food flavouring additive, the greatest potential for exposure lies in the consumption of foods that naturally contain β-myrcene or to which β-myrcene has been added ([HSDB, 2012](#)).

JECFA estimated daily intakes of β-myrcene of 138 µg/kg body weight (bw) in Europe and 3 µg/kg bw in the USA ([Pronk & Bend, 2006](#)). More recently, the Flavour and Extract Manufacturers Association estimated

daily per capita intake to be 3 µg/kg bw in the USA (assuming 10% of consumers of flavoured products only) ([Adams et al., 2011](#)), a value that is similar to other estimates ([Burdock, 2010](#); [Table 1.4](#)).

[The Working Group noted that the available information on exposure was based on estimated use of β-myrcene as a food additive, and did not include exposure resulting from the natural occurrence of β-myrcene in food and beverages. Total diet estimations were not available.]

1.4.3 Occupational exposure

The only estimate of the number of workers ($n = 25\,154$) exposed to this substance in the USA came from the National Occupational Exposure Survey (NOES) conducted in 1981–1983 ([ILS, 1997](#)).

In view of the extensive uses of β-myrcene, it is possible that workers may be exposed via dermal contact and inhalation ([NTP, 2010](#)).

[No data concerning exposure of workers were available to the Working Group].

1.5 Regulations and guidelines

The International Organization for Standardization (ISO) provided international standards with minimum and maximum percentages of β-myrcene in essential oils from various plant

Table 1.5 International standards regarding β -myrcene content in various plant essential oils

Common name	Botanical name	β -Myrcene content (% in essential oil) ^a		ISO norm no.
		Min.	Max.	
Bay	<i>Pimenta racemosa</i> (Mill.) JW Moore	20.0	30.0	3045:2004
Bergamot petitgrain	<i>Citrus bergamia</i> (Risso et Poit.)	1.2	1.8	8900:2005
Bitter fennel	<i>Foeniculum vulgare</i> Mill. ssp. <i>vulgare</i> var. <i>vulgare</i>	0.5	12.0	17 412:2007
Bitter orange	<i>Citrus aurantium</i> L.	1.5	3.0	9844:2006
Caraway	<i>Carum carvi</i> L.	0.2	0.7	8896:2016
Cardamom	<i>Elettaria cardamomum</i> (L.) Maton	Trace	2.5	4733:2004
Celery seed	<i>Apium graveolens</i> L.	0.3	1.4	3760:2002
Coriander fruits	<i>Coriandrum sativum</i> L.	0.5	1.5	3516:1997
Cumin seed	<i>Cuminum cyminum</i> L.	0.1	1.5	9301:2003
Dwarf pine	<i>Pinus mugo</i> Turra	3.0	11.0	21 093:2003
Galbanum	<i>Ferula galbaniflua</i> Boiss. et Buhse	2.5	3.5	14 716:1998
Grapefruit, obtained by expression	<i>Citrus x paradisi</i> Macfad.	1.5	2.5	3053:2004
Gum turpentine, Chinese	Mainly from <i>Pinus massoniana</i> Lamb.	Trace	1.5	21 389:2004
Juniper berry	<i>Juniperus communis</i> L.	3.0	22.0	8897:2010
Lavandin Grosso, French type	<i>Lavandula angustifolia</i> Mill. \times <i>Lavandula latifolia</i> Medik.	0.3	1.0	8902:2009
Lime (cold pressed), Mexican type	<i>Citrus aurantifolia</i> (Christm.) Swingle	1.0	2.0	3809:2004
Lime distilled, Mexican type	<i>Citrus aurantifolia</i> (Christm.) Swingle	1.1	1.5	3519:2005
Lime expressed, Persian type	<i>Citrus latifolia</i> Tanaka	1.2	2.0	23 954:2009
Mandarin, Italian type	<i>Citrus reticulata</i> Blanco	1.4	2.0	3528:2012
Molle, Argentinean type	<i>Schinus areira</i> L.	1.0	14.0	16 385:2014
Neroli	<i>Citrus aurantium</i> L., syn. <i>Citrus amara</i> Link, syn. <i>Citrus bigaradia</i> Loisel, syn. <i>Citrus vulgaris</i> Risso	1.0	4.0	3517:2012
Oregano	<i>Origanum vulgare</i> L. subsp. <i>hirtum</i> (Link) letsw	0.5	3.0	13 171:2016
Origanum, Spanish type	<i>Coridothymus capitatus</i> (L.) Rchb.f.	1.0	3.0	14 717:2008
Petitgrain, Paraguayan type	<i>Citrus aurantium</i> L. var. Paraguay (syn. <i>Citrus aurantium</i> var. <i>bigaradia</i> Hook f.)	1.3	3.0	3064:2015
Rosemary	<i>Rosmarinus officinalis</i> L.	1.0	4.5	1342:2012
Sweet orange	<i>Citrus sinensis</i> (L.) Osbeck, obtained by physical extraction of the peel	1.5	3.5	3140:2011
Thyme containing thymol, Spanish type	<i>Thymus zygis</i> (Loefl.) L.	1.0	2.8	14 715:2010
Turpentine, Iberian type	<i>Pinus pinaster</i> Sol.	0.4	1.5	11 020:1998

^a The widest possible minimum–maximum range is specified when the norm contained data on several subtypes
ISO, International Organization for Standardization
All ISO norms from ISO Standards ([ISO, 2017](https://www.iso.org/))

species; these oils are widely used in the food and perfumery industries ([ISO, 2017](#); [Table 1.5](#)).

β-Myrcene has been approved as a food additive by the United States Food and Drug Administration (FDA) ([Behr & Johnen, 2009](#)). According to FDA regulations, β-myrcene may be used as a flavouring substance or adjuvant in food in its natural form in essential oils (Code of Federal Regulations (CFR) 21, § 172.510), and as a synthetic substance (CFR 21, § 172.515) ([NTP, 2010](#)).

In 1974, the European Council included β-myrcene in the list of artificial flavouring substances that may be added to foodstuffs ([Behr & Johnen, 2009](#)), and β-myrcene is included in the most recent list of approved flavouring substances in the European Union according to Regulation No. 872/2012 ([European Commission, 2012](#)).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Oral administration

The results of studies of carcinogenicity in mice and rats treated with β-myrcene by gavage are summarized in [Table 3.1](#) ([NTP, 2010](#)).

3.1.1 Mouse

Groups of 50 male and 50 female B6C3F₁ mice (age, 6–7 weeks) were given β-myrcene (purity, > 93%; impurity: *psi*-limonene, CAS No. 499-97-8, approx. 5%) at a dose of 0 (control), 0.25, 0.5, or 1 g/kg bw by gavage in corn oil, 5 days per week for 105 (males) or 104 (females) weeks ([NTP, 2010](#)). [The Working Group noted that this was a study of commercially available β-myrcene

with a purity of > 93%. The major contaminant was *psi*-limonene and there had been no studies of carcinogenicity with this compound].

Survival of male and female mice at 1 g/kg bw was significantly lower than that of mice in the vehicle-control groups: males: 35/50 (control), 35/50, 31/50, 21/50; females: 39/50 (control), 34/50, 35/50, 17/50. The cause of the early deaths was not determined. Mean body weights of males at 1 g/kg bw, females at 0.5 g/kg bw, and females at 1 g/kg bw were less than those of controls after weeks 8, 17, and 11, respectively. Because of the number of early deaths in male and female mice at 1 g/kg bw, these groups were not considered to contain enough animals for the carcinogenesis analysis, and were not included in the statistical evaluation for the treatment-related development of tumours.

In treated male mice, there were significant increases in the incidence of epithelial hepatocellular neoplasm and of hepatoblastoma [an embryonal tumour of the liver cells], with a significant positive trend for each. These included increases in the incidence of: hepatocellular adenoma (multiple); hepatocellular adenoma (including multiple); hepatocellular carcinoma (multiple); hepatocellular carcinoma (including multiple); and hepatoblastoma (including multiple). The incidence of the combination of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma was also significantly increased, with a significant positive trend. In female mice, there were also increases in the incidence of hepatocellular tumours, but to a lesser extent than in male mice. The incidence of hepatocellular adenoma (including multiple), hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) was significantly increased at the lowest dose, without a significant positive trend.

[The Working Group noted this was a well-conducted study that complied with good laboratory practice (GLP), and was carried out in males and females. The Working Group also

Table 3.1 Studies of carcinogenicity in experimental animals treated with β -myrcene by gavage

Species, strain (sex) Age at start Duration Reference	Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Mouse, B6C3F ₁ (M) 6–7 wk 105 wk NTP (2010)	Purity, > 93% Corn oil 0, 0.25, 0.5 g/kg bw 5 d/wk for 105 wk 50, 50, 50 35, 35, 31	<i>Liver</i> Hepatocellular adenoma (multiple): 15/50*, 31/50**, 30/50** Hepatocellular adenoma (includes multiple): 26/50*, 41/50**, 43/50** Hepatocellular carcinoma (multiple): 1/50*, 4/50, 9/50** Hepatocellular carcinoma (includes multiple): 14/50*, 20/50, 28/50** Hepatocellular adenoma or carcinoma (combined): 33/50*, 44/50**, 48/50*** Hepatoblastoma: 4/50*, 6/50, 11/50** Hepatocellular adenoma, carcinoma, or hepatoblastoma: 34/50*, 45/50**, 48/50***	 * $P = 0.002$ (trend, Cochran-Armitage test)], ** $P \leq 0.01$ (poly-3 test) * $P < 0.001$ (trend, poly-3 test), ** $P < 0.001$ (poly-3 test) * [$P = 0.024$ (trend, Cochran-Armitage test)], ** $P \leq 0.01$ (poly-3 test) * $P = 0.003$ (trend, poly-3 test), ** $P = 0.004$ (poly-3 test) * $P < 0.001$ (trend, poly-3 test), ** $P = 0.003$, *** $P < 0.001$ (poly-3 test) * $P = 0.027$ (trend, poly-3 test), ** $P = 0.041$ (poly-3 test) * $P < 0.001$ (trend, poly-3 test), ** $P = 0.003$, *** $P < 0.001$ (poly-3 test) test)	Principal strengths: GLP study; study in males and females The dose of 1 g/kg bw was tested but not used for tumour analysis due to early death and effect on body weight

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Mouse, B6C3F ₁ (F) 6–7 wk 104 wk NTP (2010)	Purity, > 93% Corn oil 0, 0.25, 0.5 g/kg bw 5 d/wk for 104 wk 50, 50, 50 39, 34, 35	<i>Liver</i> Hepatocellular adenoma (multiple): 0/50, 2/50, 0/50 Hepatocellular adenoma (includes multiple): 6/50, 13/50*, 6/50 Hepatocellular carcinoma: 1/50, 7/50*, 2/50 Hepatocellular adenoma or carcinoma (combined): 7/50, 18/50*, 8/50	NS *P = 0.042 (poly-3 test) *P = 0.025 (poly-3 test) *P = 0.005 (poly-3 test)	Principal strengths: GLP study; study in males and females The dose of 1 g/kg bw was tested but not used for tumour analysis due to early death and effect (decrease) on body weight
Rat, F344/N (M) 5–6 wk 105 wk NTP (2010)	Purity, > 93% Corn oil 0, 0.25, 0.5 g/kg bw 5 d/wk for 105 wk 50, 50, 50 29, 36, 28	<i>Kidney, standard (single section) evaluation:</i> Renal tubule adenoma (multiple): 0/50, 2/50, 1/50 Renal tubule adenoma (includes multiple): 0/50*, 4/50, 8/50** Renal tubule carcinoma: 0/50, 3/50, 1/50 Renal tubule adenoma or carcinoma (combined): 0/50*, 7/50**, 9/50*** <i>Kidney, extended evaluation (step sections)</i> Renal tubule adenoma: 0/50*, 8/50**, 7/50***	NS *P = 0.002 (trend, poly-3 test), **P = 0.003 (poly-3 test) NS *P = 0.002 (trend, poly-3 test), **P = 0.010, ***P = 0.002 (poly-3 test) *P = 0.013 (trend, poly-3 test), **P = 0.005, ***P = 0.007 (poly-3 test)	Principal strengths: GLP study; study in males and females The dose of 1 g/kg bw was tested but not used for tumour analysis due to early death and effect (decrease) on body weight Historical control incidence for renal tubule carcinoma (single section): gavage studies, 0/150; all routes, 1/1394 (0.1% ± 0.5%) [range, 0–2%]

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Rat, F344/N (M) 5–6 wk 105 wk NTP (2010) (cont.)		Renal tubule carcinoma: 0/50, 3/50, 0/50	NS	
		Renal tubule adenoma or carcinoma (combined): 0/50*, 10/50**, 7/50***	* <i>P</i> = 0.017 (trend, poly-3 test), ** <i>P</i> < 0.001, *** <i>P</i> = 0.007 (poly-3 test)	
		<i>Kidney, standard (single section) evaluation and extended evaluation (step sections) (combined)</i> Renal tubule adenoma: 0/50*, 12/50**, 13/50**	* <i>P</i> < 0.001 (trend, poly-3 test), ** <i>P</i> < 0.001 (poly-3 test)	
		Renal tubule carcinoma: 0/50, 3/50, 1/50 Renal tubule adenoma or carcinoma (combined): 0/50*, 14/50**, 13/50**	NS * <i>P</i> < 0.001 (trend, poly-3 test), ** <i>P</i> < 0.001 (poly-3 test)	
Rat, F344/N (F) 5–6 wk 105 wk NTP (2010)	Purity, > 93% Corn oil 0, 0.25, 0.5, 1 g/kg bw 5 d/wk for 105 wk 50, 50, 50, 50 31, 33, 28, 33	<i>Kidney, standard (single section) evaluation</i> Renal tubule adenoma: 0/50, 1/50, 0/50, 2/50 <i>Kidney, standard (single section evaluation) and extended evaluation (step sections) (combined)</i> Renal tubule adenoma: 0/50, 2/50, 1/50, 3/50	NS NS	Principal strengths: GLP study; study in males and females Historical control incidence for renal tubule adenoma (single section): gavage studies, 0/150; all routes, 1/1340 (0.1% ± 0.4%) [range, 0–2%]

bw, body weight; d, days; F, female; GLP, good laboratory practice; M, male; NS, not significant; wk, week

noted the poor survival of male and female mice at the highest dose.]

3.1.2 Rat

Groups of 50 male and 50 female F344/N rats (age, 5–6 weeks) were given β-myrcene (purity, >93%; impurity: *psi*-limonene, CASNo. 499-97-8, approx. 5%) at a dose of 0 (control), 0.25, 0.5, or 1 g/kg bw by gavage in corn oil, 5 days per week for 105 weeks (NTP, 2010). All males in the group at 1 g/kg bw died before the end of the study as a result of renal toxicity, and this group was not included in the statistical evaluation for the treatment-related development of tumours (survival in males: 29/50 (control), 36/50, 28/50, 0/50). The mean body weights of males and females at 1 g/kg bw were less than those of controls after weeks 7 and 13, respectively. Survival of female rats was considered adequate for all exposed groups, and all three dose levels were included in the statistical analysis for tumour incidence.

Tumours of the renal tubules were seen in male and female treated rats; this tumour response was stronger in males than in females. The incidence of renal tubule adenoma in male rats at 0.5 g/kg bw was significantly increased, with a significant positive trend, compared with controls, and the incidence of renal tubule adenoma or carcinoma (combined) was significantly increased, with a significant positive trend, in male rats at 0.25 and 0.5 g/kg bw. These increases in the incidence of renal tubule tumours were confirmed by the extended evaluation (step section) of the kidneys.

According to the standard (single section) evaluation of the male rat kidney, the incidence of renal tubule tumours was: renal tubule adenoma: 0/50 (control), 4/50, 8/50; renal tubule carcinoma: 0/50 (control), 3/50 (6%), 1/50 (2%); and renal tubule adenoma or carcinoma (combined): 0/50 (control), 7/50, 9/50. According to the extended (step section) evaluation of the male rat kidney, the incidence of renal tubule tumours was: renal tubule adenoma: 0/50 (control), 8/50, 7/50; renal

tubule carcinoma: 0/50 (control), 3/50, 0/50; and renal tubule adenoma or carcinoma (combined): 0/50 (control), 10/50, 7/50. According to the original (single section) and extended evaluation (step sections) (combined) of the male rat kidney, the incidence of renal tubule tumours was: renal tubule adenoma: 0/50 (control), 12/50, 13/50; renal tubule carcinoma: 0/50 (control), 3/50, 1/50; and renal tubule adenoma or carcinoma: 0/50 (control), 14/50, 13/50. In male rats, the historical incidence (mean ± standard deviation) of renal tubule carcinoma (single section) for gavage studies was: 0/150; all routes: 2/1394 (0.1% ± 0.5%); range, 0–2%.

The evaluations of the female rat kidney also demonstrated a treatment-related carcinogenic effect. In the standard (simple section) evaluation of the female rat kidney, the incidence of renal tubule adenoma (including multiple) was: 0/50 (control), 1/50 (2%), 0/50, 2/50 (4%). According to the original (single section) and extended evaluation (step sections) (combined) of the female rat kidney, the incidence of renal tubule adenoma (including multiple) was: 0/50 (control), 2/50, 1/50, and 3/50. In female rats, the historical incidence (mean ± standard deviation) of renal tubule adenoma (single section) for oral gavage studies was: 0/150; all routes, 1/1340 (0.1% ± 0.4%); range, 0–2%. [Thus, the 4% incidence of renal tubule adenoma (single section) in female rats at 1 g/kg bw was considered by the Working Group to be related to treatment with β-myrcene.] No malignant tumours of the kidney occurred in the treated groups of female rats.

Renal toxicity was seen in treated male and female rats, as demonstrated by the occurrence of several non-neoplastic kidney lesions. The incidence of renal tubule nephrosis was increased in groups of treated male and female rats. In addition, the incidence of papillary mineralization in treated male rats was increased. Nephropathy was increased in all groups of treated female rats. The incidence of hyperplasia of the transitional epithelium lining of the pelvis and overlying

the renal papilla was significantly increased in all treated groups of male and female rats. The incidence of focal suppurative inflammation was increased in treated male rats ([NTP, 2010](#)). [The Working Group noted that this was a well-conducted study that complied with GLP, and was carried out in males and females. The Working Group also noted the poor survival of male rats at the highest dose.]

3.2 Co-carcinogenicity studies

In a study on the chemopreventive effects of terpenoids (including β -myrcene) ([Russin et al., 1989](#)), groups of female Sprague-Dawley rats (age, 6 weeks) were fed diets containing β -myrcene (purity, 94.3%) at a concentration of 0% (control, $n = 31$) or 1% ($n = 32$) for up to 20 weeks. At week 2, the rats were given a single gavage dose of 7,12-dimethylbenz[*a*]anthracene (DMBA) at 65 mg/kg bw. Starting from 5 weeks after treatment with DMBA, the rats were palpated for mammary tumours at weekly intervals until week 20. All tumours were processed for histopathology (more than 95% were mammary carcinomas). There was a total of 81 mammary tumours in the DMBA-only control group (average, 2.6 mammary tumours per rat; mean tumour latency period, 70 days) versus 72 mammary tumours (average, 2.3 mammary tumours per rat; mean tumour latency period, 77 days) in the group treated with DMBA plus β -myrcene). While the number of tumours in the group treated with DMBA plus β -myrcene was lower than in the control group, this effect was not significant when using a χ^2 test adjusted for the total number of days at risk. β -Myrcene did not significantly extend tumour latency ([Russin et al., 1989](#)). [The Working Group noted that this was a study of chemoprevention and not a study of carcinogenicity.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

No data from exposed humans were available to the Working Group.

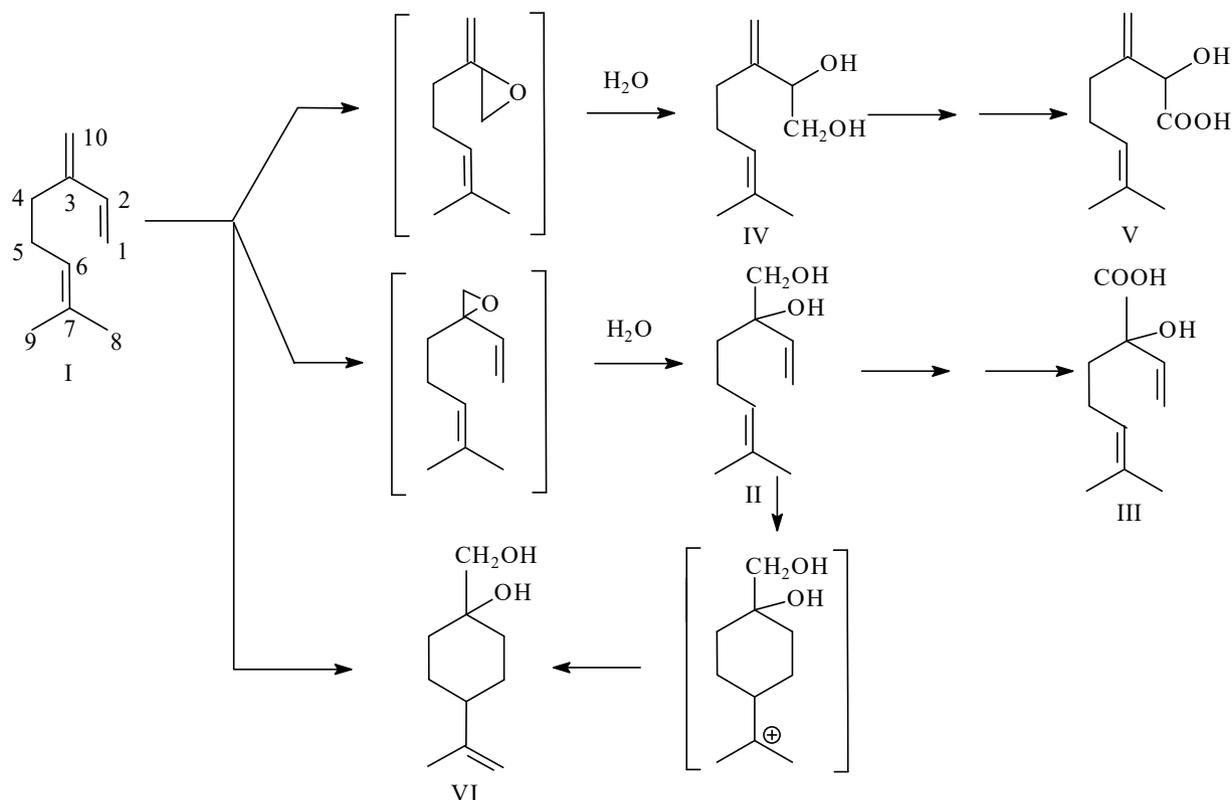
A study in vitro showed that β -myrcene permeates human skin ([Schmitt et al., 2009](#)). In a model of human intestinal absorption of xenobiotic compounds in vitro (human colon epithelial cancer cell line/Caco-2 cell monolayer), β -myrcene quickly established an equilibrium state of efflux and uptake by cells under the static conditions of the test system ([Heinlein et al., 2014](#)).

4.1.2 Experimental animals

(a) Absorption, distribution, and excretion

Few published data on the absorption, distribution, and excretion of β -myrcene in experimental animals were available to the Working Group. A study in rats indicated ready absorption through intact skin ([Valette & Cavier, 1954](#)). In rabbits and rats, β -myrcene is well absorbed after oral administration. Approximately 25% of the total dose (670 mg/kg bw per day for 2 days, by gavage) administered to male Japanese white rabbits was recovered in urine excreted over a period of 3 days after treatment ([Ishida et al., 1981](#)). In female rats treated orally with β -myrcene (1000 mg/kg bw, by gavage), blood concentrations as high as 14.1 ± 3.1 $\mu\text{g/mL}$ were detected 60 minutes after treatment ([Delgado et al., 1993a](#)). In the same study, the elimination half-life of β -myrcene was 285 minutes, and the parent compound was concentrated in the adipose tissue and in organs including the brain, liver, kidney, and testis.

Fig. 4.1 Characterized metabolites of β-myrcene



I, β-myrcene; II, 10-hydroxylylinalool; III, 10-carboxylinalool; IV, 7-methyl-3-methylene oct-6-ene-1,2-diol; V, 2-hydroxy-7-methyl-3-methylene oct-6-enoic acid, VI, 1-hydroxymethyl-4-isopropenyl cyclohexanol

Source: [Madyastha & Srivatsan \(1987\)](#). Metabolism of β-myrcene in vivo and in vitro: its effects on rat-liver microsomal enzymes, Madyastha KM, Srivatsan V, *Xenobiotica*, 1987, Taylor & Francis, by permission of the publisher (Taylor & Francis Ltd, <http://www.tandfonline.com>).

Urine was the predominant route of excretion of conjugated myrcene glycol/diol metabolites in rats and rabbits ([Ishida et al., 1981](#)). No studies examined the possibility of biliary excretion.

(b) Metabolism

The biotransformation of β-myrcene was studied in rabbits and rats (see [Fig. 4.1](#)). In male rabbits treated by gavage with β-myrcene, urinary excretion of the conjugates of two diols (10-hydroxylylinalool and 7-methyl-3-methylene-oct-6-ene-1,2-diol) was observed. Their formation involved the corresponding epoxides as intermediates, and subsequent production of two hydroxyl acids (10-carboxylinalool and

2-hydroxy-7-methyl-3-methylene-oct-6-enoic acid) ([Ishida et al., 1981](#); [Ishida, 2005](#)). Like in rabbits, male rats treated by gavage with β-myrcene (800 mg/kg bw per day) for 20 days excreted 10-hydroxylylinalool, 7-methyl-3-methylene-oct-6-ene-1,2-diol, 1-hydroxymethyl-4-isopropenyl cyclohexanol, 10-carboxylinalool, and 2-hydroxy-7-methyl-3-methylene-oct-6-enoic acid in the urine ([Madyastha & Srivatsan, 1987](#)).

A similar pattern of biotransformation of β-myrcene was also observed in vitro with rat liver microsomal fraction ([Madyastha & Srivatsan, 1987](#)). The conversion of β-myrcene into 10-hydroxylylinalool by rat liver microsomes was inhibited by several nonspecific inhibitors

of cytochrome P450 (CYP) (e.g. metyrapone, carbon monoxide, SK-525A, and *para*-chloromercuric benzoate). This indicated that the apparent oxidation of the β -myrcene carbon-carbon double bond to a 3,10-epoxide intermediate, which after hydrolysis gives rise to the corresponding 3,10-diol, is a CYP-catalysed reaction. Moreover, a higher rate of conversion of β -myrcene into 10-hydroxylinalool was seen using liver microsomal fractions from rats treated with phenobarbital than with liver microsomal fractions from rats that had or had not been treated with 3-methylcholanthrene, indicating that β -myrcene is preferentially metabolized by phenobarbital-inducible CYP forms (e.g. CYP2B) ([Madyastha & Srivatsan, 1987](#)).

The main urinary metabolites of orally administered β -myrcene found in the urine of rabbits and rats (after enzymatic hydrolysis of conjugates by treatment of urine samples with β -glucuronidase/arylsulfatase) were 10-hydroxylinalool and 7-methyl-3-methylene-oct-6-ene-1,2-diol (or myrcene-3,10-glycol and 1,2-glycol, respectively), formed by hydrolysis of the respective 3,10- and 1,2- epoxide intermediates. In both species, the epoxidation of the 3,10 carbon-carbon double bond was apparently favoured over epoxidation of the 1,2 double bond, while epoxidation of the 6,7 double bond was not observed. Further oxidation of β -myrcene primary metabolites (diols) to carboxylic acids and cyclization products was also noted in rabbits and rats ([Ishida et al., 1981](#); [Madyastha & Srivatsan, 1987](#); [Ishida, 2005](#)). Formation of a single covalent bond linking carbons 1–6 in the β -myrcene acyclic structure results in ring closure and excretion of 1-hydroxymethyl-4-isopropenyl cyclohexanol (*para*-menth-8-ene-1,7-diol) or uroterpenol (4-menth-1-ene-8,9-diol) as a minor metabolite in the rat and rabbit urine, respectively ([Ishida et al., 1981](#); [Madyastha & Srivatsan, 1987](#)).

4.2 Mechanisms of carcinogenesis

4.2.1 Genetic and related effects

The genotoxic potential of β -myrcene has been studied in different assays in vitro and in vivo that gave consistently negative results. [Table 4.1](#) summarizes studies carried out in non-human mammals in vivo, and [Table 4.2](#) summarizes studies in human cells and in various experimental systems in vitro.

(a) Humans

No data from exposed humans were available to the Working Group.

In lymphocytes isolated from nonsmoking donors (one male and one female), β -myrcene (100, 500, or 1000 $\mu\text{g/mL}$), did not induce chromosome aberrations or sister-chromatid exchange ([Kauderer et al., 1991](#)). β -Myrcene did not alter mitotic or proliferation indices.

(b) Experimental systems

(i) Non-human mammals in vivo

No changes in the incidence of metaphase cells with chromosome aberrations were detected in the bone marrow of male and female Wistar rats sampled 24 or 48 hours after oral administration of β -myrcene (0.1, 0.5, or 1.0 g/kg bw). Although not clastogenic, β -myrcene caused a dose-dependent increase in the mitotic index in bone marrow cells, indicating that the dose present in the target tissue was sufficient ([Zamith et al., 1993](#)).

No increase in the frequency of micronucleated normochromatic erythrocytes was noted at any dose level in mouse peripheral blood sampled within 24 hours after administration of the final dose in a 13-week study in which male and female B6C3F₁ mice were treated with β -myrcene (250–2000 mg/kg bw per day) by gavage ([NTP, 2010](#)).

Table 4.1 Genetic and related effects of β-myrcene in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results ^a	Dose (LED or HID)	Route, duration, dosing regimen	Reference
Chromosomal aberrations	Rat, Wistar, (M and F)	Bone marrow	–	1000 mg/kg bw	Gavage, 1×	Zamith et al. (1993)
Micronucleus formation	Mouse, B6C3F ₁ (M and F)	Peripheral blood	–	1000 mg/kg bw per day	Gavage, 13 weeks	NTP (2010)

^a –, negative; the level of significance was set at $P < 0.05$ in all cases
bw, body weight; F, female; HID, highest ineffective dose; LED, lowest effective dose; M, male

Table 4.2 Genetic and related effects of β-myrcene in experimental systems in vitro

End-point	Species, tissue, cell line	Results ^a		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
<i>Hprt</i> mutation	Chinese hamster, lung, V79	–	–	1000 µg/mL		Kauderer et al. (1991)
Reverse mutation	<i>Salmonella typhimurium</i> , TA97a, TA98, TA100, TA1535	–	–	5000 µg/plate (–S9) 1500 µg/plate (+S9)		Gomes-Carneiro et al. (2005)
Reverse mutation	<i>Salmonella typhimurium</i> , TA97, TA98, TA100, TA1535, <i>Escherichia coli</i> WP2 <i>uvrA</i>	–	–	10 000 µg/plate		NTP (2010)
Chromosomal aberrations, sister-chromatid exchange	Human, lymphocytes	–	–	1000 µg/mL		Kauderer et al. (1991)
Sister-chromatid exchange	Chinese hamster, lung, V79	–	–	500 µg/mL		Röscheisen et al. (1991)
Sister-chromatid exchange	Rat, hepatocellular carcinoma, HTC cells	±	±	100 µg/mL	Slight, reproducible increase, not concentration-related	Röscheisen et al. (1991)

^a –, negative; ±, equivocal, variable response in several experiments within an adequate study; the level of significance was set at $P < 0.05$ in all cases
HIC, highest ineffective concentration; LEC, lowest effective concentration; S9, 9000 × g supernatant

(ii) Non-human mammalian cells in vitro

β -Myrcene did not increase mutation frequencies at the hypoxanthine-guanine phosphoribosyl transferase (*Hprt*) locus or induce sister-chromatid exchange in hamster V79 cells, in the absence or presence of metabolic activation (Kauderer et al., 1991; Röscheisen et al., 1991). In a metabolically competent rat hepatocellular carcinoma cell line, β -myrcene produced a slight increase in sister-chromatid exchange at 100–250 $\mu\text{g/mL}$, but with no concentration–response relationship (Röscheisen et al., 1991).

(iii) Non-mammalian systems

In bacterial test systems, β -myrcene was not mutagenic. Two assays conducted by the National Toxicology Program (NTP) did not reveal any mutagenic activity with β -myrcene (doses ranging from 33 up to 10 000 $\mu\text{g/plate}$) in any of the *Salmonella typhimurium* strains tested (TA97, TA98, TA100, and TA1535) or in *Escherichia coli* (strain WP2 *uvrApKM101*), either in the presence or in the absence of exogenous metabolic activation (S9 fraction from Aroclor 1254-induced rat or hamster liver) (NTP, 2010). In another study, β -myrcene (10–5000 $\mu\text{g/plate}$, without metabolic activation; 1–1500 $\mu\text{g/plate}$, with metabolic activation) gave negative results in four *S. typhimurium* strains (TA100, TA98, TA97a and TA1535) (Gomes-Carneiro et al., 2005).

4.2.2 Oxidative stress

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

No study reported β -myrcene-mediated enhancement of oxidative stress in mammalian cells or tissues. Several experimental studies, however, have provided evidence that β -myrcene has antioxidant activity. In the liver of female Sprague-Dawley rats treated by gavage with

β -myrcene at a dose of up to 200 mg/kg bw per day for 30 or 60 days, there was an increase in the levels of reduced glutathione, and increases in the activities of catalase, glutathione peroxidase, and superoxide dismutase, as well as a decline in the formation of thiobarbituric acid reactive substances (lipid peroxidation) (Ciftci et al., 2011a). Moreover, Ciftci et al. (2011a) also demonstrated that concomitant administration of β -myrcene counteracted the enhancement of oxidative stress mediated by 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (2 $\mu\text{g/kg}$ bw per week by gavage) in the rat liver. Another study suggested that oral administration of β -myrcene (7.5 mg/kg bw) in male Wistar rats protected against ethanol-induced gastric ulcers, and increased the activities of glutathione reductase and glutathione peroxidase, while decreasing levels of malondialdehyde in the gastric tissue (Bonamin et al., 2014). A study in C57Bl/J6 mice showed that β -myrcene (200 mg/kg bw per day, intraperitoneal dose), given for 10 days after transient surgical occlusion of the carotid artery, attenuated the cerebral ischaemia and reperfusion-mediated enhancement of oxidative stress in brain tissue (increase in the formation of thiobarbituric acid reactive substances, and decrease in glutathione levels and activities of glutathione peroxidase and superoxide dismutase), and also attenuated the increase in incidence of histopathological damage and apoptosis induced by ischaemia (Ciftci et al., 2014).

4.2.3 Inflammation and immunosuppression

(a) Humans

No data in exposed humans were available to the Working Group.

In a primary culture of human chondrocytes, β -myrcene (25–50 $\mu\text{g/mL}$) decreased interleukin IL-1 β -induced nuclear factor- κB (NF- κB), jun terminal kinase (JNK) and p38 activation, and the expression of inflammatory inducible nitric oxide synthase (*iNOS*) and catabolic genes

(matrix metalloprotease *MMP1* and *MMP13*), while increasing the expression of anti-catabolic genes (tissue inhibitor of metalloproteases *TIMP1* and *TIMP3*) ([Rufino et al., 2015](#)).

(b) Experimental systems

β-Myrcene and nine other monoterpenoid compounds found in essential oils were tested in the rat popliteal lymph node assay (PLNA), a screening test for allergic and autoimmune-like reactions in humans ([Friedrich et al., 2007](#)). In the primary (direct) PLNA, β-myrcene induced a clear (positive) immuno-stimulatory response due to its irritant properties, but it gave a negative result and proved not to be a sensitizing agent in the secondary PLNA (a T-cell priming test) ([Friedrich et al., 2007](#)). In female Wistar rats, oral administration of β-myrcene (200 mg/kg bw per day, for 30 or 60 days) reduced (flow cytometric analysis) the percentage of CD8⁺ cells in the blood, while increasing the percentages of CD3⁺, CD4⁺, CD161⁺, CD45RA, CD4⁺CD25⁺, and the populations of total lymphocyte cells ([Ciftci et al., 2011b](#)). In the same study, β-myrcene (200 mg/kg bw per day) counteracted the immunosuppressive effects induced by 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (2 µg/kg bw per week by gavage) when administered concomitantly ([Ciftci et al., 2011b](#)).

In BALB/c mice, β-myrcene (0.8 mg/dose, injected intraperitoneally) mixed with ovalbumin or Ag85B (a protective antigen for tuberculosis) enhanced the specific antibody response to immunization with ovalbumin or Ag85B. Administration of β-myrcene alone did not enhance levels of T-helper Th1 and Th2 cytokines, nor did it cause any increase in immunoglobulin IgG subtypes ([Uyeda et al., 2016](#)).

4.2.4 Other mechanisms

In human hepatoma HepG2 cells, β-myrcene (7.4 µM) did not alter the process of repair of *tert*-butyl hydroperoxide-induced DNA damage,

as shown by data from the alkaline comet assay, performed every 30 minutes for 2.5 hours ([Mitić-Culafić et al., 2009](#)).

β-Myrcene (100, 500, or 1000 mg/kg bw, by gavage) caused a dose-dependent increase in the mitotic index in Wistar rat bone marrow cells ([Zamith et al., 1993](#)).

In cell culture, β-myrcene (1 mM and 3 mM) was more potent than limonene in inhibiting protein isoprenylation, an effect positively correlated with inhibition of cell proliferation ([Crowell et al., 1994](#)). Nonetheless, in a model of DMBA-induced mammary carcinogenesis, β-myrcene and other acyclic monoterpenes (in contrast to limonene-like monocyclic monoterpenes) did not extend mammary tumour latency and did not reduce the total number of mammary tumours in Sprague-Dawley rats fed a diet containing β-myrcene (1%) when compared with controls ([Russin et al., 1989](#)).

4.3 Data relevant to comparisons across agents and end-points

For the results of high-throughput screening assays of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA, see Section 4.3 of the *Monograph* on 1-*tert*-butoxypropan-2-ol in the present volume.

4.4 Susceptibility to cancer

No data were available to the Working Group.

4.5 Other adverse effects

4.5.1 Humans

No data were available to the Working Group.

4.5.2 Experimental systems

In male and female Wistar rats treated orally with β -myrcene for 91 days, the highest dose (500 mg/kg bw, by gavage) induced small (approximately 10%) increases in liver and kidney weights (Paumgartten et al., 1998). In male and female F344/N rats, a 14-week (GLP-compliant) study of toxicity with β -myrcene found dose-related increases in liver and kidney weights. Renal tubule necrosis, the severity of which increased in a dose-dependent manner, was augmented in all treated groups compared with control groups. The incidence of nephrosis (restricted to the outer stripe of the outer medulla) was higher in rats treated with doses of > 1000 mg/kg bw (NTP, 2010). In B6C3F₁ mice, β -myrcene (up to 1000 mg/kg bw per day, by gavage, for 14 weeks) increased liver weight in males (up to 17%) and females (up to 21%), and also increased kidney weight in females (18%) (NTP, 2010).

IARC has established seven criteria that need to be fully met in order to conclude that an agent induces tumours of the kidney by a α_{2u} -globulin-associated response (IARC, 1999). Three criteria were met for the present agent, specifically: (1) induction of the characteristic sequence of histopathological changes associated with α_{2u} -globulin accumulation; (2) identification of the accumulating protein as α_{2u} -globulin (Cesta et al., 2013); and (3) absence of genotoxicity (see Section 4.2.1). However, four of these criteria were not met for β -myrcene (NTP, 2010), specifically: (1) male rat specificity for nephropathy and renal tumorigenicity (tumours and nephropathy were induced by β -myrcene in female rats); (2) reversible binding of the chemical or metabolite to α_{2u} -globulin (no data were available on the binding of β -myrcene or its metabolites to α_{2u} -globulin); (3) induction of sustained increase in cell proliferation in the renal cortex was not demonstrated; and (4) similarities in dose-response relationships of the tumour outcome with histopathological end-points associated

with α_{2u} -globulin nephropathy (hyaline droplets were not seen at the highest dose, and α_{2u} -globulin protein was not quantified).

5. Summary of Data Reported

5.1 Exposure data

β -Myrcene is found in a wide variety of plants. It is not commonly extracted from natural materials, but is generally manufactured via the pyrolysis of β -pinene. The main use of β -myrcene is as a raw material in the manufacture of other chemicals such as menthol, although it is also used as a flavouring material in foods and cosmetics. Reliable information about global production volume was not available, but less than 100 tonnes were reported to be manufactured or imported into the European Union. The general population is mainly exposed by ingestion of foods and medicinal products containing β -myrcene, either from plant ingredients or manufactured additives. The estimated human intake from food additives is 3–138 μ g/kg bw per day. People may also be exposed by inhalation of air in forests and other natural environments containing plants that emit β -myrcene, and from inhalation of and dermal contact with consumer products containing β -myrcene. Workers may be exposed to β -myrcene by inhalation and dermal contact.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

In one well-conducted study that complied with good laboratory practice (GLP) in male and female mice treated by gavage, β -myrcene caused a significant increase, with a significant

positive trend, in the incidence of hepatocellular adenoma, hepatocellular carcinoma, hepatocellular adenoma or carcinoma (combined), hepatoblastoma, and the combination of these three tumours in males; and a significant increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) in females.

In a well-conducted GLP study in male and female rats treated by gavage, β-myrcene caused a significant increase, and a significant positive trend, in the incidence of renal tubule adenoma, and renal tubule adenoma or carcinoma (combined) in males; and rare renal tubule adenomas were also observed in treated females.

A study in rats given β-myrcene in combination with 7,12-dimethylbenz[*a*]anthracene gave negative results.

5.4 Mechanistic and other relevant data

In rabbits and rats, β-myrcene is well absorbed after oral administration, being converted into conjugated metabolites found in the urine. The parent compound undergoes oxidation by cytochrome P450 2B to 1,2- and 3,10-epoxide intermediates, with subsequent hydrolysis to diols.

No data on the absorption, metabolism, distribution, or excretion of β-myrcene in humans were available.

With respect to the key characteristics of carcinogens, it was consistently demonstrated in bacterial and mammalian assays, including tests *in vivo* and *in vitro*, that β-myrcene is not genotoxic.

Few other data on the key characteristics were available. Experimental studies demonstrated antioxidant activity.

In a long-term bioassay in rodents, the primary toxic effects were seen in the kidney. Four of the seven criteria established by IARC for

concluding that an agent induces tumours of the kidney by an α_{2u}-globulin-associated response have not been met.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of β-myrcene.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of β-myrcene.

6.3 Overall evaluation

β-Myrcene is *possibly carcinogenic to humans* (Group 2B).

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FURFURYL ALCOHOL

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 98-00-0

Chem. Abstr. Serv. name: Furanmethanol

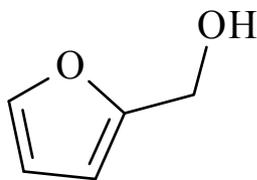
EC name: Furfuryl alcohol

IUPAC systematic name: 2-Furylmethanol

Synonyms: 2-Furanmethanol, furfurol, 2-(hydroxymethyl)furan, 2-furylcarbinol; 2-furancarbinol; α -furylcarbinol; furfuralcohol

From [NTP \(1999\)](#); [European Commission \(2011\)](#)

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: C₅H₆O₂

Relative molecular mass: 98.10 ([NTP, 1999](#))

1.1.3 Chemical and physical properties

Description: Furfuryl alcohol is a colourless or pale yellow liquid with characteristic “burning” odour and bitter taste; it turns red or brown on exposure to light and air

Boiling point: 170 °C

Melting point: -15 °C

Density: 1.1296 g/cm³ at 20 °C

Solubility: Very soluble in ethanol and ethyl ether, soluble in ketone and chloroform; it dissolves cellulose nitrate, some dyes and synthetic resins ([Ellis, 1972](#)); it is miscible with water, forming an azeotrope at atmospheric pressure (water, 80 wt%; boiling point, 98.5 °C).

Volatility: Vapour pressure, 53 kPa at 20 °C

Flash point: 65 °C (tag closed cup)

Explosive limits: 1.8–16.3 vol% in air

Stability: Pure furfuryl alcohol decomposes upon standing for extended periods; it should be stored in a dark bottle in a refrigerator at 0 °C ([NIOSH, 1994](#))

Relative vapour density: 3.4 (air = 1)

Octanol/water partition coefficient (P): log K_{ow}, 0.28

Odour threshold: About 28 mg/m³ in humans; 50% response at 8 ppm

Conversion factor: 1 ppm = 4.01 mg/m³ at normal temperature (25 °C) and pressure (103.5 kPa)

Impurities: No data on impurities were available; overall purity is usually > 98% ([NTP, 1999](#); [Hoydonckx, 2007](#)).

From [NTP \(1999\)](#); [Lide \(2005\)](#); [Hoydonckx \(2007\)](#); [European Commission \(2011\)](#).

1.2 Production and use

1.2.1 Production process

Furfuryl alcohol is produced industrially by the hydrogenation of furfural. Vapour-phase reaction or liquid-phase reaction are both used, although the vapour-phase reaction at atmospheric pressure is currently the most widely employed, except in China ([Chen et al., 2002](#); [Hoydonckx, 2007](#); [ITC, 2012](#)).

Furfural contains two kinds of reactive group – a carbonyl group and carbon–carbon double bonds; hydrogenation of the former gives furfuryl alcohol, and hydrogenation of the latter results in tetrahydrofurfural. The catalytic hydrogenation of the furfural carbonyl group requires the presence of heterogeneous or homogeneous catalysts. The heterogeneous copper chromite catalyst has been used at an industrial scale for more than six decades ([Villaverde et al., 2013](#)); however, the toxicity and carcinogenic potential associated with chromite ([IARC, 1990](#); [Chen et al., 2002](#)), and environmental problems associated with deactivated copper chromite catalyst have prompted the development of other catalysts based on copper ([Vargas-Hernández et al., 2014](#); [Jiménez-Gómez et al., 2016](#)), nickel ([Baijun et al., 1998](#); [Li et al., 2003](#); [Kotbagi et al., 2016](#)), ruthenium ([Tukacs et al., 2017](#)), platinum, and palladium ([O’Driscoll et al., 2017](#)), among others.

Production of furfuryl alcohol from xylose over a dual heterogeneous catalyst system has also been described ([Perez & Fraga, 2014](#); [Cui et al., 2016](#)).

The potential of microbial conversion of furfural for the production of furfuryl alcohol

has been explored as an alternative; however, it is still relatively understudied and not widely applied ([Mandalika et al., 2014](#)).

1.2.2 Production volume

Global production of furfuryl alcohol was estimated at about 300 000 tonnes in 2015 ([Grand View Research, 2015](#)). Furfuryl alcohol is listed by the Organisation for Economic Co-operation and Development (OECD) ([OECD, 2018](#)) and the United States Environmental Protection Agency (EPA) as a chemical with a high production volume, with more than 1 million pounds [more than 453 tonnes] produced annually ([Franko et al., 2012](#)). China is the main global manufacturer and user of furfuryl alcohol, with 80–85% of global capacity and production, and about 60% of global consumption in 2015 ([IHS Markit, 2016](#)). Between 10 000 and 100 000 tonnes are manufactured and/or imported into the European Economic Area each year ([ECHA, 2018a](#)). A single industrial plant in Belgium produced around 40 000 tonnes per year ([IFC, 2016](#)). The database ChemSources-Online lists 31 manufacturing companies worldwide ([Chemical Sources International, 2017](#)).

1.2.3 Use

It has been estimated that the production of furan resins for foundry sand binders in the metal casting industry accounted for about 85–90% of furfuryl alcohol used worldwide ([IHS Markit, 2016](#)). Furfuryl alcohol is also used as a wetting agent and as a solvent for dyes and as corrosion inhibitor in fibre-reinforced plastics, in cements and mortars, and in wood protection. Applications also include use in flavours and fragrances. Moreover, furfuryl alcohol is used as a laboratory reagent and as a chemical building block for drug synthesis ([Sriram & Yogeewari, 2010](#); [European Commission, 2011](#); [IHS Markit, 2016](#)). In addition, the product of

Table 1.1 Representative methods for the analysis of furfuryl alcohol

Sample matrix	Assay procedure	Limit of detection	Reference
Air of workplace	TDS-GC-FID	2.25 mg/m ³ (LOQ)	Tschickardt (2012)
	TDS-GC-FID	NR	ISO (2000, 2001) ; NIOSH (1994)
Dust particles	GC-UV	≤ 0.4 µg/g	Nilsson et al. (2005)
Foundry resins	GC-FID	173 µg/L	Oliva-Teles et al. (2005)
	LC-UV	5.2 mg/L	
Fruit juices	LC-UV	3 mg/L	Yuan & Chen (1999)
Environmental water	GC-MS	0.02 µg/L	Kawata et al. (2001)
Roasted coffee	SPME-GC-MS	NR	Yang & Peppard (1994)
Wine	SPME-GC-MS	7 µg/L	Carrillo et al. (2006)
Coffee	HS-SPME-GC-MS	0.59 mg/L	Petisca et al. (2013a)
Coffee	NMR	3.2 mg/L	Okaru & Lachenmeier (2017)
Deep-fried products	HS-SPME-GC-MS	1.5 mg/kg	Pérez-Palacios et al. (2012)

GC-FID, gas chromatography-flame ionization detector; GC-MS, gas chromatography-mass spectrometry; GC-UV, gas chromatography-ultraviolet spectrometry; HS, headspace; LC-UV, liquid chromatography-ultraviolet spectrometry; LOQ, limit of quantification; NMR, nuclear magnetic resonance; NR, not reported; SPME, solid-phase microextraction; TDS, thermal desorption system

the hydrogenation of furfuryl alcohol, tetrahydrofurfuryl alcohol, is used in plant protection products ([ECHA, 2018b](#)).

1.3 Analytical methods

Representative methods for the analysis of furfuryl alcohol in environmental and food matrices are summarized in [Table 1.1](#). In general, gas chromatography with mass spectrometry (GC-MS) is preferred due to its higher sensitivity.

Thermal desorption combined with GC-MS or with gas chromatography-ultraviolet spectrometry (GC-UV) has been used to analyse furfuryl alcohol adsorbed to indoor dust particles ([Nilsson et al., 2005](#)).

Extraction of furfuryl alcohol from food matrices (before chromatographic analysis) can be performed by different methods, namely solvent extraction, solid-phase extraction, simultaneous distillation extraction, and solid-phase microextraction ([Yang & Peppard, 1994](#); [Cocito et al., 1995](#); [Spillman et al., 1998](#); [Gómez Plaza et al., 1999](#); [Jerković et al., 2007](#)).

Headspace-solid phase microextraction methods are advantageous for the analysis of

volatile compounds like furfuryl alcohol ([Carrillo et al., 2006](#); [Pérez-Palacios et al., 2012](#); [2013](#); [2014](#); [Petisca et al., 2013a, 2013b, 2014](#)). The first reports on analysis indicated headspace incubation and extraction temperatures of 80 °C for at least 30 minutes ([EFSA, 2004](#)), but temperature was later reduced to 60 °C ([FDA, 2005](#)), and even lower ([Pérez-Palacios et al., 2012](#)), to avoid formation of additional amounts of furanic compounds during analysis.

1.4 Occurrence and exposure

1.4.1 Occurrence

Due to its high production volume and large number of industrial and consumer uses, furfuryl alcohol is ubiquitous in the environment.

Should furfuryl alcohol be released to the soil, it is expected to have very high mobility based upon an estimated soil adsorption coefficient, K_{oc} , of 34. If released into water, furfuryl alcohol is not expected to adsorb to suspended solids and sediment, based upon an observed degradation of 75–79% in 2 weeks. If released to air, furfuryl alcohol will exist solely as a vapour

in the atmosphere (on the basis of its vapour pressure), and will be degraded by reaction with photochemically produced hydroxyl radicals. The half-life for this reaction is estimated to be 3.7 hours. Furfuryl alcohol may also be susceptible to direct photolysis by sunlight, on the basis of its absorption of ultraviolet light at wavelengths > 290 nm ([National Library of Medicine, 2018](#)).

Furfuryl alcohol has been identified as a side product in Maillard reactions ([Schirle-Keller & Reineccius, 1992](#); [Chen & Ho, 1999](#)). Its formation from glucose in aqueous systems has been described. The mechanism involves the oxidation of glucose to gluconic acid, which is decarboxylated to a pentitol and followed by dehydration and cyclization to furfuryl alcohol ([Wnorowski & Yaylayan, 2000](#); [Yaylayan & Keyhani, 2000](#)). Likewise, sugar degradation, or hydrolysis and heating of polysaccharides containing hexoses or pentoses, can result in the formation of furfuryl alcohol. Glucose or fructose can undergo isomerization reactions at high temperatures. The intermediate compounds formed will react further by cyclization and aromatization, forming furfuryl alcohol ([Brands & van Boekel, 2001](#); [Murkovic & Swasti, 2013](#)).

Furfuryl alcohol occurs naturally in some types of fruit, and in tea, coffee, and cocoa ([European Commission, 2011](#)), and in many foods, mainly due to food processing, storage or ageing, or its addition in flavouring agents. These flavouring agents have low taste thresholds and deliver a characteristic cocoa, butter, or fruity odour. Thermal processing (e.g. roasting, baking, or deep-frying) to obtain a desirable flavour increases the formation of furfuryl alcohol ([Pérez-Palacios et al., 2012, 2013, 2014](#); [Petisca et al., 2013a, b](#)). Dried ([Giannetti et al., 2014](#); [Pasqualone et al., 2014](#)), cured or smoked ([Yu et al., 2008](#)), fermented, stored, or aged products ([Spillman et al., 1998](#); [Karagül-Yüceer et al., 2002](#); [Qian & Reineccius, 2002](#); [Morales et al., 2004](#); [Vanderhaegen et al., 2004](#); [Giri et al., 2010](#); [Lidums, et al., 2015](#), [Liang et al., 2016](#), [Harada](#)

[et al., 2017](#); [Pico et al., 2017](#)) also contain furfuryl alcohol.

Among the many heterocyclic compounds reported to be present in roasted coffee, furans were found to be abundant ([Flament & Bessiere-Thomas, 2002](#); [Petisca et al., 2013a](#)). The formation of furanic compounds in roasted coffee has been attributed to Maillard reactions; however, degradation of less volatile coffee constituents, such as quinic, caffeic, and chlorogenic acids can also result in the formation of furfuryl alcohol ([Moon & Shibamoto, 2010](#)).

During wine ageing, furfuryl alcohol is formed by microbiological reduction of the furfuryl aldehydes ([Spillman et al., 1998](#)). The concentration of furfuryl alcohol in wine in all of the sampled oak barrels was reported to be low during the first 180 days of maturation, but increased rapidly from day 180 to day 270, coinciding with spring and summer, when high temperature favours microfloral growth and enzyme activity ([Pérez-Prieto et al., 2003](#)).

Furfuryl alcohol is also found in beer. In pale beers, the concentration of furfuryl alcohol is essentially determined by the “thermal load” on wort (from heating and boiling) during brewing operations, while in dark beers a considerable fraction of furfuryl alcohol may come from the dark malts used ([Vanderhaegen et al., 2004](#)).

Products that are prepared using processes involving a short, rapid cooking method at quite high temperatures are associated with a relatively high content of furfuryl alcohol, as in the case of rice cakes and deep-fried products ([Buttery et al., 1999](#); [Pérez-Palacios et al., 2014](#)). The influence of cooking and handling conditions on the quantity of furfuryl alcohol and other furanic compounds in deep-fried breaded fish products has been studied ([Pérez-Palacios et al., 2013](#)). The content of furanic compounds in these products was lower after oven-baking or reheating in a microwave oven than after deep-frying. The content of furfuryl alcohol (and generation of furanic compounds) decreased with decreasing

temperature and duration of deep-frying, and also when there was a delay after deep-frying and before sampling. Adjusting the cooking method and conditions by using an electric oven, deep-frying in sunflower oil at 160 °C for 4 minutes, or waiting 10 minutes after cooking are strategies that could be applied to reduce the furfuryl alcohol content of breaded fish products ([Pérez-Palacios et al., 2013](#)).

1.4.2 Exposure in the general population

The general population is exposed to furfuryl alcohol mainly in food and beverages, but exposure can also occur via inhalation and the dermal route ([NIOSH, 2015](#); [IFA, 2017](#)). [The Working Group noted that there may be potential inhalation exposure from cigarette smoke or electronic cigarette aerosols, but no studies were available.]

Most reported individual foods contained low or trace amounts of furfuryl alcohol, but the cumulative amount ingested could contribute significantly to exposure. The major source of furfuryl alcohol in foods is thermal processing and ageing of alcoholic beverages ([Okaru & Lachenmeier, 2017](#)). The concentrations of furfuryl alcohol in certain items can reach several thousands of micrograms per litre or per kilogram, as summarized in [Table 1.2](#). Coffee, bread, baked goods, deep-fried fish, and some spirits may contain furfuryl alcohol at high levels; however, there is great variability according to the degree of roasting or the preparation procedure used.

Since furfuryl alcohol is an approved food flavouring additive, exposure will increase with the consumption of foods that contain added furfuryl alcohol. Daily intake of flavouring substances was evaluated by two different methods: maximized survey-derived daily intake (MSDI) estimated from annual production data for flavours, and possible average daily intake (PADI). The latter calculates an exaggerated intake, since it makes the assumption that the

flavouring agent is used at the average use level in all foods within a category of foods in which the flavour was anticipated to be used by industry. As expected, the two methods give different results, because PADI provides a substantial overestimation of the actual intake. However, this higher estimation of intake is useful to determine whether margins of safety are still adequate in a worst-case scenario ([Munro & Danielewska-Nikiel, 2006](#)). The estimated furfuryl alcohol intake was 4 µg/kg bw per day when calculated by MSDI, and 130 µg/kg bw per day by PADI ([Munro & Danielewska-Nikiel, 2006](#)).

[The Working Group estimated that, based on a furfuryl alcohol concentration of 70 mg/L, one cup of 30 mL of espresso coffee represents an intake of 2 mg of furfuryl alcohol, or about 0.03 mg/kg bw (body weight, 70 kg). Based on a consumption of 4 kg of roasted coffee for European Union inhabitants per year and average content of 250 mg/kg in roasted coffee, per capita intake would be 3 mg/day, assuming the worst-case scenario of complete extraction of furfuryl alcohol into the liquid.]

[The Working Group noted that current exposure estimates (MSDI and PADI) that are based only on added flavouring agents underestimate total intake because of the additional contribution from foods and beverages that contain furfuryl alcohol as a result of cooking or preparation processes (e.g. coffee).]

1.4.3 Occupational exposure

See [Table 1.3](#)

Workplace exposure to furfuryl alcohol can occur in the chemical industry when furfuryl alcohol is used in the manufacture of other products, and in a variety of end-user situations when furfuryl alcohol is emitted as a process-generated substance.

Furfuryl alcohol is used in polymers, laboratory chemicals, and coating products, and in the manufacture of chemicals and plastic

Table 1.2 Occurrence of furfuryl alcohol in food and beverages

Food item	Furfuryl alcohol content	Reference
<i>Liquids</i>		
Turkish coffee	14 691 µg/L	Amanpour & Selli (2016)
French press coffee	13 799 µg/L	Amanpour & Selli (2016)
Espresso coffee	31 000–70 000 µg/L	Petisca et al. (2014)
Aged wines	350–850 µg/L	Pérez-Prieto et al. (2003)
Aged wines	3500–9600 µg/L	Spillman et al. (1998)
Beer	1800–4000 µg/L	Vanderhaegen et al. (2004)
Wine vinegar	ND–594 µg/L	Tsfaye et al. (2004)
Cereal vinegar	35–40 µg/L	Liang et al. (2016)
<i>Solids</i>		
Instant coffee	267 000 µg/kg	Golubkova (2011)
Filter coffee	1 430 000 µg/kg	Golubkova (2011)
Roasted coffee	158 000–1 340 000 µg/kg	Golubkova (2011)
Roasted coffee	251 000 µg/kg	Okaru & Lachenmeier (2017)
Baked goods	110 000 µg/kg	Okaru & Lachenmeier (2017)
Bread	187 000 µg/kg	Okaru & Lachenmeier (2017)
Deep-fried coated fish	4580–22 280 µg/kg	Pérez-Palacios et al. (2012, 2013, 2014)
Toasted almonds	4400–8880 µg/kg	Vázquez-Araújo et al. (2008)
Fish miso	612–40 761 µg/kg	Giri et al. (2010)
Soy miso	4366 µg/kg	Giri et al. (2010)
Rice miso	1290 µg/kg	Giri et al. (2010)
Non-fat dried milk (stored 3 months)	14 500 µg/kg	Karagül-Yüceer et al. (2002)
Rice cake	2000–2300 µg/kg	Buttery et al. (1999)
Corn tortilla chips	540 µg/kg	Buttery & Ling (1998)
Popcorn	38.2–82.1 µg/kg	Park & Maga (2006)
Sweet potatoes	14 µg/kg	Wang & Kays (2000)
Honey	1550 µg/kg	Vazquez et al. (2007)
Citrus honeys	44–61 µg/kg	Escriche et al. (2011)
Citrus honeys	5.5–23.5 µg/kg	Castro-Vázquez et al. (2007)
Roasted cocoa powder	0–69 µg/kg	Bonvehí (2005)
Wheat bread	0.187–0.613 µg/kg	Jensen et al. (2011)

ND, not detected

products. Chemical manufacturing is carried out in enclosed process systems, which minimize potential workplace exposure. [NIOSH \(1979\)](#) described measurements made in the 1970s at two plants manufacturing furfuryl alcohol in the USA; these were mostly < 0.4 mg/m³. No recent published exposure measurements for the use of furfuryl alcohol in manufacturing industry were available to the Working Group. Occupational exposure may occur by inhalation and skin contact.

Around 85–90% of furfuryl alcohol is used to produce furan resins for use in the foundry industry ([IHS Markit, 2016](#)), and the available exposure data for this substance were mostly from foundry operations. These resins have been increasingly used in foundry operations since the 1960s. Furan binders are copolymers of furfuryl alcohol in urea–formaldehyde and phenol–formaldehyde resins ([Kim et al., 1998](#)). Unhardened resin contains free furfuryl alcohol, small amounts of free formaldehyde, and other

Table 1.3 Occupational exposure to furfuryl alcohol

Reference	Location, collection date	Description of occupation or work task	Sampling matrix; approach; N; duration	Agent, exposure level ^a	Exposure range	Comments
Low & Mitchell (1985)	Australia, around 1984	Foundry Furan mould process: mixing machine	Air; personal; N = NR; NR	Furfuryl alcohol NR	3–8 ppm [12–32 mg/m ³]	
Low & Mitchell (1985)	Australia, around 1984	Foundry Furan mould process: general foundry	Air; personal; N = NR; NR	Furfuryl alcohol NR	10–50 ppm [40–200 mg/m ³]	
Virtamo & Tossavainen (1976)	Finland, around 1976	Foundry Furan mould process	Air; personal; N = 36; 1–2 h	Furfuryl alcohol 4.6 ppm [18.4 mg/m ³]	0.2–40 ppm [0.8–160 mg/m ³]	Measurements made in the core-making areas of 10 iron and steel foundries; 22% of results exceeded 5 ppm [20 mg/m ³] (the TLV)
Pfaffli et al. (1985)	Finland, around 1984	Foundry	Urine; biological; N = 6; NR	Furoic acid in urine NR	20–1300 µmol/ mmol creatinine	Data extracted from Fig. 4 of Pfaffli et al. (1985)
Landberg et al. (2015)	Sweden, around 2015	Foundry Core-making	Air; personal; N = 3; 2 h	Furfuryl alcohol 40 mg/m ³	30–54 mg/m ³	In core-making, a core of about 0.3–1 m ³ was made by pouring sand mixed with furfuryl alcohol into a mould. This scenario was carried out for about 2 h per day, every day of the week. There were no other sources. No control measures or personal protection were used, and the work was performed in a large work room with general ventilation
Ahman et al. (1991)	Sweden, around 1991	Foundry Furan mould and core-makers	Air; personal; N = 40; 8 h	Furfuryl alcohol 7 mg/m ³	< 1–15 mg/m ³	Over short periods of time (sampling time in general, 15–30 min), the mean concentrations in six subjects exceeded the present short-term exposure limit recommended in Sweden (STEL, 40 mg/m ³). During manual filling and packing of big moulding boxes, occasional peak concentrations of up to 100 mg/m ³ were recorded on a direct-reading instrument In general, exposure concentrations of furfuryl alcohol observed in the moulding group were higher than those measured in the core-making group

Table 1.3 (continued)

Reference	Location, collection date	Description of occupation or work task	Sampling matrix; approach; N; duration	Agent, exposure level ^a	Exposure range	Comments
Westberg et al. (2001)	Sweden, 1992–1995	Foundry Furan moulds in an aluminium foundry	Air; personal; N = 3; 8 h	Furfuryl alcohol 2.4 mg/m ³ Geometric mean	0.8–23 mg/m ³	
NIOSH (1979)	USA, 1976	All Furfuryl alcohol manufacture	Air; personal; N = 24; NR	Furfuryl alcohol < 0.1 ppm [< 0.4 mg/m ³]	< 0.1–0.2 ppm [< 0.4–0.8 mg/m ³]	
NIOSH (1979)	USA, 1978	All Furfuryl alcohol manufacture	Air; personal; N = 4; NR	Furfuryl alcohol 0.3 ppm [1.2 mg/m ³]	0.2–0.4 ppm [0.8–1.6 mg/m ³]	
NIOSH (1972)	USA, 1972	Foundry Core-maker, assistant and apprentice	Air; personal; N = 3; 8 h	Furfuryl alcohol < 20 mg/m ³ Median	< 20–25 mg/m ³	
NIOSH (1973)	USA, 1973	Foundry Core-making	Air; environmental; N = 1; 8 h	Furfuryl alcohol 2.2 ppm [8.8 mg/m ³]	NA	Furfuryl alcohol was measured at: 2.2 ppm [8.8 mg/m ³] during normal conditions that day and collected over a complete core production cycle (1 h); 8.6 ppm [34.4 mg/m ³] under normal conditions and during the core preparation time only (15 min); 10.8 ppm [43.2 mg/m ³] during the core preparation when the sand was heated to a warm condition (15 min); and 15.8 ppm [63.2 mg/m ³] during the core preparation when the sand was hot (15 min)
OSHA (2018)	USA, 1984–2013	Various	Air; personal; N = 204; Various	Furfuryl alcohol 0.26 mg/m ³ Median	ND–20 mg/m ³	
INRS (2018)	France, 1987–2017	Various	Air; personal; N = 123; 61–149 min	Furfuryl alcohol 2.0 mg/m ³ Median	< 0.01–176 mg/m ³	Data from industrial manufacturing; measurement duration, > 60 min

^a Arithmetic mean unless otherwise reported
min, minute; NA, not applicable; ND, not detected; STEL, short-term exposure limit; TLV, threshold limit value

volatile agents. In foundries, furfuryl alcohol is emitted during sand mixing, moulding or core making, mould assembly, casting, knockout, shot-blasting and manual welding ([HSE, 2017](#)). Exposure levels vary according to the specific tasks being carried out. Early measurement data sets (1970s and 1980s) from foundries in Australia ([Low & Mitchell, 1985](#)) and Finland ([Virtamo & Tossavainen, 1976](#)) showed that workers could be exposed to peak concentrations of furfuryl alcohol of between 100 and 200 mg/m³, with a mean exposure of 17.2 mg/m³ reported by [Virtamo & Tossavainen \(1976\)](#). Average exposure levels were generally below 18 mg/m³. There may be coexposure to low air concentrations of formaldehyde and other volatile organic substances for core makers, with higher concentrations of these substances possibly occurring in general foundry operations. In a foundry, there could be more than one core-making process being used, and so coexposure to other volatile agents is possible.

In the United States Occupational Safety and Health Administration (OSHA) database of compliance exposure measurements, 204 measurements of exposure to furfuryl alcohol were collected between 1984 and 2014 from a variety of industries; 45% of the data were below the limit of detection and more than 95% were less than 20 mg/m³. The highest measurements were from iron foundries ([OSHA, 2018](#)). Similar data were available from France, and showed a similar pattern of general low exposure in the industrial manufacturing sector (123 measurements of more than 60 minutes duration, with 95% of the measured values being less than 35 mg/m³ ([INRS, 2018](#)).

The Finnish Institute of Occupational Health exposure database contained 16 measurements for inhalation exposure to furfuryl alcohol collected between 2012 and 2016. The measurements ranged from < 0.1 to 27 mg/m³, with the eight highest results from workers involved in

manufacturing paper and paperboard (arithmetic mean, 13 mg/m³) ([FIOH, 2018](#)).

1.5 Regulations and guidelines

For chemical use, the [ECHA \(2018a\)](#) requires the following warning: “Danger!”. According to the harmonized classification and labelling (ATP01) approved by the European Union, this substance is toxic if inhaled, harmful if swallowed, harmful in contact with skin, causes serious eye irritation, is suspected of causing cancer, may cause damage to organs through prolonged or repeated exposure, and may cause respiratory irritation.

Furfuryl alcohol is included in the most recent register of approved flavouring substances in Europe according to Regulation (EU) No 872/2012 ([European Commission, 2012](#)). However, if furfuryl alcohol is formed as a contaminant due to food processing, food legislation in Europe (Council Regulation 315/93) would demand that its content be reduced to as low as reasonably achievable (ALARA principle) ([Okaru & Lachenmeier, 2017](#)).

According to the regulations of the United States Food and Drug Administration (FDA), furfuryl alcohol is an “indirect food additive” for use only as a component of adhesives in packaging, transporting, or holding food in accordance with prescribed conditions ([FDA, 2017](#)). [According to the FDA, indirect food additives are substances that may come into contact with food as part of packaging or processing equipment, but are not intended to be added directly to food.]

In 1996, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) began a programme to evaluate the safety of food flavouring agents. To perform these evaluations, flavouring substances are first compiled into groups of structurally related materials, which are expected to present similar routes of metabolism and toxicity. JECFA has established a group

Table 1.4 Occupational exposure limits values for furfuryl alcohol as an air contaminant

Country or region	Limit value – 8 hours		Limit value – short-term		Comments
	ppm	mg/m ³	ppm	mg/m ³	
Australia	10	40	15	60	
Austria	5	20			
Belgium	10	41	15	61	
Canada (Ontario)	10	[40]	15	[60]	
Canada (Quebec)	10	40	15	60	
China		40		60*	*15 min average value
Denmark	5	20	10	40	
Finland	2	8.1	10*	41*	*15 min average value
France	10	40			
Hungary		40		40	
Ireland	5	20	15*	60*	*15 min reference period
Japan – JSOH	5	20			
New Zealand	10	40	15	60	
Poland		30		60	
Republic of Korea	10	40	15	60	
Singapore	10	40	15	60	
Spain	5	20	15	61	Skin
Sweden	5	20	10*	40*	*15 min average value
Switzerland	10	40	10	40	
United Kingdom	(5)	(20)	(15)	(61)	The United Kingdom Advisory Committee on Toxic Substances has expressed concern that, for the OELs shown in parentheses, health may not be adequately protected because of doubts that the limit was not soundly based. These OELs were included in the published United Kingdom 2002 list and its 2003 supplement, but are omitted from the published 2005 list
USA – NIOSH	10	40	15*	60*	*15 min average value
USA – OSHA	50	200			

From GESTIS international limit values (IFA, 2017)

JSOH, Japanese Society for Occupational Health; NIOSH, National Institute for Occupational Safety and Health; OEL, occupational exposure level; OSHA, Occupational Safety and Health Administration

acceptable daily intake (ADI) of 0.5 mg/kg bw for furfuryl alcohol, furfural, furfuryl acetate, and methyl 2-furoate. For all animal species, the European Food Safety Authority (EFSA) has established a maximum proposed use level of furfuryl alcohol in complete feed of 5 mg/kg (EFSA, 2016).

The exposure limits for furfuryl alcohol as an air contaminant are summarized in Table 1.4. In the USA, the National Institute for Occupational Safety and Health (NIOSH) has recommended a time-weighted average threshold limit value

(TLV–TWA) of 10 ppm (40 mg/m³) and a short-term exposure limit (TLV–STEL) of 15 ppm (60 mg/m³) for occupational exposure to furfuryl alcohol, to minimize the potential for eye and respiratory passageways irritation (NIOSH, 2016). In Germany in a 1992 reassessment, a “MAK” (TLV–TWA) value of 40 mg/m³ (10 mL/m³) was established because of data showing irritation of the respiratory tract (MAK Commission, 2008). However, in 2007, furfuryl alcohol was classified as “Carcinogen Category 3B”. In addition, workplace experience showed that irritation

of the respiratory tract and eyes occurred after exposure to furfuryl alcohol at concentrations of 1.75 mL/m³ and above, with peak concentrations of more than 10 mL/m³. Consequently, the previous MAK value of 10 mL/m³ was withdrawn ([MAK, 2016](#)).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See [Table 3.1](#)

3.1 Mouse

3.1.1 Inhalation

Groups of 50 male and 50 female B6C3F₁ mice (age, 6 weeks) were exposed to test atmospheres of furfuryl alcohol at 0 (control), 2, 8, or 32 ppm (purity, > 98%, impurities not characterized) by whole-body inhalation for 6 hours plus T₉₀ (12 minutes) per day, 5 days per week, for 105 weeks ([NTP, 1999](#)). Survival of exposed male and female mice was similar to that of the controls. Mean body weights of exposed male mice were similar to those of controls throughout the study. Mean body weights of exposed female mice were 7–14% lower than those of controls beginning at week 39 for mice at the highest dose and at week 59 for mice at the lowest and intermediate dose. Furfuryl alcohol was irritating and toxic to the nasal cavity in males and females. Nephropathy was observed in all groups of males and females. The severity of nephropathy increased with increasing exposure concentration in male mice. In male mice at the highest dose, there was an increase in the incidence of renal tubule adenoma (single section: 0/50, 0/49, 0/49, 2/50 (4%)), renal tubule carcinoma

(single section: 0/50, 0/49, 0/49, 2/50 (4%)), and renal tubule adenoma or carcinoma (combined) (single section: 0/50, 0/49, 0/49, 4/50 (8%)) that all exceeded historical control ranges for inhalation studies; there was a significant positive trend ($P = 0.002$, poly-3 test) in the incidence of renal tubule adenoma or carcinoma (combined). [Renal tubule neoplasms are uncommon in male B6C3F₁ mice.] In 2-year inhalation studies with untreated chamber controls carried out by the National Toxicology program (NTP), historical incidence (mean ± standard deviation) was: renal tubule adenoma (single section), 3/1093 (0.3% ± 0.6%); range, 0–2%; renal tubule carcinoma (single section), 1/1093 (0.1% ± 0.4%); range, 0–2%; and renal tubule adenoma or carcinoma (combined) (single section), 4/1093 (0.4% ± 1.0%); range, 0–4%. Additional analyses performed by step sectioning of the kidneys revealed an additional adenoma in males at the highest dose; the revised incidence for each group was thus 0/50 ($P = 0.009$, trend by poly-3 test), 0/49, 0/49, and 3/50 (6%). The incidence of renal tubule adenoma or carcinoma (combined) – standard (single section) evaluation and extended evaluation (step sections) combined – became 0/50 ($P < 0.001$, trend), 0/49, 0/49, 5/50 (10%), with the incidence in the group at the highest dose being significantly greater ($P = 0.036$, poly-3 test) than in the control group. There was no significant increase in the incidence of any tumours including those of the kidney in treated female mice. [The Working Group noted that this was a well-conducted study that complied with good laboratory practice (GLP), and was carried out in males and females.]

3.2 Rat

3.2.1 Inhalation

Groups of 50 male and 50 female F344/N rats (age, 6 weeks) were exposed to furfuryl alcohol at test atmospheres of 0 (control), 2, 8, or 32 ppm

Table 3.1 Studies of carcinogenicity in rodents treated with furfuryl alcohol by inhalation (whole-body exposure)

Species, strain (sex) Age at start Duration Reference	Purity Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments	
Mouse, B6C3F ₁ (M) 6 wk 105 wk NTP (1999)	Purity, > 98% 0, 2, 8, 32 ppm 6 h plus T ₉₀ (12 min) per d, 5 d/wk, for 105 wk 50, 50, 50, 50 34, 36, 30, 38	<i>Kidney, standard (single section) evaluation</i>		Principal strengths: GLP study; study in males and females	
		Renal tubule adenoma: 0/50, 0/49, 0/49, 2/50 ^a	NS	^a Exceeded historical control ranges for inhalation studies Historical incidence: 3/1093 (0.3% ± 0.6%); range, 0–2%	
		Renal tubule carcinoma 0/50, 0/49, 0/49, 2/50 ^b	NS	^b Exceeded historical control ranges for inhalation studies Historical incidence: 1/1093 (0.1% ± 0.4%); range, 0–2%	
		Renal tubule adenoma or carcinoma (combined): 0/50*, 0/49, 0/49, 4/50 ^c	*P = 0.002 (poly-3 trend test)	^c Exceeded historical control ranges for inhalation studies Historical incidence: 4/1093 (0.4% ± 1.0%); range, 0–4%	
		<i>Kidney, standard (single section) evaluation and extended evaluation (step sections) (combined)</i>			
		Renal tubule adenoma: 0/50*, 0/49, 0/49, 3/50	*P = 0.009 (poly-3 trend test)		
Renal tubule carcinoma: 0/50, 0/49, 0/49, 2/50	NS				
Renal tubule adenoma or carcinoma (combined): 0/50*, 0/49, 0/49, 5/50**	*P < 0.001 (poly-3 trend test) **Significantly greater (P = 0.036) than the control group; poly-3 test				
Mouse, B6C3F ₁ (F) 6 wk 105 wk NTP (1999)	Purity, > 98% 0, 2, 8, 32 ppm 6 h plus T ₉₀ (12 min) per d, 5 d/wk, for 105 wk 50, 50, 50, 50 34, 33, 32, 40	Any tumour type: no significant increase		Principal strengths: GLP study; study in males and females	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Rat, F344/N (M) 6 wk 105 wk NTP (1999)	Purity, > 98% 0, 2, 8, 32 ppm 6 h plus T ₉₀ (12 min) per d, 5 d/wk, for 105 wk 50, 50, 50, 50 8, 5, 9, 0	<i>Nose</i>		Principal strengths: GLP study; study in males and females
		Lateral wall adenoma: 0/50, 1/50, 0/50, 0/50	NS	
		Respiratory epithelium adenoma: 0/50, 0/50, 1/50 ^a , 0/50	NS	^a Historical control incidence for inhalation studies: 1/897 (0.1% ± 0.5%); range, 0–2%
		Respiratory epithelium carcinoma: 0/50, 0/50, 0/50, 1/50 ^b	NS	^b Exceeded historical control incidence for inhalation studies: 0/897
		Respiratory epithelium, squamous cell carcinoma: 0/50*, 0/50, 0/50, 3/50 ^b	* <i>P</i> = 0.006 (trend, poly-3 test)	
		Respiratory epithelium adenoma, carcinoma, or squamous cell carcinoma (combined): 0/50*, 1/50, 1/50, 4/50**	* <i>P</i> = 0.013 (trend) ** <i>P</i> = 0.044 (poly-3 test)	
		<i>Kidney, standard (single section) evaluation</i>		
		Renal tubule adenoma: 1/50, 1/50, 2/50 ^c , 0/50	NS	^c Historical control incidence for inhalation studies: 9/902 (1.0% ± 1.2%); range, 0–4%

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Rat, F344/N (F) 6 wk 105 wk NTP (1999)	Purity, > 98% 0, 2, 8, 32 ppm 6 h plus T ₉₀ (12 min) per d, 5 d/wk, for 105 wk 50, 50, 50, 50 26, 26, 22, 16	<i>Kidney, standard (single section) evaluation</i>		Principal strengths: GLP study; study in males and females
		Renal tubule adenoma:	NS	^a Exceeded historical control incidence for inhalation studies Historical incidence: 1/898 (0.1% ± 0.5%); range, 0–2%
		0/50, 0/49, 0/49, 2/50 ^a		
		Renal tubule carcinoma:	NS	^b Historical control incidence for inhalation studies: 4/898 (0.5% ± 0.9%); range, 0–2%
		0/50, 1/49 ^b , 0/49, 0/50		
		Renal tubule adenoma or carcinoma (combined):	NS	^c Historical control incidence for inhalation studies: 5/898 (0.6% ± 0.9%); range, 0–2%
0/50, 1/49, 0/49, 2/50 ^c				
<i>Nose</i>				
Lateral wall adenoma:	NS			
0/49, 0/50, 1/48, 0/49				
Respiratory epithelium adenoma:	NS		^d Historical control incidence for inhalation studies: 1/892 (0.1 ± 0.5%); range, 0–2%	
0/49, 0/50, 0/48, 1/49 ^d				
Adenoma (lateral wall or respiratory epithelium, combined):	NS			
0/49, 0/50, 1/48, 1/49				

d, day; F, female; GLP, good laboratory practice; M, male; NS, not significant; T₉₀, time to achieve 90% of the target concentration after the beginning of vapour generation; wk, week

(purity, > 98%, impurities not characterized) by whole-body inhalation for 6 hours plus T₉₀ (12 minutes) per day, 5 days per week, for 105 weeks (NTP, 1999). All male rats exposed at 32 ppm died by week 99. Survival of all other exposed groups of male and female rats was similar to that of the control groups. Mean body weights of males at 32 ppm were less than those of the controls beginning week 19; mean body weights of males at 2 and 8 ppm, and of all exposed females were similar to those of the control groups throughout the study. Furfuryl alcohol was irritating and toxic to the nasal cavity in males and females. All groups of exposed males and females had significantly increased incidences of non-neoplastic lesions in the nose. In the nose, one (2%) lateral wall adenoma was observed in a male at 2 ppm, and one (2%) in a female at 8 ppm; one (2%) adenoma of the respiratory epithelium was observed in a male at 8 ppm (historical incidence, 1/897; range, 0–2%) and one (2%) female at 32 ppm (historical incidence, 1/892; range, 0–2%); one male at 32 ppm (2%) developed a carcinoma of the respiratory epithelium, and three other males at 32 ppm (6%) developed squamous cell carcinomas of the respiratory epithelium. Individually, the incidence per group was not significantly greater than that in the control groups. However, in males there was a significant positive trend in the incidence of squamous cell carcinoma of the respiratory epithelium ($P = 0.006$, poly-3 test), and the incidence of adenoma, carcinoma or squamous cell carcinoma (combined) of the respiratory epithelium was significantly increased in the group at the highest dose (4/50, 8%; $P = 0.044$ by poly-3 test) with a significant positive trend ($P = 0.013$, poly-3 test). Carcinomas and squamous cell carcinomas of the respiratory epithelium were not observed in males in historical controls (0/897) in previous NTP inhalation studies.

The incidence of renal tubule hyperplasia (single section) in exposed male and female rats was not significantly different from that of

controls. Renal tubule adenomas (single section) were observed in one (2%) male in the control group, one male (2%) at 2 ppm, two males (4%) at 8 ppm, and two females (4%) at 32 ppm (historical incidence (single section) in females: 1/898 (range, 0–2%); and one female (2%) at 2 ppm had a renal tubule carcinoma. The incidence was within the historical control range for male rats, but exceeded the historical control range for female rats. Additional analyses were performed by step sectioning of the kidneys, which revealed one additional renal tubule adenoma in each group of males for the controls, at 2 ppm, and at 8 ppm, and four additional adenomas in males at 32 ppm. The incidence of renal tubule adenoma or carcinoma (combined) – standard (single section) and extended evaluation (step sections) – in males became 2/50 (4%), 2/50 (4%), 3/50 (6%), 4/50 (8%). After step sectioning, two renal tubule adenomas were observed in females at 8 ppm and one in a female at 32 ppm, and a carcinoma was observed in a female at 2 ppm. The incidence of renal tubule adenoma or carcinoma (combined) – standard (single section) and extended evaluation (step sections) – in females became 0/50, 2/50 (4%), 2/50 (4%), 3/50 (6%); [the Working Group noted that incidence was incorrectly reported (typing error) in Table 10 of NTP (1999)]. [The Working Group noted this was a well-conducted GLP study in males and females.]

3.3 Transgenic animals

3.3.1 Skin application

Spalding et al. (2000) tested the tumorigenic activity of furfuryl alcohol using the Tg.AC transgenic mouse model (Tennant et al., 1996). Groups of 15–20 hemizygous female Tg.AC transgenic mice (age, 14 weeks) were treated with furfuryl alcohol (purity, > 98%) at a dose of 0, 0.25, 0.75, or 1.5 mg per mouse in 200 mL of acetone, by skin application, five times per week for 20 weeks. 12-*O*-Tetradecanoylphorbol-13-acetate (TPA)

(1.25 µg, three times per week) was used as a positive control. At 20 weeks, survival of the mice at the highest dose (90%) was lower than in the other treated groups and negative controls (100% in all groups). No information on body weights was provided. At 26 weeks, full histopathology was performed. No significant increase in the incidence of skin tumours (papillomas) was observed in mice exposed to furfuryl alcohol. Only one mouse at the intermediate dose developed a skin papilloma, while all mice treated with TPA had skin papillomas (100%). [The Working Group noted that this was a short-term, gene-specific assay in transgenic mice, and did not provide critical information that can be obtained in longer-term bioassays (e.g. effects on multiple target organs, interactions of time and age) ([Pritchard et al., 2003](#)).]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

The absorption, distribution, metabolism, and excretion of furfuryl alcohol and some related compounds were discussed in WHO Food Additive Series No. 46 ([WHO, 2001](#)). These chemicals were also briefly addressed in WHO Technical Report Series No. 974 ([WHO, 2012](#)).

4.1.1 Absorption, distribution, and excretion

(a) Humans

No data in humans exposed to furfuryl alcohol were available to the Working Group.

In humans exposed by inhalation to furfural (the primary oxidation product of furfuryl alcohol) (see [Fig. 4.1](#)), absorption was rapid and extensive. In male volunteers exposed by inhalation to furfural at vapour concentrations of

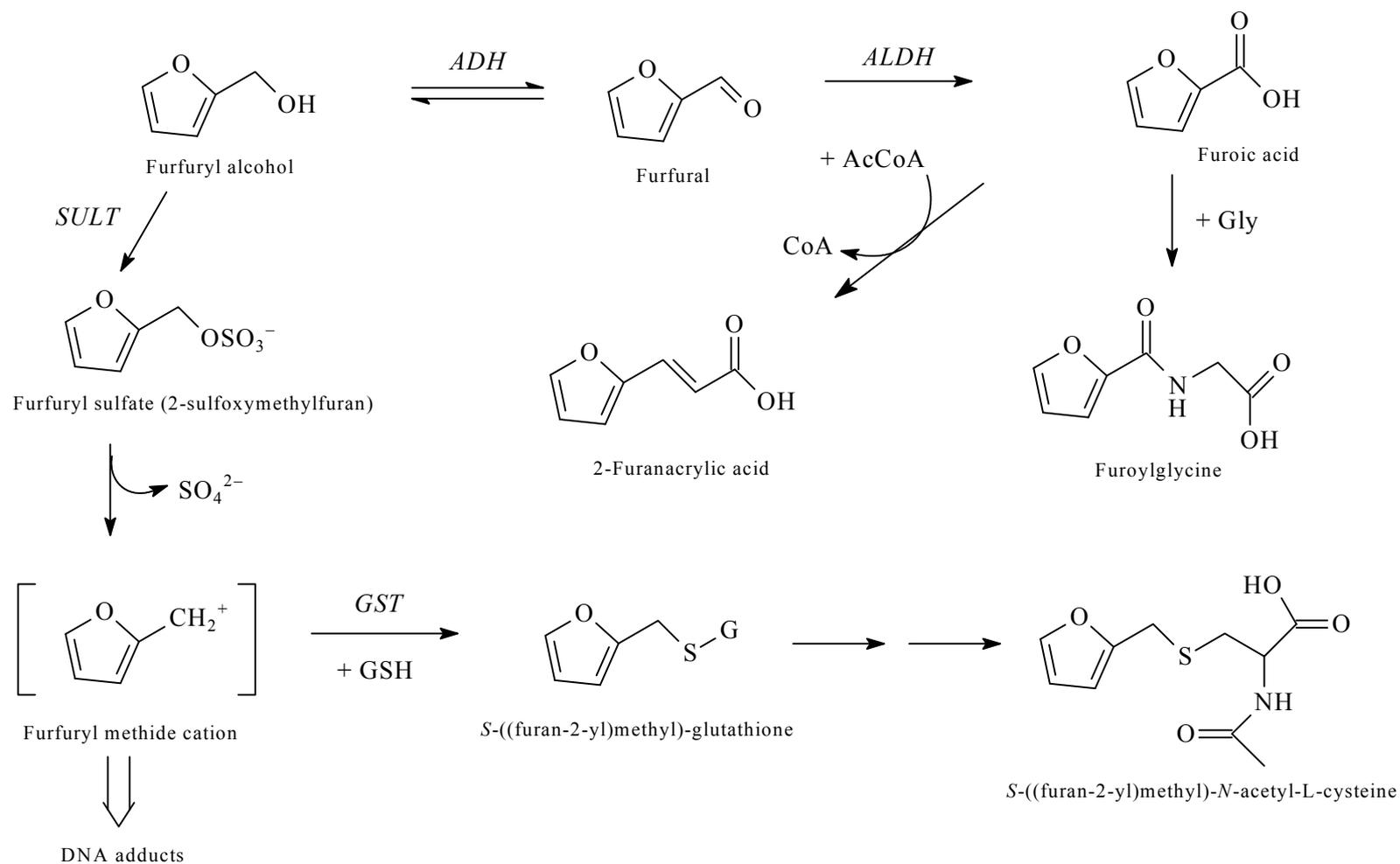
7–30 mg/m³ for 7.5 hours over an 8-hour period, pulmonary retention averaged ~78% regardless of vapour level or duration, and furfural quickly disappeared from the subjects' expired air after exposure ([Flek & Sedivec, 1978](#)). [Flek & Sedivec \(1978\)](#) also reported substantial percutaneous absorption of furfural vapour by male volunteers, particularly under warm and humid conditions.

(b) Experimental systems

Few data were available on the absorption, distribution, metabolism, and excretion of furfuryl alcohol in rodents.

Furfuryl alcohol and furfural are rapidly and extensively absorbed from the gastrointestinal tract in rodents. Furfural is converted to furfuryl alcohol by enteric bacteria under both aerobic and anaerobic conditions ([Boopathy et al., 1993](#)). In male rats treated by gavage with radiolabelled furfuryl alcohol (0.275, 2.75, or 27.5 mg/kg bw) or furfural (0.127, 1.15, or 12.5 mg/kg bw) in corn oil, an average of 86–89% of the administered dose of each compound was absorbed systemically ([Nomeir et al., 1992](#)). The liver and kidneys contained the highest levels of radiolabel at 72 hours after exposure. Both furfuryl alcohol and furfural were extensively metabolized, with 83–88% of the administered doses excreted in the urine within 72 hours. Furoylglycine, the glycine conjugate of furoic acid, was the major urinary metabolite (73–80% of the administered dose). In male and female F344 rats and CD-1 mice given a single oral dose of ¹⁴C-labelled furfural at a wide range of dose levels, the chemical was extensively absorbed and metabolized to furoylglycine and furanacryloyl-glycine, which were primarily excreted in the urine ([Parkash & Caldwell, 1994](#)). There were only minor metabolic differences according to dosage, species, and sex.

Fig. 4.1 Metabolism of furfuryl alcohol in humans and experimental animals



The predominant flux, based on recovery of urinary metabolites, is through the action of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) with conjugation to glycine to form furoylglycine. Small amounts of furfuryl alcohol can undergo sulfate conjugation with spontaneous removal of the sulfate moiety to generate a reactive and unstable intermediate (shown in brackets). Other abbreviations: AcCoA, acetyl-CoA; CoA, coenzyme A; Gly, glycine; G, glutathionyl moiety; GSH, glutathione; GST, GSH S-transferase; SULT, sulfotransferase. Adapted from [Sachse et al. \(2014\)](#). The effect of knockout of sulfotransferases 1a1 and 1d1 and of transgenic human sulfotransferases 1A1/1A2 on the formation of DNA adducts from furfuryl alcohol in mouse models, *Carcinogenesis*, 2014, volume 35, issue 10, p. 2339-2345, with permission of Oxford University Press

4.1.2 Metabolism

(a) Humans

Furfuryl alcohol appears to be rapidly and extensively metabolized by humans. The major metabolite detected in male volunteers who were treated by inhalation with furfural, as in mice and rats, was furoylglycine. A secondary urinary metabolite in humans and rodents was 2-furana-crylic acid (Flek & Sedivec, 1978). An alternate metabolic pathway involved formation of a mutagenic metabolite via sulfate conjugation. Human sulfotransferase 1A1 (SULT1A1) was efficient in catalysing the formation of 2-sulfoxymethylfuran, a reactive intermediate (Sachse et al., 2016a). SULT1A1 is found at high levels in many human tissues, including liver, lung, gastrointestinal tract, brain, and kidney (Glatt & Meinl, 2004).

(b) Experimental systems

Furfuryl alcohol is metabolized by mice and rats in much the same way as in humans. The major metabolic pathway involves the oxidation of furfuryl alcohol by alcohol dehydrogenase to furfural, which is subsequently oxidized to furoic acid. Furoic acid is excreted in the urine of mice and rats, as is its glycine conjugate (Parkash & Caldwell, 1994). Sulfate conjugation of furfuryl alcohol appears to be a minor pathway, quantitatively. In FVB/N mice given drinking-water containing furfuryl alcohol (~390 mg/kg bw) for 28 days, renal, pulmonary, and hepatic DNA adducts were detected by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MSMS) (Monien et al., 2011). Nucleoside adducts were also found in porcine liver DNA incubated with 2-sulfoxymethylfuran and also in DNA from furfuryl alcohol-exposed *Salmonella typhimurium* expressing the human sulfotransferase isoform SULT1A1 (Monien et al., 2011). In *Sult1a1* null mice given a single dose of furfuryl alcohol, levels of DNA adducts in the liver, kidney, lung, colon, and small intestine

were substantially lower than in wildtype mice (Sachse et al., 2014) (see Section 4.2.1). Sachse et al. (2016a) assessed the catalytic efficiencies of 30 sulfotransferase isoforms from mice and rats in metabolically activating furfuryl alcohol. Human SULT1A1 and mouse *Sult1a1* were considerably more efficient than the other isoforms in mediating formation of 2-sulfoxymethylfuran, a reactive DNA electrophile (Sachse et al., 2016a).

4.2 Mechanisms of carcinogenesis

4.2.1 Genetic and related effects

Furfuryl alcohol has been studied in a variety of assays for genetic and related effects. Table 4.1, Table 4.2, Table 4.3, Table 4.4, and Table 4.5 summarize the results of studies carried out in exposed humans, in human cells in vitro, in non-human mammals, in non-human mammalian cells in vitro, and in non-mammalian systems, respectively.

(a) Humans

(i) Exposed humans

See Table 4.1

Furfuryl alcohol–DNA adducts were detected in non-tumour lung tissue of patients with cancer of the lung (Monien et al., 2015).

No effect on sister-chromatid exchange was observed in workers occupationally exposed to furfuryl alcohol and furfural. Exposure levels were not described, but duration of employment, age, and possible confounding factors such as smoking, X-ray during the 2 months before blood sampling, and recent viral infections were reported (Gomez-Arroyo & Souza, 1985).

(ii) Human cells in vitro

See Table 4.2

No induction of sister-chromatid exchange was observed in cultured human lymphocytes

Table 4.1 Genetic and related effects of furfuryl alcohol in exposed humans

End-point	Tissue or cell type	Description of exposed and controls	Results	Comments	Reference
DNA adducts, N ² -MFdG and N ⁶ -MFdA, UPLC-MS/MS	Lung	Non-tumour lung tissue from 10 (4 female, 6 male) lung cancer patients	+	Smoking status not reported	Monien et al. (2015)
Sister-chromatid exchange	Blood lymphocytes	Six workers occupationally exposed to furfuryl alcohol and furfural; six unexposed workers were used as control; both smokers and non-smokers were included	(-)	Exposure levels were not reported Causative effect of furfuryl alcohol alone could not be demonstrated	Gomez-Arroyo & Souza (1985)

^a +, positive; (-), negative result in a study of limited quality

N⁶-MFdA, N⁶-((furan-2-yl)methyl)-2'-deoxyadenosine; N²-MFdG, N²-((furan-2-yl)methyl)-2'-deoxyguanosine; UPLC-MS/MS, ultra-performance liquid chromatography-tandem mass spectrometry

Table 4.2 Genetic and related effects of furfuryl alcohol in human cells in vitro

End-point	Tissue, cell line	Results ^a	Concentration (LEC or HIC)	Comments	Reference
Sister-chromatid exchange	Cultured lymphocytes	-	9.9 mM [971 µg/mL]	4 donors	Gomez-Arroyo & Souza (1985)
Sister-chromatid exchange	Cultured lymphocytes	(-)	2.0 mM [196 µg/mL]	Number of donors not specified	Jansson et al. (1986)

^a -, negative; (-), negative result in a study of limited quality; the level of significance was set at $P < 0.05$ in all cases
HIC, highest ineffective concentration; LEC, lowest effective concentration

treated with furfuryl alcohol ([Gomez-Arroyo & Souza, 1985](#); [Jansson et al., 1986](#)).

(b) Experimental systems

(i) Non-human mammals

See [Table 4.3](#)

Furfuryl alcohol–DNA adducts were detected in wildtype FVB/N mice and transgenic mice expressing human sulfotransferases SULT1A1 or SULT1A2 ([Monien et al., 2011](#); [Sachse et al., 2014, 2016b](#); [Høie et al., 2015](#)). Transgenic mice expressing human SULTs had a higher level of DNA adduct N²-((furan-2-yl)methyl)-2'-deoxyguanosine (N²-MFdG) compared with wildtype FVB/N mice ([Sachse et al., 2014, 2016b](#)).

Levels of DNA adducts were lower in FVB/N mice lacking functional mouse sulfotransferase

(*mSult1a1* null) than in wildtype mice ([Sachse et al., 2014](#)). The oral administration of ethanol or of 4-methylpyrazole (a competitive substrate and an inhibitor of alcohol dehydrogenase, respectively) before exposure to furfuryl alcohol increased the levels of furfuryl alcohol–DNA adducts in all tissues. Clear sex-specific differences were observed, with adduct levels in female mice being up to fivefold those in male mice ([Sachse et al., 2016b](#)). Negative results were reported in tests for the induction of sister-chromatid exchange, chromosomal aberrations, and micronucleus formation in bone marrow of B6C3F₁ mice treated with furfuryl alcohol ([NTP, 1999](#)).

Table 4.3 Genetic and related effects of furfuryl alcohol in non-human mammals

End-point	Species, strain	Tissue	Results ^a	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA adducts, N ² -MFdG and N ⁶ -MFdA, LC-MS/MS	Mouse, FVB/N	Liver, kidney and lung (but not colon)	+	391 (M) or 393 (F) mg/kg bw	Oral, 28 d	Only one dose tested	Monien et al. (2011)
DNA adducts, N ² -MFdG, UPLC-MS/MS	Mouse, FVB/N (wt and <i>hSULT1A1/1A2</i> transgenic) ^b	Colon, liver (wt); small intestine, colon, liver (<i>hSULT1A1/1A2</i> transgenic)	+	250 mg/kg bw	Oral, 1×		Høie et al. (2015)
DNA adducts, N ² -MFdG and N ⁶ -MFdA, UPLC-MS/MS	Mouse, FVB/N (wt, knockout, <i>hSULT1A1/1A2</i> transgenic) ^c	Liver, lung, kidney, small intestine and colon	+	400 mg/kg bw	Intraperitoneal, 1×	Only one dose tested	Sachse et al. (2014)
DNA adducts, N ² -MFdG (all tissues) and N ⁶ -MFdA (liver only), UPLC-MS/MS	Mouse, FVB/N (wt and <i>hSULT1A1/1A2</i> transgenic) ^d	Liver, lung, kidney, small intestine and colon	+	400 mg/kg bw	Intraperitoneal, 1×	Only one dose tested	Sachse et al. (2016b)
Sister-chromatid exchange, chromosomal aberrations	Mouse, B6C3F ₁	Bone marrow cells	–	300 mg/kg bw	Intraperitoneal, 1×		NTP (1999)
Micronucleus formation	Mouse, B6C3F ₁	Bone marrow cells	–	125 mg/kg bw	Intraperitoneal, 3×		NTP (1999)

^a –, negative; +, positive; the level of significance was set at $P < 0.05$ in all cases

^b Two mouse cell lines: wt, and transgenic expressing human *SULT1A1/1A2*

^c Four mouse cell lines: wt, knockout deficient in either *Sult1a1* or *Sult1d1*, and transgenic expressing human *SULT1A1/1A2* while also being deficient for mouse *Sult1a1/1d1*

^d Two mouse cell lines: wt or transgenic expressing human *SULT1A1/1A2* while also being deficient for mouse *Sult1a1/1d1*

bw, body weight; d, day; F, female; HID, highest ineffective dose; *hSULT1A1/1A2*, human sulfotransferases 1A1/1A2; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LED, lowest effective dose; M, male; N⁶-MFdA, N⁶-((furan-2-yl)methyl)-2'-deoxyadenosine; N²-MFdG, N²-((furan-2-yl)methyl)-2'-deoxyguanosine; *Sult1a1/1d1*, sulfotransferases 1a1/1d1; UPLC-MS/MS, ultra-performance liquid chromatography tandem mass spectrometry; wt, wildtype

Table 4.4 Genetic and related effects of furfuryl alcohol in non-human mammalian cells in vitro

End-point	Species, cell line	Results ^a		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA strand breaks	Chinese hamster, V79 cells	–	NT	15 mM		Huffman et al. (2016)
DNA strand breaks	Chinese hamster, V79- <i>hCYP2E1-hSULT1A1</i> cells ^b	(+)	NT	15 mM	Marginal increase ($P = 0.04$); live cell count, 64%	Huffman et al. (2016)
Chromosomal aberrations	Chinese hamster ovary cells	(+)	(+)	2.5 mM, +S9; 20 mM, –S9	Results poorly reported	Stich et al. (1981)
Chromosomal aberrations	Chinese hamster ovary cells	–	±	500 µg/mL	Aroclor 1254-induced rat liver S9	NTP (1999)
Sister-chromatid exchange	Chinese hamster ovary cells	+	–	500 µg/mL	Aroclor 1254-induced rat liver S9	NTP (1999)

^a +, positive; –, negative; ±, equivocal (variable response in several experiments within an adequate study); (+), positive result in a study that had limitations in reporting or conduct; the level of significance was set at $P < 0.05$ in all cases

^b V79-derived cells co-expressing human cytochrome P450 2E1 (*hCYP2E1*) and human sulfotransferase 1A1 (*hSULT1A1*) genes
HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; S9, 9000 × g supernatant

(ii) Non-human mammalian cells in vitro

See [Table 4.4](#)

Chromosomal aberrations were induced in Chinese hamster ovary (CHO) cells treated with furfuryl alcohol in the presence and absence of metabolic activation with S9 ([Stich et al., 1981](#)). [The Working Group noted that the results were poorly reported.] In another study, there was no induction of chromosomal aberrations in cultured CHO cells in the absence of metabolic activation; equivocal results were obtained in the presence of metabolic activation based on a positive response in one trial that was not reproduced in a second, follow-up trial ([NTP, 1999](#)). Furfuryl alcohol induced sister-chromatid exchange in cultured CHO cells without but not with metabolic activation ([NTP, 1999](#)). Furfuryl alcohol marginally increased the frequency of DNA damage measured by the comet assay (pH > 13) in Chinese hamster V79 cells expressing human cytochrome P450 2E1 (*hCYP2E1*) and *hSULT1A1*, but not in the parental V79 cell line ([Huffman et al., 2016](#)).

(iii) Non-mammalian systems

See [Table 4.5](#)

In *Drosophila melanogaster*, no mutagenic activity was observed in an assay that measured induction of sex-linked recessive lethal mutations in male germ cells or in a test for sex-chromosome loss ([Rodriguez-Arnaiz et al., 1989](#)).

Furfuryl alcohol was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537, without or with metabolic activation ([Florin et al., 1980](#); [NTP, 1999](#); [Monien et al., 2011](#); [Glatt et al., 2012](#)). However, furfuryl alcohol was mutagenic in several TA100-derived strains expressing human and rodent sulfotransferases ([Monien et al., 2011](#); [Glatt et al., 2012](#)). DNA adducts were detected in DNA of furfuryl alcohol-exposed *Salmonella typhimurium* TA100 expressing *hSULT1A1*, but not in the parental strain (TA100) ([Monien et al., 2011](#)). In an acellular system, DNA adducts were detected after incubation of porcine liver DNA with 2-sulfoxymethylfuran ([Monien et al., 2011](#)).

Table 4.5 Genetic and related effects of furfuryl alcohol in non-mammalian systems

End-point	Species, strain, tissue	Results ^a		Agent, concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Sex-linked recessive lethal mutations, sex-chromosome loss	<i>Drosophila melanogaster</i> , germ-line cells	–	NA	1300 ppm		Rodriguez-Arnaiz et al. (1989)
Reverse mutation	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537	(–)	(–)	3 µmol/plate	Only one dose tested	Florin et al. (1980)
Reverse mutation	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537	–	–	10 000 µg/plate		NTP (1999)
Reverse mutation	<i>Salmonella typhimurium</i> TA100	–	NT	10 µmol/plate		Glatt et al. (2012) ; Monien et al. (2011)
Reverse mutation	<i>Salmonella typhimurium</i> TA100-derived strains expressing human or rodent sulfotransferases	+	NT	0.1–1 µmol/plate		Glatt et al. (2012)
Reverse mutation	<i>Salmonella typhimurium</i> TA100-derived strains expressing human or rodent sulfotransferases	+	NT	25 nmol/plate		Monien et al. (2011)
DNA adducts, 2-methylfuryl adducts of dAMF, dGMF and dCMF, LC-MS/MS	DNA isolated from porcine liver	+	NT	2-sulfoxymethylfuran (sodium salt), 5 µmol/mL [5 mM]		Monien et al. (2011)
DNA adducts, N ² -MFdG and N ⁶ -MFdA, LC-MS/MS	<i>Salmonella typhimurium</i> TA100	–	NT	167 µM		Monien et al. (2011)
DNA adducts, N ² -MFdG and N ⁶ -MFdA, LC-MS/MS	<i>Salmonella typhimurium</i> TA100-derived strains expressing human sulfotransferases	+	NT	167 µM		Monien et al. (2011)

^a +, positive; –, negative; (–), negative result in a study of limited quality; the level of significance was set at $P < 0.05$ in all cases

dA, 2'-deoxyadenosine; dC, 2'-deoxycytidine; dG, 2'-deoxyguanosine; HIC, highest ineffective concentration; LEC, lowest effective concentration; LM-MS/MS, liquid chromatography-tandem mass spectrometry; N⁶-MFdA, N⁶-((furan-2-yl)methyl)-2'-deoxyadenosine; N²-MFdG, N²-((furan-2-yl)methyl)-2'-deoxyguanosine; NA, not applicable; NT, not tested; ppm, parts per million

4.2.2 Other mechanisms

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

One study reported on the immunotoxic potential of furfuryl alcohol in mice. Furfuryl alcohol was shown to be a sensitizer and an irritant in mice exposed dermally. Enhanced airway hyperreactivity, eosinophilic infiltration into the lungs, and enhanced cytokine production were observed after repeated pulmonary exposure, and the responses were augmented on dermal pre-exposure to furfuryl alcohol ([Franko et al., 2012](#)).

In 14-day and 13-week studies, lesions indicative of altered cell proliferation, cell death, and inflammation were observed in the nose (olfactory epithelium) of F344/N rats and B6C3F₁ mice treated with furfuryl alcohol at all concentrations tested (16–250 ppm for 14 days; 16–32 ppm for 13 weeks) ([Irwin et al., 1997](#); [NTP, 1999](#)). After long-term exposure, the incidence of nasal tumours was significantly increased in male F344/N rats only ([NTP, 1999](#); see Section 3).

4.3 Data relevant to comparisons across agents and end-points

For the results of high-throughput screening assays of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA, see Section 4.3 of the *Monograph* on 1-*tert*-butoxypropan-2-ol in the present volume.

4.4 Susceptibility to cancer

No data were available to the Working Group.

4.5 Other adverse effects

No data from exposed humans were available to the Working Group.

In a 2-year inhalation study of furfuryl alcohol (0, 2, 8, 32 ppm), the severity of nephropathy increased with concentration in male and female F344/N rats and in male B6C3F₁ mice. Furfuryl alcohol was irritating and toxic to the nose and induced non-neoplastic lesions of the nose in all exposed groups of rats and mice. Corneal degeneration occurred in female mice at 32 ppm ([NTP, 1999](#)).

In a 14-week study, furfuryl alcohol induced degeneration and metaplasia of the olfactory epithelium in F344/N rats and B6C3F₁ mice, and hyaline droplets in B6C3F₁ mice ([Irwin et al., 1997](#); [NTP, 1999](#)). In another short-term study in Swiss mice, hepatic and renal toxicity were observed after inhalation of furfuryl alcohol (2000 and 4000 ppm, respectively) ([Sujatha, 2008](#)). [The Working Group noted the high concentrations used in this study relative to those tested in the cancer bioassays.]

5. Summary of Data Reported

5.1 Exposure data

Furfuryl alcohol has several industrial applications, including production of furan resins, wetting agents, and as a solvent. It is listed as a chemical with a high production volume, with between 10 000 and 100 000 tonnes manufactured and/or imported into the European Economic Area each year. China is the main global manufacturer and user with around 85% of the global capacity.

The general population is exposed to furfuryl alcohol mainly in foods and beverages, since it is a contaminant that arises during food processing (such as roasting, drying, baking and deep-frying) to obtain a desirable flavour. Coffee,

deep-fried breaded products, and toasted foods may contain furfuryl alcohol at high levels. Furfuryl alcohol was included in the most recent register of approved flavouring substances by the European Commission, while the United States Food and Drug Administration regulations allow use of furfuryl alcohol only as an indirect food additive due to its occurrence in food contact materials. According to the United States Food and Drug Administration, indirect food additives are substances that may come into contact with food as part of packaging or processing equipment, but are not intended to be added directly to food. Estimates of intake from food additives are well below 0.15 mg/kg bw (body weight) per day. Consuming one cup of espresso coffee leads to an intake of furfuryl alcohol of about 0.03 mg/kg bw. An acceptable daily intake of 0.5 mg/kg bw was established for furfuryl alcohol.

Occupational exposure may occur by inhalation and skin contact. In general, exposure levels registered in the industrial manufacturing sector, both in the USA and in France, have been below 35 mg/m³.

5.2 Human carcinogenicity data

There were no data available to the Working Group.

5.3 Animal carcinogenicity data

Furfuryl alcohol was tested for carcinogenicity in one well-conducted good laboratory practice (GLP) inhalation study in male and female mice, one well-conducted GLP inhalation study in male and female rats, and in a skin application study in a female transgenic mouse model.

In male B6C3F₁ mice, furfuryl alcohol induced a significant positive trend in the incidences of renal tubule adenoma, and renal tubule adenoma or carcinoma (combined); and

a significant increase in the incidence of renal tubule adenoma or carcinoma (combined) occurred at the highest dose. In addition, the incidence of renal tubule adenoma, carcinoma, and adenoma or carcinoma (combined) in male B6C3F₁ mice exposed at the highest dose, in each case exceeded historical control ranges for inhalation studies. Renal tubule neoplasms are rare in male B6C3F₁ mice. There was no significant increase in the incidence of any neoplasm in exposed female B6C3F₁ mice.

In male F344/N rats, furfuryl alcohol induced a significant positive trend in the incidence of adenoma, carcinoma or squamous cell carcinoma (combined) of the nasal respiratory epithelium, and of squamous cell carcinoma of the nasal respiratory epithelium. Furfuryl alcohol also induced a significant increase in the incidence of adenoma, carcinoma or squamous cell carcinoma (combined) of the nasal respiratory epithelium in male rats at the highest dose. Carcinomas and squamous cell carcinomas of the nasal respiratory epithelium have not been observed in male F344/N rats in historical controls. In addition, the incidence of renal tubule adenoma in exposed female F344/N rats exceeded the range for historical controls.

No significant increase in the incidence of tumours of the skin (papillomas) was observed in a study in transgenic female mice treated with furfuryl alcohol by skin application.

5.4 Mechanistic and other relevant data

Furfuryl alcohol is well absorbed by humans and rodents. Few data were available on distribution and elimination in rodents, and no data were available in humans. Furfuryl alcohol is rapidly and extensively metabolized. The predominant metabolic route is via alcohol dehydrogenase and aldehyde dehydrogenase with conjugation to glycine. Furfuryl alcohol can undergo sulfate

conjugation to yield the electrophile 2-sulfoxymethylfuran, leading to DNA adduction.

There is *strong* evidence that furfuryl alcohol is metabolically activated to an electrophile. There were consistent results for the formation of furfuryl alcohol-specific DNA adducts in one study of non-tumorous tissue of patients with cancer of the lung, in several studies in mice, and in an assay in bacteria expressing human sulfotransferase.

There is *moderate* evidence that furfuryl alcohol is genotoxic. Only data on DNA adducts, discussed above, were available from exposed humans. In human cells in vitro, results were negative for sister-chromatid exchange. In mice, results were negative for sister-chromatid exchange, chromosomal aberrations, and micronucleus formation. In mammalian cells in vitro, results were positive for sister-chromatid exchange without (but not with) metabolic activation, but negative for chromosomal aberrations. Results were negative for mutations in *Drosophila melanogaster*. Results were positive for mutation in two studies in *Salmonella typhimurium* transfected with human or rodent sulfotransferase, but negative in the standard Ames test.

In long-term bioassays in mice and rats, renal, nasal and corneal toxicity were reported.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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MELAMINE

1. Exposure Data

This substance was considered by the Working Groups in 1985 ([IARC, 1986](#)), 1987 ([IARC, 1987](#)), and 1999 ([IARC, 1999](#)). New data have become available since that time, and these have been incorporated and taken into consideration in the present evaluation.

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 108-78-1

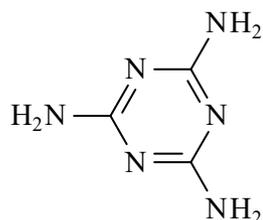
Previously used Chem. Abstr. Serv. Reg. Nos: 504-18-7; 65544-34-5; 67757-43-1; 68379-55-5; 70371-19-6; 94977-27-2

Chem. Abstr. Serv. name: 1,3,5-Triazine-2,4,6-triamine

IUPAC systematic name: Melamine

Synonyms: Cyanuramide; cyanurotriamide; cyanurotriamine; isomelamine; triaminotriazine; 2,4,6-triaminotriazine; triamino-*s*-triazine; 2,4,6-triamino-1,3,5-triazine; 2,4,6-second-triazinetriamine; 1,3,5-triazine-2,4,6(1*H*,3*H*,5*H*)-triamine

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: C₃H₆N₆

Relative molecular mass: 126.12

From [IARC \(1999\)](#); [Merck Index \(2013\)](#).

1.1.3 Chemical and physical properties

Description: Monoclinic prisms ([Merck Index, 2013](#))

Melting point: 345 °C; decomposes ([Lide, 1997](#)); emits highly toxic fumes of cyanides when heated to decomposition ([Sax, 1975](#)); non-inflammable ([Hawley, 1981](#))

Density: 1.573 g/cm³ at 16 °C ([Lide, 1997](#))

Solubility: Slightly soluble in water and ethanol; insoluble in diethyl ether ([Lide, 1997](#))

Octanol/water partition coefficient (P): log K_{ow}, -1.14 ([Verschuereen, 1996](#))

Conversion factor: 1 ppm = 5.16 mg/m³ at normal temperature (25 °C) and pressure (103.5 kPa) ([IARC, 1999](#))

Stability: Stable when stored under normal warehouse conditions ([Crews et al., 2006](#))

Impurities: The purity of melamine products is highly dependent upon the manufacturing process and the level of purification employed. Melam, melem, ammeline, ammelide, ureido-melamine, and cyanuric acid have been described as impurities, generally present at less than 0.2% ([WHO, 2008](#), [2009a](#)).

1.2 Production and use

1.2.1 Production process

Melamine was first prepared and described by Liebig in 1834 ([Liebig, 1834](#)) and has since become an increasingly important chemical commodity ([Crews et al., 2006](#)). Until about 1960, melamine was prepared exclusively from dicyandiamide. This conversion was carried out in autoclaves at 10 MPa and 400 °C in the presence of ammonia. In the early 1940s, it was discovered that melamine could also be synthesized from urea at 400 °C, with or without catalyst. Today, melamine is produced industrially almost exclusively from urea using various low- or high-pressure processes. For details, see review in [Crews et al. \(2006\)](#).

1.2.2 Production volume

Melamine is listed by the Organisation for Economic Co-operation and Development (OECD) as a chemical with a high production volume. In 1970, world production capacity was estimated at 200 000 tonnes. Production in 1994 was 610 000 tonnes/year ([Crews et al., 2006](#)).

World production in 2007 was approximately 1 200 000 tonnes ([WHO, 2009b](#)). In 2013, it was more than 1 600 000 tonnes ([Merchant Research & Consulting Group, 2015](#)). In 2017, China was the biggest producer, accounting for about 50% or more of global production and exports and 41% of global consumption ([IHS Markit, 2017](#)).

The database [Chem Sources International \(2017\)](#) lists 46 manufacturing companies

worldwide, of which 18 are located in the USA and 13 in China, including Hong Kong Special Administrative Region.

According to [ECHA \(2018\)](#), 100 000–1 000 000 tonnes of melamine are manufactured and/or imported in the European Economic Area per year.

1.2.3 Use

Melamine is a synthetically produced chemical that has many industrial uses, including the manufacture of melamine resins, laminates, glues and adhesives, surface coating resins, plastic moulding compounds, tarnish inhibitors, textile resins, textile finishes, permanent-press fabrics, bonding resins, flame-retardants, gypsum–melamine resin mixtures, orthopaedic casts, rubber additives and paper products, electrical equipment, construction materials such as plywood, and fertilizer urea mixtures ([IARC, 1999](#); [Hilts & Pelletier, 2009](#); [WHO, 2009b](#); [Tjioe & Ting, 2010](#); [IHS Markit, 2017](#); [ECHA, 2018](#)). However, it is primarily used in the production of melamine–formaldehyde resins for the manufacture of laminates, plastics, coatings (including can coatings), commercial filters, adhesives, and tableware. Important new applications are under development in the field of fire retardants for polymeric materials, especially polyurethane foams. Applications and uses of melamine differ widely among the main consumer countries or regions ([Crews et al., 2006](#); [WHO, 2009b](#); [Castle et al., 2010](#)).

Melamine has been used illegally to increase the nitrogen content in foods and animal feeds ([Lachenmeier et al., 2009](#)). Melamine contains about 66.6% nitrogen, and the addition of 1% melamine to protein leads to a false increase in the apparent protein content by 4.16%, if unspecific analytical methods are applied ([Bisaz & Kummer, 1983](#)). The first cases of melamine adulteration were detected in fish meal from Italy in the late 1970s ([Cattaneo & Cantoni, 1979, 1982](#)).

Most recently, a mass poisoning was reported in 2008 in China from contaminated milk and milk-based infant formula ([WHO, 2009b](#)).

1.3 Analytical methods

The most sensitive and selective analytical method to measure melamine, suitable for many matrices including milk, milk powder, and infant formula, as well as body fluids and tissues, is liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) ([EFSA, 2010](#)). Usable screening techniques for melamine include enzyme-linked immunosorbent assays (ELISA) and various spectroscopic techniques such as near infrared (NIR), Fourier transform infrared (FTIR), and nuclear magnetic resonance (NMR). The United States Food and Drug Administration (FDA) field laboratories use LC-MS/MS methods that are capable of determining melamine and cyanuric acid at concentrations of 0.25 ppm in powdered infant formula and other dairy-containing food products or ingredients, as well as a gas chromatography with mass spectrometry (GC-MS) method for melamine and its analogues ([FDA, 2014](#); [ECHA, 2016](#)).

Several comprehensive reviews of analytical methods for melamine are available ([Tyan et al., 2009](#); [Tittlemier, 2010](#); [Liu et al., 2012a](#); [Lu et al., 2017](#); [Nascimento et al., 2017](#); [Wang et al., 2017](#)). Representative methods for the analysis of melamine are presented in [Table 1.1](#).

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

Melamine does not occur naturally. Melamine may enter the environment from its industrial production, the processing and manufacture of resins, and from widespread use and disposal. In addition, the manufacture, use, and disposal of substances that degrade to form melamine

(i.e. triazine-based herbicides, cyromazine, and trichloromelamine) may also lead to the presence of melamine in the environment ([WHO, 2009a](#)).

Melamine does not readily biodegrade; however, its bioconcentration potential is considered to be low. A biodegradation pathway with *Pseudomonas* involves the conversion of melamine to ammeline and cyanuric acid. Volatilization from moist soil and from water surfaces, biodegradation, and hydrolysis are not expected to be important environmental fate processes. Most of the melamine present in the environment is thought to be distributed in water, based on its physiochemical properties, with minor amounts being distributed to soil, sediment, biota, and air ([HSDB, 2007](#)).

Monitoring data from rivers in Japan have indicated low concentrations of melamine in water, sediment, and fish. Data from 1986–1994 indicate melamine concentrations of < 0.0001–0.0076 mg/kg in river waters, < 0.01–0.40 mg/kg in sediment, and < 0.02–0.55 mg/kg in biota (fish). These data were considered insufficient to estimate possible concentrations in drinking-water or fish in general ([OECD, 1999](#)).

In certain provinces in China, waste water and soil near melamine-manufacturing facilities (100 m) contained high concentrations of melamine. In these situations, melamine was detected in 13 of 37 waste water samples, at concentrations ranging from 0.02 to 227 mg/L. Concentrations of melamine detected in 31 of 65 soil samples near to melamine-manufacturing facilities ranged from 0.1 to 41 mg/kg. Six of 94 irrigation water samples, collected from rivers or from underground sources, contained melamine at detectable concentrations of 0.02–0.20 mg/L. Of the 124 soil samples collected from farmlands at least 150 km from melamine factories (from 14 provinces in China), only 1 contained melamine at a detectable concentration of 0.18 mg/kg ([Qin et al., 2010](#)).

Table 1.1 Representative methods for the analysis for melamine

Sample matrix	Assay procedure	Limit of detection	Reference
Animal feed	LC-MS/MS	12.3 µg/kg	Chen et al. (2009)
Beverages	HPLC-UV	50 µg/L (limit of quantitation)	Ishiwata et al. (1987)
Chicken eggs	LC-MS/MS	8 µg/kg	Wang et al. (2012)
Chinese cabbage	GC-NPD	100 µg/kg	Bardalaye et al. (1987)
Dog food	ELISA	1 mg/L	Garber (2008)
Eggs	GC-MS/MS	3.5 µg/kg	Miao et al. (2010)
Milk powder	GC-MS/MS	3.8 µg/kg	Miao et al. (2010)
Fish and shrimp	LC-MS/MS	3.2 µg/kg	Andersen et al. (2008)
Salmon	LC-MS/MS	7.4 µg/kg	Karbiwnyk et al. (2009)
Catfish, tilapia, trout, shrimp	LC-MS/MS	3.5 µg/kg	Karbiwnyk et al. (2009)
Wheat gluten, chicken feed and processed foods	SERS	0.033 µg/mL	Lin et al. (2008)
	HPLC	1 µg/mL	
Infant formula	LC-MS/MS	8 µg/kg	Braekevelt et al. (2011)
Infant formula	HPLC-VIS	0.1 µg/L	Faraji & Adeli (2017)
Infant formula powder	NIR, FTIR-ATR, FTIR-DRIFT	1 µg/kg	Mauer et al. (2009)
Infant formula and candy	SPE-LC-MS/MS	5 µg/kg	Lachenmeier et al. (2009)
	NMR 400 MHz tube	33.26 mg/kg	
	NMR 700 MHz HRMAS	0.69 mg/kg	
Kitchenware	HPLC-FD	8 µg/L	de Lourdes Mendes Finete et al. (2014)
Liquid milk	Spectrophotometry	80 µg/L	Chansuvarn et al. (2013)
Liquid milk	CE-DAD	120 µg/kg	Sun et al. (2010a)
Milk	CE-DAD	47 µg/L	Chen & Yan (2009)
Milk and dairy products	GC-MS	20 ng/kg	Jurado-Sánchez et al. (2011)
Milk and dairy products	HPLC-DAD	35–110 µg/kg	Filazi et al. (2012)
Milk and fish feed	HPCE	80 µg/L	Wen et al. (2010)
	HPLC	50 µg/L	
Milk and infant formula	LC-MS/MS	25 µg/kg	Desmarchelier et al. (2009)
Milk products and animal feed	Immuno-chromatographic strip test	1 mg/L	Li et al. (2011a)
Milk-based products	HPLC-MS/MS	100 µg/kg	Ibáñez et al. (2009)
Fruit juice and milk blends		10 µg/L	
Muscle tissue	HPLC-MS/MS	1.7 µg/kg	Filigenzi et al. (2007)
Nutritional food ingredients	UPLC-MS/MS	100 µg/kg	Draher et al. (2014)
Tissue and body fluids	ELISA	50 µg/L	Wang et al. (2010)
	GC-MS	1 µg/L	

Table 1.1 (continued)

Sample matrix	Assay procedure	Limit of detection	Reference
Urine	UPLC-MS/MS	6 µg/L	Cheng et al. (2009)
Urine	HPLC-MS/MS	0.66 µg/L	Panuwet et al. (2012)
Urine	LC-MS/MS	10 µg/L	Zhang et al. (2010)
Various foods	LC-MS/MS	< 20.7 µg/kg	Deng et al. (2010)
Vegetable protein products	LC-MS/MS	1 µg/L	Levinson & Gilbride (2011)
Wastewater	MLC	13 µg/L	Beltrán-Martínavarro et al. (2013)

ATR, attenuated total reflectance; CE, capillary electrophoresis; DAD, diode array detector; DRIFT, diffuse reflectance; ELISA, enzyme-linked immunosorbent assay; FD, fluorescence detection; FTIR, Fourier transform infrared spectroscopy; GC, gas chromatography; HPCE, high-performance capillary electrophoresis; HPLC, high-performance liquid chromatography; HRMAS, high-resolution magic angle spinning; LC, liquid chromatography; MLC, micellar liquid chromatography; MS/MS, tandem mass spectrometry; NIR, near-infrared spectroscopy; NMR, nuclear magnetic resonance spectroscopy; NPD, nitrogen-phosphorus detector; SERS, surface-enhanced Raman spectroscopy; SPE, solid-phase extraction; UPLC, ultra-performance liquid chromatography; UV, ultraviolet; VIS, visible detection

Melamine and other triazine compounds are used as nitrogen sources in slow-release urea-based fertilizer mixtures ([Hilts & Pelletier, 2009](#)). In China, the majority of crop samples tested, including 235/246 maize samples, 141/143 soybean samples, and 166/168 wheat samples, collected from 21 provinces between October and December in 2008, contained melamine at detectable levels. However, less than 20% of crop samples contained melamine at concentrations above 0.1 mg/kg, and only 3 samples above 1 mg/kg, with the maximum of 2.05 mg/kg measured in a wheat sample ([Qin et al., 2010](#)).

1.4.2 Occurrence in food

(a) Food-contact materials

Melamine can be present in food as a result of its use in food-contact materials, including articles made of melamine–formaldehyde plastics, can coatings, paper and paperboard, adhesives, and cellophane polymers ([WHO, 2009a](#); [Bradley et al., 2011](#)).

Melamine has been shown to migrate into food and food simulants from melamine–formaldehyde tableware. The amount of melamine migration is dependent on temperature, acidity, contact time, and simulant used, as well as the quality of the product ([Ishiwata et al., 1986](#); [Sugita et al., 1990](#); [Martin et al., 1992](#); [Bradley et al., 2005](#); [Lund & Petersen, 2006](#); [Bradley et al., 2011](#); [Chien et al., 2011](#); [Chik et al., 2011](#)). Studies demonstrated that high temperatures applied to foods or simulants strongly influenced the degradation and migration of melamine, while the duration of heating and food and/or simulant acidity had only a minor influence ([Bradley et al., 2011](#)). When kitchen utensils containing melamine were tested under boiling conditions, migration was especially high. In microwave heating, high peak temperatures (“hot spots”) have been shown to result in high rates

of melamine transfer despite short contact times ([Bradley et al., 2010](#); [BfR, 2011](#)).

Melamine transfer into foods and simulants results from the migration of residual free monomers present after the manufacturing process; further transfer results from polymer breakdown, as well as chemical degradation and hydrolysis of the melamine resins ([Martin et al., 1992](#); [Lund & Petersen, 2006](#); [Bradley et al., 2010](#); [BfR, 2011](#)).

Like the migration and polymer breakdown that has been observed in melamine tableware, melamine-based resins used as can coatings and on metal closure lids of glass jars in the food industry also appear to degrade. This clearly occurs by hydrolysis of the melamine cross-linked resins, resulting in the release of additional melamine during the retort canning process ([Bradley et al., 2011](#); [Magami et al., 2015](#)).

(b) Precursor compounds that can form melamine

Melamine can occur in the environment and in food via commonly used chemicals that can form melamine (see [Table 1.2](#)).

Trichloromelamine, which decomposes to melamine, is a sanitizer and disinfectant for use on food packaging materials (except milk containers), hard food-contact surfaces, food processing equipment and utensils (except for dairy applications), and as a component of fruit and vegetable wash solutions in the USA (commercial disinfectant solutions diluted before use). It may also be used in other countries. The FDA estimated the concentration of melamine in food from disinfection to be 0.14 mg/kg based on a very conservative assumption that all disinfectants contain trichloromelamine ([WHO, 2009b](#)).

Humans are also exposed to melamine in food as a metabolite and degradation product of triazine-based pesticides, such as cyromazine ([Cook & Hütter, 1981](#); [WHO, 2009b](#)). Cyromazine can undergo metabolism in crop plants, poultry, ruminants, and other animals to form melamine.

Table 1.2 Precursor compounds that can degrade or metabolize to form melamine

Compound	Use of compound	Conditions under which the compound converts/metabolizes to melamine	Reference
Trichloromelamine (CAS No. 7673-09-8)	Sanitizer and disinfectant for use on food-packaging materials (except milk containers); food-processing equipment and utensils (except for dairy applications); hard food-contact surfaces and as a component of fruit and vegetable washes; non-food sanitizer e.g. in hospitals; pesticide	Readily decomposes to melamine during regular use as sanitizer	EPA (2005) ; WHO (2009a)
Cyromazine (CAS No. 66215-27-8)	Pesticide and herbicide; veterinary drug; insecticide (feed-through larvicide in poultry, incorporated in feed of laying hens to prevent flies hatching in manure, inhibits insect growth in cattle manure)	Undergoes metabolism in crop plants via dealkylation reactions to form melamine Undergoes metabolism via dealkylation reactions in poultry, ruminants, and other animals to form melamine Aerobic degradation to melamine in soil Photolytic degradation to melamine on soil	Sancho et al. (2005) ; Karras et al. (2007) ; Hilts and Pelletier (2009) ; WHO (2009a)
Triazine-based compounds	Pesticides, insecticides and herbicides As nitrogen sources in slow-release urea-based fertilizer mixtures (unknown whether melamine is still used); extent of use in fertilizers unknown	Certain triazine-based compounds may undergo environmental (bacterial, fungal) degradation and/or metabolism in crop plants	Hilts and Pelletier (2009) ; WHO (2009b)
Prometryn (CAS No. 7287-19-6)	Used as herbicide (triazine-based) on fruit and vegetable crops, cotton, potatoes, pastures, seed crops	Not present in fruit and vegetable crops, needs to be ingested by animals to degrade and form melamine	DAFF (2008)

CAS, Chemical Abstracts Service

Cyromazine is used in many countries as an insecticide, pesticide, or veterinary drug (EFSA, 2008; WHO, 2009b). Melamine residues on the edible part of vegetables resulting from the application of cyromazine are generally expected to be less than 1 mg/kg. These levels were found in tomatoes, lettuce, and celery plants treated with cyromazine in Japan (WHO, 2009c).

(c) *Animal feed*

Melamine is not permitted as an additive for animal feed in the European Union or USA (Hilts & Pelletier, 2009; EFSA, 2010). However, melamine can be present as an impurity in urea-based commercial feed additives used in ruminants at concentrations of up to 50 mg/kg (EFSA, 2010). Animal feed may also contain melamine as a result of its presence in the environment from approved uses of triazine pesticides and fertilizers (WHO, 2009b). On a worldwide scale, the adulteration of animal feed or feed ingredients with melamine and derivatives has been practised since 1989 and possibly earlier (Cattaneo & Ceriani, 1988; Cianciolo et al., 2008; NTP, 2008; Cruywagen & Reyers, 2009; González et al., 2009; WHO, 2009b).

Animal feed additives such as guanidinoacetic acid or urea can contain melamine as an impurity, which would result in trace amounts of melamine in feed (European Commission, 2009).

Melamine may also enter the food chain indirectly as a result of carry-over from adulterated animal feed into products of animal origin such as milk, eggs, meat, and fish (FDA, 2007; Pittet et al., 2008; WHO, 2009b). Carry-over of melamine from animal feed into animal tissues and/or products has been demonstrated in pigs (Buur et al., 2008; Wang et al., 2014), eggs and body tissues of laying hens (Bai et al., 2010; Dong et al., 2010; Valat et al., 2011; Gallo et al., 2012; Novák et al., 2012), eggs of Japanese quail (Zhang et al., 2012), dairy cows' milk (Cruywagen et al., 2009; Battaglia et al., 2010) and body tissues

(Sun et al., 2011), body tissues of sheep (Ly et al., 2010; Cruywagen et al., 2011), milk of dairy goats (Baynes et al., 2010), and fish (Andersen et al., 2008, 2011). These studies reported that melamine deposition and/or carry-over transfer rates from feed into animal products ranged from non-detectable to 3.6% in mammals and birds. Higher rates were reported in fish and shrimp.

(d) *Melamine levels in food*

Melamine concentrations measured in survey data submitted for consideration at a 2008 WHO Expert Meeting could not be easily distinguished as resulting from baseline contamination (levels occurring indirectly from approved uses of melamine or melamine precursors) or intentional adulteration, since a large number of samples were targeted as a result of potential adulteration. However, baseline levels are generally expected to be less than 1 mg/kg (Hilts & Pelletier, 2009; WHO, 2009b). The concentrations of melamine in non-adulterated foods are shown in Table 1.3.

1.4.3 Exposure in the general population

Exposure of the general population to melamine is thought to result primarily from the ingestion of melamine in non-adulterated food (HSDB, 2007). Estimates of exposure to melamine were provided by the OECD in the 1999 screening assessment, and are summarized in Table 1.4. This table presents exposure estimates from available studies in which some form of estimated exposure to melamine was provided. [The Working Group noted that the manner in which exposure was estimated differed between the studies, so direct comparisons could not be made.]

The WHO Expert Meeting estimated dietary exposure to melamine for scenarios using both baseline and adulterated concentrations in food (WHO, 2009b); the latter are discussed in Section 1.4.4. Baseline exposure estimates were made using data on concentrations of melamine

Table 1.3 Survey data on concentrations of melamine in non-adulterated food

Region or authority conducting survey	Foods surveyed	Limit of quantitation	Median melamine concentration (range) ^a	Fraction of total samples found to be positive (%)	Reference
Australia, Canada, New Zealand, Taiwan (China), USA	Infant formula	NR	All < 1 mg/kg	NR	WHO (2009b)
Health Canada Survey	Milk (<i>n</i> = 73) and soy-based (<i>n</i> = 19) infant formula (liquid ready-to-eat or concentrated [<i>n</i> = 31] and powdered [<i>n</i> = 63]) (total, <i>n</i> = 94)	4 ng/g	16 ng/g (range, 4.3–346 ng/g, “as purchased”; 5.5–69 mg/kg, calculated “as consumed”)	71/94 (76%)	Health Canada (2008) ; Tittlemier et al. (2009) ; Braekevelt et al. (2011)
Health Canada Survey ^b	Domestic and imported dairy products and soy-based dairy substitutes (<i>n</i> = 117); milk- and soy-containing items originating from Asia (<i>n</i> = 91); TDS milk and yogurt composites, 2004–2007 (<i>n</i> = 38)	4 ng/g	13 samples from China, 51 ng/g; 9 samples from North America, 8 ng/g; individual dairy and soy products (4.35–282 ng/g); TDS composite samples (95.1–7.2 ng/g)	Individual samples positive, 28/208 (13%); TDS composites positive, 4/38 (11%)	Tittlemier et al. (2010a)
Health Canada Survey ^b	A variety of egg-containing, soy-based, vegetable or fish and shrimp products (<i>n</i> = 364); TDS shrimp composites, 1993–2008 (<i>n</i> = 14) ^c	4 ng/g	Median values not reported Egg-containing items (5.1–247 ng/g); soy-based meat substitutes (4.1–47.9 ng/g); fish and shrimp products (4.1–1100 ng/g); vegetable products (4.6–688 ng/g); (5.6–29.8 ng/g) TDS shrimp composites	98/378 (26%) samples positive 8/113 (8%) egg-containing items; 8/87 (9%) soy-based items; 32/64 (50%) fish and shrimp products; 46/100 (46%) vegetable products; 6/14 (43%) TDS shrimp composites	Tittlemier et al. (2010b)
China	Eggs collected from markets	10 ng/g	(84–206 ng/g)	6/42 (14%)	Xia et al. (2009)
Germany	Protein powder, food supplements, sports food (<i>n</i> = 99)	1000 ng/g	ND	0%	Lachenmeier et al. (2017)

ND, not detected; NR, not reported; TDS, Total Diet Study (Canadian market basket survey that samples various food items from four different grocery stores and fast food restaurants in a selected Canadian city over a 5-week period each year ([Conacher et al., 1989](#)); foods are prepared as for consumption, and replicate food items from the various grocery stores or restaurants visited are combined and homogenized to form a composite sample)

^a Range of positives unless otherwise indicated

^b Many of the food items analysed were complex multi-ingredient processed foods collected at the retail level, and thus the source of the melamine in these items cannot be easily identified

^c Most TDS shrimp composites collected after 2001 were found to contain melamine, suggestive of a relatively recent exposure to melamine

Table 1.4 Estimates of exposure to melamine in the general population from various permitted uses and unintentional contamination, as reported from various sources

Source of exposure	Estimated daily exposure (µg/kg bw)	Comments ^a	Reference
Indirect exposure via the environment	1.1	Based on local monitoring data; used highest monitored concentration in drinking-water (0.0076 mg/L), and in fish (0.55 mg/kg); water intake, 2 L per d; fish intake, 0.115 kg per d; for 70 kg bw	OECD (1999)
	2.4	Based on modelled data for local level (EUSES)	
	0.05	Based on modelled data for regional level (EUSES)	
Overall exposure to consumer (i.e. general population)	10	Based on modelled data indirect via environment, dermal and inhalation from contact with polymers containing melamine (0.003 mg/kg bw, assumed 1% of occupational exposure), and from migration into food from melamine tableware (0.007 mg/kg bw; assuming average intake of hot food of 0.5 kg/d and 70 kg bw)	
Infant formula	0.54–1.60	Mean exposure; Health Canada occurrence dataset for baseline levels in infant formula (values < LOD = 1/2 LOD = 2 µg/kg); Institut National de Santé Publique du Québec, 2001 consumption estimates	Crossley et al. (2009) ; WHO (2009b)
Foods other than infant formula	0.03–0.12	Adults, mean exposure; Health Canada occurrence dataset for baseline levels in foods (values < LOD = LOD = 4 µg/kg); EFSA Concise European Food Consumption Database for 17 countries; 60 kg bw	
Disinfection in food processing	7	Adults, very conservative estimate; 0.14 mg/kg food, assumed all disinfectants contained trichloromelamine; 3 kg food consumption; 60 kg bw	
Migration from melamine-containing plastics (melamine tableware)	13	Adults, conservative estimate; assumes concentration of 1 mg/kg food; 25% of diet in contact with melamine tableware (0.25 × 3 kg = 750 g/person per d); 60 kg bw	
Migration from melamine-containing adhesives	< 0.35	Adults, conservative estimate; 3 kg food consumption; 60 kg bw	
Migration from melamine-containing paper and paperboard	0.0019	Adults, conservative estimate; 3 kg food consumption; 60 kg bw	
Residues arising from use of cyromazine as a pesticide	0.04–0.27	Adults, conservative estimate; concentration levels from STMR of the JMPR in 2007 (FAO, 2007) for cyromazine, assumed that ~10% of cyromazine residue was melamine, except for edible offal and mushrooms where assumed equal to STMR; GEMS/Food 13 cluster diets; 60 kg bw	

Table 1.4 (continued)

Source of exposure	Estimated daily exposure (µg/kg bw)	Comments ^a	Reference
Mean adult exposure from food	1.09–2.16	Using mean upper-bound melamine concentrations from industry dataset; EFSA CEFCD 19 countries, individual data	EFSA (2010)
Adult exposure from food	2.05–3.92	Using mean upper-bound melamine concentrations, 95th percentile, from industry dataset; EFSA CEFCD 19 countries, individual data	
Mean adult exposure from food	2.66–6.16	Using 95th percentile upper-bound melamine concentrations from industry dataset; EFSA CEFCD 19 countries, individual data	
Adult exposure from food	6.21–10.58	Using 95th percentile upper-bound melamine concentrations from industry dataset; EFSA CEFCD 19 countries, individual data	
Infant exposure from infant formula	1.3 (mean) and 1.8 (high)	800 g/d as mean intake, 1100 g/d as high value intake; 95th percentile upper-bound occurrence value; 6 kg bw; assume 1 part formula to 7 parts water	
Adult exposure from cyromazine use (sheep, poultry)	< 0.020 (for each)	300 g meat consumption; 60 kg bw adult	
Adult exposure from cyromazine use (eggs)	0.260–0.780	100 g egg consumption; 60 kg bw adult	
Migration from melaware (melamine tableware); scenario A; children aged 1–2 yr and 3–6 yr	Mean, 30–80; 95th percentile, 50–120	Scenario A, “typical migration levels”; assumes migration into food: 1 mg/kg acidic foods HF, 0.6 mg/kg aqueous foods HF, 0.2 mg/kg fatty foods HF, 0.05 mg/kg dry foods HF; summed exposure from all food groups; EXPOCHI consumption data, 12 Member States	
Migration from melaware (melamine tableware); scenario B; children aged 1–2 yr and 3–6 yr	Mean, 40–110; 95th percentile, 70–230	Scenario B, “high migration levels”; assumes migration into food: 5 mg/kg acidic foods, 3 mg/kg aqueous foods, 1 mg/kg fatty foods, 0.05 mg/kg dry foods; food item leading to highest exposure; EXPOCHI consumption data, 12 Member States	
Migration from coatings on metal cans and closures, infants aged 6 mo	34	Very conservative; 0.407 kg commercial baby food and drinks consumed (95th percentile) + 0.125 kg powdered infant formula; 7.8 kg bw; 0.5 mg/kg migration from coatings	
Migration from coatings on metal cans and closures, children aged 1.5 yr	92	Very conservative; 2 kg food consumed; 11 kg bw; 0.5 mg/kg migration from coatings	
Migration from coatings on metal cans and closures, adults	25	Very conservative; 3 kg food consumed; 60 kg bw; 0.5 mg/kg migration from coatings	

^a Upper-bound values < LOD, set equal to LOD

bw, body weight; CEFCD, Concise European Food Consumption Database; d, day(s); EUSES, European Union System for the Evaluation of Substances; EXPOCHI, EFSA Article 36 project, individual food consumption data and exposure assessment studies for children; FAO, Food and Agriculture Organization of the United Nations; GEMS, Global Environment Monitoring System; HF, hot filled; JMPR, Joint FAO/WHO Meeting on Pesticide Residues; LOD, limit of detection; mo, month(s); STMR, supervised trial median residue levels; yr, year(s)

Note: the Working Group considered that it was not appropriate to sum the dietary exposure assessments from different sources within each report, as the individual exposure assessments were generally very conservative

in different foods, together with food consumption data or very conservative exposure estimates. Estimates of exposure to melamine at baseline concentrations from various sources suggested: a maximum of 13 µg/kg body weight (bw) per day from the migration of melamine from tableware products such as cups, bowls, plates, or utensils; a maximum of 7 µg/kg bw per day from disinfection in food processing; a mean exposure of 0.54–1.6 µg/kg bw per day from infant formula; and a mean exposure for adults of 0.03–0.12 µg/kg bw per day from other foods.

In 2010, the European Food Safety Authority identified legitimate potential sources of melamine in food, including from food-contact materials, and estimated the associated dietary exposures ([Table 1.4](#)). Data submitted by industry, after excluding a small number of samples related to the adulteration incident, were used as the basis for dietary exposure assessment. For adult consumers of high concentrations, the dietary exposure estimates for melamine using the Concise European Food Consumption Database upper-bound occurrence values were less than 11 µg/kg bw per day ([EFSA, 2008](#)). For infants fed solely formula, the dietary exposure estimates were all less than 2 µg/kg bw per day. These estimates were considered to be conservative because many of the occurrence data were upper-bound values for samples in which melamine was found to be below the limit of detection ([EFSA, 2010](#)).

1.4.4 Exposure to melamine from contaminated food

(a) Humans

The largest incident of melamine poisoning occurred in China, beginning in the spring of 2008 ([Chen, 2009](#)). Relatively pure melamine was used in the illegal adulteration of raw milk that was subsequently used in the manufacture of infant formula and other foods ([WHO, 2009b](#); [Dorne et al., 2013](#); [Wang et al., 2013a](#)). Because of

globalization and the worldwide trade of food, melamine-contaminated foods containing milk products from China were detected in a large number of countries, including North America and the European Union ([Lachenmeier et al., 2009](#)).

[Table 1.5](#) presents estimates of exposure to melamine in infants and young children from the adulterated infant formula. Based on the median melamine concentration (1000 mg/kg), estimates of melamine exposure for Chinese children exposed to adulterated infant formula ranged from 8.6 to 23.4 mg/kg bw per day ([Jia et al., 2009](#); [WHO, 2009b](#)). Based on the mean melamine concentration (1212 mg/kg), 90th percentile concentration (2600 mg/kg), and maximum concentration (4700 mg/kg), dietary exposure estimates of melamine for children aged 3–24 months were 10.4–28.4 mg/kg bw per day, 22.3–61.0 mg/kg bw per day, and 40.3–110.2 mg/kg bw per day, respectively ([Jia et al., 2009](#)).

Dietary exposure to melamine from foods (other than infant milk formula) containing adulterated milk powder (e.g. ice cream, yoghurt, meal replacements, biscuits, chocolates) was also estimated during the WHO Expert Meeting. Using a conservative approach, assuming melamine was present in all food groups with the highest reported result for a food in that group and an average body weight of 60 kg, a dietary exposure of 0.16–0.70 mg/kg bw per day was estimated for adults consuming products adulterated with melamine ([WHO, 2009b](#)).

While limits were implemented (see Section 1.5) and food surveillance strengthened following this crisis, melamine contamination was still occasionally reported for protein-rich foods and food supplements in some countries ([Gabriels et al., 2015](#); [Deldicque & Francaux, 2016](#)). Probably due to tightened importation and market controls in Germany, no contamination was found in these products in Karlsruhe ([Lachenmeier et al., 2017](#)).

Table 1.5 Estimates of exposure to melamine from adulterated infant formula in 2008 and associated urolithiasis in infants and young children, as reported from various sources in China

Age of affected child	Age of screened child	Duration of exposure ^a	Exposure history ^b	Region	Reference
1–96 mo (mean, 25 mo)	1–126 mo (mean, 28 mo)	1.3–84 mo (mean, 19.5 mo)	12–2563 mg/kg (mean, 1295.3 mg/kg) in serum	Anhui	Hu et al. (2010) ; Hu et al. (2013)
≤ 3 yr	≤ 3 yr	≥ 30 d	High content (> 500 mg/kg): <i>n</i> = 23; moderate content (< 150 mg/kg): <i>n</i> = 19; no melamine: <i>n</i> = 8	Beijing	Guan et al. (2009)
17.5 ± 9.3 mo (stones) vs 16.9 ± 9.0 mo, mean (SD)	≤ 3 yr	Median, 6 mo (stones) vs 1 mo	0.77 (stones) vs 0.04 mg/kg bw per d, median exposure using current body weight; 2.35 (stones) vs 0.13 mg/kg bw per d, median exposure using birth weight; range of exposures, 0–51.2 mg/kg bw per d using current body weight, 0–102.4 mg/kg bw per d using birth body weight Four adulterated infant formula brands (12, 53.4, 150, and 2563 mg/kg)	Beijing	Li et al. (2010)
5–72 mo (median, 15 mo)	Mostly infants, also children	2–30 mo (mean, 13.7 ± 7.4 mo)		Gansu	Nie et al. (2013)
3 mo–4 yr; 91.7% < 3 yr (mean, 10 mo)	≤ 4 yr	1–24 mo	Melamine concentration of formula: 955–2563 mg/kg (consumed by 11 patients with stones); 6.2–17 mg/kg (consumed by 1 patient with stones). All consumed other foods or breast milk in addition to adulterated formula	Guang Dong	Zhu et al. (2009)
≤ 36 mo (mean, 19.8 mo)	≤ 36 mo	3 mo	36–220 mg/d (mean, 116 mg/d)	Yuanshi county	Liu et al. (2010b)
2–96 mo (median, 27 mo; geometric mean, 24 mo)	2–96 mo	2–96 mo (median, 20 mo; geometric mean, 17 mo)	0.01–62.67 mg/kg bw per d (median, 0.9; geometric mean, 1.28)	Shandong	Chen et al. (2009)
< 3 yr (77/79 children, 97.47%) (mean, 13.52 ± 10.13 mo)	4–72 mo (median, 15 mo)	With stones: 0.5–45 mo (mean, 12.53 ± 8.47 mo; median, 12 mo), 79 screened Without stones: mean, 8.65 ± 3.4 mo, 103 screened	5.17 ± 4.53 mg/kg bw per d (those with stones); 2.38 ± 3.39 mg/kg bw per d (103 screened without stones); exposed to 1–3 different brands of contaminated formula (those with stones)	Shandong	Sun et al. (2010b)
≤ 6 yr	≤ 6 yr	1–36 mo	High (Sanlu brand, 162–2563 mg/kg); medium (Sanlu and other brands); low (other brands, 0.09–150 mg/kg)	Shanghai	Gao et al. (2011)

Table 1.5 (continued)

Age of affected child	Age of screened child	Duration of exposure ^a	Exposure history ^b	Region	Reference
2–138 mo (median, 27.4 ± 25.5 mo)	Infants and children	1–54 mo (mean, 13.3 mo), stones; 1–96 mo (mean, 11.5 mo), no stones	Sanlu and Nanshan brand formula, > 5500 mg/kg; other formula, < 200 mg/kg; those with stones: 30.9% fed formula, 69.1% fed breast milk and formula; those without stones: 39% and 61%, respectively Those with stones: 56.7% fed Sanlu only, 13.4% fed Sanlu + others/Nanshan ± others, 29.9% fed other brands (< 200 mg/kg) Those without stones: 0.18% fed Sanlu only, 19% fed Sanlu + others/Nanshan ± others, 80.8% fed other brands	Sichuan	Wang et al. (2011)
1–60 mo (median, 16 mo) for 326 children with stones who had detailed data	1–180 mo (mean, 22 mo)	Mean, 15.7 ± 12.84 mo (stones); mean, 12.53 ± 9.49 mo (without stones)	Highest melamine concentration for brands: Sanlu, 2563 mg/kg; Shengyuan, 150 mg/kg; Yashili, 53.40 mg/kg; Shien, 17 mg/kg; Yili, 12 mg/kg	Zhejiang	Zhang et al. (2009)
88.6% ≤ 36 mo	1 mo–15 yr (mean, 22 mo)	≥ 1 mo	0.09–2563 mg/kg in formula (22 brands)	Zhejiang	He et al. (2009)
< 3 yr	NR	High exposure group: 0.67–36 mo (mean, 7.2 mo); low exposure group: 3–48 mo (mean, 17.4 mo)	High exposure: > 2.5 mg/kg; low exposure: 0.05–2.5 mg/kg; control exposure: < 0.05 mg/kg (LOD)	Taiwan, China	Wang et al. (2009a)
1.3–9 yr; high exposure: 1.3–4.8 yr; low exposure: 2.5–4 yr; control exposure: 1.9 and 9 yr	0–16 yr	High exposure: median, 12 mo (3.3–24.0); control exposure: median, 6 mo (4.0–7.0)	High exposure: > 2.5 mg/kg; low exposure: 0.05–2.5 mg/kg; control exposure: < 0.05 mg/kg (LOD)	Taiwan, China	Wang et al. (2009b)
NR	0.1–12.9 yr (mean, 6.4 yr)	≥ 1 mo	0.01–0.21 mg/kg bw per d (stones or renal deposits); 0.25 to > 1.5 L formula consumed daily; 68 mg/kg, highest concentration of melamine-adulterated formula	Hong Kong SAR	Lam et al. (2008)
3.5–32 mo	NR	3–24 mo; median, 12 mo	0.87–2002 µg/mmol creatinine (median, 21) urinary melamine levels; 1–3 brands melamine-contaminated infant formula consumed, 20–210 g daily Controls: 0.08–37 µg/mmol creatinine (median, 6.6) urinary melamine levels	Hong Kong SAR	Lam et al. (2009)

Table 1.5 (continued)

Age of affected child	Age of screened child	Duration of exposure ^a	Exposure history ^b	Region	Reference
6.7 yr, renal stones; 9.5 yr, renal deposits; 7 yr, suspected renal deposits	≤ 12 yr	26, 47, 24 pack mo	68 mg/kg, highest concentration in melamine-adulterated formula	Hong Kong SAR	Lau et al. (2012)

d, day(s); LOD, limit of detection; mo, month(s); NR, not reported; SAR, Special Administrative Region; SD, standard deviation; vs, versus; yr, year(s)

^a Those with stones unless indicated otherwise; indicated and reported as a range unless otherwise indicated

^b Those with stones unless indicated otherwise

There may be overlap between studies; cases reported by some authors may also be among the cases described by other authors in consideration of the institutional affiliation of some of the authors

(b) Companion animals

After an investigation by the FDA, it was determined in 2007 that wheat flour, presented as wheat gluten and rice protein, imported from China as pet food ingredients and subsequently incorporated into pet food manufactured in North America, had been contaminated with melamine and its analogues, cyanuric acid, ammeline, and ammelide. Melamine had been deliberately added to the wheat flour to falsely elevate the measured protein levels, in order to claim that the product was wheat gluten ([Brown et al., 2007](#); [Dobson et al., 2008](#); [Hilts & Pelletier, 2009](#); [WHO, 2009b](#); [Dorne et al., 2013](#)). The estimated number of deaths of dogs and cats attributable to exposure to pet food contaminated with melamine and cyanuric acid ranged between 2000 and 7000 ([Dorne et al., 2013](#)).

1.4.5 Biomonitoring data and biomarkers of exposure

Melamine is poorly metabolized and is mainly excreted in urine ([IARC, 1999](#); [WHO, 2009b](#)). The estimated half-life for urinary elimination of melamine in humans is approximately 6 hours ([Wu et al., 2013, 2015a](#)).

Urine samples were analysed in the general population of the USA; 76% of 492 urine samples contained melamine at detectable levels (limit of detection, 0.66 ng/mL). The geometric mean and 95th percentile concentrations were 2.4 ng/mL and 12 ng/mL [approximately 0.24 µg/mmol creatinine and 1.2 µg/mmol creatinine], respectively ([Panuwet et al., 2012](#)).

[Lin et al. \(2013\)](#) analysed 87 urine samples from 22 children aged 6–10 years and 70 urine samples from their parents in a community in Taiwan, China, and detected melamine in 98.7% of the samples. The median (and interquartile range) of melamine concentrations from the children's urine were 0.93 (0.49–1.30) µg/mmol creatinine for the first spot samples, and 1.73 (0.84–2.74) µg/mmol creatinine for the second

spot samples 24 hours later. For their parents, the corresponding melamine concentrations were 0.84 (0.51–1.97) and 0.87 (0.36–1.44) µg/mmol creatinine for the fathers ($n = 22$), and 0.87 (0.58–2.36) and 1.21 (0.65–2.14) µg/mmol creatinine for the mothers ($n = 22$).

In 2007–2008, a population survey was conducted in Hong Kong Special Administrative Region to examine the prevalence of metabolic syndrome in schoolchildren. The melamine concentrations in spot urine tests of the 502 children examined ranged from undetectable to 1467 µg/mmol creatinine (median, 0.8 µg/mmol creatinine; 58% of samples had concentrations above the limit of detection) ([Kong et al., 2011, 2013](#)). Similarly, [Wu et al. \(2015b\)](#) found that melamine was detectable in about two thirds of 264 urine samples from 88 university students in Taiwan, China. The geometric mean concentration and the highest measures were 6.5 ng/mL and 219 ng/mL [approximately 0.6 µg/mmol creatinine and 21.9 µg/mmol creatinine], respectively.

In humans, melamine reacts with uric acid to form melamine–urate crystals in the kidney ([Cruywagen et al., 2011](#)). In a study in Taiwan, China, in 211 adult patients diagnosed with calcium urolithiasis and 211 age- and sex-matched controls, urinary levels of melamine ranged from below the limit of detection to 192 ng/mL (62.1% detectable) [\sim 19.2 µg/mmol creatinine] in case patients, and from below the limit of detection to 56 ng/mL (20.4% detectable) in controls [\sim 5.6 µg/mmol creatinine] ([Liu et al., 2011](#)). In another study in 11 adults with uric acid urolithiasis, 22 adults with calcium urolithiasis, and 22 age- and sex-matched controls, measured median urinary concentrations of melamine were 0.50, 0.14, and 0.06 µg/mmol creatinine, respectively ([Wu et al., 2010a](#)).

1.4.6 Occupational exposure

Melamine has in the past been widely considered to be a low-toxicity dust except if decomposed by heat, when it emits highly toxic fumes of nitrogen oxides and hydrogen cyanide ([PubChem, 2018](#)). There are very few published exposure measurements for this substance, although occupational exposure could potentially occur by inhalation and inadvertent ingestion from hand-to-mouth contacts. Occupational exposure to melamine is most likely from its use in synthetic resins. Workers exposed to melamine may also be exposed to wood dust, phenol, formaldehyde, urea, and other hazardous substances ([Blair et al., 1990a](#)).

[Wu et al. \(2015a\)](#) investigated exposure to melamine among 44 workers in a small study at two factories manufacturing melamine tableware in Taiwan, China. Workers were involved in manufacturing and moulding, grinding and polishing, packing, and administration. In addition, a group of 105 non-exposed control workers was recruited from a neighbouring factory. Personal and area air samples at the worksite were obtained daily over 1 week for the workers exposed to melamine; pre- and post-shift spot urine samples were also acquired on each workday, as well as one spot urine sample each weekend morning and the following Monday morning. A single spot urine sample was collected on Friday morning from the control group. A blood sample was also obtained from all cases and controls. All samples were analysed for melamine. Air samples were also collected to measure exposure to formaldehyde. Exposure to melamine in the manufacturing and moulding group was consistently highest (mean personal air concentration, 97 $\mu\text{g}/\text{m}^3$; urine, 84.4 $\mu\text{g}/\text{mmol}$ creatinine; and serum, 7.2 ng/mL) compared with the administrative workers (mean personal air concentration, 0.5 $\mu\text{g}/\text{m}^3$; urine, 4.6 $\mu\text{g}/\text{mmol}$ creatinine; and serum, 1.7 ng/mL). Grinders and polishers, and packers had, on average, intermediate

exposures to melamine. The control group had the lowest average urinary concentrations of melamine (0.7 $\mu\text{g}/\text{mmol}$ creatinine). There was a high correlation between urinary and serum melamine concentration for 39 workers in the melamine tableware plants (Spearman correlation coefficient $r = 0.808$; $P < 0.001$).

1.5 Regulations and guidelines

There are no approved uses for the direct addition of melamine to food ([WHO, 2009b](#)). In the USA, melamine is an indirect food additive for use only as a component of adhesives (21 Code of Federal Regulations (CFR) 175.105). [According to the FDA, indirect food additives are substances that may come into contact with food as part of packaging or processing equipment, but are not intended to be added directly to food ([FDA, 2018a](#)).]

Melamine is approved for paper and paperboard and cellophane polymers in the USA ([WHO, 2009a](#)). Regulations for melamine-formaldehyde resins include 21 CFR sections 175.300 (resinous and polymeric coatings), 175.320 (resinous and polymeric coatings for polyolefin films), 176.170 (components of paper and paperboard in contact with aqueous and fatty foods), 176.180 (components of paper and paperboard in contact with dry food), 177.1010 (acrylic and modified acrylic plastics, semirigid and rigid), 177.1200 (cellophane), 177.1460 (melamine-formaldehyde resins in moulded articles), 177.1630 (polyethylene phthalate polymers), 177.2260 (filters, resin-bonded), and 177.2470 (polyoxymethylene copolymer) ([WHO, 2009a](#)). Melamine at the maximum allowed use level of 0.2% by weight as a stabilizer in polyoxymethylene copolymers is regulated in 21 CFR 177.2470, destined for use in the manufacture of repeat-use articles that may contact food ([FDA, 2018b](#)).

In Europe, melamine is approved for use as a monomer and as an additive in plastics ([European Commission, 2002](#); [WHO, 2009b](#)). The current

specific migration limit laid down in European Union legislation for plastics was lowered from 30 mg/kg food (EFSA, 2010) to 2.5 mg/kg food in 2011 (European Commission, 2011). In China, the migration standard for food containers is 1.2 mg/L or 0.2 mg/dm² (Ling et al., 2016).

In 2008, after findings of high levels of melamine in infant milk and milk products in China, the European Commission required Member States to check all consignments of feed and food containing milk products, soya, or soya products from China. An action level of 2.5 mg/kg was established by the European Commission to distinguish between the unavoidable background presence of melamine (from food-contact materials, pesticide use, etc.) and possible adulteration (EFSA, 2010).

WHO established a tolerable daily intake (TDI) of 0.2 mg/kg bw (WHO, 2009b), which was supported by EFSA (2010). WHO (2009b) suggested that the limits for melamine in powdered infant formula (1 mg/kg) and in other foods (2.5 mg/kg) provided a sufficient margin of safety for dietary exposure relative to the TDI of 0.2 mg/kg bw.

In 2012, the Codex Alimentarius Commission, which is jointly run by WHO and the United Nations Food and Agriculture Organization (FAO), adopted the following maximum levels for melamine: liquid infant formula, 0.15 mg/kg; powdered infant formula; 1 mg/kg; and other foods and animal feed, 2.5 mg/kg (United Nations News, 2012). In Europe, the maximum levels for powdered infant formula (1 mg/kg) and for other foods were implemented in Regulation No. 1881/2006 (Lachenmeier et al., 2017).

No occupational exposure limits for melamine were available.

2. Cancer in Humans

Melamine is often used in industry in conjunction with formaldehyde, and no occupational cohorts that were exposed to melamine and not to formaldehyde were identified by the Working Group; however, some pertinent data were available from a study of mortality among 25 619 workers in 10 industrial plants in the USA where formaldehyde was used. Exposure to formaldehyde was the focus of a series of publications based on this cohort (Blair et al., 1990b; Hauptmann et al., 2003, 2004; Beane Freeman et al., 2009, 2013), although 28% of the workers were ever exposed to melamine (Hauptmann et al., 2003). In the follow-up of this cohort to 1980, a trend in mortality from cancer of the lung with the duration of exposure to melamine was observed; this trend was statistically significant without consideration of latency ($P \leq 0.05$), but non-significant when a latency of 20 years or longer was assumed. Standardized mortality ratios (SMRs) for cancer of the lung were 1.3, 1.5, 1.9, and 2.0 for < 1, 1 to < 10, 10 to < 20, and ≥ 20 years of exposure, respectively (Blair et al., 1990b). Similar trends with standardized mortality ratios for cancer of the lung were seen for exposure to urea, and non-significant trends were seen for exposures to phenol and wood dust (which were used together with melamine in the production of resins and/or moulding compounds). No data were reported for associations between other cancers and exposure to melamine. Associations between duration of exposure to melamine and mortality from cancer of the nasopharynx, and between duration of exposure to melamine, dyes, plasticizers, and pigments [it was unclear whether exposure was to these agents in combination or separately] and mortality from all leukaemias, were reported in the text of a later publication based on further follow-up of the same cohort until 1994, but no estimates of risk or precision were given

([Hauptmann et al., 2003, 2004](#)). Exposure to melamine was analysed as a potential confounder of associations between leukaemia or cancer of the nasopharynx and exposure to formaldehyde in subsequent publications, based on extended follow-up of this cohort ([Beane Freeman et al., 2009, 2013](#)), and as a co-exposure in a re-analysis ([Marsh et al., 1992](#)), but associations for melamine were not reported. [The Working Group noted that no quantitative exposure data were available for melamine, and that the analysis was not adjusted for co-exposure to other chemicals, notably formaldehyde, or for tobacco smoking.]

Developmental and clinical effects of exposure to melamine as a contaminant in infant milk formula (melamine content, 0.1–2500 ppm) were examined in follow-up studies of fewer than 200 children in China who developed urinary stones after consuming adulterated infant milk formula. Clinical examinations carried out during 4 years of observation included ultrasound screening for cancer of the urinary system, which did not detect any tumours ([Wen et al., 2011](#); [Yang et al., 2013](#)). [The Working Group noted that this study included only children who had developed urinary stones and that the follow-up period was very short. The Working Group considered that the sensitivity of this study was low.]

3. Cancer in Experimental Animals

Melamine was previously evaluated by the Working Group with respect to its carcinogenicity in experimental animals ([IARC, 1986, 1999](#)). In its evaluation in 1999 ([IARC, 1999](#)), the Working Group concluded that there was *sufficient evidence* in experimental animals for the carcinogenicity of melamine under conditions in which it produces bladder calculi.

See [Table 3.1](#).

3.1 Mouse

3.1.1 Oral administration

In a study by the National Toxicology Program (NTP), groups of 50 male and 50 female B6C3F₁ mice (age, 6 weeks) were fed diets containing technical-grade melamine (purity, 97% [impurities were not further characterized]) at a concentration of 0, 2250, or 4500 ppm [0%, 0.225%, or 0.45%], at a dose of 0, 327, and 688 mg/day per kg bw for males and 0, 523 and 1065 mg/day per kg bw for females, ad libitum for 103 weeks, followed by a basal diet for 2 weeks ([NTP, 1983](#)). Mean body weights of males at the higher dose were slightly lower than those of the controls after week 50 of the study; mean body weights of males at the lower dose and of both treated groups of females were comparable to those of their respective controls throughout the study. The survival of males at the higher dose was significantly reduced when compared with that of the controls; survival at termination of the study was: controls, 39/49; lower dose, 36/50; and higher dose, 28/50 for males; and controls, 37/50; lower dose, 43/50; and higher dose, 41/50 for females.

No treatment-related increase in the incidence of tumours was observed in males or females. In male mice, treatment-related increases were observed in the incidence of (i) urinary bladder stones [composition unspecified]: controls, 2/45 (4.4%); lower dose, 40/47 (85%); and higher dose, 41/44 (93%); (ii) acute and chronic inflammation of the urinary bladder: controls, 0/45; lower dose, 25/47 (53%); and higher dose, 24/44 (55%); and (iii) “very mild” epithelial hyperplasia [not further specified] of the bladder: controls, 1/45 (2%); lower dose, 11/47 (23%); and higher dose, 13/44 (30%). Non-significant increases in the incidences of urinary bladder stones [composition unspecified] (4/50, 8%) and “very mild” epithelial hyperplasia (4/50, 8%) were also seen in females at the higher dose compared with controls

Table 3.1 Studies of carcinogenicity with melamine in rodents

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F ₁ (M) 6 wk 105 wk NTP (1983)	Oral Melamine, 97% Diet 0, 2250, 4500 ppm Ad libitum for 103 wk 49, 50, 50 39, 36, 28	No significant increase in tumour incidence in treated animals	NS	Principal strengths: studies in males and females, well-conducted study Urinary bladder stones were observed: 2/45 (4.4%) controls, 40/47 (85%) lower dose, 41/44 (93%) higher dose Urinary bladder hyperplasia (epithelial) was observed: 1/45 (2.2%) controls, 11/47 (23%) lower dose, 13/44 (30%) higher dose
Full carcinogenicity Mouse, B6C3F ₁ (F) 6 wk 105 wk NTP (1983)	Oral Melamine, 97% Diet 0, 2250, 4500 ppm Ad libitum for 103 wk 50, 50, 50 37, 43, 41	No significant increase in tumour incidence in treated animals	NS	Principal strengths: studies in males and females, well-conducted study Urinary bladder stones were observed: 0/42 controls, 0/49 lower dose, 4/50 (8.0%) higher dose Urinary bladder hyperplasia (epithelial) was observed: 0/42 controls, 0/49 lower dose, 4/50 (8.0%) higher dose

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Carcinogenicity with other modifying factor Mouse, BALB/c (M+F) (combined) NR (weanling) 22 wk Cremonezzi et al. (2001)	Oral	<i>Urinary bladder</i>		Principal limitations: limited number of animals, use of a single dose, data combined for sexes, limited number of organs examined, number of mice of each sex NR, short exposure duration This study was conducted to investigate the effect of dietary polyunsaturated fatty acids on mouse urinary bladder lesions induced by melamine Urinary bladder stones were observed in all groups treated with melamine (60–85% incidences, composition unspecified). No bladder stones were observed in the group without melamine In the group fed a diet containing 1.2% melamine plus 6% corn oil, the incidence of dysplasia/carcinoma in situ was significantly increased ($P < 0.05$, vs melamine group) in the urinary bladder and ureter at 13/23 (57%) and 10/23 (43%), respectively. In the group fed a diet containing 1.2% melamine plus 6% olein, the incidence of dysplasia/carcinoma in situ was significantly increased ($P < 0.05$, vs melamine group) in the urinary bladder, ureter, and renal pelvis at 11/18 (61%), 9/18 (50%), and 10/18 (56%), respectively
	Melamine, NR	Dysplasia or carcinoma in situ (combined):	NS * [$P = 0.0030$, Fisher exact test]	
	Diet	0/21, 9/27 (33%)*		
NR (weanling) 22 wk Cremonezzi et al. (2001)	Ad libitum	<i>Ureter</i>		Principal limitations: limited number of animals, use of a single dose, only one sex, low dose of application, limited number of organs examined, histopathology of tumours NR In both groups, single administration of melamine or acetone was followed by skin applications of 10 nmol TPA in 0.2 mL acetone twice weekly for 31 wk
	22, 27	Dysplasia or carcinoma in situ (combined):	NS * [$P = 0.0136$, Fisher exact test]	
	20, 27	0/21, 7/27 (26%)*		
Initiation–promotion (tested as initiator) Mouse, CD-1 (F) 8 wk 31 wk Perrella & Boutwell (1983)	Skin application	<i>Skin</i>		Principal limitations: limited number of animals, use of a single dose, only one sex, low dose of application, limited number of organs examined, histopathology of tumours NR In both groups, single administration of melamine or acetone was followed by skin applications of 10 nmol TPA in 0.2 mL acetone twice weekly for 31 wk
	Melamine, NR	Papilloma:	NS	
	Acetone	14%, 19%		
8 wk 31 wk Perrella & Boutwell (1983)	0 μ mol (followed by TPA),	Tumour multiplicity:	NS	Principal limitations: limited number of animals, use of a single dose, only one sex, low dose of application, limited number of organs examined, histopathology of tumours NR In both groups, single administration of melamine or acetone was followed by skin applications of 10 nmol TPA in 0.2 mL acetone twice weekly for 31 wk
	1 μ mol (followed by TPA),	0.14, 0.25		
	single skin application			
	20, 20			
	20, 20			

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 6 wk 105 wk NTP (1983)	Oral Melamine, 97% Diet 0, 2250, 4500 ppm Ad libitum for 103 wk 49, 50, 50 30, 30, 19	<i>Urinary bladder</i> Transitional cell carcinoma: 0/45*, 0/50, 8/49 (16%)** Transitional cell papilloma or carcinoma (combined): 0/45*, 0/50, 9/49 (18%)** Transitional cell papilloma: 0/45, 0/50, 1/49 (2.0%)	* $P < 0.001$, Cochran-Armitage and life-table trend tests; $P = 0.002$, incidental tumour trend test ** $P = 0.003$, relative to control, life-table test; $P = 0.016$, relative to control, incidental tumour test; $P = 0.002$ relative to control, Fisher exact test * $P < 0.001$, Cochran-Armitage, life-table, and incidental tumour trend tests ** $P = 0.002$, relative to control, life-table and Fisher exact tests; $P = 0.008$, relative to control, incidental tumour test NS	Principal strengths: studies in males and females, well-conducted study Urinary bladder stones were observed: 0/45 controls, 1/50 (2%) lower dose, 10/49 (20%) higher dose Urinary bladder transitional cell hyperplasia was observed: 0/45 controls, 1/50 (2%) lower dose, 2/49 (4.1%) higher dose

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (F) 6 wk 105 wk NTP (1983)	Oral Melamine, 97% Diet 0, 4500, 9000 ppm Ad libitum for 103 wk 50, 50, 50 34, 30, 27	<i>Urinary bladder</i> Transitional cell carcinoma: 0/49, 0/49, 0/47 Transitional cell papilloma: 0/49, 1/49 (2.0%), 1/47 (2.1%) <i>Thyroid gland</i> C-cell carcinoma: 0/50*, 0/49, 3/50 (6%) ^a C-cell adenoma or carcinoma (combined): 0/50, 2/49 (4.1%), 3/50 (6.0%) C-cell adenoma: 0/50, 2/49 (4.1%), 0/50	NS NS * <i>P</i> = 0.038, Cochran- Armitage trend test; <i>P</i> = 0.025, life table and incidental tumour trend tests NS	Principal strengths: studies in males and females, well-conducted study Neither urinary bladder stones nor transitional cell hyperplasia were observed in any group. No historical control data were provided for urinary bladder tumours in females ^a The incidence at the higher dose was not significantly different from the historical incidence of this thyroid tumour in untreated female F344/N rats at the same laboratory (14/689, 2.0%) or throughout the NTP bioassay programme (98/3544, 2.8%; range, 0–10%)

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Carcinogenicity with other modifying factor Rat, Wistar (M+F) (combined) NR (weanling) 36–40 wk Cremonezzi et al. (2004)	Oral Melamine, NR Diet 0, 1.5%, ad libitum 36, 20 NR, NR	<i>Urinary bladder</i> Dysplasia: 0/36, 0/20 <i>Renal pelvis</i> Dysplasia: 0/36, 2/20 (10%)	NS NS	Principal limitations: limited number of animals, dose groups, and organs examined; data combined for males and females; number of rats of each sex NR; short duration of the study This study was conducted to investigate the effect of dietary polyunsaturated fatty acid on rat urinary bladder lesions induced by melamine. In groups fed 1.5% melamine plus 6% olein diet or 6% of a mixture containing mainly stearic acid, the incidence of dysplasia in the renal pelvis was significantly increased ($P < 0.05$, vs melamine group) at 10/18 (56%) or 16/26 (62%), respectively
Carcinogenicity with other modifying factor Rat, Wistar (M+F) (combined) NR (weanling) 22–25 wk Cremonezzi et al. (2004)	Oral Melamine, NR Diet 0, 1.5%, ad libitum 22, 21 NR, NR	<i>Urinary bladder</i> Dysplasia: 0/22, 0/21 <i>Renal pelvis</i> Dysplasia: 0/22, 1/21 (4.8%)	NS NS	Principal limitations: limited number of animals, dose groups, and organs examined; data combined for males and females; number of rats of each sex NR; short exposure duration This study was conducted to investigate the effect of dietary polyunsaturated fatty acids on rat urinary bladder lesions induced by melamine Urinary bladder stones were not observed In the groups fed 1.5% melamine plus 6% corn-oil diet or 6% of a mixture containing mainly stearic acid, the incidence of dysplasia in the renal pelvis was significantly increased ($P < 0.05$, vs melamine group) at 11/20 (55%) or 14/21 (67%), respectively

F, female; M, male; NaCl, sodium chloride; NR, not reported; NS, not significant; NTP, National Toxicology Program; ppm, parts per million; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; vs, versus; wk, week(s)

(0/42 and 0/42, respectively) ([NTP, 1983](#); [Melnick et al., 1984](#)). [The Working Group considered that the strengths of this well-conducted study included the evaluation of multiple dose levels, the use of both males and females, and the study duration including most of the lifespan.]

In a study to investigate the effect of dietary polyunsaturated fatty acids on mice urinary bladder lesions induced by melamine, male and female homozygous weanling BALB/c mice [age not reported] were randomly distributed to several groups of 18–27 animals. In the group fed 1.2% melamine only [purity not reported; food intake data not provided] for 22 weeks, the incidence of dysplasia or carcinoma in situ [not further specified] (combined) was increased in the urinary bladder (9/27, 33%) [$P = 0.0030$] and the ureter (7/27, 26%) [$P = 0.0136$] compared with that in the control group receiving basal commercial diet (0/21, 0/21). Dysplasia or carcinoma in situ were also observed in the renal pelvis of mice in the group receiving melamine (4/27, 15%) and in the control group (1/21, 4.8%), without statistically significant differences. In another group receiving a diet containing melamine plus 6% corn oil for 22 weeks, there were significant ($P < 0.05$) increases in the incidence of dysplasia or carcinoma in situ (combined) of the urinary bladder (13/23, 57%) and ureter (10/23, 43%) compared with the group receiving melamine only. Finally, in a group receiving a diet containing melamine plus 6% olein for 22 weeks, there were significant ($P < 0.05$) increases in the incidence of dysplasia or carcinoma in situ (combined) of the urinary bladder (11/18, 61%), ureter (9/18, 50%), and renal pelvis (10/18, 56%) compared with the group receiving melamine only. Urinary bladder stones [composition unspecified] were observed in all groups (range, 60–85% [no further information provided]) treated with melamine ([Cremonezzi et al., 2001](#)). [The Working Group noted some limitations of the study, including the small number of animals, unspecified numbers of males and females, use of

a single dose, short duration of exposure, small number of organs examined, and lack of controls for the modifying factors used in this study, as well as the fact that only representative samples of the urothelium were investigated.]

3.1.2 Initiation–promotion

A group of 20 female CD-1 mice (age, 8 weeks) received a single topical application of melamine [purity not reported] of 1 μmol in 0.2 mL of acetone on shaved back skin, followed by twice-weekly applications of 10 nmol of 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in 0.2 mL of acetone for 31 weeks. A control group of 20 female mice received a single application of acetone alone, followed by applications of TPA. At 31 weeks, no significant increase in the incidence of skin papilloma was observed in melamine-treated mice (19%) when compared with controls (14%) ([Perrella & Boutwell, 1983](#)). [The Working Group noted the low dose of melamine used and the limited description of clinical observations and histopathology of observed tumours.]

3.2 Rat

3.2.1 Oral administration

In a study by the NTP, groups of 49–50 male and 50 female Fischer 344/N rats (age, 6 weeks) were fed diets containing technical-grade melamine (purity, 97% [impurities were not further characterized]) at a concentration of 0, 2250, or 4500 ppm for males (0, 126, and 263 mg/day per kg bw), and 0, 4500, or 9000 ppm for females (0, 262, and 542 mg/day per kg bw), ad libitum for 103 weeks, followed by a basal diet for 2 weeks ([NTP, 1983](#)). Mean body weights of male and female treated rats were lower than those of the controls after week 20 of the study. The survival of males at the higher dose was significantly reduced when compared with that of the controls;

the survival at termination of the study was: controls, 30/49; lower dose, 30/50; and higher dose, 19/50 for males; and controls, 34/50; lower dose, 30/50; and higher dose, 27/50 for females.

The incidence of transitional cell carcinoma of the urinary bladder in males was: controls, 0/45; lower dose, 0/50; and higher dose, 8/49 (16%) (control vs higher dose, $P \leq 0.016$; P (trend) ≤ 0.002). There was also a dose-related increase in the incidence of bladder stones in male rats: controls, 0/45; lower dose, 1/50 (2%); and higher dose, 10/49 (20%). Of 49 male rats at the higher dose, 7 (14%) had transitional cell carcinoma of the urinary bladder and urinary bladder stones, 1 (2%) had a carcinoma without stones, and 3 (6%) had stones without carcinoma (1 of these rats had a papilloma of the urinary bladder and 1 had epithelial hyperplasia). A statistically significant association ($P \leq 0.001$) was found between the presence of bladder stones and bladder tumours. No urinary bladder stones were reported in female rats, while one female at the lower dose and one at the higher dose had a papilloma of the urinary bladder; no data on historical controls were provided for this tumour. There was also a small but significant ($P = 0.038$) positive trend in the incidence of thyroid C-cell carcinoma in females (0/50, 0/49, 3/50); the incidence of this tumour in the group at the higher dose (3/50, 6%) was not significantly different from the historical incidence of this tumour at the laboratory (14/689, 2.0%) or throughout the NTP bioassay programme (98/3544, 2.8%; range, 0–10%) [the Working Group performed statistical tests and confirmed the lack of significance by pairwise comparison] (NTP, 1983; Melnick et al., 1984). [The Working Group noted that the strengths of this well-conducted study included the use of multiple dose levels and both males and females, and that the duration included most of the lifespan. The Working Group also noted that there may have been a relationship between the presence of stones and tumours of the urinary bladder. See also Sections 4.2 and 4.5.]

Four groups of 20 male Fischer 344 rats (age, 6 weeks) were fed diets containing melamine (purity, > 99%) at a concentration of 0% (control), 0.3%, 1.0%, or 3.0% (food intake, 15.3, 15.0, 14.7, and 11.7 g/rat per day) for 36 weeks, followed by a basal diet for 4 weeks. Mean body weight of rats at the highest dose was significantly lower than that of the controls ($P < 0.001$). Transitional cell carcinomas of the urinary bladder were observed in 0/20, 0/20, 1/20 (5%), and 15/19 (79%) ($P < 0.01$, increase) rats at the control, low, intermediate, and highest doses, and transitional cell papillomas in 0/20, 0/20, 1/20 (5%), and 12/19 (63%) ($P < 0.01$, increase) rats, respectively. One (5.3%) rat at the high dose developed a carcinoma of the ureter and 3 (16%) rats at the high dose developed papillomas of the ureter. The findings of tumours correlated ($P = 0.0065$) [correlation coefficient not provided] with the formation of urinary bladder calculi [composition unspecified] (Okumura et al., 1992). [The Working Group noted the short duration of the study, the use of one sex only, the small number of animals at start, and the small number of organs examined.]

In a study in which the effects of urinary volume on melamine-induced urinary bladder calculi formation were examined by administration of a diet supplemented by sodium chloride (NaCl), six groups of 20 male Fischer 344 rats (age, 6 weeks) were fed diets containing melamine (purity, 99.9%) at a concentration of 1% or 3% (food intake, 14.8 or 12.2 g/rat per day for the rats receiving melamine at 1% or 3%, respectively), with or without NaCl at 5% or 10% for 36 weeks, followed by a basal diet for 4 weeks. No transitional cell papillomas or carcinomas of the urinary bladder were observed in 10 control rats fed only the basal diet (food intake, 15.0 g/rat per day). Transitional cell carcinomas of the urinary bladder were observed in 4/19 (21%), 18/20 (90%) [$P < 0.0001$, increase compared with basal diet controls], and 18/20 (90%) rats given 1% melamine only, 3% melamine only, or 3%

melamine plus 5% NaCl, respectively. No transitional cell carcinomas of the urinary bladder were observed in the groups receiving 3% melamine plus 10% NaCl (0/20), or 1% melamine plus 5% (0/19) or 10% NaCl (0/19). The incidence of transitional cell papilloma of the urinary bladder was similarly decreased by NaCl. The incidence of transitional cell papilloma of the urinary bladder was 10/20 (50%) [$P < 0.02$, increase compared with basal diet controls] in the group given 3% melamine only, but 5/20 (25%) and 3/20 (15%) in the rats receiving 3% melamine plus 5% NaCl or 10% NaCl, respectively. Transitional cell papillomas of the urinary bladder developed in 8/19 (42%) rats receiving 1% melamine only [$P < 0.03$, increase compared with basal diet controls]. The occurrence of tumours correlated with calculus (melamine–uric acid salt, determined by high-performance liquid chromatography) formation and papillomatosis. The total combined contents of melamine and uric acid in the calculi obtained from four rats in the group treated with 1% melamine only were 61.1–81.2%, and the molar ratios of uric acid to melamine were 0.99–1.05 (Ogasawara et al., 1995). [The Working Group noted the use of males only, the short duration of the study, the small number of animals at the start, and the small number of organs examined.]

In a study to investigate the effect of dietary polyunsaturated fatty acids on lesions of the urinary bladder induced by melamine, male and female weanling Wistar rats [age not reported] were randomly distributed into several groups of 18–36 animals. In two groups fed diets containing melamine [purity not reported] at a concentration of 1.5% [food intake data not provided] for 22–25 weeks or 36–40 weeks, dysplasia [not further specified] was observed in the renal pelvis of 1/21 (4–8%) and 2/20 (10%) rats, respectively, but not in the urinary bladder. No dysplasia of the renal pelvis or urinary bladder was observed in the respective control groups receiving basal commercial diet (0/22 and 0/36, respectively). In

other groups treated with melamine, additional dietary administration of 6% corn oil or 6% of a mixture containing mainly stearic acid for 22–25 weeks significantly increased ($P < 0.05$) the incidence of dysplasia in the renal pelvis (11/20 (55%) and 14/21 (67%), respectively). Additional administration of 6% olein or 6% of a mixture containing mainly stearic acid for 36–40 weeks significantly increased ($P < 0.05$) the incidence of dysplasia in the renal pelvis (10/18 (56%) and 16/26 (62%), respectively). Urolithiasis was not observed in any group (Cremonezzi et al., 2004). [The Working Group noted the small number of animals, the unspecified number of males and females, the use of a single dose, the short duration of the study, the short exposure duration, the small number of organs examined, the lack of controls for the modifying factors used in this study, and the fact that only representative samples of the urothelium were investigated.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Absorption

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In rats and monkeys, melamine was rapidly absorbed after oral administration (Liu et al., 2010a; Jacob et al., 2012).

The apparent efficiency of absorption of melamine was 76% in eight Dohne Merino rams. After a 10-day period during which all animals received a forage-based diet supplemented with control pellets, six rams received pellets containing melamine and two rams received control pellets

for 8 days. Melamine intake for the treated rams was 0.69 g/day ([Cruywagen et al., 2011](#)).

4.1.2 Distribution

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Monkeys

When given to three rhesus monkeys as a single oral dose at 1.4 mg/kg bw, melamine was rapidly absorbed and cleared, and mainly distributed in body fluids. The maximum concentration of melamine in plasma was 1767 ± 252 $\mu\text{g/L}$. The time to maximum concentration was 2.67 ± 1.16 hours, and the half-life of melamine in plasma was 4.41 ± 0.43 hours ([Liu et al., 2010a](#)).

(ii) Rats

In several studies in rats, melamine was distributed to the kidney and urinary bladder, among other organs. In Fischer 344 rats given a single oral dose of [^{14}C]-labelled melamine (0.025 mCi; ~ 1.3 mg/kg bw), the only organs showing concentrations of radiolabel much higher than those in the plasma were the kidney and bladder ([Mast et al., 1983](#)). In Sprague-Dawley rats treated with melamine (50 mg/kg, gavage), melamine concentrations were highest in the bladder, while almost no melamine was found in the brain ([Wu et al., 2010b](#)). In groups of six Sprague-Dawley rats randomly assigned to receive a single oral dose of melamine at 5 mg/kg, or a single intravenous dose at 2 mg/kg, melamine was predominantly restricted to blood or extracellular fluid and was not extensively distributed to organ tissues ([Yang et al., 2009](#)).

When administered at a daily dose of 40 or 400 mg/kg bw by gavage on days 13–20 of gestation in pregnant female F344 rats, melamine passed the placental barrier to reach the fetus in a dose-dependent manner ([Jingbin et al., 2010](#)).

Similarly, in pregnant and neonatal Sprague-Dawley rats treated with melamine at a single oral dose of 21.4 mg/kg per day, melamine was able to pass through the placenta and reach the fetus, and to accumulate in the lactating mammary gland and neonatal kidney. Moreover, melamine was eliminated via the kidneys for the neonates and via the placenta for the fetus, and later excreted into the amniotic fluid ([Chu et al., 2010](#)).

(iii) Pigs

Melamine residues were detected in the brain, duodenum, liver, heart, muscle, and kidney of fattening pigs given a diet supplemented with melamine at a concentration of 500 or 1000 mg/kg diet. Tissue concentrations declined 5 days after the withdrawal of melamine from the diet, to less than 2.5 mg/kg ([Wang et al., 2014](#)).

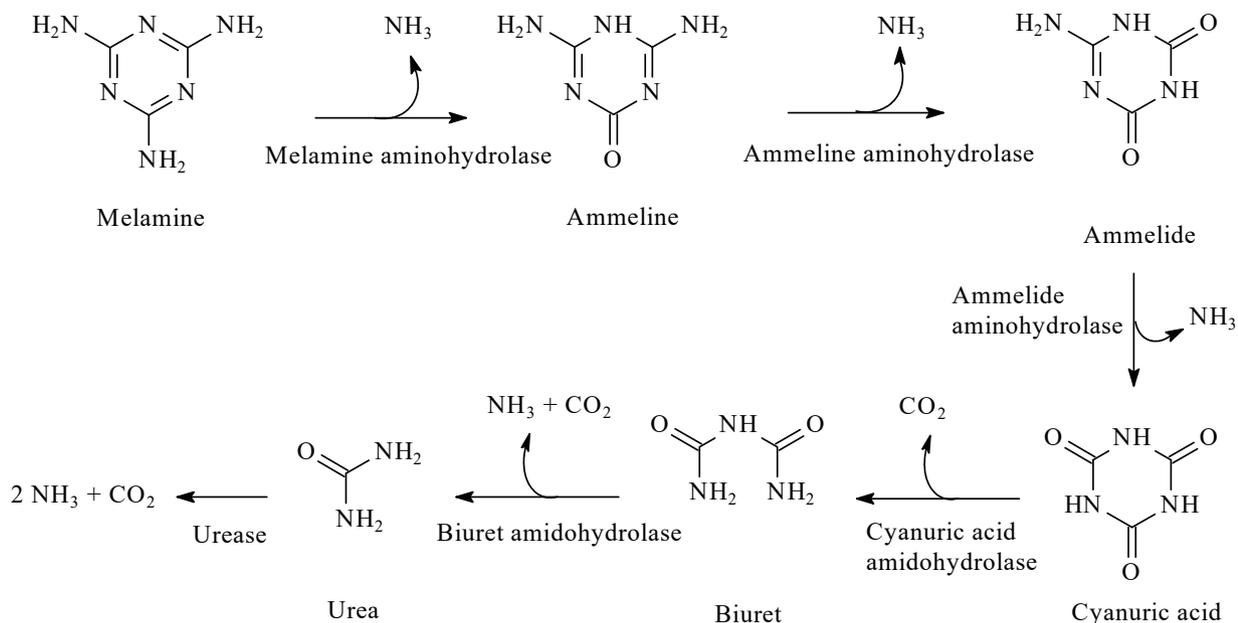
In five weanling pigs given melamine intravenously at a dose of 6.13 mg/kg bw, with plasma samples being collected for 24 hours, the data best fitted a one-compartment model with a half-life of $4.04 (\pm 0.37)$ hours, clearance of $0.11 (\pm 0.01)$ L/h per kg, and volume of distribution of $0.61 (\pm 0.04)$ L/kg ([Baynes et al., 2008](#)).

(iv) Sheep

In a study involving rams fed pellets containing melamine (described in Section 4.1.1(b)), melamine was detected in the urine, blood, muscle, and fat tissue of all rams that received melamine. Melamine concentrations reached 5.4 mg/kg in serum on day 8 of the collection period, and 9.6 mg/kg in meat ([Cruywagen et al., 2011](#)).

(v) Goats

Five lactating goats were given melamine as a single oral dose at 40 mg/kg bw. Blood samples were collected for 144 hours. The apparent plasma half-life (11.12 hours) was 3 times as long in these ruminants compared with monogastrics such as pigs and rodents ([Baynes et al., 2010](#)).

Fig. 4.1 The bacterial degradation of melamine

Source: [Eaton & Karns \(1991\)](#), amended with permission from the American Society for Microbiology.

4.1.3 Metabolism

Multiple studies, including in rats and in non-human primates, have indicated that melamine is not metabolized in mammalian tissue (e.g. [Mast et al., 1983](#); [Yang et al., 2009](#); [Liu et al., 2010a](#)). Comparable data were not available for humans, but a similar lack of metabolism is recognized from inferences that can be made ([Wu & Zhang, 2013](#)).

Melamine may be metabolized by bacteria, such as *Klebsiella terrigena* or *Pseudomonas*, to several metabolites, including cyanuric acid (see [Fig. 4.1](#)).

(a) Humans

[Zheng et al. \(2013\)](#) reported a correlation between melamine-induced toxicity in humans ([Liu et al., 2010b](#)) and the incidence of *K. terrigena* colonization in humans. [The Working Group noted that metabolism of melamine in the human gut has not been shown to be mediated by *K. terrigena* or other bacteria.]

(b) Experimental systems

Rats that had been colonized with *K. terrigena* exhibited exacerbated melamine-induced nephrotoxicity. Melamine-induced toxicity in rats was attenuated, and melamine excretion increased, after antibiotic suppression of gut microbial activity. Cyanuric acid was detected in the kidney of rats given melamine only, and the concentration was increased after *K. terrigena* colonization ([Zheng et al., 2013](#)). [The Working Group noted that the role of *K. terrigena* in the metabolism of melamine in the human gut has not been established.]

4.1.4 Excretion

See Section 4.5 for a discussion of the formation of precipitates containing melamine in the urinary tract.

(a) *Humans*

Urinary concentrations of melamine have been measured in children not specifically known to have been exposed to melamine.

In 2007–2008 in Hong Kong Special Administrative Region, 502 schoolchildren aged 6–20 years participated in a primary and secondary school survey that used a cluster sampling method. A high urinary level of melamine was defined as urine melamine/creatinine ratio > 7.1 $\mu\text{g}/\text{mmol}$. In 213 children (42%), melamine was undetectable. In 47 children (9%), urinary levels of melamine were high. The median urine melamine/creatinine ratio for all the schoolchildren tested was 0.76 $\mu\text{g}/\text{mmol}$ ([Kong et al., 2011](#)).

Melamine was detectable in all urine samples collected from schoolchildren aged 6–10 years (7 girls and 16 boys) in Taiwan, China. The median melamine concentrations in one-spot overnight urine samples on the mornings of the first and second day were 0.93 and 1.73 $\mu\text{g}/\text{mmol}$ creatinine, respectively. Melamine concentrations on the second morning were highly correlated with the total melamine excretions in urine during the previous 8 and 24 hours ([Lin et al., 2013](#)).

In a pilot study, 16 healthy volunteers (age range, 20–27 years) consumed 500 mL of hot noodle soup (initial temperature, 90 °C) served in melamine bowls. Postconsumption mean urinary melamine concentrations (corrected for urinary creatinine) initially increased sharply, peaked at 4–6 hours, and then declined (sharply for 2 hours, and then less steeply) until 12 hours after consumption. In another experiment in the same study, groups of three men and three women fasted for 8 hours before consuming 500 mL of hot noodle soup (initial temperature, 90 °C) served in either melamine bowls or ceramic bowls. Total urinary excretion of melamine in the urine over 12 hours was 8.35 ± 1.91 μg for those who were served soup in melamine bowls and 1.31 ± 0.44 μg for those who were served soup in ceramic bowls ($P < 0.001$) ([Wu et al., 2013](#)).

(b) *Experimental systems*

(i) *Monkeys*

In three rhesus monkeys given melamine as a single oral dose at 1.4 mg/kg bw, melamine was rapidly excreted, mainly through urinary clearance ([Liu et al., 2010a](#)).

(ii) *Rats*

In adult male Fischer 344 rats, more than 90% of a single oral dose of [^{14}C]-labelled melamine (0.025 mCi; ~ 1.3 mg/kg bw) was excreted within 24 hours via urine, exhaled air, and faeces, with 99% total recovery after 96 hours. The elimination half-life, urinary-excretion half-life, and renal clearance for melamine were 2.7 hours, 3.0 hours, and 2.5 mL/min , respectively. No residual radiolabel was observed in the blood or plasma after 24 hours. At this time point, residual radiolabel in the liver and kidney was 1.8 and 1.3 μg equivalents/kg tissue, respectively; radiolabel concentrations were much higher in the bladder and ureter (31 and 12 μg equivalents/kg tissue, respectively) ([Mast et al., 1983](#)).

In pregnant Sprague-Dawley rats given a single dose of melamine at 21.4 mg/kg bw by gavage at day 16–18 of gestation, 80% of the administered dose was found in the dams' serum at 0.5 hours. The peak melamine concentration of 7.15 ppm was reported in the fetuses after 2 hours, with 4.36 ppm reported in amniotic fluid after 3 hours. In the lactating rats, 40% of maternal intake of melamine was transferred to the milk, with peak concentrations at 3 hours ([Chan et al., 2011](#)).

(iii) *Sheep*

In a study in rams fed pellets containing melamine, urine was the major excretion route, accounting for 53.2% of ingested melamine; faeces accounted for 23.3% ([Cruywagen et al., 2011](#); described in Section 4.1.1(b)).

Table 4.1 Genetic and related effects of melamine in exposed humans

End-point	Tissue	Cell type, if specified	Description of exposed and controls	Response ^a , significance	Comments	Reference
DNA oxidation	Urine	NA	Infants exposed to melamine in contaminated powdered formula Four exposure groups: high: > 90% intake from contaminated formula; moderate: 50–90% intake from contaminated formula; low: < 50% of intake from contaminated formula; reference group, > 90% intake from imported milk powdered formula not containing melamine	–	Groups 1–3 are the observation groups, and Group 4 is the reference group	Ke et al. (2010)

^a –, negative

NA, not applicable

(iv) *Cows and goats*

Melamine was shown to distribute to the milk in lactating goats ([Baynes et al., 2010](#)) and in lactating cows (e.g. [Cruywagen et al., 2009](#); [Sun et al., 2012](#)).

4.2 Mechanisms of carcinogenesis

4.2.1 Genetic and related effects

The data on tests for genotoxicity with melamine were reviewed previously by the [NTP \(1983\)](#) and [IARC \(1999\)](#). New data have become available since then, and these have been incorporated into this Section.

(a) *Humans*

(i) *Exposed humans*

See [Table 4.1](#).

Urinary levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) were measured in a cross-sectional study in China of 73 male and 66 female infants (age, 0.5–1.5 years) who presented urinary problems and who were grouped by intake of melamine-contaminated infant formula milk ([Ke et al., 2010](#)). Even in the group with highest

exposure (infants who received more than 90% of their intake from contaminated formula), no increases in 8-OHdG levels were seen. [The Working Group noted that the study did not evaluate the relationship between 8-OHdG levels and the occurrence of urinary tract stones.]

(ii) *Human cells in vitro*

No evidence of malignant transformation was observed in a human liver cell line, L02, up to 6 months after treatment with melamine (doses up to 4000 µM) ([Zhang et al., 2011](#)).

(b) *Experimental systems*

(i) *Non-human mammals in vivo*

See [Table 4.2](#).

In male Sprague-Dawley rats treated by gavage, melamine did not induce DNA damage (as measured by the comet assay) in urothelial bladder cells or liver, even in the presence of some histopathological changes suggestive of cytotoxicity ([Wada et al., 2014](#)). In male F344 rats given drinking-water containing melamine, there was no increase in levels of γH2AX, a marker of DNA double-strand breaks, in the urinary bladder ([Toyoda et al., 2015](#)). No increases in the

Table 4.2 Genetic and related effects of melamine in rodents in vivo

End-point	Species, strain (sex)	Tissue	Results ^a	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Chromosomal damage	Mouse, B6C3F ₁ (M)	Bone marrow (PCE)	-	2000 mg/kg bw per day for 3 day	i.p.		Shelby et al. (1993)
Chromosomal damage	Mouse, NIH (M and F)	Bone marrow (PCE)	-	1600 mg/kg bw per day for 2 days	i.p., sampling 6 h after last dose		Zhang et al. (2011)
Chromosomal damage	Mouse, Kunming (M and F)	Bone marrow	-	Melamine + cyanuric acid, 294.5 mg/kg bw	Gavage; 2 doses, 24 h interval	Dose levels not clearly described	Liu et al. (2014)
DNA strand breaks (comet assay)	Rat, Sprague-Dawley (M)	Urothelial bladder and liver cells	-	2000 mg/kg bw	Gavage, 2 doses on 2 consecutive days		Wada et al. (2014)
DNA damage	Rat, F344 (M)	Urinary bladder epithelial cells	-	3% or ~2089 mg/kg bw per day	Diet, 4 wk with and without a 2-wk recovery period		Toyoda et al. (2015)
Mutation	Rat, Sprague-Dawley (M)	Peripheral blood	-	2000 mg/kg bw per day for 3 days	Gavage; sampling at 15, 29, and 60 days after treatment		Tu et al. (2015)
Mutation	Rat, Crl:CD(SD) (M)	Peripheral blood	-	2000 mg/kg bw	Gavage; 1×, sampling after 1, 2, or 4 wk		Kyoya et al. (2016)
Chromosomal damage	Mouse, B6C3F ₁ (M)	Bone marrow	±	300 mg/kg bw	i.p.; 1×, sampling at 36 h after injection		NTP (2017a)
Chromosomal damage	Mouse, B6C3F ₁ (M)	Bone marrow	+	87.5 mg/kg bw	i.p.; 1×, sampling at 23 and 42 h after injection		NTP (2017b)

^a +, positive; -, negative; ±, equivocal (variable response in several experiments within an adequate study); the level of significance was set at P < 0.05 in all cases
bw, body weight; F, female; h, hour(s); HID, highest ineffective dose; i.p., intraperitoneal; LED, lowest effective dose (units as reported); M, male; PCE, polychromatic erythrocytes; wk, week(s)

Table 4.3 Genetic and related effects of melamine in rodent cells in vitro

End-point	Species, cell line	Results ^a		Concentration (LEC or HIC)	Comments	Reference
		Results without metabolic activation	Results with metabolic activation			
Chromosomal aberrations	CHO	–	–	300 µg/mL	Highest dose, non-toxic; limited by solubility	Galloway et al. (1987)
Sister-chromatid exchange	CHO	±	–	225 µg/mL	Highest dose limited by solubility	Galloway et al. (1987)
Gene mutation	Mouse, L5178Y <i>Tk</i> ^{+/-} lymphoma cells	–	–	160 µg/mL		McGregor et al. (1988)
Chromosomal aberrations	CHO	–	–	4 mM		Zhang et al. (2011)
Micronucleus formation	CHO-K1	–	–	300 µg/mL		Tu et al. (2015)

^a –, negative; ±, equivocal (variable response in several experiments within an adequate study); the level of significance was set at $P < 0.05$ in all cases

CHO, Chinese hamster ovary; HIC, highest ineffective concentration; LEC, lowest effective concentration

frequency of *Pig-a* mutations or of micronucleus formation were seen in male Sprague-Dawley rats given melamine as three daily doses (up to 2000 mg/kg bw) by gavage ([Tu et al., 2015](#); [Kyoya et al., 2016](#)).

No induction of micronucleus formation was observed in bone marrow cells of male B6C3F₁ or NIH mice after intraperitoneal injection of melamine ([Shelby et al., 1993](#); [Zhang et al., 2011](#)). In bone marrow cells of male B6C3F₁ mice given a single intraperitoneal injection of melamine, both chromosomal aberrations and sister-chromatid exchange were reported ([NTP, 2017a, b](#)). In the test for induction of chromosomal aberrations, a significant increase was observed 36 hours after administration of melamine at the intermediate dose (300, but not 150 or 600 mg/kg bw); the trend test was not significant ($P = 0.358$) ([NTP, 2017a](#)). In the test for sister-chromatid exchange, significant increases were seen in two trials (four mice per group) at 23 hours (but not at 42 hours) after injection. In the first trial, only the group given an intermediate dose (175 mg/kg bw) gave

a positive result; in the second trial, the groups given a low dose (87.5 mg/kg bw) and intermediate dose (175 mg/kg bw) gave positive results ([NTP, 2017b](#)).

(ii) *Non-human mammalian cells in vitro*

See [Table 4.3](#).

No induction of gene mutation was observed in mouse lymphoma L5178Y *Tk*^{+/-} cells ([McGregor et al., 1988](#)). No induction of chromosomal aberrations, or of micronucleus formation, was observed in Chinese hamster ovary cells exposed to melamine with or without metabolic activation from induced rat liver S9 ([Galloway et al., 1987](#); [Zhang et al., 2011](#); [Tu et al., 2015](#)). In Chinese hamster ovary cells tested in the absence of metabolic activation, one of two trials yielded a small increase in the frequency of sister-chromatid exchange; no increases in the frequency of sister-chromatid exchange were seen in a single trial with rat liver S9 ([Galloway et al., 1987](#)).

(iii) *Non-mammalian systems*

See [Table 4.4](#).

Table 4.4 Genetic and related effects of melamine in non-mammalian experimental systems

Test system (species, strain)	End-point	Results ^a		Agent, concentration (LEC or HIC)	Comments	Reference
		Results without metabolic activation	Results with metabolic activation			
<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	Melamine, 1111 µg/plate	Tested in four strains in two laboratories	Haworth et al. (1983)
<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	Cyanuric acid, 10 000 µg/plate		Haworth et al. (1983)
<i>Drosophila melanogaster</i> Canton-S	Sex-linked recessive lethal mutations	±		Melamine, 1000 ppm	Feeding administration, equivocal results; injection, negative results	Foureman et al. (1994)
<i>Salmonella typhimurium</i> TA97, TA98, TA100, TA102	Reverse mutation	–	–	Melamine, 5000 µg/well		Zhang et al. (2011)
<i>Salmonella typhimurium</i> TA97, TA98, TA100, TA102	Reverse mutation	–	–	Melamine and cyanuric acid in combination, 500 µg/plate	Highest dose was limited by toxicity	Liu et al. (2014)
<i>Salmonella typhimurium</i> TA97a, TA98, TA100, TA102, TA1537	Reverse mutation	–	–	Melamine, 1000 µg/well	Highest concentration limited by solubility	Tu et al. (2015)

^a –, negative; ±, equivocal (variable response in several experiments within an adequate study)
HIC, highest ineffective concentration; LEC, lowest effective concentration; ppm, parts per million

The results of a test for sex-linked recessive lethal mutation in *Drosophila melanogaster* fed with melamine were equivocal; a second sex-linked recessive lethal assay using injection as the route of exposure yielded negative results ([Foureman et al., 1994](#)).

Although in cell-free systems melamine interacted with native DNA via minor groove binding by hydrogen bonds ([Shen et al., 2011](#); [Xie et al., 2015](#)), melamine (doses up to 5000 µg/plate) did not induce reverse mutation in any of several strains of *Salmonella typhimurium* in the presence or absence of exogenous metabolic activation ([Haworth et al., 1983](#); [Zhang et al., 2011](#); [Tu et al., 2015](#)).

(iv) Metabolites

[Haworth et al. \(1983\)](#) reported that cyanuric acid (doses up to 10 000 µg/plate) gave negative results in bacterial assays for mutagenicity in several strains of *S. typhimurium*, with and without metabolic activation with a preincubation protocol. [Liu et al. \(2014\)](#) also reported negative results in bacterial assays for mutagenicity in *S. typhimurium* and in tests for micronucleus formation in mouse bone marrow in vivo when cyanuric acid was administered in fixed combinations with melamine.

4.2.2 Inflammation

(a) Humans

After an outbreak of melamine-associated renal stones in children in 2008 in China, [Lau & Tu \(2013\)](#) examined clinical differences between children who had been highly exposed to contaminated infant formula milk in Sichuan and children who had been less exposed in Hong Kong Special Administrative Region. [Lau & Tu \(2013\)](#) reported that children exposed to milk that was highly contaminated with melamine were younger, were diagnosed with more numerous and larger renal stones, and showed a significantly higher urinary interleukin-8 (IL-8)/creatinine ratio than children exposed to milk that was less contaminated. However, after a 12-month follow-up, the urinary IL-8/creatinine ratio for highly exposed children declined, reaching levels similar to those in children whose renal stones had been completely passed in urine. WHO reported that the contaminant in the milk associated with this outbreak was primarily melamine; however, samples of infant formula collected at random from homes in 2008 showed that although 93% contained melamine (150–4700 mg/kg), 73% also contained cyanuric acid (0.4–6.3 mg/kg) ([WHO, 2009b](#)). [The Working Group noted that the ratio of melamine to cyanuric acid was much lower than the 1:1 mixtures examined in studies in experimental systems.]

(b) Experimental systems

(i) Non-human mammals *in vivo*

The association between melamine-induced renal or bladder inflammation and carcinogenicity in experimental animals is not clear. For example, in a 2-year study of carcinogenicity, the kidney from female F344 rats and the bladder from male B6C3F₁ mice showed evidence of chronic inflammation with no significant increase in the incidence of neoplasms.

Conversely, the degree of inflammation of the kidneys of male F344 rats was not significantly different from that of controls, but a significant increase in the incidence of bladder neoplasms was observed ([NTP, 1983](#)).

More recently, the inflammatory effects of short-term exposure to melamine have been further investigated. For example, melamine (60, 300, or 600 mg/kg bw per day in drinking-water for 3 months) induced an overexpression of inflammatory markers in male Sprague-Dawley rats. Specifically, treatment-related increases in bone morphogenic protein 4 (BMP4) and cyclooxygenase-2 (COX-2) were observed in the kidneys and renal arteries of treated rats at all doses ([Tian et al., 2016](#)). Proteomic analyses of urinary bladder stones from male Sprague-Dawley rats fed diets containing 2% melamine (~1000 mg/kg bw per day) for 13 weeks suggested that most of the proteins in the bladder stones were from damaged or dead cells, and some were associated with an inflammatory response ([Liu et al., 2012b](#)).

Pregnant Sprague-Dawley rats exposed to melamine at 800 mg/kg bw per day by gavage on days 6–20 of gestation showed inflammatory cells in the renal tubules associated with tubular necrosis or degeneration ([Kim et al., 2011](#)). The kidney tissue of male Sprague-Dawley rat offspring, who were exposed *in utero* (dams exposed at a dose of 600 mg/kg bw per day from 2 weeks before mating until gestation) and at 600 mg/kg bw per day in drinking water for 3 months after parturition, showed increased mRNA expression of chemokine ligand 2 (CCL2), tumour necrosis factor (TNF), and interleukin-1 β (IL1 β) ([Tian et al., 2016](#)).

In a study of male Sprague-Dawley rats given melamine and cyanuric acid (1.26:1; 0.0315–315 mg/kg bw per day for 7 days by gavage), crystal formation in the kidneys was associated with tubular damage and secondary inflammation ([Choi et al., 2010](#)). Similarly, female Sprague-Dawley rats exposed to melamine and

cyanuric acid (1:1) during days 6–19 of gestation showed an increase in the incidence of inflammatory cells in the renal tubules and tubular necrosis or degeneration when dams were exposed at 30 mg/kg bw per day. No changes were seen in the kidneys of pups ([Kim et al., 2013](#)).

Twelve of thirteen cats exposed to pet food contaminated with melamine and cyanuric acid for 4–6 days showed histopathological signs of renal tubular necrosis and perivascular inflammation (indicated by the presence of neutrophils, macrophages, eosinophils, and lymphocytes) involving the renal subcapsular veins ([Cianciolo et al., 2008](#)). Similarly, pigs exposed to contaminated feed containing melamine and various derivatives showed evidence of chronic inflammation (indicated by infiltrates of macrophages, lymphocytes, plasma cells, and multinucleated, foreign-body-type giant cells) associated with crystals in the cortex and medulla of the kidneys, which caused flattening of the renal tubular epithelial cells ([González et al., 2009](#)). Due to the inadvertent nature of the poisonings, the amount of melamine and cyanuric acid consumed by the cats and pigs was uncertain.

(ii) *Non-human mammalian cells in vitro*

Indicators of inflammation have been measured in murine macrophages ([Kuo et al., 2013](#)) and canine kidney cells ([Choi et al., 2010](#)) exposed to melamine. Similarly, indicators of inflammation have been measured in canine kidney cells exposed to melamine and cyanuric acid (1.26:1) ([Choi et al., 2010](#)).

(iii) *In silico*

Using docking and molecular dynamics simulation, [Rajpoot et al. \(2016\)](#) showed that melamine may bind with some known arachidonic acid-binding sites of albumin.

4.2.3 Oxidative stress

(a) *Humans*

No significant increase in levels of oxidative DNA damage, as measured by urinary 8-OHdG concentrations, was observed in a cross-sectional study of infants exposed to powdered formula contaminated with melamine ([Ke et al., 2010](#); see Section 4.2.1). [The Working Group noted that the study did not evaluate the relationship between 8-OHdG levels and the occurrence of urinary tract stones.]

(b) *Experimental systems*

(i) *In vivo*

Oral exposure to melamine affects various parameters associated with oxidative stress in rat kidneys ([El Rabey et al., 2014](#); [Al-Seeni et al., 2015](#)). For instance, exposure to melamine decreased glutathione *S*-transferase (GST) activity and increased lipid peroxidation (malondialdehyde; MDA) in the kidney tissue homogenate of male rats (*Rattus norvegicus*) fed a diet containing melamine at 20 000 ppm (~1000 mg/kg bw per day) for 28 days. Compared with controls, melamine induced a decrease of approximately 35% in GST activity and a 53% increase in MDA concentration. Signs of impaired kidney function were also apparent ([Al-Seeni et al., 2015](#)). Similarly, [El Rabey et al. \(2014\)](#) showed that melamine significantly decreased GST activity and increased MDA concentration in the kidney tissue homogenate of male Wistar rats fed diets containing melamine at 30 000 ppm (~1500 mg/kg bw per day). Compared with controls, melamine induced a decrease in GST activity of approximately 47% and an increase in MDA concentration of approximately 49%.

In the ovary of Sprague-Dawley rats, melamine (20 or 40 mg/kg bw per day in corn oil, for 28 consecutive days, via oral gavage) decreased mRNA expression of superoxide dismutase 1 (SOD1), glutathione peroxidase 1 (GPx1), and

glutathione peroxidase 2 (GPx2) in the granulosa cells (Sun et al., 2016a). Oxidative stress was also induced in the hippocampus of male Wistar rats exposed to melamine at 300 mg/kg bw per day orally for 28 consecutive days (An et al., 2012).

Similarly, melamine plus cyanuric acid (1:1) was shown to affect parameters associated with oxidative stress in rodent kidney (Lv et al., 2013a, b; Li et al., 2015), testis (Lv et al., 2013b), and ovary (Sun et al. 2016b).

(ii) *In vitro*

In rodent kidney cells *in vitro*, combined treatment with trolox, a water-soluble analogue of vitamin E, significantly attenuated the effects of melamine on intracellular production of reactive oxygen species (ROS), SOD and GPx activities, and MDA concentrations (Guo et al. (2012). Similarly, Wang et al. (2015) showed that an ROS scavenger (i.e. *N*-(mercaptopropionyl)-glycine) can attenuate melamine-induced (1980 µg/mL) increases in intracellular hydrogen peroxide production in rat mesangial cells (HBYZ-1). Indicators of oxidative stress have also been measured in rat pheochromocytoma cells exposed to melamine (e.g. Han et al., 2011).

4.2.4 Immunosuppression

(a) *Humans*

After an outbreak of melamine-associated renal stones in Chinese children in 2008, Zhou et al. (2010) investigated the effects of melamine-contaminated milk on the cellular immunity of a cohort of exposed children. Young children (age, 1–3 years) exposed to heavily contaminated milk and who presented with renal stones had decreased levels of circulating CD3+ and CD4+ lymphocytes compared with children without stones, but the CD4/CD8 ratio for children with stones was within a normal functioning range (~2) and not significantly different from that for children without stones. Additionally, with the exception of IgM (higher

in infants with stones than infants without), Zhou et al. (2010) did not observe any difference in humoral immunity (i.e. IgA, IgG, C3, or C4) between children with and without stones.

(b) *Experimental systems*

Evidence of toxicity has been observed in the organs of the immune system of experimental systems *in vivo*. For example, rats (Choi et al., 2010) and mice (Yin et al., 2014, 2016; Abd-Elhakim et al., 2016) exposed orally to melamine have shown evidence of altered immune parameters and/or histopathology. In particular, Abd-Elhakim et al. (2016) showed that exposure of male Swiss mice to melamine at 50 mg/kg bw per day, by gavage for 60 days, induced hyperplasia in the white pulp and degeneration of megakaryocytes in the red pulp of the spleen. These histopathological effects were accompanied by an increased presence of splenic CD4+ and CD8+ cells, decreased circulating leukocytes, lymphocytes, and basophils, and significantly decreased IgM, IgG, phagocytic indices of the circulating leukocytes, and lysozyme activity. Similarly, mice (Yin et al., 2014, 2016) and rats (Choi et al., 2010) exposed orally to 1:1 mixtures of melamine plus cyanuric acid have shown evidence of altered immune parameters and/or histopathology.

4.2.5 Altered cell proliferation or death

(a) *Humans*

No data were available to the Working Group.

(b) *Experimental systems*

(i) *Non-human mammals in vivo*

Melamine has been shown to have effects on cell proliferation and apoptosis in the urinary tract (NTP, 1983; Kim et al., 2011; Early et al., 2013; Toyoda et al., 2015; Tian et al., 2016), testis (Yin et al., 2013; Chang et al., 2014), ovary (Sun et al., 2016b), and spleen (Yin et al., 2014) of

experimental animals. For instance, in male and female Sprague-Dawley rats treated with melamine at a dose of ≥ 700 mg/kg bw per day by gavage for 14 consecutive days, melamine induced hyperactive regeneration of the renal tubular epithelium associated with multifocal necrosis and degeneration. Moderate tubular degeneration or regeneration was also observed in 1 out of 3 monkeys exposed to melamine at a dose of 700 mg/kg bw per day for 13 weeks ([Early et al., 2013](#)).

Similarly, melamine plus cyanuric acid has been shown to affect cell proliferation and apoptosis in the urinary tract ([Lu et al., 2012](#)), testis ([Yin et al., 2013](#); [Chang et al., 2014](#)), and ovary ([Sun et al., 2016b](#)) of exposed rodents. Melamine plus cyanuric acid (1:1) increased the number of apoptotic renal tubular cells in the cortex and medulla of male Sprague-Dawley rats fed a diet containing melamine at a dose of 250 mg/kg bw per day for 4 weeks. The increase in apoptosis in male rats was accompanied by crystal formation and tubular necrosis ([Lu et al., 2012](#)). Co-exposure with melamine and sodium citrate has been shown to significantly attenuate crystal formation and proliferating cell nuclear antigen (PCNA) levels in Sprague-Dawley rats ([Chen et al., 2013](#)).

Male rats appear to be more sensitive to proliferative changes induced by melamine in the urinary tract than are female rats or male and female mice. For example, in studies by the NTP, melamine induced hyperplasia of the bladder epithelium in most male F344 rats fed diets containing melamine at a concentration of 750–18 000 ppm (~ 37.5 –900 mg/kg bw per day) for 13 weeks. Conversely, female F344 rats and male and female B6C3F₁ mice did not show hyperplasia of the bladder epithelium after feeding with diets containing melamine at concentrations of 12 000 ppm or less (~ 600 mg/kg bw per day for rats; 1560 mg/kg bw per day for mice) for 13 weeks ([NTP, 1983](#)). The incidence of epithelial hyperplasia of the urinary bladder was higher in

male and female mice treated with melamine for 2 years than in concurrent controls ([NTP, 1983](#)). [The Working Group noted that hyperplasia of the transitional epithelium is a common response to mechanical irritation from a foreign body in the urinary bladder of rats or mice, and that there is a very strong correlation between the occurrence of hyperplasia and the presence of bladder stones in weanling male F344 rats exposed to melamine at dietary concentrations ranging from 0.2% to 1.9% for 4 weeks ([Heck & Tyl, 1985](#)).]

(ii) *Non-human mammalian cells in vitro*

Melamine caused dose-dependent suppression of cell proliferation and/or increased apoptosis in rodent kidney (e.g. [Guo et al., 2012](#); [Wang et al., 2015](#)), canine kidney ([Choi et al., 2010](#)), porcine kidney ([Yiu et al., 2017](#)), rodent pheochromocytoma (e.g. [Han et al., 2011](#)), and rodent testis ([Chang et al., 2017](#)) cells. Similarly, melamine plus cyanuric acid caused dose-dependent suppression of cell proliferation and/or increased apoptosis in canine kidney cells ([Choi et al., 2010](#)).

4.2.6 Other mechanisms

Few studies were available concerning receptor-mediated effects, immortalization, or DNA repair. Regarding epigenetic effects, melamine decreased DNA methylation in the ovary of female ICR mice given drinking-water containing melamine at a dose of 10 or 50 mg/kg bw per day for 8 weeks ([Duan et al., 2015](#)).

4.3 Data relevant to comparisons across agents and end-points

For the results of high-throughput screening assays of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA, see Section 4.3 of the *Monograph on 1-tert-butoxypropan-2-ol* in the present volume.

4.4 Susceptibility to cancer

No data were available to the Working Group. Susceptibility to stone formation in the urinary tract is described in Section 4.5.1.

4.5 Other adverse effects

4.5.1 Humans

In 2008 in China, an incident in which infant milk formula was deliberately adulterated with melamine caused illness in approximately 300 000 infants, including 50 000 hospitalizations and 6 confirmed deaths ([WHO, 2009b](#)). The concentrations of melamine found in 111 samples of infant formula produced by one of the main manufacturers in China ranged from < 0.05 mg/kg to 4700 mg/kg (median, 1000 mg/kg), with an estimated infant exposure of 8.6–110.2 mg/kg bw per day ([WHO, 2009b](#)). Cyanuric acid was also found in samples of infant formula from this manufacturer, albeit at much lower levels (range, 0.4–6.3 mg/kg; median, 1.2 mg/kg) ([WHO, 2009b](#)).

The exposed infants presented symptoms indicative of pathology of the urinary tract, including dysuria, haematuria, proteinuria, and passage of “sand-like” precipitates in the urine ([Zhu et al., 2009](#); [Sun et al., 2010c](#)). Nephrolithiasis and hydronephrosis were approximately 3.1 times as frequent in male infants younger than 1 year compared with female infants of that age, but this difference was not observed in older infants ([Liu et al., 2010b](#)). [The Working Group noted that the increased incidence of nephrolithiasis and hydronephrosis was not necessarily attributable to age, but could instead have been caused by differences in the amount of formula consumed with age.] The nephroliths (stones) were primarily located in the ureter and kidney, often bilaterally, and varied considerably in gross morphology and colour, ranging from sand- or granule-like shapes to stones exceeding 15 mm

in diameter. A detailed analysis of these stones revealed the presence of variable proportions of melamine (0.2–339 mg/g), uric acid, ammonium urate, ammonium magnesium phosphate, and calcium carbonate apatite ([Chang et al., 2012](#)). In a surgically resected stone, calcification increased from the core to the surface of the stone ([Li et al., 2011b](#)). In biopsy samples obtained from the kidney of a boy aged 8 months who had received melamine-contaminated formula and who had complete obstruction of the right ureter, there was evidence of generalized lymphocytic infiltration, sclerosis, and fibrosis of the glomeruli, and swelling of the tubular cells, with crystal accumulation observed in the lumen ([Sun et al., 2010d](#)). [The Working Group noted that there was epidemiological evidence that cancer of the urinary tract in humans is associated with a history of calculi in the bladder ([Capen et al., 1999](#)).]

In a survey of 589 children in China in 2009, as melamine content in formula increased, the percentage of infants with stones in the urinary tract increased ([Guan et al., 2009](#); see [Table 4.5](#)).

In a 4-year follow-up study of 45 infants with melamine-related urinary stones who underwent conservative treatment for urolithiasis, 34 infants had no detectable stones at the end of the study period, 6 infants had stones that had partially dissolved, 4 infants had stones that had not changed in size, and a single infant had a stone that had increased in size ([Yang et al., 2013](#)).

Follow-up studies examining the incidence of cancer in children with melamine-related urinary stones are described in Section 2.

Few data were available on the toxic effects of melamine in organs other than those of the urinary tract of the infants. While some studies reported alterations in clinical chemistry markers of liver function in infants exposed to melamine (e.g. [Hu et al., 2013](#)), other studies did not report such alterations (e.g. [Wang et al., 2013b](#)). The occurrence of liver lesions, hepatomegaly, and

Table 4.5 Characteristics of children exposed to infant formula contaminated with melamine, according to the presence or absence of stones in the urinary tract

Presence of stones in the urinary tract	Age (years)			Sex		Birth type ^a		Melamine content in formula ^b		
	0 to ≤ 1 (n = 160)	> 1 to ≤ 2 (n = 224)	> 2 to ≤ 3 (n = 205)	Male (n = 341)	Female (n = 248)	Preterm (n = 36)	Term (n = 431)	High (n = 121)	Moderate (n = 300)	None (n = 168)
No. with stones (%)	11 (6.9)	24 (10.7)	15 (7.3)	30 (8.8)	20 (8.1)	7 (19.4)	29 (6.7)	23 (19.0)	19 (6.3)	8 (4.8)
No. with suspected stones (%)	30 (18.8)	36 (16.1)	46 (22.4)	64 (18.8)	48 (19.4)	7 (19.4)	87 (20.2)	30 (24.8)	58 (19.3)	24 (14.3)
No. without stones (%)	119 (74.4)	164 (73.2)	144 (70.2)	247 (72.4)	180 (72.6)	22 (61.1)	315 (73.1)	68 (56.2)	223 (74.3)	136 (81.0)

^a Birth type was known for only 467 of the 589 children studied

^b High melamine content was defined as > 500 ppm and moderate content was defined as < 150 ppm

Adapted from [Guan et al. \(2009\)](#). Melamine-contaminated powdered formula and urolithiasis in young children, Volume No. 360, issue 11, page no 1069. Copyright © (2009) Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

gallstones in children who had been exposed to melamine-contaminated infant formula has been reported, but no comparison with unexposed children was included (e.g. [Hu et al., 2013](#)).

4.5.2 Experimental systems

In a study in male and female cynomolgus monkeys (*Macaca fascicularis*) dosed daily with melamine at 60, 200, or 700 mg/kg bw per day by the nasogastric route for 13 weeks, the primary target organ for toxicity was the kidney ([Early et al., 2013](#)). The range of observations, including kidney hypertrophy, renal tubular degeneration or regeneration, tubular dilatation, and tubular necrosis observed at the highest dose, was consistent with observations reported in the kidney of Sprague-Dawley rats exposed to melamine. No adverse histopathological changes were observed at the lowest dose. Several extra-renal effects were noted, including an elevation in alanine aminotransferase activity suggestive of hepatocellular injury, and pericarditis, interpreted to be secondary to uraemia.

The NTP conducted 13-week and 103-week studies in F344 rats and B6C3F₁ mice fed diets containing melamine ([NTP, 1983](#); [Melnick et al., 1984](#)). In the 103-week study, the accumulation of stones was observed in the urinary bladder in male (but not female) rats; a statistically significant correlation was observed between the formation of stones and transitional cell carcinoma of the urinary bladder in male rats (see [Table 3.1](#), Section 3). In contrast, despite a substantial increase in the incidence of stones and of epithelial hyperplasia in the urinary bladder in male mice, there was no increase in the incidence of tumours of the urinary bladder. In female mice, stones and epithelial hyperplasia in the urinary bladder were only reported in the group fed the diet with the higher dose.

In the 103-week study, there was an increase in the incidence of chronic inflammation in the kidney of female rats receiving melamine at

the lowest (4500 ppm; 262 mg/kg bw per day) and highest (9000 ppm; 542 mg/kg bw per day) doses. In addition, a dose–response relationship was reported for the deposition of “calcareous deposits” in the straight segments of the proximal tubules of the kidney of female rats in one of the 13-week studies (dose range: 750–12 000 ppm; 560–1600 mg/kg bw per day). There were no reported effects in the kidney of male rats, or male and female mice ([Melnick et al., 1984](#)).

After incidents involving the adulteration of pet food with melamine and derivatives in the USA in 2007, and the adulteration of infant milk formula in China in 2008, a renewed interest in the toxicology of melamine led to a re-evaluation of archived histology slides from the NTP 103-week and 13-week studies with melamine in F344 rats ([Hard et al., 2009](#)). In contrast to the previous histopathological evaluation, the results of the re-evaluation indicated a range of kidney lesions, extending from the papilla to the cortex, and included tubule dilatation and basophilia at the 13-week end-point in male and female rats. The incidence and severity of these lesions were higher in male rats than in female rats in the 13-week studies. After exposure to melamine for 103 weeks, fibrotic scars and tubule loss were noted from the superficial cortex into the medulla of the kidney.

Several other studies have investigated the effects of melamine in the kidney of Sprague-Dawley, CD IGS, F344, and Wistar rats. Renal inflammation, fibrosis, tubular dilation, necrosis, degeneration, regeneration, transitional cell hyperplasia, ischaemic changes, hypertrophy, and elevated levels of blood urea nitrogen (BUN) and serum creatinine have been reported, without any clear evidence of strain-dependent susceptibility ([Ogasawara et al., 1995](#); [Kim et al., 2011](#); [Wong et al., 2013](#); [Bandelet et al., 2014](#); [El Rabey et al., 2014](#); [Stine et al., 2014](#); [Tian et al., 2016](#)).

In rats, stones were not detected in the kidney after exposure to melamine, but small crystals

or crystal clusters accumulated in the lumen of the renal tubules ([Bandelet al., 2014](#); [Stine et al., 2014](#)). The term “stone” is often misused in the literature to classify small intratubular crystals derived from melamine, as noted by [Reimschuessel & Puschner \(2010\)](#). While the stones formed in the kidney of infants exposed to melamine ranged from “sand-like” in size to more than 15 mm in diameter ([Chang et al., 2012](#)), the crystals formed in the renal tubules of rats treated with melamine were substantially smaller, in the order of only tens of micrometres. These crystals are soluble in formalin, and thus the true extent of their accumulation can only be ascertained in non-fixed kidney tissue with a wet-mount technique ([Stine et al., 2014](#)). Unlike the stones found in infants exposed to melamine, the precipitates formed in the kidneys of Sprague-Dawley rats exposed to melamine for 4 weeks were reported to be devoid of uric acid, and composed essentially of melamine ([Cong et al., 2014](#)); however, in F344 rats exposed for 36 weeks, the stones consisted of melamine and uric acid in equal molar ratio ([Ogasawara et al., 1995](#)).

Melamine has been studied in pigs [a model relevant for human renal physiology]. In one study in a male Yorkshire-cross pig (age, ~16 weeks) treated orally with melamine at a dose of 400 mg/kg bw per day for 3 days, there were no signs of nephrotoxicity according to blood clinical chemistry and there was no evidence of accumulation of crystals in the kidney, or of any histopathological lesion ([Reimschuessel et al., 2008](#)). In contrast, small numbers of crystals were found in the kidney of one of two weanling cross-bred Barrow pigs treated orally with melamine at a dose of 200 mg/kg bw per day for 7 days. Mass spectral analysis revealed that the crystals were composed of melamine and cyanuric acid at a ratio of approximately 1:1. In a subsequent study reported in the same publication, no crystals were detected in the kidney of eight pigs treated orally with melamine at a

dose of 200 mg/kg bw per day for 28 days ([Stine et al., 2011](#)).

Nephrotoxicity (in some instances accompanied by the accumulation of renal crystals) has also been observed after exposure to melamine in other species, including sheep ([Clark, 1966](#)), broiler chickens ([Brand et al., 2012](#)), and Jinding laying ducks ([Gao et al., 2010](#)). In contrast, cats ([Puschner et al., 2007](#)) and fish ([Reimschuessel et al., 2008](#)) treated with melamine failed to show signs of nephrotoxicity under the experimental conditions used.

Although the kidney seems to be the primary organ affected by toxicity associated with exposure to melamine, toxicity has also been reported in the reproductive organs ([Yin et al., 2013](#); [Sun et al., 2016b](#)), spleen ([Yin et al., 2014](#)), and immune system ([Yin et al., 2014, 2016](#); [Abd-Elhakim et al., 2016](#)) of rodents.

In an incident in the USA in 2007, the adulteration of pet food ingredients with “scrap melamine” (an industrial residue from the production of melamine) containing melamine and other oxytriazines, including cyanuric acid, led to kidney disease in and the death of large numbers of cats and dogs ([WHO, 2009b](#)). Early research demonstrated that cats and F344 rats fed diets containing both melamine and cyanuric acid showed an accumulation of crystalline spherulites of a highly insoluble complex of melamine cyanurate in the lumen of the nephron, leading to obstructive nephropathy and potentially renal failure ([Puschner et al., 2007](#); [Dobson et al., 2008](#)). A considerable number of studies have since investigated the effects of combined exposure to melamine and cyanuric acid in F344 rats ([Gamboa da Costa et al., 2012](#); [Yasui et al., 2014](#)), Sprague-Dawley rats ([Choi et al., 2010](#)), Wistar rats ([Xie et al., 2010](#)), Kunming mice ([Chang et al., 2014, 2015](#)), C57BL/6 mice ([Peng et al., 2012](#)), pigs ([Reimschuessel et al., 2008](#); [Stine et al., 2011](#)), and fish ([Reimschuessel et al., 2008](#)). These studies indicated that the kidney is the primary target organ of toxicity in

a broad range of species, and that the mechanism of toxicity involves the formation and accumulation of melamine cyanurate crystals in the lumen of the renal tubules. The range of reported effects associated with the obstructive nephropathy stemming from co-exposure to melamine and cyanuric acid were in general qualitatively comparable to those reported after exposure to melamine alone; however, the nephrotoxic potency of the mixture was higher than that of melamine alone, and more intense nephrotoxic effects were observed at lower doses in rats ([Jacob et al., 2011](#); [Son et al., 2014](#)), mice ([Peng et al., 2012](#); [Chang et al., 2014](#)), pigs ([Reimschuessel et al., 2008](#)), and fish ([Reimschuessel et al., 2008](#)) exposed to a 1:1 mixture than to melamine alone. As a result of the kinetics of absorption, distribution, and renal elimination of melamine and cyanuric acid, the timing of administration of melamine and cyanuric acid, and the mode of administration (gavage vs feed) can modulate the intensity of the nephrotoxicity of the combination in rats ([Sprando et al., 2012](#)). There was a greater accumulation of melamine cyanurate crystals in the kidney in male rats than in female rats exposed to melamine and cyanuric acid at the same doses ([Gamboa da Costa et al., 2012](#)).

Although the kidney seems to be the primary organ of toxicity associated with combined exposure to melamine and cyanuric acid, toxicity has also been reported in the gastrointestinal tract and liver ([Chang et al., 2015](#)), the reproductive organs ([Yin et al., 2013](#)), and immune system ([Yin et al., 2016](#)) of rodents. [The Working Group noted that effects were reported in studies where acute kidney toxicity, and in some instances animal mortality, was observed.]

5. Summary of Data Reported

5.1 Exposure data

Melamine has been available commercially since the late 1930s and it is primarily used in the production of certain plastic materials, including coatings, filters, adhesives, and tableware. Melamine is a chemical with a high production volume, and has world production of more than 1 million tonnes. Melamine has been used to illegally adulterate foods and animal feeds in order to increase the apparent protein content. Exposure of the general population comes from the environment, migration from food-contact materials, and from the degradation of some pesticides or disinfectants. Background exposure is generally less than 0.1 mg/kg body weight (bw) per day. Average exposures of 10–30 mg/kg bw per day have been estimated in Chinese children exposed to infant milk formula adulterated with melamine. WHO has established a tolerable daily intake (TDI) of 0.2 mg/kg bw. Specific limits for melamine have been established in several pieces of legislation as migration limits for plastic food-contact materials, and the WHO/FAO Codex Alimentarius adopted maximum levels for several food categories and for animal feeds. Occupational exposure to melamine may occur by inhalation of melamine dust during its production and its use in the manufacture of laminates, surface coatings, moulding compounds, and textiles.

5.2 Human carcinogenicity data

Two studies of cancer in humans exposed to melamine were available. A large cohort study in the USA of cancer among workers exposed to formaldehyde also identified workers exposed to other chemicals, including melamine. A positive trend in mortality attributable to cancer of the lung and duration of exposure to melamine was

observed; however, the quantitative level of exposure to melamine was not measured, and the analysis was not adjusted for tobacco smoking or exposure to other chemicals. Positive associations were also reported for leukaemia and cancer of the nasopharynx, but these also lacked adjustment for chemical exposures or other risk factors and were not reported in subsequent follow-up of the cohort.

A study of a small cohort of children who developed urinary stones after exposure to infant milk formula adulterated with melamine was considered uninformative because of its small size and short follow-up period.

5.3 Animal carcinogenicity data

In one well-conducted 103-week feeding study in male and female rats, melamine significantly increased the incidence (with a significant positive trend) of transitional cell carcinoma and of transitional cell papilloma or carcinoma (combined) of the urinary bladder in males.

In two feeding studies in male rats, melamine significantly increased the incidence of transitional cell carcinoma and of transitional cell papilloma of the urinary bladder.

In one feeding study in male and female mice (combined), melamine significantly increased the incidence of dysplasia or carcinoma in situ (combined) of the urinary bladder, and of dysplasia or carcinoma in situ (combined) of the ureter.

One well-conducted 103-week feeding study in male and female mice gave negative results. One feeding study in male and female rats (combined) gave negative results.

One initiation–promotion study in which melamine was tested as an initiator in female mice treated by skin application gave negative results.

5.4 Mechanistic and other relevant data

No data are available on the absorption or distribution of melamine in humans. In non-human primates, farm animals, and rodents, melamine is rapidly and widely distributed.

Melamine is not metabolized by mammalian tissue. Melamine is metabolized by bacteria, with production of multiple intermediates including ammeline, ammelide, cyanuric acid, and ultimately urea. Relevant bacteria, including *Klebsiella* species, are found in the human gut. Melamine has been detected in the urine of children not known to have been exposed to adulterated infant formula. In a variety of animal species, melamine is rapidly excreted.

There is evidence that melamine is not genotoxic. In the single study conducted in humans, no differences in urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were seen. In experimental animals, melamine did not induce DNA damage, γ H2AX and *Pig-a* gene mutations, or micronucleus formation. In mammalian cells in vitro, melamine did not induce gene mutations, micronucleus formation, or chromosomal aberrations. Melamine interacted with DNA in cell-free systems, but was not mutagenic in bacterial assays.

There is *strong* evidence that melamine induces chronic inflammation in the urinary tract. In human infants, a biomarker for renal inflammation was elevated and resolved after the cessation of exposure. Inflammation was seen in the urinary tract of rats and mice in the 103-week feeding studies, and in additional studies of shorter-term exposure; however, only male rats developed tumours of the bladder in the 103-week feeding study.

Oxidative stress was not observed in the single available study in exposed infants. Oral exposure to melamine in rodents induces oxidative stress in various organs, including kidney, ovary, and

the hippocampus. More pronounced effects were seen with melamine plus cyanuric acid.

There is *weak* evidence that melamine is immunosuppressive. Oral exposure to melamine induced immunosuppression in a few rodent studies.

Although no data in humans were available, melamine induced cell proliferation and increased apoptosis in the urinary tract of monkeys and rodents, with more pronounced effects with melamine plus cyanuric acid.

In infants exposed to melamine in milk formula, stones composed primarily of melamine and uric acid were found in the kidney, ureter, and urinary bladder; in most cases these stones resolved upon cessation of exposure. Nephrotoxicity was observed in some of these children. In the 103-week feeding study, male (but not female) rats developed stones in the urinary bladder (but not in the kidney), and the incidence of these stones was associated with the incidence of transitional cell carcinoma of the urinary bladder. Male mice also developed stones in the urinary bladder, accompanied by epithelial cell hyperplasia but not tumours. Unlike human infants, rats exposed to melamine do not accumulate kidney stones but instead accumulate substantially smaller intratubular crystals that are composed primarily of melamine.

Melamine was nephrotoxic in experimental animals, including cynomolgus monkeys, pigs, and rats. In a range of mammalian and fish species, co-exposure to melamine and cyanuric acid induced a nephrotoxic response at lower exposure levels than exposure to melamine alone.

Precipitates and inflammation of the urinary tract were observed in highly exposed humans and in experimental animals. Overall, inconsistent findings of inflammation, stones, and carcinogenesis were seen in different rodent sexes and species.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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PYRIDINE

1. Exposure Data

Pyridine was considered by the Working Group in 2000 ([IARC, 2000](#)). New data have become available since that time, and these have been incorporated and taken into consideration in the present evaluation.

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 110-86-1

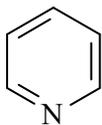
EC/List No.: 203-809-9

Chem. Abstr. Serv. name: Pyridine

IUPAC systematic name: Pyridine

Synonyms: Azabenzene; azine ([IARC, 2000](#))

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: C₅H₅N

Relative molecular mass: 79.10

1.1.3 Chemical and physical properties

Description: Colourless liquid with a characteristic, disagreeable odour ([IARC, 2000](#); [IPCS, 2000](#)), also reported as colourless to yellow liquid with a nauseating, fish-like odour ([NIOSH, 2016](#))

Boiling point: 115 °C ([IPCS, 2000](#))

Melting point: -42 °C ([IPCS, 2000](#))

Density: 0.9819 g/cm³ at 20 °C ([Lide & Milne, 1996](#))

Solubility: Miscible with water, acetone, benzene, chloroform, diethyl ether, and ethanol ([Lide & Milne, 1996](#)); solubility in water, 1000 g/L at 20 °C ([ECHA, 2017](#))

Volatility: Vapour pressure, 2.67 kPa at 20 °C ([ECHA, 2017](#))

Relative vapour density: 2.73 (air = 1); relative density of the vapour/air mixture at 20 °C, 1.03 ([IPCS, 2000](#))

Stability: Flammable; lower flammability limit, 1.8%; upper flammability limit, 12.4% ([IPCS, 2000](#); [ECHA, 2017](#))

Flash point: 20 °C (close cup) ([IPCS, 2000](#); [ECHA, 2017](#))

Auto-ignition temperature: 482 °C ([IPCS, 2000](#)); 900 °C at standard atmospheric pressure of 101.3 kPa ([ECHA, 2017](#))

Specific gravity: 0.98 at 20 °C ([NIOSH, 2016](#))

Ionization potential: 9.27 eV ([NIOSH, 2016](#))

Octanol/water partition coefficient (P): $\log K_{ow}$, 0.65 ([IPCS, 2000](#))

pH: 8.81 ([ECHA, 2017](#))

Odour threshold: 0.2 ppm (0.65 mg/m³) ([SCOEL, 2004](#))

Conversion factor: 1 ppm = 3.24 mg/m³ ([NIOSH, 2016](#))

Impurities: Specifications of pyridine vary according to country but are usually of > 99.8% purity by gas chromatographic analysis ([Shimizu et al., 2012](#))

1.2 Production and use

1.2.1 Production process

Historically, pyridine was extracted from coal tar or obtained as a by-product of coal gasification. The process was labour-consuming and inefficient: coal tar contains only about 0.1% pyridine, and therefore a multistage purification was required, which further reduced the output ([Gossauer, 2006](#)).

Today, most pyridine is produced synthetically using various reactions. The Tchichibabin synthesis, which is a condensation reaction of aldehydes with ammonia ([Tchichibabin, 1924](#)), is especially suitable for mass production. The reaction of acetaldehyde and formaldehyde with ammonia is one of the most widely used for pyridine production. It is usually carried out at 350–550 °C and a space velocity of 500–1000 h⁻¹ in the presence of a solid acid catalyst (e.g. silica–alumina). Alkylpyridines of low commercial value, obtained as by-products of pyridine base synthesis, can be converted into useful pyridine bases by dealkylation. These and other syntheses are described in detail by [Shimizu et al. \(2012\)](#).

1.2.2 Production volume

Major companies producing pyridine are located in China, India, and the USA ([Murugan & Scriven, 2013](#)). A directory of chemicals, chemical suppliers, and producers lists 28 international suppliers and manufacturers of pyridine: 13 of them are in China, 6 in Germany, 5 in the USA, 2 in Belgium, 1 in Sweden, and 1 in Switzerland ([BuyersGuideChem, 2018](#)). Another directory listed 37 manufacturers of pyridine, including 18 in the USA, 5 in the United Kingdom, 3 in China, 3 in Japan, 2 in Germany, 2 in Hong Kong Special Administrative Region, 1 in Belgium, 1 in Canada, 1 in Mexico, and 1 in Switzerland ([Chemical Sources International, 2017](#)).

According to the European Chemicals Agency (ECHA) database, pyridine is registered under a 1000–10 000 tonnes/year usage with seven active registrants and/or suppliers in Europe ([ECHA, 2017](#)).

Pyridine appears on the 2007 Organisation for Economic Co-operation and Development (OECD) list of high production volume chemicals ([OECD, 2009](#)), which contains those chemicals which are produced or imported at levels greater than 1000 tonnes/year in at least one member country and/or region. [The OECD has 35 member countries, including many of the world's most industrialized countries in the Asia-Pacific region, Europe, and North and South America.] Global domestic pyridine capacity reached 145 000 tonnes in 2011, 60% (87 000 tonnes) of which was accounted for by China ([CCPIA, 2012](#)).

1.2.3 Use

Pyridine as a solvent is used in organic chemistry and in industry. Pyridine is used as a denaturant in alcohol and antifreeze mixtures, as a solvent for paint, rubber, and polycarbonate resins, and as an intermediate in the manufacture of insecticides, herbicides, and fungicides

([NTP, 2000](#)). It is also used in the production of piperidine and as an intermediate and solvent in the preparation of vitamins and drugs, dyes, textile water repellents, and flavouring agents in food ([NTP, 2000](#)). Agricultural chemicals, mainly the non-selective contact herbicide paraquat, account for most consumption of pyridine ([SCOEL, 2004](#); [IHS Markit, 2014](#)).

1.3 Analytical methods

Methods of detection and quantification of pyridine are reported in [Table 1.1](#). The methods used to evaluate pyridine concentrations in different media such as air, water, soil, or food are based on gas chromatography with flame ionization detection, nitrogen–phosphorus detection, or mass spectrometry.

No methods for biological matrix are available for this compound.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

Pyridine is reported to occur naturally in wood oil, in the leaves and roots of *Atropa belladonna*, and in other plants, for example, coffee and tobacco ([Furia & Bellanca, 1975](#)). Pyridine can be formed from the breakdown of many natural materials in the environment. Moreover, due to its variety of applications, pyridine can be released to the environment since it presents high volatility and evaporates into the air very easily. If pyridine is released into the air, it may take several months to years to break down into other compounds. Pyridine also mixes very easily with water. If it is released into water or soil, it may break down in a few days to a few months ([ATSDR, 1992](#)).

In 2015, according to the United States Environmental Protection Agency Toxic Release Inventory (TRI), about 529 200 pounds [240 tonnes] of pyridine were released into the

environment through on- and offsite disposal or other releases. Onsite air emissions accounted for 38 000 pounds [17.2 tonnes, 7% of total releases]; of this total, land [soil] releases represented 92% of total releases, and offsite disposal or other represented 1% ([EPA-TRI, 2018](#)).

The fate of pyridine in the environment is a function of both abiotic and biotic processes, including photochemical transformations, volatilization, complexation, surface attenuation, transport, and biological degradation. Pyridine and several pyridine derivatives such as hydroxypyridines and pyridinecarboxylic acids can be degraded in soil by bacteria. Data suggest that pyridine and some substituted pyridines are degraded via different mechanisms, possibly involving initial reductive steps and lacking hydroxylated intermediates ubiquitous in the metabolism of other aromatic compounds. Perhaps least understood are the mechanisms for catabolism of alkyl- and chloropyridines, two of the most important classes of pyridine derivatives detected in environmental samples ([Sims et al., 1989](#)).

Pyridine and its derivatives can be formed in foods from carbonyl compounds derived from lipids (or reducing sugars) and ammonia released from amino acids ([Maga, 1981](#); [Kim et al., 1996](#)). However, other pathways are described for pyridine formation in foods; for example, during coffee roasting trigonelline is degraded, producing a variety of volatile compounds including pyridines (46%), pyrroles (3%), and pyrazines ([Farah, 2012](#)). Little attention has been given to pyridine derivatives, although many of them have been found in a large number of foods. Moreover, many food flavour additives are complex mixtures that contain pyridine or pyridine-ring structures that may decompose into pyridine ([WHO, 2012](#)) (see also Section 1.5).

Table 1.1 Methods of detection and quantification of pyridine

Media	Method	Technique	Target concentrations	Remarks
Air	NIOSH 1613, issue 2 (NIOSH, 1994)	GC-FID	Estimated LOD, 0.02 mg per sample; working range, 1–14 ppm (3–45 mg/m ³) for 100-L air sample	Replaces method S161 of 1977
	IRSST 199-1 (IRSST, 2012)	GC-NPD	Minimum reported value, 4 µg	
	OSHA PV2295 (OSHA, 1991)	GC-FID	5 ppm (15 mg/m ³)	As of December 1991, the method was partially validated and presented for information and trial use only
Soil and water	EPA 8260B-3 Revision 2 (EPA, 1996)	GC-MS	EQL: ~5 µg/kg for soil samples (wet weight), ~0.5 mg/kg for wastes (wet weight), and ~5 µg/L for groundwater samples	Generic method; adequate for preparation technique 5031 (injection of sample concentrated by azeotropic distillation) and direct injection
	(Peters & van Renesse von Duivenbode, 1994)	GC-MS	Detection limit is 0.01 mg/kg for soil samples and 0.2 µg/L for water samples	Minimum weight for soil samples, 20 g; minimum volume for water samples, 500 mL
Waste water in pharmaceutical manufacturing	EPA Method 1665 (EPA, 1995)	GC-MS	Minimum level, 5 µg/L	Accuracy, 7–12 µg/L
Mainstream cigarette smoke	Saha et al. (2010)	RP-HPLC, ESI-MS/MS	Limit of detection, 1.74–14.32 ng per cigarette	The yields measured by standard machine-smoking tests are misleading and have little value in the assessment of human exposure (IARC, 2004)
<i>Foods and beverages</i>				
Fried bacon, fried pork loin	Timón et al. (2004)	GC-MS	0.0567 ± 0.0072 µg/kg	Pyridine found in headspace volatiles of fried bacon expressed as µg/kg of sample
Coffee	Amanpour & Selli (2016)	GC-FID, GC-MS	3904–4360 µg/kg	Liquid–liquid extraction with dichloromethane; mean concentrations of coffee obtained from two different brewing methods were analysed; limit of detection not reported

EPA, Environmental Protection Agency; EQL, estimated quantitation limits; ESI, electrospray ionization; FID, flame ionization detection; GC, gas chromatography; HPLC, high-performance liquid chromatography; IRSST, Institut de recherche Robert-Sauvé en santé et en sécurité du travail; LOD, limit of detection; MS, mass spectrometry; NIOSH, National Institute for Occupational Safety and Health; NPD, nitrogen-phosphorous detection; OSHA, Occupational Safety and Health Administration; ppm, parts per million; RP, reverse-phased

1.4.2 Exposure in the general population

Humans may be exposed to pyridine by ingestion, inhalation, or dermal contact; food and tobacco smoke are thought to be the major sources of exposure to pyridine for the general population ([Maga, 1981](#); [Eatough et al., 1989](#); [IARC, 2000](#)). Pyridine was rarely detected in ambient rural or urban air in the 1980s in the USA, except in the vicinity of industrial or waste-treatment facilities ([ATSDR, 1992](#)). Exposure to low concentrations of pyridine may also occur by ingesting pyridine-contaminated water. Pyridine was rarely detected in rivers or other natural waters in the 1970s and 1980s in the USA ([ATSDR, 1992](#)). Since the publication of the previous *Monograph* ([IARC, 2000](#)), no further data on pyridine in food or water were available to the Working Group.

Pyridine was identified in cigarette smoke constituents ([Eatough et al., 1989](#); [Kulshreshtha & Moldoveanu, 2003](#); [Saha et al., 2010](#); [Wright, 2015](#)). Pyridine may be produced from nicotine degradation, and its quantity in mainstream cigarette smoke has been reported to range from 3 to 28 µg per cigarette ([Kulshreshtha & Moldoveanu, 2003](#); [IARC, 2004](#); [Herrington & Myers, 2015](#); [Kibet et al., 2016](#)). In 30 brands of cigarettes sold in China, the average pyridine yield was 17 µg per cigarette (standard deviation, 3.9 µg per cigarette) ([Xie et al., 2012](#)). The yields measured by standard machine-smoking tests are misleading and have little value in the assessment of human exposure. Mean concentrations of pyridine in second-hand tobacco smoke in different studies ranged from 6.5 to 23.8 µg/m³ ([IARC, 2004](#)). Pyridine was detected but not quantified in an electronic cigarette (e-cigarette) ([Margham et al., 2016](#)). Analysis of e-cigarette solutions identified several pyridine derivatives, three of which were also identified in resultant aerosols ([Herrington & Myers, 2015](#)).

[EPA \(1978\)](#) reported that total pyridine ingested in the USA is estimated at about

500 mg/year per person, mainly from food. Pyridine was detected among the volatile components of several foods, including fried chicken ([Jayasena et al., 2013](#)), fried bacon ([Timón et al., 2004](#)), French fries ([van Loon et al., 2005](#)), corn tortilla chips ([Buttery & Ling, 1998](#)), roasted duck or goose ([Baruth & Ternes, 2011](#)), tea ([Ho et al., 2015](#)), and mango fruit ([Pino et al., 2005](#)). Pyridine is also a coffee aroma constituent ([Farah, 2012](#); [Petisca et al., 2013](#); [Amanpour & Selli, 2016](#); [Lee et al., 2017](#)). Few articles describe pyridine content in foods; most of the above-mentioned articles describe only pyridine identification. The concentration of pyridine is 4360 µg/kg in French press coffee and 3904 µg/kg in Turkish coffee ([Amanpour & Selli, 2016](#)). Pyridine is also found in corn tortilla chips at the approximate concentration of 30 µg/kg ([Buttery & Ling, 1998](#)), fried bacon at 0.06 µg/kg ([Timón et al., 2004](#)), and mango fruit from non-detectable amounts to 80 µg/kg ([Pino et al., 2005](#)).

The assessment of 33 derivatives of pyridine, pyrrole, indole, and quinoline was undertaken by [EFSA \(2008\)](#). The daily per capita intakes for these flavourings were estimated on the basis of the annual volumes of production reported. More than 50% of the total annual volume of production for the 33 candidate substances is accounted for by the following three flavourings: 4-methylpyridine, 1-methylpyrrole, and 2-methylpyridine. The estimated daily per capita intakes of these three substances from use as flavourings are 0.73, 0.3, and 0.21 µg, respectively. The daily per capita intakes for each of the 30 remaining substances (including pyridine derivatives) are less than 0.2 µg. [The Working Group noted that this assessment was only for pyridine derivatives, which could degrade to pyridine in the food preparation or metabolize to pyridine in the body.]

1.4.3 Occupational exposure

Pyridine is produced in closed and open systems ([Vertellus, 2018](#)). Exposure may occur by inhalation and dermal contact during its production, or when used as an intermediate or as a solvent. Exposure can also occur at coke ovens, oil-shale plants, coffee processing facilities, sewage treatment plants, polymer combustion plants and other similar industries.

In the previous *Monograph* ([IARC, 2000](#)), occupational exposure data from the 1970s were summarized for workplaces in the USA where pyridine was manufactured, used as a chemical intermediate, or used as a solvent. Workers were exposed to 8-hour time-weighted average (TWA) pyridine concentrations ranging from 0.026 to 3.240 mg/m³. A similar range of exposure levels was reported from coke works (0.005–2.980 mg/m³) in Czechia, with lower levels in blast furnaces, steel works, rolling mills, and foundries (≤ 0.63 mg/m³) and in a Polish coke by-products plant (≤ 0.7 mg/m³). However, in a pyridine production area of a coal-tar plant in the Russian Federation, pyridine levels were reportedly 7.5–10 mg/m³ and occasionally reached 20 mg/m³ ([Izmerov, 1984](#)). According to data from the second half of the 20th century in Poland, pyridine air concentration in various workplaces ranged from 0.002 mg/m³ to about 20 mg/m³ ([Sapota & Skrzypińska-Gawrysiak, 2013](#)).

Technicians working in quality control and research and development laboratories of a pyridine manufacturer were exposed to low TWA pyridine concentrations (measured over 6-hour periods) of up to 0.29 mg/m³ ([IARC, 2000](#)). Similarly, based on three personal air exposure measurements made in the work environment, an 8-hour TWA pyridine exposure of 0.3 mg/m³ was estimated for a smell tester who used pyridine as one of their test substances ([NIOSH, 1983](#)).

Air samples were collected in the moulding and pouring departments of a United States iron foundry using a phenolic urethane binder. The

2-day average level of pyridine, which was emitted as a breakdown product of 4-phenylpropylpyridine, used as a binder catalyst, was 19 mg/m³ in the moulding area ([NIOSH, 1982](#)). In an investigation near a nylon injection moulding operation at an electrical components plant in the USA, 10 air samples for pyridine were collected. All of these measurements were below the analytical limit of detection, that is, 0.32 mg/m³ ([NIOSH, 1985](#)).

In the United States Occupational Safety and Health Administration (OSHA) database of compliance exposure measurements, 96 measurements of pyridine exposure were collected between 1984 and 2012 from a variety of industries. Seventy percent of the data were below the limit of detection, and more than 90% were less than 0.5 mg/m³ ([OSHA, 2017](#)).

Up to 70 workplace air analyses were performed in the laboratory of the Institut de recherche Robert-Sauvé en santé et en sécurité du travail, Canada, during 1997–2017; only five results were over the limit of detection of their method (0.8 mg/m³) ([IRSST, 2017](#)).

A total of 22 measurements of workplace exposure were collected from the Finnish Institute of Occupational Health during 2012–2016, ranging between 0.0006 and 0.5 mg/m³. The two highest measurements were recorded at a coffee roasting factory and a waste treatment plant ([FIOH, 2017](#)).

1.5 Regulations and guidelines

The Committee of Experts on the Transport of Dangerous Goods and Globally Harmonized System of Classification and Labelling of Chemicals of the United Nations Economic Commission for Europe identified pyridine as United Nations No. 1282, Hazard Class 3, United Nations Packing Group II ([UNECE, 2015](#)).

In 2012, the Ministry of the Environment and Climate Change of the province of Ontario, Canada, developed the Ambient Air Quality Criteria (AAQC), which quantifies the desirable

Table 1.2 Eight-hour and short-term limit values for pyridine in different countries or regions

Country or region	8-hour limit value		Short-term limit value	
	ppm	mg/m ³	ppm	mg/m ³
Australia	5	16		
Austria	5	15	20	60
Belgium	1	3.3		
Canada (Ontario)	1			
Canada (Quebec)	5	16		
China		4		
Denmark	5	15	10	30
Finland	1	3	5 ^a	16 ^a
France	5	15	10	30
Hungary		15		60
Ireland	5	15	10 ^b	30 ^b
Latvia	5	15		
Netherlands		0.9		
New Zealand	5	16		
Poland		5		30
Republic of Korea	2	6		
Romania	5	15		
Singapore	5	16		
Spain	1	3		
Sweden	2	7	3 ^a	10 ^a
Switzerland	5	15	10	30
Turkey	5	15		
United Kingdom	5	16	10	33
United States of America (NIOSH)	5	15		
United States of America (OSHA)	5	15		

NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; ppm, parts per million

^a 15-minute average value

^b 15-minute reference period

Source: [GESTIS \(2017\)](#)

concentration of a contaminant in the air based on protection against adverse effects. For pyridine the AAQC are 150 µg/m³ for 24 hours (health) and 80 µg/m³ for 10 minutes (odour) ([Ontario Ministry of the Environment and Climate Change, 2012](#)).

In 2004, the European Union (EU) Scientific Committee on Occupational Exposure Limits recommended that occupational exposures to pyridine should be maintained well below 5 ppm (15 mg/m³), but could not derive a health-based limit value, either 8-hour TWA or short-term (15 minutes) exposure limit, from the available data. However, it was noted that because pyridine

could be absorbed through the skin, it could pose a threat of systemic toxicity ([SCOEL, 2004](#)). Pyridine must not penetrate the sewer system or come into contact with surface water or ground-water ([ECHA, 2017](#)).

Pyridine 8-hour limit values (0.9–16.0 mg/m³) and short-term (15 minutes) limit values (10–60 mg/m³) from the GESTIS international limit values database ([GESTIS, 2017](#)) are presented in [Table 1.2](#) for different countries.

From 2004, the American Conference of Governmental Industrial Hygienists has recommended a threshold limit value (TLV) 8-hour

TWA of 1 ppm [3.1 mg/m³] for occupational exposures to pyridine in workplace air. The National Institute for Occupational Safety and Health (NIOSH) recommended exposure limits (RELs) of 5 ppm [15 mg/m³] for pyridine are for up to 10-hour TWAs during a 40-hour working week. The OSHA permissible exposure limit (PEL, 8-hour TWA) for pyridine is 5 ppm (15 mg/m³) ([OSHA, 2018](#)).

Pyridine is listed in the EU Register of Flavouring Substances in accordance with Article 3(1) of EC 2232/96. FL No: 14.008; FEMA No.: 2966; CoE No.: 604; Chemical Group 28 ([Vertellus, 2018](#)). Thirty-two pyridine derivatives are included in the list of approved flavouring substances of Commission Implementing Regulation (EU) No. 872/2012 ([EU, 2012](#)).

The European Medicines Agency classified pyridine as a class 2 solvent in new veterinary medicinal products with a permitted daily exposure (PDE) of 2.0 mg/day and a concentration limit of 200 ppm ([EMA, 2000](#)). The United States Department of Health and Human Services Food and Drug Administration also issued a guidance for the industry on pharmaceuticals for human use with a similar classification, PDE, and concentration limit ([FDA, 2012](#)).

2. Cancer in Humans

A cohort study of mortality of 729 men manufacturing 4,4'-bipyridyl [4,4'-bipyridine] in England ([Paddle et al., 1991](#)) was reviewed in the previous evaluation of pyridine by the Working Group in 2000 ([IARC, 2000](#)) (pyridine is used in the manufacture of 4,4'-bipyridyl, which is used to make paraquat). Standardized mortality ratios (SMRs) for cancer outcomes were reported only for all cancers combined and for cancer of the lung. For all cancers combined, the standardized mortality ratio was 1.1 (95% confidence interval (CI), 0.7–1.5; 29 deaths) and for cancer of the lung it was 1.2 (95% CI, 0.7–2.1; 13 deaths).

The cancer of the lung risks were investigated in a nested case-referent study and various sub-cohort analyses, but no quantitative results of these analyses or data on the relationship between cancer of the lung and exposure to pyridine were reported.

An earlier case-series study of skin lesions in the same plant had identified 99 chemicals used in the 4,4'-bipyridyl manufacturing process ([Bowra et al., 1982](#)). A total of 6 cases of Bowen's disease and 6 cases of squamous cell carcinoma were observed, but no cancer risk data were reported.

[No quantitative exposure data were available from these studies, and associations between cancer risk and exposure to pyridine were not reported.]

3. Cancer in Experimental Animals

Pyridine was evaluated by the Working Group in 2000 ([IARC, 2000](#)), which concluded that there was *limited evidence* for the carcinogenicity of pyridine in experimental animals.

Studies of the carcinogenicity of pyridine, given in drinking-water, in mice and rats have been conducted ([NTP, 2000](#)), the results of which are summarized in [Table 3.1](#).

3.1 Mouse

3.1.1 Oral administration

Groups of 50 male and 50 female B6C3F₁ mice (age, 7 weeks) were given pyridine (purity, > 99%) in drinking-water at doses of 0 (control), 125 (females only), 250, 500, or 1000 (males only) ppm, equivalent to average daily doses of 0, 35, 65, or 110 mg/kg body weight (bw) in males and 0, 15, 35, or 70 mg/kg bw in females, for 104 weeks (males) or 105 weeks (females) ([NTP, 2000](#)).

The survival of exposed males and females was similar to that of controls. Final mean body weights of females given pyridine at doses of

Table 3.1 Studies of carcinogenicity with pyridine in rodents

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F ₁ (M) 7 wk 104 wk NTP (2000)	Drinking-water Pyridine, > 99% Deionized water 0, 250, 500, 1000 ppm (0, 35, 65, 110 mg/kg bw/d) continuously 50, 50, 50, 50 35, 28, 34, 35	<i>Liver</i> Hepatocellular adenoma (multiple): 16/50*, 29/50**, 29/49**, 28/50** Hepatocellular adenoma (includes multiple): 29/50*, 40/50**, 34/49, 39/50*** Hepatocellular carcinoma (multiple): 3/50*, 19/50**, 26/49**, 18/50** Hepatocellular carcinoma (includes multiple): 15/50*, 35/50**, 41/49**, 40/50** Hepatoblastoma (multiple): 1/50, 4/50, 6/49*, 2/50 Hepatoblastoma (includes multiple): 2/50*, 18/50**, 22/49**, 15/50** Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined): 38/50*, 47/50**, 46/49***, 47/50****	*[<i>P</i> = 0.018 (trend), Cochran-Armitage test] ** <i>P</i> ≤ 0.05 * <i>P</i> = 0.031 (trend) ** <i>P</i> = 0.003 *** <i>P</i> = 0.011 *[<i>P</i> < 0.001 (trend), Cochran-Armitage test] ** <i>P</i> ≤ 0.01 * <i>P</i> < 0.001 (trend) ** <i>P</i> < 0.001 * <i>P</i> ≤ 0.05 * <i>P</i> = 0.005 (trend) ** <i>P</i> < 0.001 * <i>P</i> < 0.001 (trend) ** <i>P</i> = 0.002 *** <i>P</i> = 0.003 **** <i>P</i> < 0.001	Principal strengths: GLP study in both males and females Statistical test, poly-3 test if not otherwise specified

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F ₁ (F) 7 wk 105 wk NTP (2000)	Drinking-water Pyridine, > 99% Deionized water 0, 125, 250, 500 ppm (0, 15, 35, 70 mg/kg bw/d) continuously 50, 50, 50, 50 32, 30, 22, 29	<i>Liver</i> Hepatocellular adenoma (multiple): 24/49, 34/50*, 37/50**, 30/50 Hepatocellular adenoma (includes multiple): 37/49, 39/50, 43/50*, 34/50 Hepatocellular carcinoma (multiple): 3/49*, 11/50**, 14/50***, 30/50*** Hepatocellular carcinoma (includes multiple): 13/49*, 23/50**, 33/50***, 41/50*** Hepatoblastoma (multiple): 0/49, 0/50, 3/50, 4/50 Hepatoblastoma (includes multiple): 1/49*, 2/50, 9/50**, 16/50*** Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined): 41/49*, 42/50, 45/50**, 44/50***	 * <i>P</i> ≤ 0.05 ** <i>P</i> ≤ 0.01 * <i>P</i> = 0.015 *[<i>P</i> < 0.001 (trend), Cochran-Armitage test] ** <i>P</i> ≤ 0.05 *** <i>P</i> ≤ 0.01 * <i>P</i> < 0.001 (trend) ** <i>P</i> = 0.014 *** <i>P</i> < 0.001 NS * <i>P</i> < 0.001 (trend) ** <i>P</i> = 0.007 *** <i>P</i> < 0.001 * <i>P</i> = 0.009 (trend) ** <i>P</i> = 0.042 *** <i>P</i> = 0.045	Principal strengths: GLP study in both males and females Statistical test, poly-3 test if not otherwise specified

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (F) 7 wk 105 wk NTP (2000)	Drinking-water Pyridine, > 99% Deionized water 0, 100, 200, 400 ppm (0, 7, 14, 33 mg/kg bw/d) continuously 50, 50, 50, 50 32, 37, 29, 26	<i>All organs</i> , mononuclear cell leukaemia: 12/50*, 16/50, 22/50**, 23/50***	* <i>P</i> = 0.013 (trend) ** <i>P</i> = 0.043 *** <i>P</i> = 0.020	Principal strengths: GLP study in both males and females Statistical test, poly-3 test
Full carcinogenicity Rat, Wistar (M) 7 wk 104 wk NTP (2000)	Drinking-water Pyridine, > 99% Deionized water 0, 100, 200, 400 ppm (0, 8, 17, 36 mg/kg bw/d) continuously 50, 50, 50, 50 22, 14, 11, 7	<i>Testis</i> , testicular (interstitial cell) adenoma: 5/50*, 6/49, 4/49, 12/50**	* <i>P</i> = 0.008 (trend) ** <i>P</i> = 0.012	Principal strengths: GLP study Statistical test, poly-3 test

bw, body weight; d, day(s); F, female; GLP, good laboratory practice; M, male; NS, not significant; wk, week(s)

250 ppm and 500 ppm were 73% and 70% that of controls, respectively. Final mean body weights of all groups of treated male mice and the group of female mice given pyridine at 125 ppm were within 10% of that of controls. Water consumption by males exposed to pyridine at 250 or 500 ppm was generally greater than that by controls during the second year of the study; male mice exposed to pyridine at 1000 ppm consumed less water than controls throughout the entire study. Water consumption by exposed females was generally lower than that by controls during the first year of the study, but greater than controls during the second year of the study. [The Working Group noted that water consumption in exposed females was at least 30% greater than that of controls during the second year of the study; the authors did not suggest that this had any influence on study results.]

Treated male and female mice had significant increases in the incidences (generally with a significant positive trend) of hepatocellular neoplasms and hepatoblastoma [an embryonal tumour of the liver cells]. Compared with controls, in male mice given pyridine at 250, 500, and 1000 ppm, these included increases in the incidence of: hepatocellular adenoma (multiple): 16/50 (control), 29/50 ($P \leq 0.05$), 29/49 ($P \leq 0.05$), and 28/50 ($P \leq 0.05$); hepatocellular adenoma (includes multiple): 29/50 (control), 40/50 ($P = 0.003$), 34/49, and 39/50 ($P = 0.011$); hepatocellular carcinoma (multiple): 3/50 (control), 19/50 ($P \leq 0.01$), 26/49 ($P \leq 0.01$), and 18/50 ($P \leq 0.01$); hepatocellular carcinoma (includes multiple): 15/50 (control), 35/50 ($P < 0.001$), 41/49 ($P < 0.001$), and 40/50 ($P < 0.001$); hepatoblastoma (multiple): 1/50 (control), 4/50, 6/49 ($P \leq 0.05$), and 2/50; and hepatoblastoma (includes multiple): 2/50 (control), 18/50 ($P < 0.001$), 22/49 ($P < 0.001$), and 15/50 ($P < 0.001$). The incidences for the combination of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma were: 38/50 (control), 47/50 ($P = 0.002$), 46/49 ($P = 0.003$), and 47/50 ($P < 0.001$).

Compared with controls, in female mice given pyridine at 125, 250, and 500 ppm there were increases in the incidences (generally with a significant positive trend) of: hepatocellular adenoma (multiple): 24/49 (control), 34/50 ($P \leq 0.05$), 37/50 ($P \leq 0.01$), and 30/50; hepatocellular adenoma (includes multiple): 37/49 (control), 39/50, 43/50 ($P = 0.015$), and 34/50; hepatocellular carcinoma (multiple): 3/49 (control), 11/50 ($P \leq 0.05$), 14/50 ($P \leq 0.01$), and 30/50 ($P \leq 0.01$); hepatocellular carcinoma (includes multiple): 13/49 (control), 23/50 ($P = 0.014$), 33/50 ($P < 0.001$), and 41/50 ($P < 0.001$); hepatoblastoma (multiple): 0/49 (control), 0/50, 3/50, and 4/50; and hepatoblastoma (includes multiple): 1/49 (control), 2/50, 9/50 ($P = 0.007$), and 16/50 ($P < 0.001$). The incidences for the combination of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma were: 41/49 (control), 42/50, 45/50 ($P = 0.042$), and 44/50 ($P = 0.045$) ([NTP, 2000](#)). [The Working Group noted this was a well-conducted good laboratory practice (GLP) study and the use of both sexes.]

3.1.2 Transgenic models

Transgenic mouse models were developed to characterize carcinogens, including a p53^{+/-} mouse model that responds to genotoxic chemicals, and a Tg.Ac model that was reported to respond to genotoxic and non-genotoxic carcinogens ([Tennant et al., 1996](#)). Pyridine was tested in both of these models for evidence of treatment-related lesions ([Spalding et al., 2000](#)). In the Tg.Ac model, pyridine was administered to hemizygous females (age, 14 weeks) by skin application at doses of 0, 1.5, 3.0, or 6.0 mg for 20 weeks. Pyridine was added to the feed of the p53^{+/-} mice (age, 8–11 weeks) at doses of 0, 250, 500, or 1000 ppm (males) and 0, 125, 250, or 500 ppm (females) for 26 weeks. Gross necropsy was performed on all animals in both transgenic models at 26 weeks. Tissues from multiple organs of control mice and mice given the highest dose

were examined microscopically. In addition, in the Tg.Ac model, a section of the skin at the site of application was examined microscopically. Tg.Ac mice treated three times a week with 1.25 µg 12-*O*-tetradecanoylphorbol-13-acetate (used as a positive control) had a 100% incidence of skin papillomas. No significant increase in the incidence of neoplasms was observed in either of the transgenic mouse models exposed to pyridine (Spalding et al., 2000). [The Working Group noted the difficulty in evaluating these short-term gene-specific transgenic assays because they may not provide critical information that can be obtained from longer-term bioassays (e.g. effects on multiple target organs, effects with time and age) (Pritchard et al., 2003).]

3.2 Rat

3.2.1 Oral administration

Groups of 50 male and 50 female F344/N rats (age, 7 weeks) were given pyridine (purity, > 99%) in drinking-water at doses of 0 (control), 100, 200, or 400 ppm, equivalent to average daily doses of 0, 7, 14, or 33 mg/kg bw for 104 weeks (males) or 105 weeks (females) (NTP, 2000).

The survival of exposed males and females was similar to that of controls. Final mean body weights of male and female rats given pyridine at 200 ppm were 81% and 89% of those of male and female controls, respectively. Final mean body weights of male and female rats given pyridine at 400 ppm were 80% and 84% those of male and female controls, respectively. The final mean body weights of both male and female rats given pyridine at 100 ppm were within 5% of the final mean body weight of controls. Water consumption by male and female rats given pyridine at 400 ppm was greater than that of controls throughout the study, and water consumption by males and females given pyridine at 200 ppm was greater during the second year of the study.

Incidences of renal tubule adenoma and renal tubule adenoma or carcinoma (combined) in male rats exposed to pyridine at 400 ppm were significantly increased (with a significant positive trend) compared with controls. Compared with controls, in the standard kidney evaluation (single section) of male rats given pyridine at 100, 200, and 400 ppm there were observed incidences of: renal tubule adenoma: 1/50 (2%, control), 0/48, 2/50 (4%), and 6/49 (12%, $P = 0.042$); renal tubule carcinoma: 0/50 (control), 1/48 (2%), 0/50, and 0/49; and renal tubule adenoma or carcinoma (combined): 1/50 (2%, control), 1/48 (2%), 2/50 (4%), and 6/49 (12%, $P = 0.042$). The incidences of renal tubule neoplasms in all groups of exposed male rats equalled or exceeded the historical control ranges (single section) for drinking-water studies of 1/327 (renal tubule adenoma only) ($0.3\% \pm 0.8\%$), range 0–2%. In this standard kidney evaluation, increased incidence of renal tubule hyperplasia was observed in the groups of males given pyridine at 200 ppm (4/50, 8%) and 400 ppm (7/49, 14%, $P \leq 0.05$) compared with controls (1/50, 2%) and those given pyridine at 100 ppm (0/48). In the extended evaluation of the kidney (step sections), the incidences of renal tubule adenoma were 1/50 (2%, control), 3/48 (6%), 5/50 (10%), and 9/49 (18%, $P \leq 0.01$) for male rats given pyridine at 0, 100, 200, and 400 ppm, respectively. There were no additional rats with carcinomas found in the extended evaluation.

In the original (single section) and extended (step sections) evaluations (combined) of the kidney of male rats, there were observed incidences of: renal tubule adenoma: 2/50 (4%, control), 3/48 (6%), 6/50 (12%), and 10/49 (20%, $P = 0.008$); renal tubule carcinoma: 0/50 (control), 1/48 (2%), 0/50, and 0/49; and renal tubule adenoma or carcinoma (combined): 2/50 (4%, control), 4/48 (8%), 6/50 (12%), and 10/49 (20%, $P = 0.008$).

The incidences of mononuclear cell leukaemia in female rats were significantly increased with a significant positive trend in the 200 and 400 ppm

groups: 12/50 (24%, control), 16/50 (32%), 22/50 (44%, $P = 0.043$), and 23/50 (46%, $P = 0.020$). The incidence in the group given pyridine at 400 ppm exceeded the historical control range. In female rats, the historical incidence of mononuclear cell leukaemia for drinking-water studies was 102/330 ($30.9 \pm 10.0\%$), range 16–44% (NTP, 2000). [The Working Group noted that mononuclear cell leukaemia can occur spontaneously in female rats. The strengths of this study were its well-conducted GLP design and the use of both sexes.]

Groups of 50 male Wistar rats (age, 7 weeks) were given pyridine (purity, > 99%) in drinking-water at doses of 0 (control), 100, 200, or 400 ppm, equivalent to average doses of 0, 8, 17, or 36 mg/kg bw per day, for 104 weeks (NTP, 2000). Pyridine was shown to increase the incidence of leukaemia in a transplant model for leukaemia in male F344/N rats (Dieter et al., 1989), and male Wistar rats were used to evaluate the effects of pyridine in a rat model with a low spontaneous incidence of mononuclear cell leukaemia.

The survival of exposed rats given pyridine at 200 or 400 ppm was significantly less than that of controls; the numbers of rats surviving at the end of the study were 22/50 (control), 14/50, 11/50 ($P = 0.020$), and 7/50 ($P < 0.001$). Final mean body weights of rats exposed to 200 ppm or 400 ppm were significantly less than that of controls at 83% and 84%, respectively. Water consumption by control and exposed rats was similar.

There was a significant increase in the incidence of testicular (interstitial cell) adenoma in rats exposed to 400 ppm, with observed incidences of 5/50 (10%, control), 6/49 (12%), 4/49 (8%), and 12/50 (24%, $P = 0.012$) with a significant positive trend. [There were no historical control data for this tumour for male Wistar rats.] The incidences of interstitial cell hyperplasia observed in exposed groups were numerically greater than those in controls, but these increases – 3/50 (6%, control), 4/49 (8%), 7/49 (14%), and 7/50 (14%) – were not significant.

[The Working Group noted that in this male Wistar rat model there were no statistical increases in the incidence of mononuclear cell leukaemia that were related to treatment. The Working Group also noted that the male Wistar rat kidney was evaluated in a manner similar to that for the male F344/N rat, but the incidences of renal cell tumours in exposed male Wistar rats compared with control rats were not significantly increased.]

There were treatment-related liver non-neoplastic lesions in exposed male Wistar rats, including centrilobular degeneration and necrosis, fibrosis, and pigmentation (NTP, 2000). [The Working Group noted this was a well-conducted GLP study.]

3.2.2 Subcutaneous injection

Groups of male and female Fischer 344 rats (age, ~6 weeks) were injected subcutaneously with pyridine [commercial product] in saline at 0, 3, 10, 30, or 100 mg/kg bw twice a week for 52 weeks. The animals were then kept in observation for an additional 6 months. The number of animals in each group varied with the dose level: there were 60 rats per sex (negative control, no treatment), 60 rats per sex at 0 mg/kg bw (saline control), 10 rats per sex at 3 mg/kg bw, 20 rats per sex at 10 mg/kg bw, 30 rats per sex at 30 mg/kg bw, and 40 rats per sex at 100 mg/kg bw. There was no treatment-related effect on survival, and the final mean body weights of the male and female rats given pyridine at 100 mg/kg bw were 1–6% less than those of the negative or saline controls. All spontaneous deaths, moribund rats, and rats showing gross microscopic changes were examined histologically. Selected histopathological results were reported with findings from all dose levels combined. There was no significant increase in tumour incidence (Mason et al., 1971).

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Absorption, distribution, and excretion

(a) Humans

As noted in previous reports ([ATSDR, 1992](#); [European Commission, 2004](#)), human data for pyridine are sparse and no data are available from exposure by inhalation. Pyridine at a dose of 3.4 mg of [¹⁴C]-labelled pyridine (~0.04 mg/kg bw) was administered orally in orange juice to two healthy male subjects and 24-hour urine samples were collected ([D'Souza et al., 1980](#); [Damani et al., 1982](#)). Only 65% and 68% of the dose was recovered in the two volunteers, about half of which was recovered as pyridine *N*-oxide and about 10% and 20% of which was recovered as *N*-methylpyridinium ion.

(b) Experimental systems

No studies of exposure by inhalation were available to the Working Group. However, the absorption, distribution, and excretion of pyridine have been described after either oral exposure or intraperitoneal injection in multiple experimental animal species, including rats, mice, rabbits, gerbils, and hamsters. As in humans, pyridine is absorbed by various tissues in a dose-dependent manner, but there is no tissue accumulation due to rapid elimination in urine, faeces, and exhaled breath ([ATSDR, 1992](#)).

Significant species- and dose-dependent differences have been reported. For example, urinary excretion of pyridine *N*-oxide after mice, hamsters, rats, guinea-pigs, rabbits, and ferrets were given pyridine by intraperitoneal injection varied from 10% of the dose in rats to almost 40% in mice and guinea-pigs ([Gorrod & Damani, 1980](#)). A comparison of urinary

excretion in several species (i.e. rats, guinea-pigs, mice, gerbils, hamsters, rabbits, and cats) given intraperitoneal injections of [¹⁴C]-labelled pyridine (7 mg/kg bw) showed marked species differences in the extent of recovery, ranging from 48% of the total dose in rats to 75% in cats ([D'Souza et al., 1980](#)). In the same study, comparisons of urinary excretion after either oral or intraperitoneal administration revealed similar rates of recovery for a given species regardless of route of administration. This observation is consistent with the rapid and virtually complete absorption of pyridine regardless of route of administration.

4.1.2 Metabolism

(a) Humans

As shown in [Fig. 4.1](#), the initial metabolic reaction is catalysed primarily by cytochrome P450 (CYP) 2E1. In the human study described above (Section 4.1.1a), formation of pyridine *N*-oxide was the predominant route of metabolism. An *in vitro* study in microsomes from human liver, kidney, and lung ([Wilke et al., 1989](#)) showed tissue-specific patterns of metabolism. Pyridine *N*-oxide was the predominant metabolite produced from liver microsomes, the second most abundant from kidney microsomes, and the equally abundant metabolite from lung microsomes. Evidence for formation of 2,5-dihoxypyridine, discussed below in Section 4.1.2(b), is only available from studies in experimental systems.

(b) Experimental systems

As shown in [Fig. 4.1](#), pyridine metabolism yields a diversity of potential metabolites. Pyridine *N*-oxide is generally the predominant metabolite recovered in urine in all species studied. However, the distribution of metabolites varies markedly across species and tissues. The metabolite *N*-methylated pyridinium ion is particularly variable among species and according to pyridine dose. [Damani et al. \(1982\)](#)

showed that *N*-methylation is the preferred initial metabolic step at low doses, whereas *N*-oxidation can account for up to 10% in rats to as much as 20–40% in other species, including rabbits, mice, hamsters, guinea-pigs, and ferrets, at higher doses.

Oxidative pyridine metabolism is initiated by CYPs, as evidenced by generation of metabolites in preparations of tissue (primarily liver) microsomes that require a reduced nicotinamide adenine dinucleotide phosphate generating system, and produces spectral shifts characteristic of substrate binding to heme moieties on CYPs (Hlavica et al., 1982). CYP2E1 is the primary enzyme that catalyses the primary reaction, *N*-oxide formation; other CYPs only seem to play a quantitatively significant role at high pyridine concentrations (Kim et al., 1991a). Competitive inhibition of pyridine *N*-oxidation by the presence of *para*-nitrophenol is also indicative of the major role of CYP2E1.

One metabolite of potential toxicological importance because of its chemical reactivity is 2,5-dihydroxypyridine, which can undergo redox cycling, generating reactive oxygen species. Rabbit liver microsomes metabolized both the 3-hydroxy and 2-hydroxy metabolites of pyridine to 2,5-dihydroxy metabolite (Kim & Novak, 1990a). Three CYPs catalyse the reaction, with markedly different relative contributions and rates by substrate. The rate of CYP2E1 catalysis was 15- to 30-fold greater than that of either CYP2B1 or CYP1A2 catalysis with 3-hydroxypyridine as substrate. With 2-hydroxypyridine as substrate, the rate of CYP2E1 activity was 10-fold that of CYP1A2, with no detectable activity by CYP2B1.

4.1.3 Modulation of metabolic enzymes

(a) Humans

Pyridine or some of its metabolites can alternately induce or inhibit expression of CYP1A1/1A2 and/or CYP2E1. *CYP1A1* mRNA

transcripts were detected in all human lung samples from 27 subjects, and were induced by both pyridine (12.4 mM) or 2-hydroxypyridine (10 mM) (Wei et al., 2002). In contrast, *CYP1A2* mRNA was variably detected and was only inducible in some of the tissue samples. In HepG2 cells, only 2-hydroxypyridine (among four metabolites tested, and the parent compound) induced *CYP1A1* mRNA expression and increased ethoxyresorufin-*O*-deethylase activity (Iba et al., 2002). Pyridine only induced *CYP1A1* expression when cells were first engineered to express human CYP2E1, indicating that a pyridine metabolite is responsible for the induction.

(b) Experimental systems

(i) CYP2E1 induction

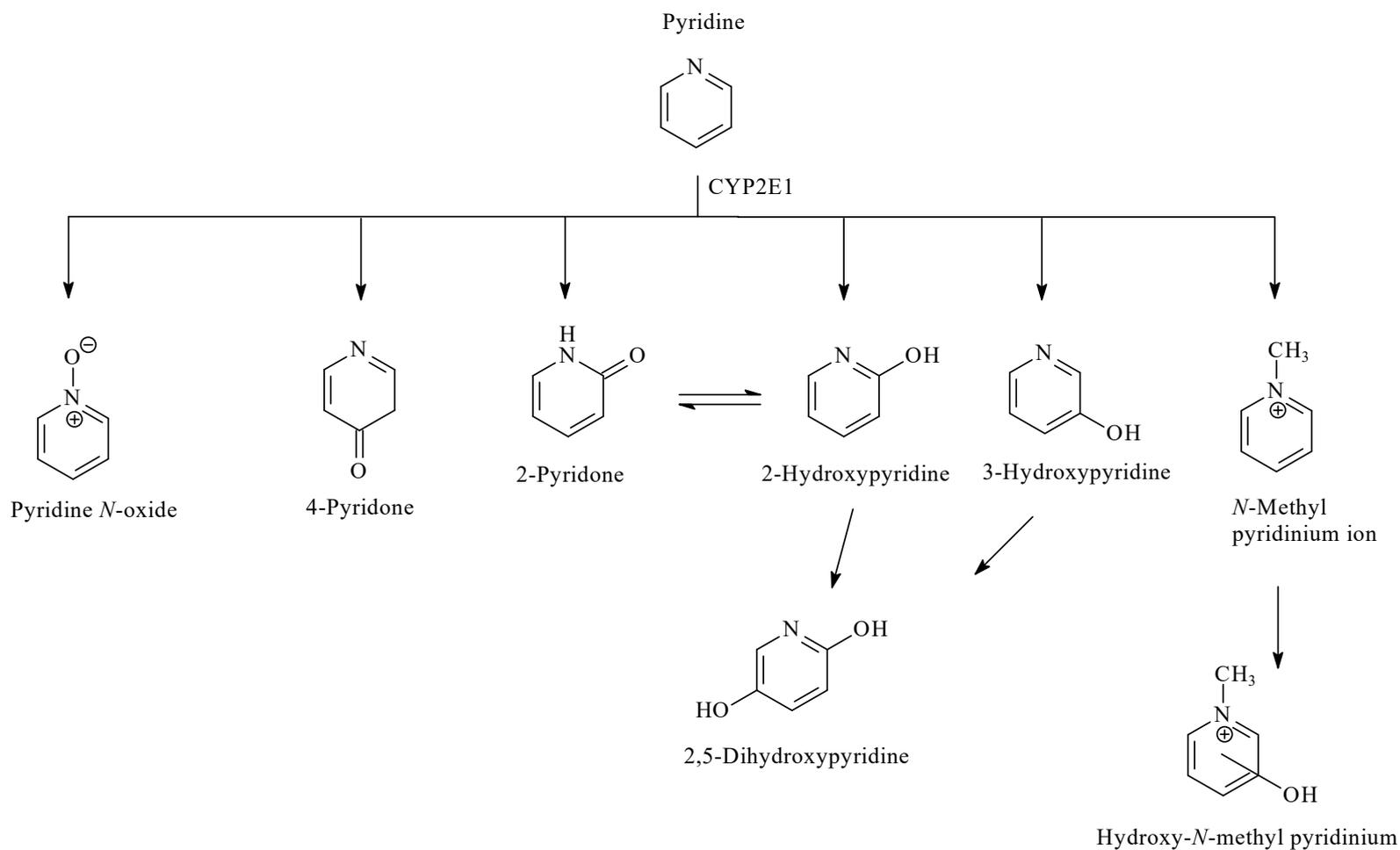
Considerable data are available on the use of pyridine as an inducing agent for CYPs. While CYP2E1 is the primary focus of most studies, other CYPs, including CYP2B1 and CYP1A1/1A2, are also induced in certain tissues and certain species.

Induction of rat liver CYP2E1 has been extensively studied, primarily by intraperitoneal injection of pyridine (e.g. Carlson & Day, 1992; Kim et al., 1993; Cummings et al., 2001; González-Jasso et al., 2003). Hotchkiss et al. (1993) studied pyridine inhalation in rats, reporting significant hepatic induction of CYP2E1 at concentrations equivalent to the threshold limit value of 5 ppm.

Pyridine induction of CYP2E1 has been characterized in other rodent tissues, including rat kidney (Kim et al., 1992; Hotchkiss et al., 1995; Cummings et al., 2001), rat lung (Carlson & Day, 1992; Page & Carlson, 1994), rat prostate and testis (Jiang et al., 1998), rat peripheral lymphocytes (González-Jasso et al., 2003), and mouse liver and lung (Page & Carlson, 1994).

Pyridine increases CYP2E1 protein levels and enzymatic activity, but not mRNA levels (e.g. Kim & Novak, 1990a). The mechanism involves increased translational efficiency with no effect

Fig. 4.1 Metabolic pathways for pyridine



The scheme illustrates the identified metabolites of pyridine, most of which derive from the initial catalysis by cytochrome P450 2E1 (CYP2E1). The rate of formation of each metabolite of pyridine differs among tissues and species. Whereas most of the metabolites have been identified in all species studied, 2,5-dihydroxypyridine has only been characterized in rabbit liver microsomes. 2-Pyridone and 2-hydroxypyridine are in rapid equilibrium with each other. Compiled by the Working Group.

on transcription. As examples of its impact on the metabolism of other chemicals, the intraperitoneal administration of pyridine increased carbon tetrachloride metabolism in rat liver ([Gruebele et al., 1996](#)), styrene metabolism and styrene-induced pneumo- and hepatotoxicity in mice ([Gadberry et al., 1996](#); [Carlson, 1997](#)), and CYP2E1-catalysed reductive dehalogenation and subsequent hepatotoxicity of 1,1-dichloro-1-fluoroethane (HCFC-141b) ([Zanovello et al., 2001](#)). In contrast, pyridine administration had no effect on pulmonary or hepatic cell injury in rats due to acrylonitrile ([Felten et al., 1998](#)), on benzene-induced clastogenicity in mice ([Harper et al., 1984](#)), or on benzene-induced pneumotoxicity and hepatotoxicity in rats ([Chaney & Carlson, 1995](#)).

(ii) Other CYPs

[Iba et al. \(1999a\)](#) showed that pyridine produced complex and tissue-, sex-, and enzyme-specific effects on CYP1A enzymes in Sprague-Dawley rats. Pyridine treatment of rats induced activities of CYP1A1 and CYP1A2, but the response varied according to sex and tissue, and was generally greater for CYP1A1 ([Kim et al., 1991b](#); [Kim et al., 1995](#)). [Fung et al. \(1999\)](#) observed induction of CYP1A1, but not CYP1A2, in rat peripheral blood lymphocytes that was associated with an increase in the *in vivo* bioactivation and bacterial mutagenicity of benzo[*a*]pyrene.

CYP2B enzymes in experimental animal tissues are also induced by pyridine, but the underlying mechanism, kinetics, and tissue specificity differ from that by which induction of CYP2E1 occurs ([Park et al., 1992](#); [Kim et al., 1993](#)).

4.2 Mechanisms of carcinogenesis

4.2.1 Genetic and related effects

See [Table 4.1](#), [Table 4.2](#), and [Table 4.3](#).

The genotoxicity data for pyridine were reviewed by the [NTP \(2000\)](#) and [IARC \(2000\)](#).

A summary of those data and of one report published since then, describing a study in *Drosophila melanogaster* ([Muñoz & Barnett, 2003](#)), is provided in the following section.

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Mammalian systems

In a study *in vivo*, pyridine did not induce micronuclei in orally exposed male ICR mice ([Harper et al., 1984](#)) and did not induce micronuclei or chromosomal aberrations in intraperitoneally injected male B6C3F₁ mice ([NTP, 2000](#)). In male B6C3F₁ mice given pyridine by oral gavage (175, 350, and 700 mg/kg bw), no indication of unscheduled DNA synthesis was detected in hepatocytes harvested 2 and 16 hours after dosing ([MacGregor et al., 2000](#)).

In a study *in vitro*, no significant increases in mutant frequencies were seen in L5178Y *Tk*^{+/-} mouse lymphoma cell cultures after incubation with pyridine (≤ 5000 µg/mL), with or without rat liver S9 ([McGregor et al., 1988](#)). Pyridine was also negative for induction of chromosomal aberrations in Chinese hamster cells in the absence or presence of S9 ([Abe & Sasaki, 1977](#); [Ishidate & Odashima, 1977](#); [NTP, 2000](#)). Sister-chromatid exchanges were increased in one study in Chinese hamster cells (without exogenous metabolic activation) ([Abe & Sasaki, 1977](#)), but not in another study of Chinese hamster ovary cells with or without S9 ([NTP, 2000](#)).

(ii) Non-mammalian systems

Pyridine yielded mixed results in experiments for induction of sex-linked recessive lethal (SLRL) mutations in adult male *D. melanogaster* ([Valencia et al., 1985](#); [Mason et al., 1992](#); [Fouremant et al., 1994](#)). [Valencia et al. \(1985\)](#) reported negative results for pyridine administered by intraperitoneal injection (at 7000 ppm in aqueous 0.7% saline solution),

Table 4.1 Genetic and related effects of pyridine in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results ^a	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Micronuclei	Mouse, ICR (M)	Bone marrow (PCE)	-	1000 mg/kg bw	Gavage, 1×	1000 PCEs scored per each of 5 mice per dose group	Harper et al. (1984)
Unscheduled DNA synthesis	Mouse, B6C3F ₁ (M)	Liver	-	700 mg/kg bw	Gavage, 1×		MacGregor et al. (2000)
Chromosomal aberrations	Mouse, B6C3F ₁ (M)	Bone marrow	-	600 mg/kg bw	i.p., 1×; sampling at 17 and 36 h		NTP (2000)
Micronuclei	Mouse, B6C3F ₁ (M)	Bone marrow	-	500 mg/kg bw	i.p., 3× at 24 h intervals; sampling 24 h after final injection		NTP (2000)

bw, body weight; h, hour(s); HID, highest ineffective dose; i.p., intraperitoneal; LED, lowest effective dose; M, male; PCE, polychromatic erythrocyte

^a -, negative

Table 4.2 Genetic and related effects of pyridine in non-human mammalian cells in vitro

End-point	Species, cell line	Results ^a		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Chromosomal aberrations	Chinese hamster, Don cells	-	NT	5 mM [396 µg/mL]		Abe & Sasaki (1977)
Sister-chromatid exchange	Chinese hamster, Don cells	+	NT	1 mM [79.10 µg/mL]		Abe & Sasaki (1977)
Chromosomal aberrations	Chinese hamster, lung	-	NT	505.7 mg/mL		Ishidate & Odashima (1977)
Mutation	Mouse, L5178 <i>Tk</i> ^{+/-} , lymphoma cells	-	-	5000 µg/mL	Little cytotoxicity at the HIC (relative total growth, 62–77%)	McGregor et al. (1988)
Chromosomal aberrations	Chinese hamster, ovary	-	-	5000 µg/mL		NTP (2000)
Sister-chromatid exchange	Chinese hamster, ovary	-	-	5020 µg/mL	HIC without S9, 1673 µg/mL	NTP (2000)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; S9, 9000 × g supernatant from rat liver; *Tk*^{+/-}, thymidine kinase locus

^a +, positive; -, negative

Table 4.3 Genetic and related effects of pyridine and its metabolites in non-mammalian experimental systems

Test system (species, strain)	End-point	Results ^a		Agent, concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
<i>Pyridine</i>						
<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537	Reverse mutation	-	-	Pyridine, 3 µmol/plate [237 µg/plate]		Florin et al. (1980)
<i>Salmonella typhimurium</i> TA98 and TA100, TA1535, TA1537	Reverse mutation	-	-	Pyridine, 10 000 µg/plate		Haworth et al. (1983)
<i>Drosophila melanogaster</i>	Sex-linked recessive lethal mutations	+/-	NA	Pyridine, 700 ppm	Equivocal results by feeding (<i>P</i> = 0.043); negative results by injection (HIC, 7000 ppm)	Valencia et al. (1985)
<i>Saccharomyces cerevisiae</i> D61.M	Aneuploidy	+	NT	Pyridine, 0.99%		Zimmermann et al. (1986)
φX-174 RF double-stranded plasmid DNA	DNA strand breaks	-	NT	Pyridine, 1 mM [79 µg/mL]	Only a single dose tested	Kim & Novak (1990b)
<i>Drosophila melanogaster</i>	Sex-linked recessive lethal mutations	+	NA	Pyridine, 4300 ppm	Injection; negative results by feeding (500 ppm)	Mason et al. (1992)
<i>Drosophila melanogaster</i>	Sex-linked recessive lethal mutations	-	NA	Pyridine, 500 ppm	Negative results by injection (500 ppm) or by feeding (730 ppm)	Foureman et al. (1994)
<i>Drosophila melanogaster</i>	Aneuploidy	+	NA	Pyridine, 0.05%		Muñoz & Barnett (2003)
<i>Pyridine metabolites</i>						
<i>Escherichia coli</i> W3110 and AB1157 and DNA repair- deficient derivatives; <i>Bacillus</i> <i>subtilis</i> wild-type and UV- sensitive mutants	Other	-	NT	Pyridine 1-oxide [pyridine <i>N</i> -oxide], 500 µg		Nagao & Sugimura (1972)
<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537	Reverse mutation	-	-	3-Hydroxypyridine [3-pyridinol], 3 µmol/plate [285 µg/plate]		Florin et al. (1980)
<i>Salmonella typhimurium</i> TA98, TA100; <i>Klebsiella</i> <i>pneumoniae</i> , <i>E. coli</i>	Mutation	-	NT	Pyridine 1-oxide [pyridine <i>N</i> -oxide], 100 mM [9510 µg/mL]		Voogd et al. (1980)

Table 4.3 (continued)

Test system (species, strain)	End-point	Results ^a		Agent, concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
ϕ X-174 RF double-stranded plasmid DNA	DNA strand breaks	+	NT	2.5-Dihydroxypyridine EC ₅₀ , 60 μ M [6.7 μ g/mL]	Catalase (0.5–1.0 μ g) inhibited formation of DNA strand breaks	Kim & Novak (1990b)
ϕ X-174 RF double-stranded plasmid DNA	DNA strand breaks	-	NT	3-Hydroxypyridine, 1 mM [95 μ g/mL]	Only a single dose tested	Kim & Novak (1990b)

EC₅₀, half maximal effective concentration; HIC, highest ineffective concentration; LEC, lowest effective concentration; NA, not applicable; NT, not tested; ppm, parts per million; RF, replicative form; UV, ultraviolet radiation

^a +, positive; -, negative; +/-, equivocal (variable response in several experiments within an adequate study)

whereas feeding (at 700 ppm pyridine in aqueous 5% sucrose) modestly increased recessive lethal mutations ($P = 0.043$). A second experiment using both intraperitoneal injection (at 500 ppm) and feeding (at 730 ppm) routes yielded negative results ([Foureman et al., 1994](#)). In a third study ([Mason et al., 1992](#)), results of a feeding (at 500 ppm) experiment were negative, but administration of pyridine by intraperitoneal injection (at 4300 ppm) significantly increased the frequency of SLRL mutations. A follow-up test for induction of reciprocal translocations in germ cells of male *D. melanogaster* given pyridine produced negative results ([Mason et al., 1992](#)). Finally, increased frequencies of nondisjunction were observed in *D. melanogaster* broods arising from nearly mature oocytes, but not early-stage or mature oocytes, after females were fed pyridine (at 0.05, 0.1, 0.2, and 0.3%) and mated to untreated males ([Muñoz & Barnett, 2003](#)).

[Zimmermann et al. \(1986\)](#) reported induction of aneuploidy, likely from disruption of microtubule assembly, in *Saccharomyces cerevisiae* D61.M after treatment with up to 1.09% pyridine. Pyridine was negative in bacterial reverse mutation assays in various *Salmonella typhimurium* strains tested with and without S9 mix ([Florin et al., 1980](#); [Haworth et al., 1983](#)).

Pyridine (1 mM, single dose) did not induce DNA strand breaks in ϕ X-174 phage DNA ([Kim & Novak, 1990b](#)).

(iii) Metabolites of pyridine

Few mutagenicity data are available for metabolites of pyridine. One metabolite, pyridine 1-oxide [pyridine *N*-oxide], was not mutagenic in *S. typhimurium* strains TA98 and TA100, and negative in tests for growth inhibition due to DNA damage in *Klebsiella pneumoniae* and *Escherichia coli* K12 ([Voogd et al., 1980](#)). Pyridine 1-oxide was also negative for growth inhibition resulting from DNA damage in *E. coli* and *Bacillus subtilis* ([Nagao & Sugimura, 1972](#)). These tests were all conducted in the absence of S9. Another

metabolite, 3-pyridinol [3-hydroxypyridine], was not mutagenic in several strains of *S. typhimurium* tested with and without induced rat liver S9 ([Florin et al., 1980](#)). 2,5-Dihydroxypyridine (at 10–1000 μ M) induced dose-dependent increases in DNA strand breaks in ϕ X-174 phage DNA ([Kim & Novak, 1990b](#)), an effect that was mitigated in the presence of catalase, suggesting a role for oxidative damage in the production of DNA strand breaks. In this study, the structural analogue 3-hydroxypyridine (1 mM, single dose) did not induce DNA strand breaks ([Kim & Novak, 1990b](#)).

4.2.2 Other mechanisms

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Chronic inflammation, fibrosis, and necrosis were seen in the liver of male and female F344/N rats and male Wistar rats exposed to pyridine at 0, 50, 100, 250, 500, or 1000 ppm in drinking-water for 13 weeks ([NTP, 2000](#)). Dose-related increases in hepatic fibrosis and necrosis were seen in male and female F344/N rats and male Wistar rats exposed to pyridine at 0, 100, 200, or 400 ppm in drinking-water for 2 years. Renal tubule hyperplasia was increased in the male F344/N rats given pyridine at 400 ppm ([NTP, 2000](#)).

In Sprague-Dawley rats, pyridine given at 100 or 150 mg/kg bw by intraperitoneal injection induced heme oxygenase-1 and CYP1A1 mRNA and protein in liver, lung, and kidney. Lipid peroxidation as assessed by thiobarbituric acid reactive substances increased in the liver, lung, and kidney ([Iba et al., 1999](#)). In male and female Syrian hamsters, pyridine given at 400 mg/kg bw by intraperitoneal injection increased CYP1A1, inducible nitric oxide synthase, and metallothionein I-II, responses indicative of the induction of oxidative stress ([Tunca et al., 2009](#)).

4.3 Data relevant to comparisons across agents and end-points

For the results of high-throughput screening assays of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA ([Kavlock et al., 2012](#); [Tice et al., 2013](#); [EPA, 2016a, b](#); [Filer et al., 2016](#)), see Section 4.3 of the *Monograph* on 1-*tert*-butoxypropan-2-ol in the present volume.

4.4 Susceptibility to cancer

No data were available to the Working Group.

4.5 Other adverse effects

4.5.1 Humans

Effects of pyridine include irritation of the eyes, skin, and respiratory tract (in the form of coughing and shortness of breath), as well as liver and kidney damage ([Jori et al., 1983](#); [ATSDR, 1992](#); [OSHA, 2006](#)).

4.5.2 Experimental systems

In a 2-year study in F344/N rats, dose-related non-neoplastic liver lesions, including centrilobular cytomegaly, cytoplasmic vacuolization, periportal fibrosis, fibrosis, centrilobular degeneration and necrosis, and pigmentation, were observed in both sexes. In male Wistar rats, a dose-related increase in non-neoplastic liver lesions, including centrilobular degeneration and necrosis, fibrosis, periportal fibrosis, and pigmentation, was observed ([NTP, 2000](#)).

In male F344/N rats given pyridine at 500 and 1000 ppm in drinking-water for 13 weeks, increased incidences of kidney lesions, including casts, chronic inflammation, and mineralization, were observed. There was also a dose-related increase in the incidences of granular casts and

hyaline degeneration (hyaline droplets), lesions consistent with α_{2u} -globulin nephropathy. There was no increase in non-neoplastic kidney lesions observed in Wistar rats ([NTP, 2000](#)).

IARC established seven criteria for the induction of kidney tumours to have occurred by an α_{2u} -globulin-associated response ([Capen et al., 1999](#)). Four criteria have been met for pyridine, specifically: (i) lack of genotoxic activity of the agent and/or metabolite (Section 4.2.1); (ii) male rat specificity for nephropathy and renal tumorigenicity ([NTP, 2000](#)); (iii) induction of the characteristic sequence of histopathological changes associated with α_{2u} -globulin accumulation ([NTP, 2000](#)); and (iv) identification of the accumulating protein as α_{2u} -globulin (α_{2u} -globulin protein was detected by immunohistochemistry; [NTP, 2000](#)). However, the remaining three of these criteria have not been met for pyridine, specifically: (i) reversible binding of the chemical or metabolite to α_{2u} -globulin (not measured); (ii) similarities in dose–response relationships of the tumour outcome with histopathological end-points associated with α_{2u} -globulin nephropathy (tumours occurred in the absence of α_{2u} -globulin; [NTP, 2000](#)); and (iii) induction of sustained increases in cell proliferation in the renal cortex (not measured).

5. Summary of Data Reported

5.1 Exposure data

Pyridine has several applications in organic chemistry and in industrial practice. It is a high production volume chemical. Pyridine can be formed from the breakdown of many natural materials in the environment. Due to its variety of applications, pyridine can be released in air, water, and soil. The major sources of exposure to pyridine for the general population are foods and cigarette smoke. Information about pyridine content in specific foods is scarce, but was

quantified in the volatile components of coffee and in fried or roasted food. The estimated pyridine intake in the USA was less than 1 g/year per person.

Occupational exposure may occur by inhalation and dermal contact during the production or use of pyridine as an intermediate or as a solvent. Exposure can also occur at coke ovens, oil-shale plants, and other similar industries. People working in quality control and research laboratories can also be exposed to pyridine.

5.2 Human carcinogenicity data

One small cohort study of mortality in workers exposed to pyridine and numerous other chemicals did not show any excess of mortality from cancer of the lung or all cancers combined. Six cases of squamous cell carcinoma of the skin were observed in the study population, but no risk data were reported.

5.3 Animal carcinogenicity data

In one well-conducted good laboratory practice (GLP) study in male and female mice given drinking-water containing pyridine, there was a significant increase, with a significant positive trend, in the incidence of hepatocellular adenoma, hepatocellular carcinoma, hepatoblastoma, and the combination of these tumours in males and females.

In another well-conducted GLP drinking-water study in male and female F344/N rats, pyridine significantly increased the incidence of renal tubule adenoma and renal tubule adenoma or carcinoma (combined) in males, and of mononuclear cell leukaemia in females, with a significant positive trend. In a third well-conducted GLP drinking-water study in male Wistar rats, pyridine significantly increased the incidence of testicular cell adenoma with a significant positive trend.

One study in male and female rats given pyridine by subcutaneous injection gave negative results. One feeding study and one skin-application study in transgenic mice gave negative results.

5.4 Mechanistic and other relevant data

Few data on absorption, distribution, metabolism, or excretion of pyridine in humans were available. Pyridine is absorbed following oral exposure in humans and other species, as well as by other routes in experimental animals. Pyridine *N*-oxide is the primary metabolite in humans and other species, and is generated through cytochrome P4502 E1-mediated oxidation. Pyridine induces multiple cytochrome P450s, and affects the metabolism and toxicity of other chemicals, such as carbon tetrachloride.

Regarding the key characteristics of carcinogens, there is *weak* evidence that pyridine is genotoxic. No human data are available. Pyridine did not induce chromosome or DNA damage in mice. It gave positive results in a few tests in *Drosophila melanogaster*, and in a single test of sister-chromatid exchange induction in Chinese hamster cells in the absence of metabolic activation from S9. Pyridine did not induce mutations in bacterial test systems.

There is *weak* evidence that pyridine induces oxidative stress. Two short-term studies in which pyridine was given by intraperitoneal injection, one in rats and one in hamsters, demonstrated oxidative stress. There is *moderate* evidence that pyridine induces chronic inflammation in rat liver from 13-week and chronic studies, in which necrosis and fibrosis were additionally shown. Renal tubule hyperplasia was observed in male rat kidney.

In the chronic drinking-water study in male rats, toxic effects and carcinogenicity were seen in the kidney. Three of the seven criteria established

by IARC for the induction of kidney tumours to have occurred by an α_{2u} -globulin-associated response have not been met.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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TETRAHYDROFURAN

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 109-99-9

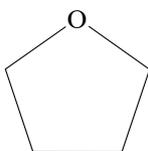
EC/List No.: 203-726-8

Chem. Abstr. Serv. name: Tetrahydrofuran

IUPAC systematic name: Oxolane

Synonyms: Butane alpha,delta-oxide; butane, 1,4-epoxy-; cyclotetramethylene oxide; diethylene oxide; 1,4-epoxybutane; furan, tetrahydro-; furanidine; hydrofuran; oxacyclopentane; tetramethylene oxide; THF

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: C₄H₈O

Relative molecular mass: 72.11

1.1.3 Chemical and physical properties

Description: Tetrahydrofuran is a colourless, volatile liquid with an ethereal or acetone-like odour ([EPA, 2012](#))

Boiling point: 65–66 °C ([EPA, 2012](#))

Melting point: –108.44 °C ([ECHA, 2018](#))

Relative density: 0.883 at 25 °C (water, 1) ([ECHA, 2018](#))

Solubility: Miscible in water ([ECHA, 2018](#))

Volatility: Vapour pressure, 19.3 kPa at 20 °C ([IPCS, 1997](#))

Relative vapour density: 2.5 (air = 1); relative density of the vapour/air mixture at 20 °C (air = 1): 1.28 ([IPCS, 1997](#))

Stability: Tetrahydrofuran is prone to oxidation to peroxides, butyric acid, butyraldehyde, and related compounds, mainly on ageing and in the presence of light, heat, and moisture. The formation of peroxides can be retarded by adding stabilizers such as hydroquinone or 2,6-di-*tert*-butyl-*p*-cresol at 250 mg/kg ([Coetzee & Chang, 1985](#); [Müller, 2012](#)).

Flash point: –14.5 °C ([IPCS, 1997](#))

Explosive limits: 2.0–11.8 vol% in air ([IPCS, 1997](#))

Auto-ignition temperature: 321 °C ([NIOSH, 2014](#))

Octanol/water partition coefficient (P): log K_{ow}, 0.45 at 25 °C ([ECHA, 2018](#))

Odour threshold: 2–7.4 ppm; 60–150 mg/m³ ([EPA, 2012](#))

Conversion factor: 1 ppm = 2.95 mg/m³ ([EPA, 2012](#))

1.2 Production and use

1.2.1 Production process

A process developed by Reppe in the 1930s was for many years the preferred synthetic route to 1,4-butanediol and tetrahydrofuran, and is still the most common approach in Europe and the USA. The Reppe process involves a reaction between acetylene and formaldehyde to give 2-butyne-1,4-diol, with subsequent hydrogenation to 1,4-butanediol, which is further dehydrated and cyclized by acid catalysis at temperatures above 100 °C to tetrahydrofuran ([Müller, 2012](#)). This and other industrial routes to produce tetrahydrofuran (e.g. butadiene acetoxylation, propylene oxide process, maleic anhydride hydrogenation, *n*-butane–maleic anhydride process, and pentosan/furfural processes) are described in more detail by [Müller \(2012\)](#).

1.2.2 Production volume

Tetrahydrofuran appears in the Organisation of Economic Co-operation and Development (OECD) 2007 list of high production volume chemicals ([OECD, 2009](#)), which contains those chemicals which are produced or imported at quantities greater than 1000 tonnes/year in at least one member country or region.

According to the European Chemicals Agency (ECHA) database, more than 100 000 tonnes of tetrahydrofuran are manufactured and/or imported in the European Economic Area per year ([ECHA, 2018](#)).

World consumption of tetrahydrofuran was approximately 439 000 tonnes in 2006 ([Müller, 2012](#)). It grew by about 40% during 2012–2015 ([IHS Markit, 2016](#)) and is projected to exceed 1 million tonnes in 2020 ([Global Industry Analysts, 2018](#)).

1.2.3 Use

Tetrahydrofuran has two primary industrial uses. Its main use is as a monomer in the production of polytetramethylene ether glycol (PTMEG), a component of cast and thermoplastic urethane elastomers, polyurethane stretch fibres, and high-performance copolyester-polyether elastomers. In 2015, the production of PTMEG accounted for almost 90% and about 80% of total use in Asia and in the USA, respectively. A smaller amount of tetrahydrofuran is used as a solvent in polyvinyl chloride (PVC) cements, pharmaceuticals and coatings, precision magnetic tape manufacture, and as a reaction solvent ([IHS Markit, 2016](#)). The National Industrial Chemicals Notification and Assessment Scheme of the Australian Government assessed tetrahydrofuran ([NICNAS, 2016](#)) and, similarly to the ECHA assessment, identified many domestic and industrial uses of tetrahydrofuran. Domestic uses include as: polish and cleaning agents; adhesives; stain, paint, and varnish removers; sealants; lubricating oils; coating products; and pharmaceuticals. Industrial uses include as solvent in the production of polymers (e.g. PTMEG); reagent for chemical reactions; bulk pharmaceutical manufacturing; synthetic perfumes; insecticides; printing inks, dyes, adhesives, lacquers, and other coatings; synthesis of motor fuels; PVC cement; fabrication of articles for packaging, transporting, or storing food (if residual amount does not exceed 1.5% of the film); and metal-working fluids ([ECHA, 2009, 2018](#); [NICNAS, 2016](#)).

1.3 Analytical methods

All methods used to analyse tetrahydrofuran in ambient air are derived from the United States Environmental Protection Agency (EPA) method 8260B, which is a general method used to determine tens of different volatile organic compounds in nearly all types of samples using

Table 1.1 Analytical methods for measurement of tetrahydrofuran in the workplace

Method	Technique	Target concentration	Remarks
IRSST 179-1 (IRSST, 1996)	GC-FID	Precision, 0.8 Minimum reported value, 53 µg	Flow rate, maximum 0.2 L/min TWA sampling volume, 9 L
IRSST 369 (IRSST, 1996)	GC-MS	Analytical uncertainty (CVa), 5.4%	
NIOSH 1609 (NIOSH, 1994)	GC-FID	Estimated LOD, 50 µg/sample Range studied, 323–1240 mg/m ³ Overall precision, 0.055 Accuracy, ±12.6%	Flow rate: minimum 0.01 L/min; maximum 0.2 L/min Sampling volume, 1–9 L Working range, 100–2600 mg/m ³ for a 5-L air sample This is also the primary method used by OSHA
MTA/MA-049/ A01 (2001) (GESTIS (2004))	GC-FID	Working range, 13–275 mg/m ³	Flow rate, 0.2 L/min Recommended sampling time, 60 min Recommended air volume, 12 L Validated according to INSHT protocol

CVa, coefficient of variation; FID, flame ionization detection; GC, gas chromatography; INSHT, Instituto Nacional de Seguridad e Higiene en el Trabajo; IRSST, Institut de recherche Robert-Sauvé en santé et en sécurité du travail; LOD, limit of detection; min, minute(s); MS, mass spectrometry; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; TWA, time-weighted average

gas chromatography in combination with mass spectrometry (GC-MS) ([EPA, 1996](#)). Analytical methods for tetrahydrofuran measurement in the workplace are reported in [Table 1.1](#).

There are also European Union guidelines on testing conditions for articles in contact with foodstuffs, with a focus on kitchenware ([European Commission, 2009](#)). United States EPA method 524.2 is a general-purpose method to evaluate the concentration of tetrahydrofuran and other volatile organic compounds in water ([EPA, 1995](#)).

Finally, there is at least one method to evaluate the concentration of residual solvents in pharmaceutical products. [Li et al. \(1998\)](#) described a capillary gas chromatographic procedure for the analysis of nine common residual solvents, including tetrahydrofuran, in water-insoluble bulk pharmaceuticals.

1.3.1 Exposure assessment and biological markers

In 1991, it was demonstrated that the associations between occupational exposure to tetrahydrofuran and its concentrations in exhaled air, in blood, and in urine had correlation coefficients

of $r = 0.61$, 0.68 , and 0.88 , respectively ([Ong et al., 1991](#)). Laboratory methodological considerations, together with the good correlation between the concentration of tetrahydrofuran in the environment and urinary tetrahydrofuran concentration, suggest that urinary tetrahydrofuran concentration is a useful biological marker of occupational exposure to tetrahydrofuran ([Ong et al., 1991](#)).

According to the American Conference of Governmental Industrial Hygienists (ACGIH), there is inadequate information to set biological exposure indices (BEI) for tetrahydrofuran in venous blood and in exhaled air ([ACGIH, 2008](#)).

Using a headspace GC-MS technique, [Prado et al. \(2010\)](#) demonstrated a detection limit low enough to quantify tetrahydrofuran in urine at occupational exposure levels. The established occupational exposure limit value, measured at the end of the working day, was 2 mg/L ([INSHT, 2011](#)).

1.4 Occurrence and exposure

Exposure to tetrahydrofuran may occur as a result of its release into the environment or its potential occurrence in some foods and consumer products.

1.4.1 Environmental occurrence

Tetrahydrofuran is a synthesized organic compound that is not found in the natural environment ([ACGIH, 2001](#)). Release to the environment from the manufacture of PTMEG is no more than 1% of the tetrahydrofuran produced or handled. Other environmental exposures during regular use are also low ([OECD, 2000](#)).

Fugacity models, distribution-based models incorporating all environmental compartments and based on steady-state fluxes of pollutants across compartment interfaces, suggest that tetrahydrofuran will be found in the environmental compartment in which it was released. Photodegradation by hydroxyl radicals in air is estimated to be rapid; hydroxyl radical reaction half-life is estimated to be 7.3 hours. Tetrahydrofuran released to the environment could partition to the water compartment where it is readily biodegradable, but it would not degrade through hydrolysis. Bioaccumulation of tetrahydrofuran is not expected because of its very low octanol/water partition coefficient. Based upon its physical and chemical properties, production, use patterns, and low levels in the environment at a magnitude of parts per billion, the potential of environmental exposure is expected to be low ([OECD, 2000](#)).

Release of tetrahydrofuran to the environment is likely to occur from industrial use, for example, in processing aids at industrial sites and in the manufacturing of the substance in closed systems with minimal release. For example, total production of tetrahydrofuran in 1999 was 551 million pounds [250 000 tonnes], of which 78% was used for the synthesis of PTMEG in

closed systems ([OECD, 2000](#)). Other release to the environment of this substance is likely to occur from outdoor and indoor use (e.g. machine wash liquids and/or detergents, automotive care products, paints and coatings, adhesives, fragrances, and air fresheners) ([ECHA, 2018](#)).

1.4.2 Occurrence in food

According to the United States Hazardous Substances Data Bank ([HSDB, 2011](#)), tetrahydrofuran was detected in some natural materials such as roasted coffee ([Heins et al., 1966](#); [Stoffelsma & Pypker, 1968](#); [Stoffelsma et al., 1968](#); [Walter & Weidemann, 1969](#); [Furia & Bellanca, 1975](#); [Ross, 2005](#)), floured chickpea (*Cicer arietinum* L.) seed ([Rembold et al., 1989](#)), and chicken breast muscle ([Grey & Shrimpton, 1967](#); [Shahidi et al., 1986](#)).

[The Working Group noted that the literature on potential occurrence of tetrahydrofuran in food is extremely limited, dated, and most likely erroneous. Tetrahydrofuran is commonly used in typical laboratory environments, so contamination during analysis may occur. It is also possible that the occurrences in food were from environmental contamination of samples. None of the studies included quantification. For all these reasons, there is currently insufficient evidence to assume a natural occurrence of tetrahydrofuran.]

1.4.3 Exposure in the general population

Non-occupational exposure to tetrahydrofuran has been described as uncommon ([EPA, 2011](#)), but the general population may be exposed to it by various media. Tetrahydrofuran often occurs in effluent from the production of synthetic textiles, and at solvent recycling facilities ([Isaacson et al., 2006](#)). It has been detected in ambient air, groundwater, drinking-water, and landfill sludge, and in some common household products. In groundwater, it has been measured

at concentrations that exceed the water quality criteria and guidelines set by different states of the USA (50–1300 µg/L) ([Isaacson et al., 2006](#)). Tetrahydrofuran may migrate into foods when present in the contact surface of materials intended for use in food processing ([NTP, 1998](#)).

Qualitative data have indicated that the general population may be exposed to tetrahydrofuran via the inhalation of ambient air, the ingestion of food and drinking-water, and by dermal contact with products containing tetrahydrofuran ([HSDB, 2011](#)).

In four urban areas of the USA, tetrahydrofuran was qualitatively detected in one out of eight samples of breast milk ([Pellizzari et al., 1982](#)). The United States Consumer Product Information Database lists 56 liquid or paste products for home maintenance that contain tetrahydrofuran in concentrations of 10–75% ([National Library of Medicine, 2017](#)).

[No quantitative exposure data in the general population were available to the Working Group.]

1.4.4 Occupational exposure

Exposure to tetrahydrofuran is most likely to occur in occupational settings through inhalation or by dermal contact. In an initial assessment profile, the OECD reported in 2000 that worker exposure at production and use facilities were well below any designated exposure limits, with average concentrations in air less than 10 ppm. Plumbers who use plastic pipe solvent cements can be exposed to tetrahydrofuran; however, workplace exposure monitoring is well below United States standards/guidelines of 200 ppm 8-hour daily values, and the short-term exposure limit (STEL; 15 minutes) of 250 ppm. Monitoring of the plumbing workplace during the use of plastic pipe solvent cements revealed that exposure to tetrahydrofuran did not exceed the above values ([OECD, 2000](#)).

In Germany, a report compiled data from 357 measurements made at approximately 120

companies from the year 1990 onwards. Exposure levels per shift were measured as: 166 mg/m³ (95% value) in the manufacture of plastics adhesives (20 measurements from 7 companies); 89 mg/m³ (95% value) in the manufacture of plastics and plastic foams (19 measurements from 13 companies); and 182 mg/m³ and 290 mg/m³ in plastic coating with ventilation (149 measurements from 47 companies) and without ventilation (140 measurements from 50 companies), respectively ([BGAA, 1999](#)).

Twenty measurements of workplace exposure from the Finnish Institute of Occupational Health, collected between 2012 and 2016, range between 0.01 and 14 mg/m³ with a mean of 1 mg/m³. The highest measurements were from gluing plastic products ([FIOH, 2017](#)).

In a study of 78 plumbers installing plastic pipe, 4 of 29 workers who had significant skin contact with primer and cement had urinary tetrahydrofuran concentrations 1.4–6.7-fold higher than the maximum measured among plumbers with little skin contact but with similar airborne exposures, suggesting the likelihood of significant skin contribution ([ACGIH, 2005](#)).

1.5 Regulations and guidelines

The Committee of Experts on the Transport of Dangerous Goods and the Globally Harmonized System of Classification and Labelling of Chemicals of the United Nations Economic Commission for Europe identified tetrahydrofuran as: United Nations No. 2056, Hazard Class 3, United Nations Packing Group II ([UNECE, 2015](#)).

Tetrahydrofuran 8-hour (140–590 mg/m³) and short-term (15 minutes, 280–737 mg/m³) limit values for different countries, from the GESTIS international limit values database, are presented in [Table 1.2](#) ([GESTIS, 2017](#)).

For the ACGIH, the time-weighted average (TWA) threshold limit value (TLV) was 200 ppm during 1957–2004. A TLV-STEL value of

Table 1.2 Eight-hour and short-term limit values for occupational exposure to tetrahydrofuran in different countries or regions

Country or region	8-hour limit value		Short-term limit value	
	ppm	mg/m ³	ppm	mg/m ³
Australia	100	295		
Austria	50	150	100	300
Belgium	50	150	100	300
Canada (Ontario)	50		100	
Canada (Quebec)	100	300		
China		300		
Denmark	50	148	100	296
European Union	50	150	100	300
Finland	50	150	100 ^a	300 ^a
France	50 ^b	150 ^b	100 ^b	300 ^b
Germany (AGS)	50	150	100 ^a	300 ^a
Germany (DFG)	50	150	100 ^a	300 ^a
Hungary		150		300
Ireland	50	150	100 ^c	300 ^c
Italy	50 ^d	150 ^d	100 ^d	300 ^d
Japan	50			
Japan (JSOH)	50	148		
Latvia	50	150	100 ^a	300 ^a
Netherlands		300		600
New Zealand	100	295		
Poland		150		300
Republic of Korea	50	140	100	280
Romania	50	150	100 ^a	300 ^a
Singapore	200	590	250	737
Spain	50 ^d	150 ^d	100 ^d	300 ^d
Sweden	50	150	80 ^a	250 ^a
Switzerland	50	150	100	300
Turkey	50	150	100 ^a	300 ^a
United Kingdom	50	150	100	300
USA (NIOSH)	200	590	250 ^a	735 ^a
USA (OSHA)	200	590		

AGS, Ausschuff für Gefahrstoffe; DFG, Deutsche Forschungsgemeinschaft; JSOH, Japan Society for Occupational Health; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; ppm, parts per million

^a 15-minute average value

^b Restrictive statutory limit values

^c 15-minute reference period

^d Dermal exposure

Source: [GESTIS \(2017\)](#)

250 ppm was added in 1976. In 2005, the ACGIH committee approved a TLV-TWA of 50 ppm and a TLV-STEL of 100 ppm ([ACGIH, 2005](#)).

The United States National Institute for Occupational Safety and Health proposed an immediately dangerous for life and health limit of 2000 ppm, based on 10% of the lower explosive limit ([NIOSH, 2014](#)).

The American Industrial Hygiene Association published Emergency Response Planning Guidelines (ERPGs) for various chemicals, including tetrahydrofuran. Values for ERPG-1, 2, and 3 are 100, 500, and 5000 ppm, respectively, where the maximum 1-hour airborne exposure limit concentration classifications are: ERPG-1 for mild, transient adverse health effects or clearly objectionable odour; ERPG-2 for irreversible or other serious health effects; and ERPG-3 for life-threatening health effects (25% of the lower explosion limit) ([AIHA, 2008](#)).

For use in pharmaceutical products, tetrahydrofuran is classified in class 2 of table 2 of the United States Food and Drug Administration Guidance for Industry. Based on chronic toxicity and/or carcinogenicity data, the permitted daily exposure (PDE) to tetrahydrofuran as a residual solvent is 7.2 mg/day and the concentration limit is 720 ppm ([FDA, 2017](#)).

In 1993, the German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area set the biological tolerance value (BAT) of 8 mg tetrahydrofuran per litre of urine for samples collected at the end of exposure or shift. In 2001, for exposure to a tetrahydrofuran concentration of 50 mg/m³ (the maximum permissible concentration in the workplace (MAK) value), the BAT value was re-evaluated and lowered to 2.0 mg tetrahydrofuran per litre of urine ([DFG, 2016](#)).

In 2000, the ACGIH adopted a BEI value of 8 mg tetrahydrofuran per litre of urine at the end of shift ([ACGIH, 2005](#)).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See [Table 3.1](#).

3.1 Mouse

Groups of 50 male and 50 female B6C3F₁ mice (age, 7 weeks) were exposed to tetrahydrofuran (purity, ~99%; no impurities > 0.1%) by whole-body inhalation at 0 (control), 200, 600, or 1800 ppm for 6 hours plus T₉₀ (time to achieve 90% of the target concentration after the beginning of vapour generation; 12 minutes) per day, 5 days per week, for 105 weeks ([Chhabra et al., 1998](#); [NTP, 1998](#)). After week 36, the survival of the males exposed at 1800 ppm was significantly less than that of controls. The survival of males exposed at 200 or 600 ppm, and of all exposed females, was similar to that of controls. Mean body weights of males and females exposed to tetrahydrofuran were similar to those of controls throughout the study. Necropsies were performed on all mice and major organs were investigated by light microscopy.

Increased incidences of hepatocellular adenoma and carcinoma were reported in exposed female mice relative to controls. The incidences of hepatocellular adenoma or carcinoma (combined) were 17/50, 24/50, 26/50, and 41/48, and the incidences of hepatocellular carcinoma were 6/50, 10/50, 10/50, and 16/48 for groups exposed to tetrahydrofuran at 0, 200, 600, and 1800 ppm, respectively. The increases in the incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) were statistically significant by the trend test ($P < 0.001$), and statistically significant ($P < 0.001$) by pairwise comparison

Table 3.1 Studies of carcinogenicity with tetrahydrofuran in rodents

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F ₁ (M) 7 wk 105 wk NTP (1998)	Inhalation (whole-body exposure) Tetrahydrofuran, ~99% Clean air 0, 200, 600, 1800 ppm for 6 h + T ₉₀ (12 min) per d, 5 d/wk for 105 wk 50, 50, 50, 50 32, 31, 28, 12	<i>Liver</i> Hepatocellular adenoma: 24/50, 19/50, 16/50, 14/50 Hepatocellular carcinoma: 14/50, 13/50, 14/50, 9/50 Hepatocellular adenoma or carcinoma (combined): 35/50, 31/50, 30/50, 18/50	NS NS NS	Principal strengths: GLP study in both males and females Overall historical control incidence in chamber controls for hepatocellular adenoma or carcinoma (combined), 358/947 (37.8 ± 12.5%); range, 11–60% Statistical test: logistic regression
Full carcinogenicity Mouse, B6C3F ₁ (F) 7 wk 105 wk NTP (1998)	Inhalation (whole-body exposure) Tetrahydrofuran, ~99% Clean air 0, 200, 600, 1800 ppm for 6 h + T ₉₀ (12 min) per d, 5 d/wk for 105 wk 50, 50, 50, 50 29, 33, 26, 32	<i>Liver</i> Hepatocellular adenoma: 12/50*, 17/50, 18/50, 31/48** Hepatocellular carcinoma: 6/50*, 10/50, 10/50, 16/48** Hepatocellular adenoma or carcinoma (combined): 17/50*, 24/50, 26/50, 41/48**	*P < 0.001 (trend), **P < 0.001 *P = 0.012 (trend), **P = 0.014 *P < 0.001 (trend), **P < 0.001	Principal strengths: GLP study in both males and females Statistical test: logistic regression
Full carcinogenicity Rat, F344/N (M) 7 wk 105 wk NTP (1998)	Inhalation (whole-body exposure) Tetrahydrofuran, ~99% Clean air 0, 200, 600, 1800 ppm for 6 h + T ₉₀ (12 min) per d, 5 d/wk for 105 wk 50, 50, 50, 50 12, 6, 5, 6	<i>Kidney</i> Renal tubule adenoma: 1/50, 1/50, 4/50, 3/50 Renal tubule carcinoma: 0/50, 0/50, 0/50, 2/50 Renal tubule adenoma or carcinoma (combined): 1/50*, 1/50, 4/50, 5/50	NS NS *P = 0.037 (trend)	Principal strengths: GLP study in both males and females Historical incidence for 2-yr NTP inhalation studies in chamber controls: renal tubule adenoma or carcinoma (combined), 6/652 (0.9 ± 1.3%) [range, 0–4%]; renal tubule carcinoma, 0/652 Statistical test: logistic regression The NTP (1998) original slides were reviewed by Bruner et al. (2010)

Table 3.1 (continued)

Study design	Route	Tumour incidence	Significance	Comments
Species, strain (sex)	Agent tested, purity			
Age at start	Vehicle			
Duration	Dose(s)			
Reference	No. of animals at start			
	No. of surviving animals			
Full carcinogenicity Rat, F344/N (F)	Inhalation (whole-body exposure) Tetrahydrofuran, ~99%	<i>Mammary gland</i> Fibroadenoma:		Principal strengths: GLP study in both males and females
7 wk	Clean air	23/50*, 22/50, 29/50, 31/50	*P = 0.031 (trend)	Historical incidence for 2-yr NTP inhalation studies in chamber controls: mammary gland fibroadenoma, 180/653 (27.6 ± 7.7%) [range, 16–42%]
105 wk	0, 200, 600, 1800 ppm for 6 h + T ₉₀ (12 min) per d, 5 d/wk for 105 wk			Statistical test: logistic regression
NTP (1998)	50, 50, 50, 50 25, 25, 26, 26			

d, day(s); F, female; GLP, good laboratory practice; h, hour(s); M, male; min, minute(s); NS, not significant; NTP, National Toxicology Program; ppm, parts per million; T₉₀, time to achieve 90% of the target concentration after the beginning of vapour generation; wk, week(s); yr, year(s)

between the controls and the group exposed to the highest dose. The incidences of hepatocellular adenoma or carcinoma (combined) in male mice were 35/50 (70%), 31/50 (62%), 30/50 (60%), and 18/50 (36%) for groups exposed to tetrahydrofuran at 0, 200, 600, and 1800 ppm, respectively. No statistically significant increase in the incidences of hepatocellular adenoma, carcinoma, or adenoma or carcinoma (combined) were observed in treated males compared with the control group; however, the incidence of hepatocellular adenoma or carcinoma (combined) for the control group (70%) was above the maximum value for historical controls ($37.8 \pm 12.5\%$, with a range of 11–60%). In addition, the rates of incidence for the groups given the middle dose (60%) and low dose (62%) were at the high end or slightly above the range for historical controls. The low tumour incidence in the group given the highest dose was attributed to lower survival in this group; the survival-adjusted incidence was comparable between the group given the highest dose (73.4%) and the control group (77.2%) (NTP, 1998). [The Working Group noted this was a well-conducted good laboratory practice (GLP) study and that both sexes were used.]

3.2 Rat

Groups of 50 male and 50 female F344 rats (age, 7 weeks) were exposed to tetrahydrofuran (purity, 99%; no impurities > 0.1%) by whole-body inhalation at 0 (control), 200, 600, or 1800 ppm for 6 hours plus T_{90} (12 minutes) per day, 5 days per week, for 105 weeks (Chhabra et al., 1998; NTP, 1998). The survival of males and females exposed to tetrahydrofuran was similar to that of controls. Mean body weights of exposed groups of males and females were similar to those of controls throughout the study. Necropsies were performed on all rats and major organs were investigated by light microscopy.

In males, NTP (1998) reported slight (but non-significant) increases in renal tubular

epithelial tumours at exposure concentrations of 600 ppm (adenoma, 4/50) and 1800 ppm (adenoma, 3/50; carcinoma, 2/50) compared with controls (adenoma, 1/50; carcinoma, 0/50). The incidences of renal tubule adenoma or carcinoma (combined) were 1/50, 1/50, 4/50, and 5/50 at 0, 200, 600, and 1800 ppm, respectively; statistical analysis revealed a significant positive trend ($P = 0.037$). The original slides from the NTP (1998) study were subsequently reviewed by an additional group of experts, who examined the slides for which kidney proliferative lesions had been reported (Bruner et al., 2010). This re-evaluation confirmed the presence of treatment-related renal tubule proliferative lesions, but failed to reproduce the results of the original NTP (1998) study; two carcinomas were downgraded to adenomas in the re-evaluation (Bruner et al., 2010). [The Working Group analysed the three relevant articles cited above, critically reviewed the results, and weighed the interpretations; in consideration of all data, the Working Group agreed with the conclusions of the original NTP (1998) study.] In females, there was a small but significant positive trend ($P = 0.031$) in the incidence of mammary gland fibroadenoma (23/50, 22/50, 29/50, and 31/50) (Chhabra et al., 1998; NTP, 1998). [The Working Group noted this was a well-conducted GLP study and that both sexes were used.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Absorption, distribution, and excretion

(a) Humans

Tetrahydrofuran is extensively absorbed following inhalation ([Ong et al., 1991](#)); it rapidly appears in the blood, demonstrating rapid systemic absorption from the lungs of exposed workers. Although dermal uptake does occur, the degree of absorption through the skin was negligible compared with inhalation ([Brooke et al., 1998](#)).

The half-life of tetrahydrofuran in humans is estimated to be approximately 30 minutes. Analysis of hepatic blood flow and clearance values suggests that tetrahydrofuran is extensively metabolized in human liver during first-pass metabolism ([Fowles et al., 2013](#)).

Several studies of workers exposed to tetrahydrofuran by inhalation were summarized by [Droz et al. \(1999\)](#) and were used to develop a physiologically based pharmacokinetic model for the biomonitoring of exposed workers.

(b) Experimental systems

Chronic exposure of rats to tetrahydrofuran vapour (at 200, 1000, or 2000 ppm) initially resulted in a dose-dependent increase in tetrahydrofuran content in brain and perirenal fat; exposure for up to 18 weeks showed a decrease with time in tetrahydrofuran content in the body, consistent with rapid metabolism and the low potential for bioaccumulation ([Elovaara et al., 1984](#)). In a study in which rats and mice were exposed to [¹⁴C]-labelled tetrahydrofuran by gavage and monitored for up to 168 hours after dosing ([Fowles et al., 2013](#)), rapid absorption and metabolism of tetrahydrofuran, with

the majority recovered as carbon dioxide, were observed in rats. Generally similar kinetics were observed for mice; the main difference between observed results for rats and mice was that the maximal plasma concentration in rats of both sexes was achieved at about 4 hours, whereas that in male and female mice was achieved at 0.8 and 1 hour, respectively, consistent with faster overall pharmacokinetics in mice compared with rats.

Tissue distribution of tetrahydrofuran after oral dosing was analysed in male and female Fischer 344 rats or B6C3F₁ mice given [¹⁴C]-labelled tetrahydrofuran by single gavage at target concentrations of 50 or 500 mg/kg body weight (bw) ([DuPont Haskell Laboratory, 1998](#)). The liver exhibited the highest concentrations of radioactivity, followed by fat and adrenal glands. Relatively high amounts of tetrahydrofuran were detected in the spleen, suggesting distribution through the lymphatic circulation. Differences in distribution between the sexes were not evident.

4.1.2 Metabolism

Few data were available on the metabolism of tetrahydrofuran in human or other mammalian systems. The initial step is oxidative metabolism by cytochrome P450 (CYP) enzymes with further hydrolysis by paraoxonase 1 and further action of cytoplasmic dehydrogenases. The specific CYPs have not been clearly identified. One reaction results in hydroxylation of the ring structure, whereas the other results in ring-opening to form a hydroxylated butanal. A major metabolite that is detected is gamma-hydroxybutyrate (GHB; 4-hydroxybutyrate), a neurotoxicant that can arise either from 4-hydroxyl-butanal or from γ -butyrolactone (GBL; 4-butyrolactone) ([ECHA, 2010](#)). GHB is oxidized to succinic semialdehyde, which is then converted to succinate and processed through the citric acid cycle to yield carbon dioxide ([Fowles et al., 2013](#)).

Both GHB and carbon dioxide have been recovered ([Fowles et al., 2013](#)), consistent with

the pathway. In a tetrahydrofuran poisoning case ([Cartigny et al., 2001](#); [Imbenotte et al., 2003](#)), analysis of urine and serum samples by ¹H-nuclear magnetic resonance spectroscopy showed very high concentrations of GHB in urine and serum. Urinary and serum concentrations of tetrahydrofuran were 850 and 813 mg/L (11.8 and 11.3 mM), respectively, and those of GHB were 2977 and 239 mg/L (28.6 and 2.3 mM), respectively.

4.1.3 Modulation of metabolic enzymes

As reviewed by [Moody \(1991\)](#), the effects of tetrahydrofuran on mixed-function oxidation were observed to range from increased, decreased, or no change in activities or processes in studies in vivo. Male Sprague-Dawley rats were exposed to tetrahydrofuran for 16 hours, resulting in the induction of activities dependent upon CYP (CYP2E1). Total CYP content and ethoxycoumarin deethylase (ECOD) activity (marker for CYP1A1) increased in liver microsomes isolated from male Wistar rats exposed by inhalation to tetrahydrofuran for 18 weeks ([Moody, 1991](#)). Increased total CYP content and activities of ethoxyresorufin-*O*-deethylase and pentoxyresorufin-*O*-deethylase, suggesting induction of CYP1A1 and CYP2B1, respectively, was reported in the liver of female B6C3F₁ mice 5 days after exposure by inhalation to tetrahydrofuran at 5400 mg/m³ (1800 ppm) ([Gamer et al., 2002](#)). Tetrahydrofuran both stimulates and inhibits other enzyme systems in rats, including inhibition of hepatic alcohol and formaldehyde dehydrogenase activities ([Elovaara et al., 1984](#)), and also both stimulates and inhibits rat and rabbit phosphorylase activity ([Moody, 1991](#)).

In female B6C3F₁ mice, exposure to a high concentration of tetrahydrofuran (15 000 mg/m³) by inhalation markedly induced hepatic microsomal enzymes ([van Ravenzwaay et al., 2003](#)). [Choi et al. \(2017\)](#) reported a 1.6-fold increase in total CYP content and a 1.4–1.7-fold increase in

mRNA expression of *Cyp1a1/1a2* and *Cyp2b10* in wildtype [C57BL/6] female mice exposed orally to tetrahydrofuran at 1500 mg/kg bw. The oral exposure of constitutive androstane receptor/pregnane X receptor (*Car/Pxr*) knockout female mice to tetrahydrofuran at 1500 mg/kg bw had no effect on CYP expression.

In vitro studies, as reviewed by [Moody \(1991\)](#), showed varying degrees of inhibition, primarily in rat but also in pig liver microsomes. In particular, tetrahydrofuran inhibited benzo[*a*]pyrene metabolism in liver microsomes from phenobarbital-induced rats, and markedly inhibited glutathione *S*-transferase activity in rat liver cytosol with benzo[*a*]pyrene, styrene, or 1,2-dichloro-4-nitrobenzene as substrates. Liver microsomes from female rats were notably more sensitive to the inhibition of ECOD by tetrahydrofuran than those from male rats.

4.2 Mechanisms of carcinogenesis

4.2.1 Genetic and related effects

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

No change was seen in micronucleated polychromatic and normochromatic erythrocytes in female B6C3F₁ mice and in polychromatic erythrocytes in male mice exposed to tetrahydrofuran by inhalation at up to 5000 ppm for 14 weeks. However, an increase in the frequency of micronucleated normochromatic erythrocytes was observed in male mice at the end of the 14-week exposure period: a significant increase ($P = 0.004$) was observed in male mice exposed at 1800 ppm relative to the control group, but the trend test was not significant ($P = 0.074$) ([NTP, 1998](#)).

Although induction of sister-chromatid exchange (SCE) was seen in the bone marrow of male B6C3F₁ mice 23 hours (but not 42 hours) after exposure to tetrahydrofuran by intra-peritoneal injection at 2000 mg/kg bw, this was not reproduced in a second trial at doses up to 2500 mg/kg bw (lethal dose) (NTP, 1998). No increase in chromosomal aberrations was induced in the bone marrow of male B6C3F₁ mice by exposure to tetrahydrofuran by intra-peritoneal injection in phosphate-buffered saline or corn oil [the Working Group noted that the vehicle was not clearly reported] at up to 2000 mg/kg bw (NTP, 1998).

(ii) *Non-human mammalian cells in vitro*

[The Working Group noted that, due to its volatility, the concentration of tetrahydrofuran in the cell culture medium may decline rapidly in the absence of specific controls for volatility. Tetrahydrofuran concentrations in the cell culture medium at different time points from the beginning of the incubations were not assessed in the in vitro studies cited below.]

Tetrahydrofuran (at up to 5000 µg/mL) was negative in the assays for SCE and chromosomal aberrations in Chinese hamster ovary cells (Galloway et al., 1987; NTP, 1998). No increase in micronuclei was seen in metabolically active Syrian hamster embryo cells exposed to tetrahydrofuran (at 3000, 3500, and 4000 µg/mL) for 24 hours (Gibson et al., 1997).

(iii) *Non-mammalian systems*

Assays for the mutagenicity of tetrahydrofuran (oral exposure at 125 000 ppm in feed or exposure by injections at 40 000 ppm) were negative in *Drosophila melanogaster* (Valencia et al., 1985; NTP, 1998) and in different strains of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) (Mortelmans et al., 1986; NTP, 1998).

Tetrahydrofuran is a solvent prone to oxidation to peroxides, butyric acid, butyraldehyde,

and related compounds, mainly on ageing and in the presence of light, heat, and moisture (Coetzee & Chang, 1985). Tetrahydrofuran containing 2-hydroxy-tetrahydrofuran as a result of its natural oxidation reacted with DNA bases, giving four adducts as products of the reaction of 4-hydroxy-butanal with the DNA bases (Hermida et al., 2006). The adducts were also detected in calf thymus DNA samples after in vitro reactions with oxidized tetrahydrofuran. Rat liver microsomes oxidized tetrahydrofuran to the reactive 4-hydroxy-butanal, assessed by the formation of the dGuo-THF 1 adduct (Hermida et al., 2006).

4.2.2 Altered cell proliferation or death

(a) *Humans*

No data in exposed humans were available to the Working Group.

In one study using human embryonic lung fibroblasts, tetrahydrofuran (at 0.2% for 24 hours, used to dissolve β-carotene) reduced the percentage of cells in S-phase (Stivala et al., 1996).

(b) *Experimental systems*

Marginal increased incidences of hyperplasia of the bone marrow in male B6C3F₁ mice during the 2-year inhalation study of tetrahydrofuran were reported by NTP (1998).

In the NTP 13-week study (Chhabra et al., 1990; NTP, 1998), inhalation exposure to tetrahydrofuran increased liver weight in male (at 600, 1800, and 5000 ppm) and female (at 1800 and 5000 ppm) B6C3F₁ mice, and in female F344 rats (at 5000 ppm). Minimal to mild hepatic centrilobular hypertrophy occurred in male and female mice (at 5000 ppm).

Absolute and relative liver weights increased in female mice exposed to tetrahydrofuran at 1800 ppm (5400 mg/m³) for 20 days (Gamer et al., 2002). Cell proliferation, assessed by the number of cells in S-phase, increased mainly in the subcapsular region of the renal cortex in male

rats exposed to tetrahydrofuran at 1800 ppm for 5 days and at 600 and 1800 ppm for 20 days. The number of apoptotic cells increased in the renal cortex of male rats exposed to 1800 ppm for 20 days and also for 5 days (the latter evaluated 21 recovery days later).

Cell proliferation in liver was increased in female B6C3F₁ mice exposed to tetrahydrofuran by inhalation at 5000 ppm for 6 hours per day, for 5 days ([van Ravenzwaay et al., 2003](#)).

In B6C3F₁ and C57BL/6 female mice given tetrahydrofuran by oral gavage at 300, 1000, or 1500 mg/kg bw per day for 7 days, hepatocellular proliferation was observed, assessed by 5-bromo-2'-deoxyuridine staining. The same effect was not observed in constitutive androstane receptor/pregnane X receptor (*Car/Pxr*) double-knockout C57BL/6 mice that received tetrahydrofuran by oral gavage at 1500 mg/kg bw per day, for 7 days ([Choi et al., 2017](#)).

In a study in vitro, tetrahydrofuran at 30–100 µL per 5 mL culture medium inhibited metabolic cooperation, an indication of gap-junctional intercellular communication inhibition, in Chinese hamster V79 lung fibroblast cells ([Chen et al., 1984](#)).

4.2.3 Other mechanisms

Tetrahydrofuran was negative when tested for induction of cell transformation in cultured Syrian hamster embryo cells ([Kerckaert et al., 1996](#)) and mouse fibroblast BALB/c-3T3 cells ([Matthews et al., 1993](#)).

4.3 Data relevant to comparisons across agents and end-points

For the results of high-throughput screening assays of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA ([Kavlock et al., 2012](#); [Tice et al., 2013](#); [EPA, 2016a, b](#); [Filer et al., 2017](#)), see Section 4.3 of the

Monograph on 1-*tert*-butoxypropan-2-ol in the present volume.

4.4 Susceptibility to cancer

No data were available to the Working Group.

4.5 Other adverse effects

4.5.1 Humans

The only available data came from case reports of occupational exposure to a glue containing tetrahydrofuran (8 hours per day, for 3 days) during plastic pipe repair in a confined space and without the use of any protective device; reported effects were consistent with liver toxicity ([Garnier et al., 1989](#)).

4.5.2 Experimental systems

In the NTP 2-year inhalation study ([NTP, 1998](#)), effects observed in male B6C3F₁ mice exposed to tetrahydrofuran at 1800 ppm were hyperplasia of the bone marrow and iliac lymph nodes, haematopoietic cell proliferation of the spleen, and thymic atrophy, considered to be consequences of the inflammation of the urinary and urogenital tracts. Liver necrosis was increased in female mice exposed to tetrahydrofuran at 1800 ppm ([NTP, 1998](#)). In the NTP 13-week study ([Chhabra et al., 1990](#); [NTP, 1998](#)), serum bile acids in female F344 rats were increased. Mild degeneration of the X-zone of the innermost cortex of the adrenal glands and uterine atrophy were observed in the female B6C3F₁ mice exposed to tetrahydrofuran at 5000 ppm. Tetrahydrofuran also induced narcosis in mice and ataxia in rats.

A concentration-dependent accumulation of α_{2u} -globulin, assessed by immunohistochemistry, was observed in the renal cortex of male F344 rats exposed to tetrahydrofuran for 5 and 20 days, with no regression in the animals that

were killed 21 recovery days after 5 days of exposure. However, renal cortex α_{2u} -globulin accumulation in the animals that were killed 21 recovery days after 5 days of exposure (concentrations were close to or higher than those observed after 20 days of exposure to tetrahydrofuran) was not accompanied by increased cell proliferation ([Gamer et al., 2002](#)).

IARC established seven criteria for the induction of kidney tumours to have occurred by an α_{2u} -globulin-associated response ([IARC, 1999](#)). The criterion that was met was the identification of the accumulating protein as α_{2u} -globulin (by immunohistochemical staining, in the short-term studies of [Gamer et al., 2002](#)). However, six criteria were not met, specifically: (i) lack of genotoxic activity of the agent and/or metabolite (tetrahydrofuran containing 2-hydroxy-tetrahydrofuran was reactive towards DNA bases in vitro, giving different DNA adducts; see Section 4.2.1); (ii) reversible binding of the chemical or metabolite to α_{2u} -globulin (no data are available); (iii) induction of sustained increases in cell proliferation in the renal cortex (no demonstration of sustained cell proliferation); (iv) induction of the characteristic sequence of histopathological changes associated with α_{2u} -globulin accumulation (the histopathological changes were not detected); (v) male rat specificity for nephropathy and renal tumorigenicity (there was no increase in the incidence or severity of nephropathy in exposed male rats; [NTP, 1998](#)); and (vi) similarities in dose–response relationships of the tumour outcome with histopathological end-points associated with α_{2u} -globulin nephropathy (no evidence of histopathological end-points associated with α_{2u} -globulin nephropathy in chronic and subchronic studies; [NTP, 1998](#)).

5. Summary of Data Reported

5.1 Exposure data

Tetrahydrofuran is a solvent that is used in a variety of plastics, dyes, elastomers, and glues for joining plastic components. It is also used in the synthesis of motor fuels, and in the manufacture of pharmaceuticals, synthetic perfumes, organometallic compounds, and insecticides. Global consumption is about 650 000 tonnes per year. The general population may be exposed by using household products containing tetrahydrofuran; however, reliable information on the exposure of the general population is unavailable. Workers may be exposed by inhalation and skin contact, particularly in professional use of glues, paints, and other similar products containing tetrahydrofuran.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Tetrahydrofuran was tested by whole-body inhalation in one well-conducted good laboratory practice (GLP) study in male and female mice, and in one well-conducted GLP study in male and female rats.

Tetrahydrofuran caused a significant increase in the incidence of, and a positive trend in the incidence of, hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) in female mice. In male rats, tetrahydrofuran caused a significant positive trend in the incidence of renal tubule adenoma or carcinoma (combined). In female rats, tetrahydrofuran caused a significant positive trend in the incidence of fibroadenoma of the mammary gland. There was no

significant increase in the incidence of any tumours in male mice.

5.4 Mechanistic and other relevant data

In humans and experimental animals, tetrahydrofuran is extensively absorbed. Tetrahydrofuran is oxidized by cytochrome P450 (CYP) enzymes with further hydrolysis by para-oxonase 1. Major metabolites include 4-butyrolactone and 4-hydroxybutyrate, which is neurotoxic. Tetrahydrofuran inhibits or induces multiple CYPs. Tetrahydrofuran induces CYPs through activation of constitutive androstane receptor.

Regarding the key characteristics of carcinogens, the evidence is *weak* that tetrahydrofuran is genotoxic. No data are available in humans. Tetrahydrofuran did not induce chromosome damage in mice or in mammalian cells. It also gave negative results in tests in *Drosophila* and in different strains of *Salmonella typhimurium*. However, oxidized metabolites of tetrahydrofuran react with DNA in vitro, yielding DNA adducts; one such adduct was formed in vitro from tetrahydrofuran when activated by rat liver microsomes.

The evidence is *moderate* that tetrahydrofuran alters cell proliferation. Cell proliferation was increased in several studies in female mouse liver and in one study in male rat kidney. One study reported inhibition of gap-junctional intercellular communication in vitro. No induction of cell transformation was reported in vitro.

In the carcinogenicity bioassay, toxic effects were not seen in the kidney of male rats. Only one of the seven criteria established by IARC for the induction of kidney tumours to have occurred by an α_{2u} -globulin-associated response was met.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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VINYLDENE CHLORIDE

1. Exposure Data

Data were previously reviewed by the Working Group in 1985 ([IARC, 1986](#)) and vinylidene chloride was classified in *Monographs Supplement 7* as *not classifiable as to its carcinogenicity to humans* (Group 3) ([IARC, 1987](#)). This substance was further considered by the Working Group in 1998 ([IARC, 1999](#)). New data have become available since that time, and these have been incorporated and taken into consideration in the present evaluation.

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 75-35-4

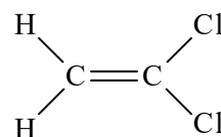
EC/List No.: 200-864-0

Chem. Abstr. Serv. name: 1,1-Dichloroethene

IUPAC systematic name: 1,1-Dichloroethene

Synonyms: Vinylidene chloride; 1,1-dichloroethylene; 1,1-DCE; vinylidene dichloride; ethene, 1,1-dichloro-

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: C₂H₂Cl₂

Relative molecular mass: 96.94

1.1.3 Chemical and physical properties

Description: Volatile, colourless, oily liquid with sweet, chloroform-like odour ([Budavari, 1996](#); [IPCS, 2014](#); [NIOSH, 2014](#))

Melting point: -122.5 °C ([Lide, 1995](#))

Boiling point: 31.6 °C ([Lide, 1995](#))

Relative density: 1.2 (water = 1) ([IPCS, 2014](#))

Solubility: Insoluble in water (2.5 g/L) ([IPCS, 2014](#)); soluble in acetone, ethanol, and many organic solvents; very soluble in diethyl ether ([IARC, 1999](#); [WHO, 2003](#))

Volatility: Vapour pressure: 67 kPa at 20 °C ([WHO, 2003](#))

Relative vapour density: 3.3 (air = 1) ([IPCS, 2014](#)); relative density of the vapour/air mixture at 20 °C (air = 1): 3.25 ([Verschueren, 1996](#))

Octanol/water partition coefficient (P): log K_{ow}, 1.32 ([WHO, 2003](#))

Flash point: –19 °C (closed cup); –15 °C (open cup) ([IARC, 1999](#))

Explosive limits: Lower limit, 5.6%; upper limit, 16% ([IPCS, 2014](#))

Auto-ignition temperature: 530 °C ([IPCS, 2014](#))

Stability: In the absence of an added inhibitor, monomethyl ether of hydroquinone (up to 200 ppm), vinylidene chloride readily polymerizes; in the presence of air or oxygen, shock-sensitive and explosive peroxides are formed ([NTP, 2015](#))

Odour threshold: 190 ppm in air ([Amoore & Hautala, 1983](#))

Henry constant: 23.2 kPa·m³/mol at 20 °C ([WHO, 2003](#))

Conversion factor: 1 ppm = 3.96 mg/m³ ([IARC, 1999](#))

1.2 Production and use

1.2.1 Production process

Vinylidene chloride is produced in a closed system using a stainless steel reactor. It is almost exclusively produced from 1,1,2-trichloroethane that is made from 1,2-dichloroethane or vinyl chloride. 1,1,2-Trichloroethane is converted to vinylidene chloride by dehydrochlorination, which is carried out by one of two different routes: liquid-phase or gas-phase reaction. Vinylidene chloride can be obtained from thermal cracking of 1,1,1-trichloroethane. Other production methods use vinyl chloride oxychlorination or tetrachloroethane dehydrochlorination and the high-temperature reaction of methane with chlorinating agents. Patents exist for the catalytic hydrogenation of 1,1,1,2-tetrachloroethane and the reaction of ethane with hexachloroethane to produce a mixture including vinylidene chloride ([Dow Chemical, 2002](#); [Dreher et al., 2014](#)).

1.2.2 Production volume

Vinylidene chloride is classified by the Organisation for Economic Co-operation and Development (OECD) as a high production volume substance, that is, it is produced or imported at levels greater than 1000 tonnes per year in at least one OECD member country and/or region ([OECD, 2017](#)).

Non-confidential estimates of production capacity for vinylidene chloride are difficult to obtain due to the limited number of global producers and the fact that there is only one producer in the USA. In the early 1980s, estimated annual production of vinylidene chloride was over 91 700 tonnes in the USA ([Grayson, 1983](#)) and 306 000 tonnes globally ([IPCS, 1990](#)). Current and future demand have been impacted by the phasing-out of hydrochlorofluoro-carbon (HCFC)-141b [1,1-dichloro-1-fluoroethane] (banned in the European Union in 2002 and in the USA in 2003 according to the Montreal Protocol; [UNEP, 1989](#)), which used significant quantities of vinylidene chloride as a precursor in one of the commercial routes of manufacture ([Dow Chemical, 2002](#)).

In 2012, the global production capacity of vinylidene chloride was estimated at 502 000 tonnes and the global demand was estimated at 354 000 tonnes ([Dreher et al., 2014](#)).

1.2.3 Use

Globally, vinylidene chloride is used primarily as an intermediate in the manufacture of 1,1,1-trichloroethane, polyvinylidene chloride polymers, copolymers, and terpolymers (latex and resin), which may in turn be used in a variety of end products such as food plastic wrap, carpet latex backing, fire- and ignition-resistant clothing, vapour barriers for insulation, steel pipe coating, outdoor furniture, paper and board coatings, adhesives, and photographic film. Vinylidene chloride may persist as an unintended

manufacturing residue in some of these items. It may also be used in the production of chloroacetyl chloride and of latex and resins, as an aid in ore flotation, as a solvent in paint and varnish remover, and as a vapour degreaser and industrial cleaning agent (EPA, 2000; WHO, 2003; Health Canada, 2013; Dreher et al., 2014). Vinylidene chloride is also used in the production of certain HCFCs (except HCFC-141b; see Section 1.2.2).

1.3 Analytical methods

Because of its volatility, vinylidene chloride is well suited to determination by gas chromatography using a variety of detectors, including flame ionization, electron capture, electrolytic conductivity detection, and mass spectrometry. Methods are available for quantifying vinylidene chloride in environmental samples (air, water, soil, and sediment) and in biological samples (breath, food, and body tissues) (see Table 1.1) (WHO, 2003).

1.4 Occurrence and exposure

Vinylidene chloride does not occur naturally (IARC, 1986; WHO, 2003; Health Canada, 2013; NTP, 2015). It can be found in the environment from release during its manufacture and use, the breakdown of polyvinylidene chloride products, and the biotic or abiotic breakdown of 1,1,1-trichloroethane, tetrachloroethene, 1,1,2-trichloroethene, and 1,1-dichloroethane. It is frequently found at hazardous waste sites (WHO, 2003), and also appears as an impurity in trichloroethylene (classified as *carcinogenic to humans*, Group 1; IARC, 2013).

1.4.1 Environmental occurrence

(a) Water

Environmental levels of vinylidene chloride in water are very low. In raw industrial wastewater in the USA, mean concentrations ranged from 18 to 760 µg/L; a concentration of 32 µg/L was reported from the Netherlands. Vinylidene chloride concentrations of 0.3–80 µg/L have been measured from the River Rhine. The level in untreated drinking-water is generally not detectable. Median concentrations of vinylidene chloride were 0.28–1.2 µg/L in treated potable water taken from groundwater sources; in public drinking-water supplies, concentrations were generally less than 1 µg/L, although levels of up to 20 µg/L have been detected (IPCS, 1990; Kubota & Tsuchiya, 2010).

A national assessment in 2006 by the United States Geological Survey (USGS) reported the detection of vinylidene chloride in 19 (of 1207) domestic well samples (ATSDR, 2009).

An earlier USGS assessment of untreated, ambient groundwater wells between 1985 and 1995 identified vinylidene chloride in 3% of wells in urban areas ($n = 406$) and in 0.3% of wells in rural areas ($n = 2542$). Areas of known point-source contamination were excluded from the assessment (Squillace et al., 1999).

In an analysis of 70 private residential wells in 21 counties in South Carolina, USA, from August 2000 to February 2001, vinylidene chloride was detected in 2 wells (Aelion & Conte, 2004).

Vinylidene chloride was not detected in any of 14 Canadian surveys of drinking-water among different cities between 2003 and 2008 (Health Canada, 2013).

(b) Air

Up to approximately 5% of manufactured vinylidene chloride (a maximum of ~23 000 tonnes) is emitted into the atmosphere annually. The high vapour pressure and low water solubility of

Table 1.1 Analytical methods for the analysis of vinylidene chloride

Media	Method	Remarks	Reference
Ambient air	EPA Reference Method 23	Analysis by GC-FID: column temperature, 100 °C; flow rate, 20 mL/min; working range, 0.4–800 mg/m ³ The method has not been validated by the EPA for vinylidene chloride, but a similar analytical procedure has been used to measure occupational exposures	EPA (1985b)
Workplace air	NIOSH Analytical Method 1015	Analysis by GC: for vinylidene chloride, a 100 (front) + 50 (back) mg charcoal tube should be used; desorption solvent, CS ₂ General method for volatile organic compounds	Health and Safety Executive (2000)
Workplace air	OSHA Organic Method 19	Analysis by GC-FID: detection limit and limit of reliable quantification, 0.2 mg/m ³ (0.05 ppm) based on a 3-L sample at 0.2 L/min; target concentration, 1 ppm (4.0 mg/m ³); analytical solvent, CS ₂ Fully validated specific method; collection on charcoal tubes	OSHA (1980)
Workplace air	NIOSH Analytical Method 1015, issue 2	Analysis by GC/FID: limit of detection, 7 µg; working range, 0.5–5 ppm (2–20 mg/m ³) for a 5-L air sample using a charcoal tube with a flow rate of 0.01–0.2 L/min Validated for concentrations of 7–10 mg/m ³ in air; the capacity of charcoal for vinylidene chloride decreases rapidly with increasing relative humidity, and was also found to be a function of concentration; a capillary column such as 105 m Rtx® 502.2 is required	NIOSH (1994)
Water	EPA Method 524.2	Analysis by GC-MS: detection limit, 0.05 µg/L General-purpose method for purgeable volatile organic compounds in surface water, groundwater, and drinking-water in any stage of treatment; cryogenic trapping, narrow-bore capillary column	EPA (1995)
Water	–	Detection by purge and gas trap chromatography followed by FID or MS; minimum practical quantitation limit, 5 µg/L Based on similarity to seven other volatile organics	Otson et al. (1982) , Health Canada (2015)
Soil/sediment	–	Extraction with an organic solvent or purging with an inert gas, trapping, and GC-MS; sample size, 1–3 g; detection limit, 10 µg/g (DeLeon et al., 1980) or 0.40 µg/g (Amaral et al., 1994)	DeLeon et al. (1980) , Amaral et al. (1994) , WHO (2003)
Films, food, body tissues	–	Detection limit in the 5–10 µg/kg range using a headspace technique, purge and trap, and GC-ECD or GC-MS; vacuum distillation and GC-MS using fused-silica capillary column for fish tissue Quantification limit: 1 µg/m ² in PVC-containing films, 20 µg/m ³ in foodstuffs, at least 0.14 µg/g of tissue (wet weight)	Gilbert et al. (1980) , Easley et al. (1981) , Lin et al. (1982) , Hiatt, 1983 , WHO (2003)

CS₂, carbon disulfide; ECD, electrolytic conductivity detection; EPA, United States Environmental Protection Agency; FID, flame ionization detection; GC, gas chromatography; MS, mass spectrometry; NIOSH, National Institute for Occupational Safety and Health; ppm, parts per million; PVC, polyvinyl chloride

vinylidene chloride favour its relatively higher concentration in the atmosphere compared with that in other environmental compartments. Vinylidene chloride in the atmosphere is expected to have a half-life of approximately 2 days. The measured half-life of 80 mg/m³ vinylidene chloride in sealed quartz flasks exposed outdoors was 56 days (IPCS, 1990). [The relevance of this result to the environmental persistence of vinylidene chloride is difficult to interpret.]

Singh et al. (1981) and Brodzinski & Singh (1983) reported data on vinylidene chloride in ambient air in 30 locations in the USA, and Guicherit & Schulting (1985) measured vinylidene chloride in ambient air at three sites in the Netherlands. According to the World Health Organization (WHO), the three datasets are consistent, indicating typical mean concentrations of 20–120 µg/m³ and maximum concentrations of 40–560 µg/m³ (WHO, 2003). Measurements taken in industrial areas near sources of vinylidene chloride yielded a much higher mean value of 120×10^3 µg/m³ and a maximum of 270×10^3 µg/m³. The median concentration of vinylidene chloride in different areas in the USA were reported as: approximately 0.02 µg/m³ in urban and/or suburban ambient air; about 8.7 µg/m³ in industrial areas; and about 90–100 µg/m³ and 25–50 µg/m³ in the atmosphere around vinylidene chloride monomer and polymer manufacturing plants, respectively (EPA, 1985a). Ambient air quality data were compiled from 1982 to 2001 for vinylidene chloride at 87 individual locations throughout the USA; the range in the arithmetic mean values for these locations was 0.004–4 µg/m³ (WHO, 2003).

Health Canada (2013) provided detailed summaries of concentrations of vinylidene chloride in ambient and indoor air from studies carried out during 1986–2008, mainly from Canada and the USA. It was suggested that the highest median concentration in Canada, measured at 0.076 µg/m³ in both outdoor and indoor air, be used to derive estimates of

environmental intake. Mean indoor concentrations in three out of four studies in the USA in which vinylidene chloride was quantifiable were reported as 12.06 µg/m³, 1.81 µg/m³, and 5.02 µg/m³.

(c) Food

Vinylidene chloride may migrate into foods when plastic food-contact materials containing this monomer are used (HSDB, 2009). Concentrations of vinylidene chloride in food are usually not detectable; in analyses of various food products in Japan and the United Kingdom, a maximum concentration of 20 µg/kg was observed (Gilbert et al., 1980; IPCS, 1990; Ohno & Kawamura, 2006). Vinylidene chloride was not detected in any of the 4 samples of 34 food composites analysed in Canada in several studies in the early 1990s (Health Canada, 2013).

1.4.2 Exposure in the general population

The general population may be exposed by environmental contamination of air or drinking-water, or eating contaminated food. The main exposure is from indoor air (Health Canada, 2013).

Based on personal air sampling in the general population in urban areas, the estimated mean vinylidene chloride exposure was 6.5 µg/m³ (Wallace, 1991). Levels of vinylidene chloride in the breath of residents in North Carolina and New Jersey, USA, ranged from undetectable to 26 µg/m³ (Wallace et al., 1982, 1984). In the early 1980s, vinylidene chloride was found in 3% of approximately 347 overnight personal air samples and in 6% of approximately 340 daytime personal air samples in Bayonne and Elizabeth, New Jersey, USA (Wallace et al., 1986a), and 12% of approximately 300 breath samples from residents of the same cities contained quantifiable levels (0.2–2.0 µg/m³) of vinylidene chloride (Wallace et al., 1986b).

The National Health and Nutrition Examination Survey 2003–2004 conducted by the National Center for Health Statistics in the USA did not detect vinylidene chloride in any of 1367 samples of human blood from adults aged 20–59 years (detection limit, 0.009 ng/mL) (NCHS, 2009). Vinylidene chloride was qualitatively detected in 1 of 12 breast milk samples acquired from four cities in the USA [detection limit unspecified] (Pellizzari et al., 1982). In a related study, vinylidene chloride was qualitatively detected in one of eight samples of breast milk acquired from four cities in the USA [detection limit unspecified] (EPA, 1980).

It has been estimated that the maximum possible intake of vinylidene chloride from food as a result of the use of packaging materials is no more than 1 µg per person per day in the United Kingdom (MAFF, 1980).

Consumers may be exposed via migration of vinylidene chloride from the films and coatings of packaging materials into foods in contact with the packaging (NTP, 2015). However, food sources are expected to be negligible (WHO, 2003). [No reliable data were available to the Working Group to estimate the exposure from food.]

Environment Canada and Health Canada provided a detailed upper-bounding deterministic estimate of vinylidene chloride intake from ambient air, indoor air, drinking-water, food and beverages, and soil based on various studies measuring the occurrence in these matrices. The total intake from all routes of exposure according to age group was 7.67×10^{-2} µg/kg body weight (bw) per day for formula-fed infants, 1.340 µg/kg bw per day for infants not fed formula, 0.911 µg/kg bw per day for those aged 0.5–4 years, 0.591 µg/kg bw per day for those aged 5–11 years, 0.344 µg/kg bw per day for those aged 12–19 years, 0.260 µg/kg bw per day for those aged 20–59 years, and 0.214 µg/kg bw per day for those aged 60 years and older (Health Canada, 2013).

Based on air measurements in the homes of smokers and non-smokers (8-hour average exposures) in Hong Kong Special Administrative Region, Guo et al. (2004) provided exposure estimates of 38.3 ng/kg bw per day and 4.45 ng/kg bw per day, respectively.

Lee et al. (2002) estimated the exposure to vinylidene chloride for residents exposed to groundwater contaminated by a hazardous waste site. The exposure routes included inhalation during showering and dermal absorption during showering and other activities involving skin contact with water. The chronic intake was estimated as 2.64×10^{-2} mg/kg bw per day for inhalation and 3.74×10^{-4} mg/kg bw per day for dermal absorption.

1.4.3 Occupational exposure

No new data on occupational exposure were available to the Working Group. Industrial use of vinylidene chloride is currently within closed systems, minimizing potential occupational exposure. Significant occupational exposure is usually only by inhalation, although skin or eye contamination can also occur. Small numbers of chemical workers are exposed to vinylidene chloride. From the 1980s workers have been exposed to concentrations of less than 20 mg/m³, although exposure levels could have been as high as about 8000 mg/m³ until the 1970s.

Exposure to concentrations of about 80 000 mg/m³ can occur from spillage, and worker exposure before the 1970s may have exceeded 1200–4000 mg/m³ (Fishbein, 1981).

Ott et al. (1976) reported exposure data from a fibre production plant in the USA where vinylidene chloride was used with ethyl acrylate. Between 1955 and 1968, the measured time-weighted average (TWA) exposure levels for production-related jobs ranged from about 40 to 280 mg/m³, although individual measured exposures ranged from 0 up to about 7500 mg/m³. Thiess et al. (1979) reported average exposure

measurements of about 40 mg/m³ in a German plant manufacturing vinylidene chloride from 1975 onwards. In the associated polymerization plant, average quarterly concentrations of vinylidene chloride were between 2.6 and 6.7 mg/m³, with 220 peak exposures in excess of 120 mg/m³ during 1976.

The British Health and Safety Executive reported that, during the 1980s, occupational exposure to vinylidene chloride was limited to about 100 people in the country, and that 95% of exposure measurements were less than 20 mg/m³ (HSE, 1996). Fishbein (1981) confirmed that daily average vinylidene chloride exposures were most frequently measured at trace levels at that time.

1.5 Regulations and guidelines

In 1993, WHO established an international drinking-water quality guideline for vinylidene chloride of 30 µg/L (WHO, 2003). WHO did not set a formal guideline value for vinylidene chloride in its latest guidelines for drinking-water quality, however, because it occurs in drinking-water at concentrations well below those at which it is a concern to health (WHO, 2011).

The Committee of Experts on the Transport of Dangerous Goods and Globally Harmonized System of Classification and Labelling of Chemicals of the United Nations Economic Commission for Europe identified vinylidene chloride as: United Nations No. 1303, Hazard Class 3, United Nations Packing Group I (UNECE, 2017).

Commission Regulation (EU) No. 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food includes vinylidene chloride in the list of substances not authorized to be used as an additive or polymer production aid. It is authorized to be used as a monomer, or other starting substance or macromolecule obtained from microbial fermentation (European Commission, 2011).

In 2008 in the European Union, the Scientific Committee on Occupational Exposure Limits proposed an 8-hour TWA of 2 ppm [8 mg/m³] and a short-term exposure limit (15 minutes) of 5 ppm [20 mg/m³] without any notation (SCOEL, 2008).

The European Chemicals Agency hazard classification and labelling, approved by the European Union, warns that vinylidene chloride is an extremely flammable liquid and vapour, is harmful if inhaled, and is suspected of causing cancer. Additionally, vinylidene chloride causes damage to organs through prolonged or repeated exposure, is harmful if swallowed, causes serious eye irritation, and is harmful to aquatic life with long-lasting effects (ECHA, 2017).

The Institute for Occupational Safety and Health of the German Social Accident Insurance association has an information system on hazardous substances (GESTIS) and a database (GESTIS, 2017), with international limit values for different countries for vinylidene chloride as presented in Table 1.2.

In 2014, the French National Institute for Industrial Environment and Risks generated a series of environmental guideline concentration values for vinylidene chloride (INERIS, 2015), namely: fresh water used for the production of drinking-water, 3 µg/L (annual mean concentration); fresh water not used for the production of drinking-water, 8 µg/L (annual mean concentration); maximum concentration acceptable in fresh water, 91 µg/L; based on human health protection via fishing product consumption, 55 µg/kg biota; maximum concentration acceptable in seawater, 9.1 µg/L; and based on the protection of organisms of the aquatic environment, 7.8 µg/L (annual mean concentration).

In 2015, the Canadian Government established a maximum acceptable concentration for vinylidene chloride in drinking-water of 14 µg/L (Health Canada, 2015).

Table 1.2 Eight-hour and short-term limit values for vinylidene chloride in the workplace in different countries and regions

Country or region	8-hour limit value ^a		Short-term limit value ^a	
	ppm	mg/m ³	ppm	mg/m ³
Australia	5	20	20	79
Austria	2	8	8	32
Belgium	5	20	20	80
Canada (Ontario)	1	4	20	80
Canada (Quebec)	1	4		
Denmark	2	8	4	16
European Union	2	8	5 ^b	20 ^b
Finland	2	8	5 ^b	20 ^b
France	5	20		
Germany (AGS)	2	8	4 ^b	16 ^b
Germany (DFG)	2	8	4	16
Hungary		8		32
Ireland	5	20		
New Zealand	5	20	20	79
Poland		12.5		
Republic of Korea	5	20	20	80
Romania	5	20	20 ^b	80 ^b
Singapore	5	20	20	79
Spain	5	20		
Sweden	5	20	10 ^b	40 ^b
Switzerland	2	8	4	16
United Kingdom	10	40		

AGS, Ausschuff für Gefahrstoffe; DFG, Deutsche Forschungsgemeinschaft; ppm, parts per million

^a Range of values for an 8-hour period: 1–10 ppm (4–40 mg/m³); range of values for a short period (15 minutes): 4–20 ppm (16–80 mg/m³)

^b 15-minute average value

Source: [GESTIS \(2017\)](#)

In 1999, the California Environmental Protection Agency (CalEPA) established a non-cancer chronic reference exposure level of 0.07 mg/m³ for vinylidene chloride. The CalEPA reference exposure level is a concentration at or below which adverse health effects are not likely to occur ([CalEPA, 2000](#)).

In 1999, the American Conference of Governmental Industrial Hygienists recommended a TWA threshold limit value of 5 ppm for occupational exposures to vinylidene chloride in workplace air ([ACGIH, 1999](#)).

The United States Food and Drug Administration categorizes vinylidene chloride as

Class 1 in the list of solvents included in the Guidance for Industry table (Q3C). It should not be used in the manufacture of drug substances, excipients, and drug products, because of its unacceptable toxicity. If its use is unavoidable in the production of a drug product with a significant therapeutic property, the concentration limit is 8 ppm ([FDA, 2017](#)).

2. Cancer in Humans

[Waxweiler et al. \(1981\)](#) investigated an excess of lung cancer in a cohort of 4806 workers ever employed in a synthetic plastics plant in the USA. Workers were exposed to vinyl chloride, polyvinyl chloride (PVC) dust, vinylidene chloride, and several other chemicals. A statistically significant excess of mortality from lung cancer was observed among all workers at the plant with a standardized mortality ratio (SMR) of 149 ($P < 0.01$). Associations between cancer of the lung and estimates of exposure generated by plant personnel for 19 chemicals, including vinylidene chloride, were analysed using a serially additive expected dose model. A significant association between cancer of the lung and exposure to PVC dust, but not to vinylidene chloride, was observed.

Two other small cohort studies of workers were generally uninformative about the cancer risks from exposure to vinylidene chloride. [Ott et al. \(1976\)](#) studied 138 chemical workers in the USA who were exposed to vinylidene chloride in processes that did not involve vinyl chloride. A total of 5 deaths, of which the only death from cancer was due to cancer of the lung (0.3 deaths expected), were observed during 28 years of follow-up. A statistically significant SMR for cancer of the lung based on 5 deaths was reported in a study of 629 workers exposed to vinylidene chloride in Germany ([Thiess et al., 1979](#)). Two of the bronchial carcinoma cases were found in young men (both aged 37 years) who had been employed at the plant for short periods (14 months and 25 months). Workers at this plant were also exposed to vinyl chloride monomer and acrylonitrile. [The Working Group noted that neither of these smaller studies included adjustment for smoking or potential confounders related to occupation.]

3. Cancer in Experimental Animals

Vinylidene chloride was reviewed in *IARC Monographs* Volume 39 ([IARC, 1986](#)), Supplement 7 ([IARC, 1987](#)), and Volume 71 ([IARC, 1999](#)). The *IARC Monographs* Volume 71 Working Group concluded that there was *limited evidence* in experimental animals for the carcinogenicity of vinylidene chloride. This section provides an evaluation of the studies of carcinogenicity in animals reviewed in previous *Monographs* and the Supplement, and a review of any studies published since then.

See [Table 3.1](#).

3.1 Mouse

3.1.1 Inhalation

Groups of 36 male and 36 female CD-1 mice (age, 2 months) were exposed to vinylidene chloride (purity, 99%) at a concentration of 55 ppm in air for 6 hours per day, 5 days per week for up to 12 months, at which point the experiment was terminated. Four mice were killed at 1, 2, 3, 6, and 9 months from the start of the experiment. An increase [not significant] in the incidence of bronchioloalveolar adenoma (1/26 controls vs 6/35 exposed) and an increase [not significant] in the incidence of haemangiosarcoma of the liver (0/26 control vs 2/35 exposed) was observed in males. Three hepatomas [hepatocellular carcinomas] (two in males and one in females) and two skin keratoacanthomas [a benign tumour of the follicular epithelium] were also reported to occur in treated mice [sex unspecified] ([Lee et al., 1977, 1978](#)). [The Working Group concluded that this was an inadequate study for the evaluation because of the unsatisfactory study design, the use of only one dose, the limited reporting, and the short duration of exposure.]

Groups of 8–12 male and 8–12 female CD-1 mice (age, 2 months) were exposed to vinylidene chloride (purity, 99%) in air at 55 ppm for

Table 3.1 Studies of carcinogenicity with vinylidene chloride in rodents

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, Swiss (M) 16 wk Lifetime (up to 121 wk) Maltoni et al. (1984)	Inhalation (whole-body) Vinylidene chloride, 99.9% Air 0 (non-chamber controls), 10, 25 ppm, 4 h/d, 4–5 d/wk for 52 wk and observed for life 100, 30, 30 NR, NR, NR	<i>Kidney</i> , adenocarcinoma: 0/54, 0/24, 3/21 (14.3%)* <i>Lung</i> , pulmonary adenoma: 3/80 (3.7%), 11/28 (39.3%)*, 7/28 (25%)*	*[$P < 0.02$, Fisher exact test] * $P < 0.05$, Fisher exact test	Principal limitations: short duration of exposure; small number of animals in some of the exposure groups; lack of a concurrent chamber control Experiment BT402: initial experiment (using control group A)
Full carcinogenicity Mouse, Swiss (F) 16 wk Lifetime (up to 121 wk) Maltoni et al. (1984)	Inhalation (whole-body) Vinylidene chloride, 99.9% Air 0 (non-chamber controls), 10, 25 ppm, 4 h/d, 4–5 d/wk for 52 wk and observed for life 100, 30, 30 NR, NR, NR	<i>Mammary gland</i> , tumours (mainly carcinomas): 2/98 (2%), 6/30 (20%)*, 4/30 (13.3%)* <i>Lung</i> , pulmonary adenoma: 4/92 (4.3%), 3/30 (10%), 7/29 (24.1%)*	* $P < 0.05$, Fisher exact test * $P < 0.05$, Fisher exact test	Principal limitations: short duration of exposure; lack of a concurrent chamber control Experiment BT402: initial experiment (using control group A)
Full carcinogenicity Mouse, Swiss (M) 9 wk Lifetime (up to 121 wk) Maltoni et al. (1984)	Inhalation (whole-body) Vinylidene chloride, 99.9% Air 0 (non-chamber controls), 25 ppm, 4 h/d, 4–5 d/wk for 52 wk and observed for life 90, 120 NR, NR	<i>Kidney</i> , adenocarcinoma: 0/66, 25/98 (25.5%)* <i>Lung</i> , pulmonary adenoma: 3/74 (4%), 16/113 (14.2%)*	*[$P < 0.0001$, Fisher exact test] * $P < 0.05$, Fisher exact test	Principal limitations: short duration of exposure; lack of a concurrent chamber control Experiment BT402: additional control group (B) and additional group exposed at 25 ppm
Full carcinogenicity Mouse, Swiss (F) 9 wk Lifetime (up to 121 wk) Maltoni et al. (1984)	Inhalation (whole-body) Vinylidene chloride, > 99.9% Air 0 (non-chamber controls), 25 ppm, 4 h/d, 4–5 d/wk for 52 wk and observed for life 90, 120 NR, NR	<i>Lung</i> , pulmonary adenoma: 3/86 (3.5%), 11/118 (9.3%)* <i>Mammary gland</i> , tumours (mainly carcinomas): 1/89 (1.1%), 12/118 (10.2%)*	* $P < 0.05$, Fisher exact test * $P < 0.05$, Fisher exact test	Principal limitations: short duration of exposure; lack of a concurrent chamber control Experiment BT402: additional control group (B) and additional group exposed at 25 ppm

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F ₁ /N (M) 9 wk 104 wk NTP (1982)	Gavage Vinylidene chloride, 99% Corn oil 0, 2, 10 mg/kg bw, 1×/d, 5 d/ wk for 104 wk 50, 50, 50 33, 35, 36	All sites No significant increase in tumour incidence in treated animals	NS	Principal limitations: MTD does not appear to have been achieved
Full carcinogenicity Mouse, B6C3F ₁ /N (F) 9 wk 104 wk NTP (1982)	Gavage Vinylidene chloride, 99% Corn oil 0, 2, 10 mg/kg bw, 1×/d, 5 d/ wk for 104 wk 50, 50, 50 40, 32, 42	<i>Haematopoietic system [haematopoietic and lymphoid tissues]</i> Malignant lymphoma (all): 2/48 (4%), 9/49 (18%)*, 6/50 (12%) Lymphoma or leukaemia (all): 7/48 (15%), 15/49 (31%)*, 7/50 (14%)	* <i>P</i> = 0.028, Fisher exact test; <i>P</i> = 0.012, life table test * <i>P</i> = 0.050, Fisher exact test; <i>P</i> = 0.037, life table test	Principal limitations: MTD does not appear to have been achieved
Initiation– promotion (tested as initiator) Mouse, Ha:ICR Swiss (F) 6–8 wk 428–576 d Van Duuren et al. (1979)	Skin application Vinylidene chloride, NR Acetone Single application of vinylidene chloride (in 0.2 mL acetone) then 14 d later 5 µg TPA in 0.2 mL acetone 3×/wk for 428–576 d Five groups: no treatment (control); acetone only (vehicle control); 121.0 mg vinylidene chloride + TPA; TPA only (TPA-treated control); 20 µg DMBA + TPA (positive control) 100, 30, 30, 90, 30 NR, NR, NR, NR, NR	<i>Skin, papilloma:</i> 0/100, 0/30, 8/30 (27%)*, 6/90 (7%), 29/30 (97%)	* <i>P</i> < 0.005, χ^2 test vs TPA control	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk Lifetime Cotti et al. (1988)	Inhalation (whole-body) Vinylidene chloride, > 99.9% Air 0, 100 ppm, 4 h/d, 5 d/wk for 7 wk, then 7 h/d, 5 d/wk for 97 wk 60, 54 NR, NR	<i>Mammary gland</i> Malignant tumours: 2/60 (3.3%), 4/54 (7.4%) Benign and malignant tumours: 24/60 (40%), 29/54 (53.7%)	[NS] [NS]	Principal limitations: unsatisfactory study design (use of only one dose); unsatisfactory reporting (no detailed information on survival or description of observed tumours) Breeders of the Cotti et al. (1988) transplacental plus inhalation exposure experiment (see below)
Full carcinogenicity Rat, Sprague-Dawley (M) Embryo Lifetime Cotti et al. (1988)	Inhalation (whole-body) Vinylidene chloride, > 99.9% Air 0, 100 ppm transplacentally at d 12 of gestation, then 4 h/d, 5 d/wk for 7 wk then 7 h/d, 5 d/wk for 97 wk 158, 62 NR, NR	<i>Haematopoietic system</i> , leukaemia: 12/158 (7.6%), 10/62 (16.1%)	[NS]	Principal limitations: unsatisfactory study design (use of only one dose); unsatisfactory reporting (no detailed information on survival or description of observed tumours) Offspring of the Cotti et al. (1988) breeders (see above)
Full carcinogenicity Rat, Sprague-Dawley (F) Embryo Lifetime Cotti et al. (1988)	Inhalation (whole-body) Vinylidene chloride, > 99.9% Air 0, 100 ppm transplacentally at d 12 of gestation, then 4 h/d, 5 d/wk for 7 wk then 7 h/d, 5 d/wk for 97 wk 149, 61 NR, NR	<i>Haematopoietic system</i> , leukaemia: 1/149 (0.7%), 4/61 (6.5%)*	*[$P < 0.03$, Fisher exact test]	Principal limitations: unsatisfactory study design (use of only one dose); unsatisfactory reporting (no detailed information on survival or description of observed tumours) Offspring of the Cotti et al. (1988) breeders (see above)
Full carcinogenicity Rat, Sprague-Dawley (M) 6–7 wk 24 mo Quast et al. (1986)	Inhalation Vinylidene chloride, 99% Air 0, 25, 75 ppm for 6 h/d, 5 d/wk for 18 mo 86, 85, 86 13, 13, 8	All sites No significant increase in tumour incidence in treated animals	NS	Principal limitations: short duration of exposure (18 mo total) Exposure to 0, 10, 75 ppm the first month

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 6–7 wk 24 mo Quast et al. (1986)	Inhalation Vinylidene chloride, 99% Air 0, 25, 75 ppm, 6 h/d, 5 d/wk for 18 mo 84, 86, 84 19, 11, 16	<i>Mammary gland</i> , adenocarcinoma: 2/84, 7/86*, 4/84 (total no. tumours: 2, 8, 4)	* $P < 0.05$, Fisher exact test [NS; $P = 0.0898$, 1-tail Fisher exact test]	Principal limitations: short duration of exposure (18 mo total); poor survival in high-dose females Exposure to 0, 10, 40 ppm the first month
Full carcinogenicity Rat, F344/N (M) 5–6 wk 105 wk NTP (2015)	Inhalation (whole-body) Vinylidene chloride, > 99.9% Air 0, 25, 50, 100 ppm, for (6 h + T ₉₀ (10 min))/d, 5 d/wk for 105 wk 50, 50, 50, 50 25, 27, 22, 19	<i>Mesothelium</i> , malignant mesothelioma: 1/50 (2%)*, 12/50 (24%)**, 28/50 (56%)**, 23/50 (46%)** <i>Nose</i> , respiratory epithelium, adenoma: 0/49*, 0/50, 1/50 (2%), 4/50 (8%) <i>Kidney</i> , renal tubule carcinoma: 0/50, 2/50, 1/49, 1/50	* $P < 0.001$ (trend), poly-3 test; ** $P < 0.001$, poly-3 test * $P = 0.004$ (trend), poly-3 test NS	Principal strengths: well-conducted GLP study Historical incidence of nasal respiratory epithelium adenoma (mean ± standard deviation): inhalation studies, 0/198; all routes: 0/697 Historical incidence of renal tubule carcinoma: inhalation studies, 0/200; all routes, 1/697

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 9 wk 104 wk NTP (1982)	Gavage Vinylidene chloride, 99% Corn oil 0, 1, 5 mg/kg bw, 1×/d, 5 d/ wk for 104 wk 50, 50, 50 20, 24, 37	<i>Adrenal gland</i> , pheochromocytoma: 6/50 (12%)*, 5/48 (10%), 13/47 (28%)** <i>Pancreas</i> , islet cell adenoma or carcinoma (combined): 4/49 (8%)*, 1/47 (2%), 8/48 (17%) <i>Skin, subcutaneous</i> , fibroma: 0/50*, 1/48 (2%), 4/48 (8%)	* <i>P</i> = 0.010 (trend), Cochran- Armitage test, but NS by life table analysis; ** <i>P</i> = 0.045, Fisher exact test, but NS by life table analysis * <i>P</i> = 0.025 (trend), Cochran- Armitage test, but NS by life table analysis * <i>P</i> = 0.024 (trend), Cochran- Armitage test, but NS by life table analysis	Principal strengths: well-conducted study Principal limitations: MTD does not appear to have been achieved 12 controls and 10 low-dose animals were killed accidentally during wk 82 of the study; one low-dose animal was killed accidentally during wk 42 of the study
Full carcinogenicity Rat, F344/N (F) 9 wk 104 wk NTP (1982)	Gavage Vinylidene chloride, 99% Corn oil 0, 1, 5 mg/kg bw, 1×/d, 5 d/ wk for 104 wk 50, 50, 50 27, 28, 29	<i>Pituitary gland</i> , adenoma, NOS: 16/48 (33%)*, 20/49 (41%), 24/43 (56%)**	* <i>P</i> = 0.017 (trend), Cochran- Armitage test, but NS by life table analysis; ** <i>P</i> = 0.026, Fisher exact test, but NS by life table analysis	Principal strengths: well-conducted study

bw, body weight; d, day(s); DMBA, 12-dimethylbenz[*a*]anthracene; F, female; GLP, good laboratory practice; M, male; mo, month(s); MTD, maximum tolerated dose; NOS, not otherwise specified; NR, not reported; NS, not significant; T₉₀, time to achieve 90% of the target concentration after the beginning of vapour generation; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; vs, versus; wk, week(s)

6 hours per day, 5 days per week for 1, 3, or 6 months, and maintained without treatment for a further 12-month observation period. Unexposed control groups consisted of 16–28 mice of each sex. There was a decrease in survival in exposed males and females. When control groups were pooled and exposed groups were pooled, the incidence of hepatocellular tumours was 10/60 (17%) in male controls and 4/28 (14%) in exposed males. Bronchioloalveolar tumours were observed in 8/60 (13%) male controls, 8/60 (13%) female controls, 4/28 (14%) exposed males, and 1/28 (3%) exposed females. One treated male had a haemangiosarcoma of the mesentery [a rare tumour] ([Hong et al., 1981](#)). [The Working Group considered that this study was inadequate for the evaluation because of the unsatisfactory study design (e.g. the use of only one dose, the small number of mice of each sex per exposure group, the short treatment periods, the poor survival, and the limited reporting).]

Two groups of 30 male and 30 female Swiss mice (age, 16 weeks) were exposed to vinylidene chloride (purity, 99.9%; 0.04% 1,2-dichloroethylene and 0.002% mono- and dichloroacetylene; stabilized with 200 ppm paramethoxyphenol) at concentrations of 10 or 25 ppm in air for 4 hours per day, 4–5 days per week, for 52 weeks and observed for their lifespan (up to 121 weeks) ([Maltoni et al., 1984](#)). A group of 100 mice of each sex (age, 16 weeks) not kept in inhalation chambers served as one group of controls (control A). Compared with control A mice, increased tumour incidences were seen for groups exposed at 10 and 25 ppm for: kidney adenocarcinoma in male mice (0/54, 0/24, 3/21 (14.3%) [$P = 0.02$, Fisher exact]); pulmonary adenoma in male mice (3/80 (3.7%), 11/28 (39.3%; $P < 0.05$, Fisher exact), 7/28 (25%; $P < 0.05$, Fisher exact)) and female mice (4/92 (4.3%), 3/30 (10%), 7/29 (24.1%; $P < 0.05$, Fisher exact)); and mammary tumours (mainly carcinomas) in female mice (2/98 (2%), 6/30 (20%; $P < 0.05$, Fisher exact), 4/30 (13.3%; $P < 0.05$, Fisher exact)). To increase the power of

the study, additional groups of 120 Swiss mice of each sex (age, 9 weeks) were then exposed to vinylidene chloride at a concentration of 25 ppm for their lifespan (up to 121 weeks) and observed concurrently with separate control groups of 90 mice of each sex (age, 9 weeks) not kept in inhalation chambers (control B). Comparisons of tumour incidences between control B mice and the groups of mice exposed concurrently at 25 ppm showed increases in the incidences of tumours at several sites: kidney adenocarcinoma in male mice (0/66 vs 25/98 (25.5%) [$P < 0.0001$, Fisher exact test]); pulmonary adenoma in male mice (3/74 (4%) vs 16/113 (14.2%; $P < 0.05$, Fisher exact test) and female mice (3/86 (3.5%) vs 11/118 (9.3%; $P < 0.05$, Fisher exact); and mammary tumours (mainly carcinomas) in female mice (1/89 (0.1%) vs 12/118 (10.2%; $P < 0.05$, Fisher exact) ([Maltoni et al., 1984](#)). [The Working Group noted the short exposure duration and the lack of a concurrent chamber control group.]

In another study, groups of 50 male and 50 female B6C3F₁/N mice (age, 5–6 weeks) were exposed by whole-body inhalation to vinylidene chloride (purity, > 99.9%; stabilized with 300 ppm monomethyl ether hydroquinone) vapour at concentrations of 0 (control), 6.25, 12.5, or 25 ppm, for 6 hours plus T₉₀ (time to achieve 90% of the target concentration after the beginning of vapour generation; 10 minutes) per day, 5 days per week for 105 weeks ([NTP, 2015](#)). The survival of male mice exposed to the low concentration was significantly greater than that of controls; the survival of males exposed to the high concentration and the survival of females exposed to the low and high concentrations were significantly lower than that of the controls. Mean body weights of males exposed to the medium and high concentrations and females exposed to the high concentration were at least 10% lower than those of controls during the study.

The incidences of renal tubule adenoma, renal tubule carcinoma, and renal tubule adenoma or carcinoma (combined) were significantly

increased in all exposed groups of male mice, with a significant positive trend in the incidence of these tumours. The incidences of renal tubule hyperplasia were also significantly increased in all exposed groups of males. The incidences of haemangioma of the vascular system in all exposed groups of females were non-significantly increased (0/50, 2/50, 2/50, 2/50) compared with controls. There was a significant positive trend ($P = 0.044$) in the incidence of haemangiosarcoma of the vascular system (4/50, 4/50, 4/50, 9/50) in females. Compared with controls, the incidence of haemangioma or haemangiosarcoma (combined) of the vascular system (4/50, 6/50, 6/50, 11/50) in females exposed to the high concentration was significantly greater, and a significant positive trend was observed. Compared with controls, the incidence of liver haemangiosarcoma (1/50, 1/50, 1/50, 6/50) in females exposed to the high concentration was significantly greater, and a significant positive trend was observed. The incidences of hepatocellular adenoma in females exposed to medium concentrations, of hepatocellular carcinoma in females exposed to high concentrations, and in hepatocellular adenoma or carcinoma (combined) in females exposed to medium and high concentrations were significantly greater than those in the control groups, with significant positive trends. In addition, hepato-cholangiocarcinoma occurred in all exposed groups of females (0/50, 1/50, 1/50, 2/50). In female B6C3F₁ mice, this neoplasm is very rare and has not been observed in 300 inhalation controls or 948 controls from all routes of exposure in studies conducted by the National Toxicology Program (NTP). [The Working Group considered that the occurrence of hepato-cholangiocarcinomas may have been related to treatment.] The incidences of hepato-cholangiocarcinoma in exposed groups of males were also non-significantly increased compared with that in the control group (1/50, 2/50, 2/50, 3/50) and exceeded the historical control range for inhalation studies (0–2%). In

males, hepato-cholangiocarcinoma has been reported in 2/299 (0.7%) inhalation controls and in 10/949 (1.1%) controls from all routes of exposure. The incidence of bronchioloalveolar carcinoma was significantly increased in females exposed to medium concentrations with a significant positive trend. In females exposed to high doses, it was also reported that the incidence of carcinoma of the small intestine (ileum) (3/50, 6%) exceeded the historical control ranges for inhalation studies and all routes of administration. Historical rates for this tumour are 2/300 (range, 0–2%) for inhalation studies and 2/950 (range, 0–2%) for all routes of administration (NTP, 2015). [The Working Group noted that this was a well-conducted good laboratory practice (GLP) study, and both sexes were used.]

3.1.2 Oral administration

Groups of 50 male and 50 female B6C3F₁/N mice (age, 9 weeks) were given vinylidene chloride (purity, 99%; 0.15% *trans*-dichloroethylene and the stabilizer hydroquinone monomethyl ether [0.02%]) in corn oil by gavage at doses of 2 or 10 mg/kg bw once per day, 5 days a week for 104 weeks (NTP, 1982). Groups of 50 mice of either sex were given corn oil alone and served as vehicle controls. Compared with controls, the exposure to vinylidene chloride had no effect on survival in male and female mice. Mean body weights of the female mice given the high dose were comparable with those of controls; however, the mean body weights of male mice given either dose and of female mice given the low dose were slightly lower than those of controls. Significant increases in the incidence of tumours of the haematopoietic system [haematopoietic and lymphoid tissues] were observed in female mice given the low dose: these tumours were malignant lymphoma (2/48; 9/49, $P = 0.012$ by life table test, and $P = 0.028$ by Fisher exact test; 6/50) and lymphoma or leukaemia (combined) (7/48; 15/49, $P = 0.037$ by life table test, and $P = 0.050$

by Fisher exact test; 7/50). [The Working Group noted the absence of compound-related effects on survival or clinical signs, which suggests that the maximum tolerated dose (MTD) was not reached.]

3.1.3 Subcutaneous injection

A group of 30 female Ha:ICR Swiss mice (age, 6–8 weeks) received subcutaneous injections of 2.0 mg vinylidene chloride [purity unspecified] (Aldrich Chemical Co.) in 0.05 mL trioctanoin into the left flank once per week for 548 days [78 weeks] (Van Duuren et al., 1979). A group of 30 mice received similar treatment with trioctanoin (vehicle control) only. An additional group of 100 mice served as untreated controls. No local sarcomas were observed in the controls or mice treated with vinylidene chloride. [The Working Group noted some limitations of the study, including the use of only one dose, a lack of body weight data, and the fact that only tissues from the injection site and the liver were examined histologically.]

3.1.4 Skin application

Van Duuren et al. (1979) also tested vinylidene chloride on mouse skin. Two groups of 30 female Ha:ICR Swiss mice (age, 6–8 weeks) were treated three times per week for 440–594 days [not further reported] with skin applications of 40.0 or 121.0 mg vinylidene chloride [purity unspecified] (Aldrich Chemical Co.) in 0.2 mL acetone on the dorsal skin. Controls received no treatment ($n = 100$) or treatment with acetone only ($n = 30$). No skin papillomas were observed in the controls or mice treated with vinylidene chloride. [The Working Group noted some limitations of the study, including the lack of body weight data and the uncertain dose due to the volatility of vinylidene chloride.]

3.1.5 Initiation–promotion

Vinylidene chloride was tested for its initiating activity in a two-stage mouse-skin assay (Van Duuren et al., 1979). A group of 30 female Ha:ICR Swiss mice (age, 6–8 weeks) received a single skin application of 121.0 mg vinylidene chloride [purity unspecified] (Aldrich Chemical Co.) in 0.2 mL acetone on the dorsal skin, followed 14 days later by applications of 5 μ g 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in 0.2 mL acetone three times per week for 428–576 days [not further reported]. Four other groups of mice received no treatment ($n = 100$), treatment with acetone only ($n = 30$), treatment with TPA only ($n = 90$), or treatment with 20 μ g 7,12-dimethylbenz[*a*]anthracene (DMBA) plus TPA ($n = 30$), and served as untreated, vehicle, TPA-treated, or positive controls, respectively. Complete necropsies were performed at termination of the study or at death, and all abnormal-appearing tissues and organs were examined histologically. Routine sections of certain tissues and organs were examined [not further specified]. In the 90 TPA-only control mice, seven skin papillomas were observed in six mice; two mice had skin squamous cell carcinomas. In the vinylidene chloride plus TPA group of 30 mice, nine skin papillomas were observed in eight mice ($P < 0.005$ versus TPA controls); one mouse had a skin squamous cell carcinoma. No skin papilloma or carcinoma was observed in the untreated or acetone controls. In the positive control group (DMBA+TPA), 317 skin papillomas developed in 29 mice ($P < 0.0005$ vs TPA controls); 18 mice had skin squamous cell carcinomas. [The Working Group noted some limitations of the study, including the use of only one dose, a lack of body weight data, and the uncertain dose due to the volatility of vinylidene chloride.]

3.2 Rat

3.2.1 Inhalation

In an exposure experiment in utero, groups of 60 or 54 pregnant female Sprague-Dawley breeder rats (age, 13 weeks) were exposed by whole-body inhalation to 0 (controls) or 100 ppm vinylidene chloride (purity, > 99.9%; 1,2-dichloroethylene, 0.40 g/kg; mono- and dichloroethylene, 0.02 g/kg; stabilized with 200 ppm paramethoxyphenol) for 4 hours per day, 5 days per week for 7 weeks, then for 7 hours per day, 5 days per week for 97 weeks, and then kept under observation until spontaneous death. Concurrently, groups of 62 male and 61 female offspring were exposed transplacentally beginning at day 12 of gestation, and by whole-body inhalation postnatally with the same regimen as the breeders described above. Along with 158 male and 149 female rats serving as unexposed controls, all were kept under observation until spontaneous death. Exposure to vinylidene chloride did not affect survival, but caused a slight decrease in body weights in all exposed groups. In breeders, vinylidene chloride caused non-significant increases in the incidences of benign and malignant tumours of the mammary gland and malignant tumours of the mammary gland. Compared with controls, an increased incidence of leukaemia was found in exposed male (control, 12/158, 7.6%; exposed, 10/62, 16.1% [not significant]) and female (control, 1/149, 0.7%; exposed, 4/61, 6.5% [$P < 0.03$]) offspring (Cotti et al., 1988). [The Working Group noted the unsatisfactory study design with limited reporting, the use of only one dose, and the lack of detailed information on survival or observed tumours.]

Lee et al. (1977, 1978) exposed two groups of 36 male and 36 female CD rats (age, 2 months) to vinylidene chloride (purity, 99% pure) in air at 0 (controls) or 55 ppm for 6 hours per day, 5 days per week for up to 12 months (with interim terminations of 4 rats after 1, 2, 3, 6, and 9 months), at

which time the experiment was terminated. Of the 36 exposed male rats, 2 developed haemangiosarcomas [not significant], 1 in a mesenteric lymph node and 1 in the subcutaneous tissue. No haemangiosarcomas were observed in 35 male controls. There was no treatment-related increase in tumour incidence in females. [The Working Group concluded this was an inadequate study for the evaluation because of the unsatisfactory study design, use of only one dose, short durations of exposure, and limited reporting.]

Groups of male and female CD rats (age, 2 months) were exposed to 55 ppm vinylidene chloride (purity, 99%) in air for 6 hours per day, 5 days per week, for 6 months (20 males and 20 females) or 10 months (14 males and 16 females). After treatment, all exposed groups were maintained without further exposure for 12 months, at which time the remaining rats were killed. Corresponding control groups of 20 and 16 rats (a total of 36 control rats per sex) were maintained on filtered air for the same treatment periods and then maintained for a further 12-month period. There was a decrease in survival in exposed males. A single hepatic haemangiosarcoma was observed in a male rat that had been exposed to vinylidene chloride for 6 months (Hong et al., 1981). [The Working Group concluded this was an inadequate study for the evaluation because of the unsatisfactory study design (e.g. the use of only one dose, the small number of rats of each sex per exposure group, and the short duration of exposure) and the limited reporting.]

Maltoni et al. (1984) exposed groups of 30 male and 30 female Sprague-Dawley rats (age, 16 weeks) to vinylidene chloride (purity, 99.9%; 0.04% 1,2-dichloroethylene and 0.002% mono- and dichloroacetylene; stabilized with 200 ppm paramethoxyphenol) at 10, 25, 50, or 100 ppm for 4 hours per day, 4–5 days per week for 52 weeks, followed by observation for lifetime (up to 137 weeks). An additional group of 60 rats of each sex was initially exposed at 200 ppm for 2 days,

then 150 ppm for 4 hours per day, 4–5 days per week for 52 weeks, followed by observation for lifetime; the dosing frequency was reduced periodically to four times per week due to toxicity. Groups of 100 rats of each sex (age, 16 weeks) not kept in inhalation chambers were used as controls. The pattern of neoplasms and their incidences were comparable among treated and control rats. [The Working Group noted the poor reporting of survival and body weight information for treated and control rats, as well as the short duration of exposure.]

Groups of 85–86 male and 84–86 female Sprague-Dawley rats (age, 6–7 weeks) were exposed to vinylidene chloride (purity, 99%; stabilized with hydroquinone monomethyl ether) at 0 (control), 10, or 40 ppm for 6 hours per day, 5 days per week for 1 month. Exposure was then increased to 25 or 75 ppm vinylidene chloride for 17 months because of the lack of treatment-related effects at 10 and 40 ppm after 1 month of treatment. Surviving rats were held for an additional 6 months. There were no treatment-related effects on body weight gain or survival, except for a significant increase in mortality among females exposed at 75 ppm during months 14–24 of the study. Compared with controls, vinylidene chloride caused a [non-significant] increase in the incidence of mammary gland adenocarcinoma in the females exposed at low concentrations (2/84; 7/86, $P < 0.05$, Fisher exact test [$P = 0.0898$, 1-tail Fisher exact test]; 4/84). There was no significant increase in the incidence of any tumours in males (Quast et al., 1986). [The Working Group noted some limitations of the study, including the short duration of exposure, incorrect statistics, and poor survival in females exposed at high concentrations.]

In another study (NTP, 2015), groups of 50 male and 50 female F344/N rats (age, 5–6 weeks) were exposed by whole-body inhalation to vinylidene chloride (purity, > 99.9%; stabilized with 300 ppm monomethyl ether hydroquinone) vapour at concentrations of 0 (control), 25, 50,

or 100 ppm for 6 hours plus T_{90} (10 minutes) per day, 5 days per week for 105 weeks. The survival of exposed groups of males was similar to that of controls. The survival of females exposed at 100 ppm was significantly less than that of controls. Mean body weights of exposed groups of male and female rats were similar to those of controls throughout the study. In male rats, the incidences of malignant mesothelioma (mainly from the tunica vaginalis, then pleura, pericardium, and peritoneum) occurred with a significant positive trend and were significantly increased in all exposed groups compared with the control group. A significant positive trend in the incidence of adenoma of the nasal respiratory epithelium was observed in male rats (0/49, 0/50, 1/50, 4/50); no nasal respiratory epithelium adenomas have been seen in male historical controls. The incidence of adenoma of the nasal respiratory epithelium in females exposed to the high concentration (1/50, 2%) also exceeded the historical control range for inhalation studies (0/200; all routes, 1/697, 0–2%). Significantly increased incidences, with significant positive trends, were seen for C-cell adenoma of the thyroid gland in females exposed to the high concentration, and for C-cell adenoma or carcinoma (combined) of the thyroid gland in females exposed to low and high concentrations. Significantly increased incidence was seen for C-cell carcinoma of the thyroid gland in females exposed to the low concentration. The incidence of mononuclear cell leukaemia was significantly increased in females exposed to the high concentration, with a significant positive trend. Renal tubule carcinomas were observed in four males exposed to vinylidene chloride (0/50, 2/50, 1/49, 1/50); these neoplasms are rare in male F344/N rats (historical incidence: inhalation studies, 0/200; all routes, 1/697). Rare malignant mesotheliomas occurred in one female exposed to the low concentration (pleura and pericardium) and one female exposed to the medium concentration (peritoneum) (historical incidence:

inhalation studies, 0/200; all routes, 0/700) ([NTP, 2015](#)). [The Working Group noted that this was a well-conducted GLP study, and that both sexes were used.]

3.2.2 Oral administration

A group of 24 female BD IV rats [age unspecified] were given a single dose of 150 mg/kg bw vinylidene chloride (purity, 99%; containing 0.03% 4-methoxyphenol) in olive oil by gavage on day 17 of gestation. Their progeny (89 males and 90 females) were given doses of 50 mg/kg bw vinylidene chloride in 0.3 mL olive oil once per week for life, beginning at weaning. A vehicle-control group of 14 dams were given 0.3 mL olive oil on day 17 of gestation, and their progeny (53 males and 53 females) were given 0.3 mL olive oil once per week for life, beginning at weaning. All survivors were killed at 120 weeks or when moribund. Litter sizes, pre-weaning mortality, survival rates, and body weight gain were similar between the group treated with vinylidene chloride and the vehicle-control group. No statistically significant increase in the incidence of any tumours was noted in exposed male or female offspring or in exposed dams ([Ponomarev & Tomatis, 1980](#)). [The Working Group noted the use of only one dose.]

The [NTP \(1982\)](#) exposed groups of 50 male and 50 female Fischer 344/N rats (age, 9 weeks) to vinylidene chloride (purity, 99%; 0.15% *trans*-dichloroethylene and the stabilizer, hydroquinone monomethyl ether [0.02%]) at doses of 0 (control), 1, or 5 mg/kg bw in corn oil by gavage once a day, 5 days per week for 104 weeks. Survival and body weight throughout the study were similar in all treated and control groups. Twelve control male rats and 10 male rats exposed to the low dose were killed accidentally during week 82 of the study, and one male exposed to the low dose was killed accidentally during week 42. [The absence of compound-related effects on survival or clinical signs suggests that the rats could have

tolerated higher doses.] No significant increase in tumour incidence was observed in male and female rats treated with vinylidene chloride in this study. [The Working Group noted that the ability of this study to assess the carcinogenicity of vinylidene chloride was limited by the accidental deaths of the control rats and male rats exposed to the low dose, and by not achieving the MTD.]

[Maltoni et al. \(1984\)](#) exposed groups of 50 male and 50 female Sprague-Dawley rats (age, 9–10 weeks) to vinylidene chloride (purity, 99.9%; 0.04% 1,2-dichloroethylene and 0.002% mono- and dichloroacetylene; stabilized with 200 ppm paramethoxyphenol) at 0.5, 5, 10, or 20 mg/kg bw in olive oil by gavage once per day for 4–5 days per week for 52 weeks, followed by observation for their lifespan (up to 147 weeks). A group of 82 males and 77 females served as vehicle controls for the group exposed to the lowest dose, and a separate group of 100 rats of each sex served as vehicle controls for the remaining exposed groups. The pattern and incidences of tumours observed in this study were comparable among treated and control rats. [The Working Group noted the poor reporting on survival and body weight information for treated and control rats, and the short duration of exposure.]

Groups of 47–48 male and 48 female Sprague-Dawley rats (age, 6–7 weeks) were given vinylidene chloride (purity, $\geq 99.5\%$; with 1–5 mg/L hydroquinone monomethyl ether) at 50, 100, or 200 mg/L in drinking-water ad libitum for 2 years. A group of 80 male and 80 female controls received drinking-water only. Mortality and body weight gain were similar in the treated and control groups; no statistically significant increase in tumour incidence was reported in treated rats ([Quast et al., 1983](#)). [The Working Group noted that the stability of drinking-water solutions was not determined. The MTD does not appear to have been achieved.]

3.3 Hamster

3.3.1 Inhalation

Groups of 30 male and 30 female Chinese hamsters (age, 29 weeks) were exposed to vinylidene chloride (purity, 99.9%; with 0.04% 1,2-dichloroethylene and 0.002% mono- and dichloroacetylene; stabilized with 200 ppm para-methoxyphenol) at 25 ppm in air for 4 hours per day, 4–5 days per week for 52 weeks, and observed for their lifetime (up to 164 weeks). A group of 18 males and 17 females, not housed in inhalation chambers, were used as controls. The pattern of neoplasms and their incidences were comparable among treated and control hamsters ([Maltoni et al., 1984](#)). [The Working Group noted the use of only one dose, the short duration of exposure, and the small number of controls, making this study inadequate for evaluating the carcinogenicity of vinylidene chloride.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

The toxicokinetics of vinylidene chloride have been reviewed by several groups over the past 30 years, including *IARC Monographs Working Groups* in 1985 and 1998 ([IARC, 1986, 1999](#)). Several major vinylidene chloride review documents ([EPA, 2002](#); [WHO, 2003](#); [ATSDR, 2009](#); [Health Canada, 2015](#)) have been published since 1999.

4.1.1 Absorption

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Rodents

Vinylidene chloride is well absorbed from the lungs and gastrointestinal (GI) tract as it is a small, uncharged, lipophilic molecule. The lungs are an optimal site for absorption of the volatile organic chemical (VOC), due to their large surface area, high blood perfusion rate, and intimate alveolar–capillary interfaces ([Bruckner et al., 2013](#)). Arterial blood levels rapidly reach and remain at near steady-state for the duration of inhalation exposures of rats. Percentage systemic uptake of a series of vapour concentrations in rats was as high as 80% ([Dallas et al., 1983](#)). Equivalent vapour exposures should result in higher systemic doses of VOCs in rodents than in humans because of the higher alveolar ventilation rate, blood:air partition coefficient, cardiac output, and metabolic rate of rodents ([NAS, 2009](#)). Vinylidene chloride is also well absorbed from the GI tract ([Putcha et al., 1986](#)), but the administration of equivalent oral and inhaled doses to rats results in significantly higher arterial blood levels and nephrotoxicity in animals inhaling the chemical ([Bruckner et al., 2010](#)). Fatty foods retard its GI absorption, and the VOC is subject to extensive first-pass hepatic and pulmonary elimination as it is extensively metabolized and volatile ([Bruckner et al., 2010, 2013](#)).

4.1.2 Distribution and excretion

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Rat

Vinylidene chloride was rapidly distributed to all tissues examined following a single oral dose of the [¹⁴C]-labelled compound to rat. The highest levels of radioactivity were found in the liver and kidneys 30 minutes after dosing ([Jones](#)

& Hathway, 1978a). Preferential distribution to the liver, kidneys, and lungs was seen in rats following inhalation of vinylidene chloride at 10 or 200 ppm (McKenna et al., 1978).

Vinylidene chloride is rapidly eliminated by rats. Putcha et al. (1986) reported the half-life of vinylidene chloride in rats to be up to 60 minutes. Metabolic clearance is primarily responsible for systemic elimination. Metabolic saturation was manifest at an oral dose of 50 mg/kg bw by reduction in exhaled carbon dioxide and urinary metabolites, and increased vinylidene chloride exhalation (Jones & Hathway, 1978b).

4.1.3 Metabolism

(a) Humans

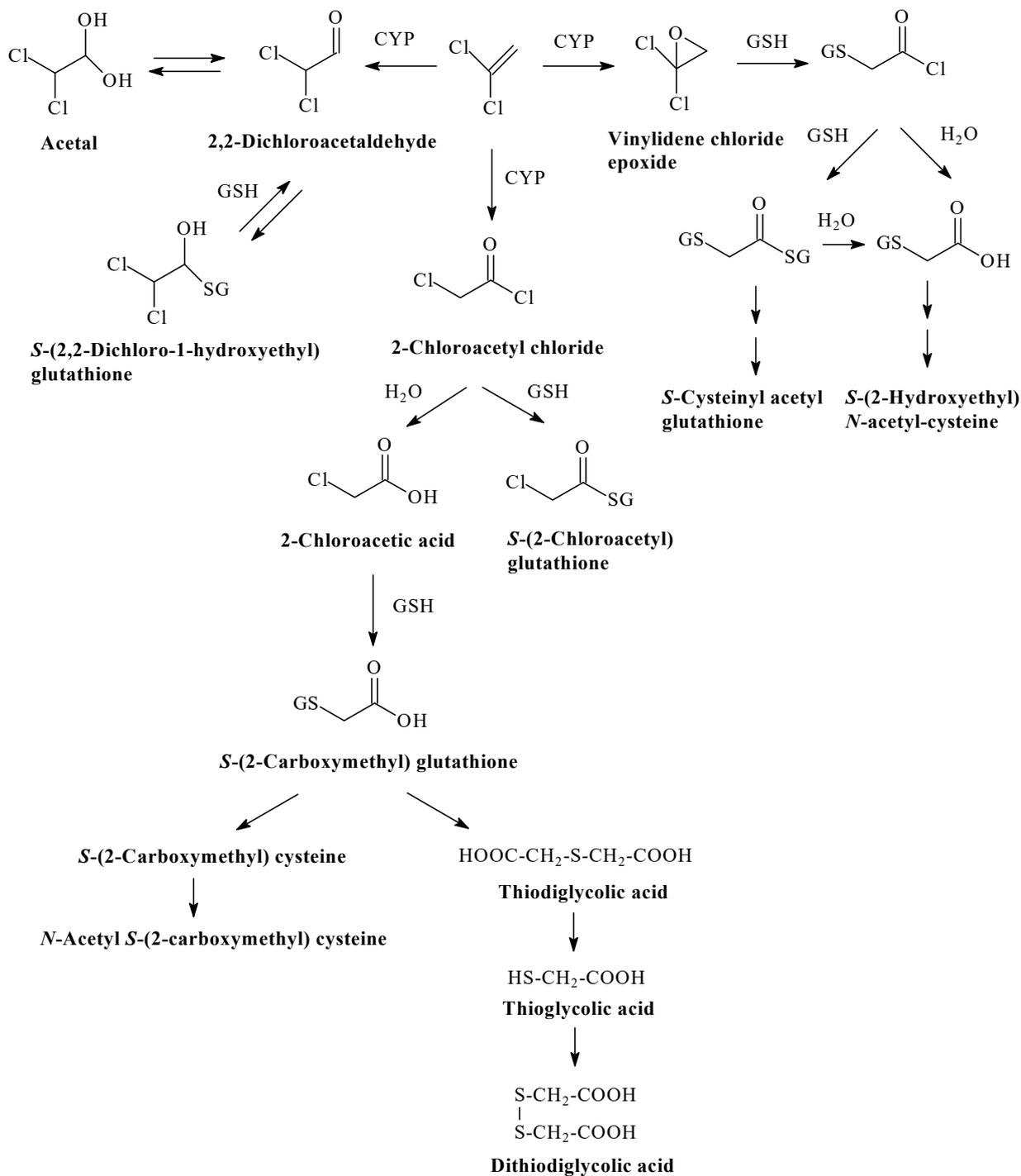
Cytochrome P450 (CYP) 2E1 is the predominant enzyme in liver (Hakkola et al., 1994), lung, and kidney responsible for the oxidation of vinylidene chloride. The formation of vinylidene chloride epoxide and 2,2-dichloroacetaldehyde was demonstrated in human lung and liver microsomes (Dowsley et al., 1999). Human lung has low and variable levels of CYP2E1 activity (Shimada et al., 1996), primarily because of the rarity of Clara cells (Forkert, 2001). CYP2E1 activity was low or not detectable in human kidney microsomal samples (Amet et al., 1997; Caro & Cederbaum, 2004; Sasso et al., 2013).

(b) Experimental systems

Vinylidene chloride is metabolized largely by CYP-catalysed oxidation in rat and mouse liver as illustrated in Fig. 4.1 (NTP, 2015). CYP2E1 is primarily responsible for the oxidative metabolism and metabolic activation of vinylidene chloride in rodents. The enzyme inducers enhance both the metabolic activation of vinylidene chloride and cytotoxicity, while certain inhibitors decrease its biotransformation and toxicity as described in Section 4.5.2. Vinylidene chloride is metabolized by CYP2E1 in rodents to at least three reactive metabolites, including vinylidene

chloride epoxide, 2-chloroacetyl chloride, and 2,2-dichloroacetaldehyde (Forkert, 2001; Forkert et al., 2001). These products undergo glutathione (GSH) conjugation and/or hydrolysis. Relatively high levels of CYP2E1 are present in three primary target organs of vinylidene chloride in rodents: liver, kidney, and lung. The epoxide, an electrophilic intermediate, is an important cytotoxic metabolite of vinylidene chloride (Forkert, 2001; Forkert et al., 2001; Simmonds et al., 2004); it binds covalently to proteins and nucleic acids, and can damage hepatocytes (Jones & Liebler, 2000) and renal tubular cells (Brittebo et al., 1993).

Variance in the expression of CYP2E1 is an important factor in tissue, species, and sex susceptibility to vinylidene chloride. Levels of GSH and epoxide hydrolase are also important determinants of the extent of injury. For example, human kidney has very low or non-detectable renal CYP2E1 activity. The rate of formation of vinylidene chloride epoxide and 2,2-dichloroacetaldehyde was much lower in human lung and liver microsomes compared with that of mouse. Vinylidene chloride cytotoxicity and covalent binding are greatest in murine cells with the highest CYP2E1 content, namely centrilobular hepatocytes, followed by bronchiolar Clara cells and renal proximal tubular cells (Speerschneider & Dekant, 1995; Forkert, 2001). Biotransformation of vinylidene chloride, and presumably its metabolic activation, is about six times higher in liver microsomes from mice compared with those from rats (Dowsley et al., 1995). Sex difference in CYP2E1-mediated metabolism of vinylidene chloride correlates with the occurrence of renal tumours induced by vinylidene chloride (Speerschneider & Dekant, 1995). Metabolism of vinylidene chloride by kidney microsomes from male mice was six times greater than that by females. The rank order (adult female > weanling male = weanling female > adult male) of CYP2E1-catalysed metabolic activation of vinylidene chloride in mouse

Fig. 4.1 Proposed metabolic pathway of vinylidene chloride in rodents

CYP, cytochrome P450; GSH, glutathione
Adapted from [NTP \(2015\)](#)

lung microsomes ([Lee & Forkert, 1995](#); [Forkert et al., 1996a](#)) correlates with the severity of injury of mouse bronchiolar Clara cells ([Forkert et al., 1996a](#)). [Simmonds et al. \(2004\)](#) subsequently demonstrated that CYP2F2, as well as CYP2E1, was capable of bioactivation of vinylidene chloride in murine lung. Expression of CYP2F in the lung is much higher in mice than in humans ([Chen et al., 2002](#)).

GSH plays an important role in the metabolism of vinylidene chloride ([Fig. 4.1](#)). This is consistent with the observation that exposure to vinylidene chloride depletes liver GSH levels ([Jaeger et al., 1974](#); [Reichert et al., 1978](#); [Reynolds et al., 1980](#)). GSH depletion by fasting or xenobiotics permits reactive oxidative metabolites, such as vinylidene chloride epoxide, 2-chloroacetyl chloride, and 2,2-dichloroacetyl chloride, to bind to and alkylate cellular macromolecules instead of being detoxified ([Jaeger et al., 1974](#); [Reynolds et al., 1980](#); [Kanz et al., 1988](#)). GSH conjugates formed in the liver can also reach target cells in the kidney, where they undergo metabolic activation by β -lyase to reactive, cytotoxic thiols ([Ban et al., 1995](#)). Pretreatment with aminooxyacetic acid, an inhibitor of cysteine conjugate β -lyase, decreased the number of damaged tubules in the kidneys of mice given a single oral dose of vinylidene chloride at 200 mg/kg bw ([Ban et al., 1995](#)). Aminooxyacetic acid pretreatment also protected rats exposed to vinylidene chloride vapour at 150–180 ppm for 4 hours from liver and kidney injury ([Cavelier et al., 1996](#)). [The Working Group noted that the metabolism of vinylidene chloride is similar to that of other vinyl halides such as vinyl chloride.]

4.2 Mechanisms of carcinogenesis

4.2.1 Genetic and related effects

Metabolic transformation of vinylidene chloride in the liver produces a highly reactive and short-lived epoxide, along with other reactive metabolites.

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Mammalian systems in vivo

See [Table 4.1](#).

No evidence of genotoxicity was seen with vinylidene chloride in vivo. Bone marrow micronucleus tests (24 hours after treatment) in ddY male mice following a single exposure (25–200 mg/kg bw) or multiple exposures (25–100 mg/kg bw per day for 4 days) to vinylidene chloride by gavage were negative ([Sawada et al., 1987](#)). In a transplacental exposure study in pregnant ICR mice given vinylidene chloride at 25–100 mg/kg bw by a single intraperitoneal injection on gestational day 18, no increases in micronucleated cells in fetal liver and blood cells were seen 24 hours after treatment ([Sawada et al., 1987](#)). [The Working Group noted that these studies assessed micronuclei in 1000 binucleated lymphocytes, whereas 2000 are recommended ([OECD, 2016](#)).] Similarly, no increases in the frequencies of micronucleated erythrocytes were observed in the peripheral blood of male or female B6C3F₁/N mice exposed to 100 ppm vinylidene chloride for 3 months by inhalation ([NTP, 2015](#)). Negative results were also reported in dominant lethal tests (assays for mutagenicity in germ cells) in male CD-1 mice exposed to vinylidene chloride by inhalation at 10–50 ppm for 6 hours per day for 5 days, followed by mating ([Anderson et al., 1977](#)), and in male CrI:CD(SD) rats exposed to vinylidene chloride by inhalation at 55 ppm for 6 hours per

Table 4.1 Genetic and related effects of vinylidene chloride and its metabolite 2,2-dichloroacetaldehyde in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results ^a	Dose (LED or HID)	Route, duration, dosing regimen	Reference
<i>Vinylidene chloride</i>						
Dominant lethal test	Mouse, CD-1 (M)	Sperm	–	50 ppm	Inhalation, 6 h/d for 5 d	Anderson et al. (1977)
Dominant lethal test	Rat, Crl:CD(SD) (M)	Sperm	–	55 ppm	Inhalation, 6 h/d, 5 d/wk for 11 wk	Short et al. (1977)
DNA alkylation, DNA repair	Mouse, CD-1 (M); rat, Sprague-Dawley (M)	Liver and kidney	–	50 ppm	Inhalation, 6 h	Reitz et al. (1980)
Micronuclei	Mouse, ddY (M)	Bone marrow	–	25–200 mg/kg bw (1×) or 25–100 mg/kg bw (4×)	Gavage, sampling after 24 h	Sawada et al. (1987)
Micronuclei	Mouse, ICR (F)	Fetal liver/blood (transplacental MN test)	–	25–100 mg/kg bw	Intraperitoneal injection (in pregnant mice), 1×; sampling after 24 h	Sawada et al. (1987)
Micronuclei	Mouse, B6C3F ₁ /N (M, F)	Peripheral blood erythrocytes	–	100 ppm	Inhalation, 5 d/wk for 3 mo	NTP (2015)
<i>2,2-Dichloroacetaldehyde</i>						
DNA strand breaks (alkaline unwinding assay)	Mouse, B6C3F ₁ (M); rat, F344 (M)	Liver	–	Mouse, 5 mmol/kg bw [565 mg/kg]; rat, 10 mmol/kg bw [1130 mg/kg]	Oral, single dose, 4 h duration	Chang et al. (1992)

bw, body weight; d, day(s); F, female; h, hour(s); HID, highest ineffective dose; LED, lowest effective dose; M, male; MN, micronuclei; mo, month(s); ppm, parts per million; wk, week(s)

^a –, negative

day, 5 days per week for 11 weeks before mating ([Short et al., 1977](#)).

Alkylated DNA was recovered from the livers and kidneys of CD-1 mice and Sprague-Dawley rats exposed by inhalation to radiolabelled vinylidene chloride (10 or 50 ppm for 6 hours). However, compared with animals exposed to intraperitoneal injection of dimethylnitrosamine, few alkylated nucleotides were recovered and DNA repair synthesis was only modestly elevated (to 6% of the level induced by dimethylnitrosamine) ([Reitz et al., 1980](#)). [The Working Group noted the low specific activity of the test article, limiting the sensitivity of the assay.]

(ii) *Mammalian systems in vitro*

See [Table 4.2](#).

Inconsistent mutagenic responses were seen in L5178Y mouse lymphoma cells with vinylidene chloride in the absence of metabolic activation; with activation, both cytotoxicity and mutagenicity were consistently positive in repeat experiments ([McGregor et al., 1991](#)).

Vinylidene chloride (2 and 10% in air) did not induce 8-azaguanine and ouabain resistance in Chinese hamster V79 cells in the presence of S15 (15 000 g liver supernatant) from phenobarbital-treated rats and mice; although exposures were conducted within a closed environment to control for volatility, no mutagenic activity was reported in this assay with either species with or without S15 ([Drevon & Kuroki, 1979](#)).

Strong, dose-related increases in chromosomal aberrations were seen in cultured Chinese hamster lung cells exposed to vinylidene chloride in tightly sealed bottles over a concentration range of 0.125–1.5 mg/mL in the presence of S9 from Kanechlor 400-induced male F344 rat liver. In addition, sister-chromatid exchanges were increased in the presence of S9 at 0.075 mg/mL ([Sawada et al., 1987](#)).

Vinylidene chloride (exposure period, 2.5 hours) induced unscheduled DNA synthesis

in isolated rat hepatocytes using a method that does not require the blocking of semi-conservative DNA synthesis ([Costa & Ivanetich, 1984](#)). [The Working Group noted the sparse experimental details.]

(iii) *Non-mammalian systems*

See [Table 4.3](#).

Vinylidene chloride did not induce increases in sex-linked recessive lethal mutations in germ cells of adult male *Drosophila melanogaster* exposed via feeding or injection ([Fouerman et al., 1994](#)).

In bacterial test systems, vinylidene chloride consistently demonstrated mutagenic activity when tested in the presence of a metabolic activation system, in a closed environment to control for volatility ([Jacobson-Kram, 1986](#)). Vinylidene chloride (0.2, 2, and 20% in air (v/v) in a closed environment) was mutagenic in *Salmonella typhimurium* strains TA100 and TA1530 in the presence of non-induced rat or mouse liver S9. Mutagenicity was higher in the presence of mouse S9, but lower when mouse kidney or lung S9 fractions were used ([Bartsch et al., 1975](#)). Phenobarbital induction increased mutagenic responses in tests using mouse liver, kidney, and lung S9 ([Bartsch et al., 1975](#)). Similarly, vinylidene chloride is mutagenic in strains TA100 and TA1535 ([Baden et al., 1978, 1982](#)), in which pretreatment with CYP inducers increased the effectiveness of mouse liver and kidney S9, with mouse liver S9 also being more effective than rat liver S9 ([Jones & Hathway, 1978c](#)). Vinylidene chloride (2.5 mM) also induced a mutagenic response in *Escherichia coli* K-12 in the presence, but not the absence, of mouse S9 ([Greim et al., 1975](#)). Positive results were also observed in *S. typhimurium* strains TA92, TA98, TA100, TA1535, and TA1537 and *E. coli* strain WP2 *uvrA* in the presence of human S9 or Swiss mouse liver S9 (uninduced or induced by vinylidene chloride) ([Oesch et al., 1983](#)). In the absence of S9, comparable and low responses were observed in

Table 4.2 Genetic and related effects of vinylidene chloride and its metabolite 2,2-dichloroacetaldehyde in mammalian cells in vitro

End-point	Species, tissue, cell line	Results ^a		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
<i>Vinylidene chloride</i>						
Gene mutation	Chinese hamster, fibroblast V79 cells	-	-	10% (v/v) in air	Exposures carried out in a desiccator	Drevon & Kuroki (1979)
Unscheduled DNA synthesis	Rat, Long Evans, hepatocytes	+	NT	2.1 mM [203 µg/mL]	Sparse experimental details; unclear reporting of results	Costa & Ivanetich (1984)
Chromosomal aberrations, sister-chromatid exchange	Chinese hamster, lung	-	+	0.125–1.5 mg/mL (CA); 0.075 mg/mL (SCE)	Sealed bottles to control for volatility	Sawada et al. (1987)
Gene mutation	Mouse, L5178Y lymphoma cells	+/-	+	0.16% (v/v) in air		McGregor et al. (1991)
<i>2,2-Dichloroacetaldehyde</i>						
DNA strand breaks, alkaline unwinding assay	Human, CCRF-CEM, lymphoblastic leukaemia cell line	+	NT	1 mM [113 µg/mL]		Chang et al. (1992)
DNA strand breaks, alkaline unwinding assay	Rat, Fischer F334, primary hepatocytes	-	NT	10 mM [1130 µg/mL]	Cells treated for 4 h	Chang et al. (1992)

CA, chromosomal aberration; h, hour(s); HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; SCE, sister-chromatid exchange; v/v, volume per volume

^a +, positive; -, negative; +/-, equivocal (variable response in several experiments within an adequate study)

Table 4.3 Genetic and related effects of vinylidene chloride and its metabolite 2,2-dichloroacetaldehyde in non-mammalian systems

Test system (species, strain)	End-point	Results ^a		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
<i>Vinylidene chloride</i>						
<i>Salmonella typhimurium</i> TA100, TA1530	Reverse mutation	NT	+	0.2, 2, 20% (v/v) in air	S9 from uninduced BDVI female rat, and uninduced and PB-induced male OF-1 mouse liver, kidney, or lung	Bartsch et al. (1975)
<i>Escherichia coli</i> K-12	Reverse mutation	-	+	2.5 mM [242 µg/mL]	3 reverse mutation targets were tested: arg+, gal+, nad+; only the arg+ system produced a positive response	Greim et al. (1975)
<i>Salmonella typhimurium</i> TA100, TA1535	Reverse mutation	NT	+	3%		Baden et al. (1978)
<i>Salmonella typhimurium</i> TA100, TA1535	Reverse mutation	NT	+	5% (v/v) in air	Tested as a vapour in a closed system	Jones & Hathway (1978c)
<i>Saccharomyces cerevisiae</i> D7	Gene conversion	-	+	20 mM [1939 µg/mL]		Bronzetti et al. (1981)
<i>Saccharomyces cerevisiae</i> D7, host-mediated assay	Reverse mutation and gene conversion, host-mediated assay	+	NA	400 mg/kg bw, single oral dose; 100 mg/kg bw/d for 5 d/wk, total of 23 dosings		Bronzetti et al. (1981)
<i>Salmonella typhimurium</i> TA100, TA1535	Reverse mutation	(+)	+	3%	Few experimental details	Baden et al. (1982)
<i>Salmonella typhimurium</i> TA92, TA98, TA100, TA1535, TA1537; <i>E. coli</i> WP2 <i>uvrA</i>	Reverse mutation	-	+	375 ppm	In TA100, various species (mouse, rat, hamster, human) and tissue (kidney, liver) sources of S9 examined	Oesch et al. (1983)
<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537	Reverse mutation	-	-	6666 µg/plate	No controls for volatility	Mortelmans et al. (1986)
<i>Saccharomyces cerevisiae</i> D7	Gene conversion	-	+	50.3 µM [4876 µg/mL]	S9 from Aroclor 1254-induced male mice	Koch et al. (1988)
<i>Saccharomyces cerevisiae</i> D7	Reverse mutation	-	+	25.1 mM [2433 µg/mL]	S9 from Aroclor 1254-induced male mice	Koch et al. (1988)
<i>Saccharomyces cerevisiae</i> D61.M	Aneuploidy	+	+	25.1 mM [2433 µg/mL]		Koch et al. (1988)

Table 4.3 (continued)

Test system (species, strain)	End-point	Results ^a		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
<i>Drosophila melanogaster</i> Canton-S	Sex-linked recessive lethal mutations	–	NA	25 000 ppm (feeding); 5000 ppm (injection)		Foureman et al. (1994)
<i>Salmonella typhimurium</i> RSJ100, TPT100	Reverse mutation	(+)	NT	500 ppm		Granville et al. (2005)
<i>2,2-Dichloroacetaldehyde</i>						
<i>Salmonella typhimurium</i> TA100, TA1535	Reverse mutation	+ (TA100)	+ (TA100)	10 µL/plate	Controlled for volatility	Bignami et al. (1980)
<i>Streptomyces coelicolor</i> A3	Reverse mutation	+	NT	10 µL/plate	Controlled for volatility	Bignami et al. (1980)
<i>Aspergillus nidulans</i> Haploid strain 35	Forward mutation	+	NT	10 µL/plate		Bignami et al. (1980)
<i>Aspergillus nidulans</i> diploid strain 35 × 17	Chromosomal damage	+	NT	10 mM [1130 µg/mL]	+, mitotic non-disjunction and haploidization; –, mitotic crossing-over	Crebelli et al. (1984)

bw, body weight; d, day; HIC, highest ineffective concentration; LEC, lowest effective concentration; NA, not applicable; NT, not tested; PB, phenobarbital; ppm, parts per million; v/v, volume per volume; wk, week

^a +, positive; –, negative; (–), negative in a study of limited quality

S. typhimurium strains that express rat *GSTT1-1* (strain RSJ100) or contain an unexpressed rat *GSTT1-1* gene (strain TPT100), indicating a lack of activation of vinylidene chloride by rat *GSTT1-1* (Granville et al., 2005). Vinylidene chloride (tested at up to 6666 µg/plate) was not mutagenic in strains TA98, TA100, TA1535, or TA1537, with or without induced S9, when a preincubation protocol was used (Mortelmans et al., 1986), illustrating the importance of controlling for volatility.

Vinylidene chloride was toxic but not mutagenic in the diploid yeast *Saccharomyces cerevisiae* strain D7 in the absence of exogenous metabolic activation. However, in the presence of Aroclor-1254-induced liver S10 (10 000 g supernatant) from male Swiss albino CD mice, dose-related increases in both point mutations and mitotic gene conversions were observed (Bronzetti et al., 1981). Vinylidene chloride also induced significant increases in both point mutations and mitotic gene conversion in logarithmic phase *S. cerevisiae* D7 cells with a high level of CYP (Koch et al., 1988), and vinylidene chloride induced a highly significant, dose-related increase in aneuploidy in *S. cerevisiae* D7 strain D61.M, with and without S9 mix (Koch et al., 1988).

Point mutations and mitotic gene conversion were seen in *S. cerevisiae* D7 recovered from kidney and liver, but not lung, tissues in a host-mediated assay in male Swiss albino mice treated with vinylidene chloride (Bronzetti et al., 1981).

(iv) Metabolites

See Table 4.1, Table 4.2, and Table 4.3.

Vinylidene chloride is metabolized in isolated hepatocytes to several reactive metabolites (Costa & Ivanetich, 1984), including vinylidene chloride epoxide, 2-chloroacetyl chloride, and 2,2-dichloroacetaldehyde (NTP, 2015).

2,2-Dichloroacetaldehyde induced DNA strand breaks in cultured CCRF-CEM human lymphoblastic leukaemia cells in the absence

of S9, but not in primary rat hepatocytes or in livers of rats and mice treated orally (Chang et al., 1992). Positive results were reported in *S. typhimurium* TA100 (but not TA1535) with and without induced rat liver S9; S9 markedly attenuated the response (Bignami et al., 1980). 2,2-Dichloroacetaldehyde was also mutagenic in assays for forward (streptomycin resistance) and reverse (histidine independence) mutation in the bacterium *Streptomyces coelicolor* in the absence of S9 (Bignami et al., 1980). In a haploid strain of the mould *Aspergillus nidulans*, methionine suppression, requiring multilocus point mutations, was induced by 2,2-dichloroacetaldehyde in the absence of S9, and 8-azaguanine resistance, which results from a single locus point mutation, was weakly positive (Bignami et al., 1980). Mitotic nondisjunction and haploidization, but not mitotic crossing-over, was induced by 2,2-dichloroacetaldehyde in a diploid strain of *A. nidulans* in the absence of S9 (Crebelli et al., 1984).

4.2.2 Altered cell proliferation or death

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In male CD-1 mice, whole-body exposure to vinylidene chloride vapour at 10 or 50 ppm for 6 hours stimulated the incorporation of [³H]-labelled thymidine in kidney DNA, but not in liver DNA. A lesser extent of [³H]-labelled thymidine incorporation was induced in kidney DNA of male Sprague-Dawley rats (Reitz et al., 1980).

In the lungs of male CD-1 mice given a single intraperitoneal injection of [¹⁴C]-labelled vinylidene chloride at 125 mg/kg bw, there was higher macromolecular binding in the Clara cells (associated with higher CYP activity (7-ethoxycoumarin deethylase activity) and cellular damage) than that in the alveolar type II cells

([Forkert et al., 1990](#)). Following a rapid exfoliation of non-ciliated Clara cells from the bronchiolar epithelium in male C57BL/6 mice treated with a single oral dose of vinylidene chloride at 200 mg/kg bw, [³H]-labelled thymidine incorporation was transiently increased primarily in non-ciliated bronchiolar epithelial cells ([Forkert et al., 1985](#)).

In female CD-1 mice, vinylidene chloride given by intraperitoneal injection at 75 or 125 mg/kg bw decreased the membrane potential of hepatic as well as lung mitochondria, accompanied by activation of caspase-3 and DNA fragmentation characteristic of apoptosis ([Martin & Forkert, 2004, 2005](#)).

4.2.3 Other mechanisms

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Global gene expression profiles of mesotheliomas (mainly testicular) induced in male F344/N rats by whole-body exposure to vinylidene chloride vapour at 25, 50, or 100 ppm for 6 hours per day, 5 days per week for 2 years revealed overrepresentation of DNA damage and repair, as well as of pathways associated with immune dysfunction and inflammation, that were not observed in spontaneous mesotheliomas arising in control male F344/N rats from several NTP studies ([NTP, 2015](#)). The overexpressed pathways included pro-inflammatory pathways (e.g. the nuclear factor κ -light-chain-enhancer of activated B-cells (NF- κ B) signalling pathway), interleukin responses (IL-8, IL-12, and IL-17), Fc receptor signalling, dendritic and natural killer cell signalling, and phosphatidylinositol 3/protein kinase B (PI3/AKT) signalling ([Blackshear et al., 2015](#)).

Global gene expression profiles of renal cell carcinoma induced in male B6C3F₁ mice by whole-body exposure to vinylidene chloride

vapour at 6.25, 12.5, or 25 ppm for 6 hours per day, 5 days per week for 2 years ([NTP, 2015](#)) demonstrated overrepresented gene categories associated with oxidative stress, including the nuclear factor-erythroid-related factor 2 (Nrf2) pathway ([Hayes et al., 2016](#)). A trend analysis showed a correlation of oxidative stress pathway modulation between exposed non-tumour and tumour tissue in the mice. Several other pathways, including those associated with cell cycle checkpoint regulation, cell growth, and cell proliferation, were overexpressed in non-tumour kidney tissue of exposed mice compared with unexposed mice ([Hayes et al., 2016](#)).

A significant increase in T helper cell type 2 (Th2) cytokine production (IL-4, IL-5, IL-13, and interferon- γ) in single-cell suspensions obtained on day 25 from lung-associated lymph nodes and cultured in the presence of concanavalin A was seen after whole-body exposure of female BALB/c mice to vinylidene chloride vapour at 10 ppm for 6 hours per day for 4 days. Vinylidene chloride had no effect on blood levels of immunoglobulin E, on the influx of inflammatory cells into alveolar spaces, or on goblet cell proliferation ([Ban et al., 2006](#)).

Serum levels of cytokines (tumour necrosis factor α (TNF α) and IL-6) increased by 6 hours and then tended to decrease with time in male Swiss OF1 mice given a single dose of vinylidene chloride at 100, 150, or 200 mg/kg bw by gavage. Maximum renal and hepatic damage occurred at 16 and 24 hours, respectively, after treatment. There was an inverse correlation between renal tubule damage percentage and serum antibody-forming cell response and natural killer cell activity ([Ban et al., 1998](#)).

4.3 Data relevant to comparisons across agents and end-points

For the results of high-throughput screening assays of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA ([Kavlock et al., 2012](#); [Tice et al., 2013](#); [EPA, 2016a, b](#); [Filer et al., 2017](#)), see Section 4.3 of the *Monograph on 1-tert-butoxypropan-2-ol* in the present volume.

4.4 Susceptibility to cancer

No data were available to the Working Group.

4.5 Other adverse effects

4.5.1 Humans

Acute, high-level (~4000 ppm) exposures to vinylidene chloride can cause neurological effects including depression of the central nervous system in humans, while long-term exposures to lower concentrations may cause liver and kidney toxicity ([ATSDR, 1994](#); [IARC, 1999](#); [EPA, 2002](#); [NTP, 2015](#)).

4.5.2 Experimental systems

The primary target organs of toxicity induced by vinylidene chloride in experimental animals are the liver, lungs, and kidneys. Liver toxicity was induced in mice and rats by inhalation ([Lee et al., 1977](#); [Plummer et al., 1990](#); [NTP, 2015](#)) or by oral ([Forkert & Reynolds, 1982](#); [NTP, 1982](#); [Wang et al., 1999](#)) exposure; liver inflammation and degeneration was observed in rats ([NTP, 2015](#)). [Despite the hepatotoxic effects of vinylidene chloride in both sexes of rats and mice, increased incidences of liver tumours were detected only in exposed female mice.] Mechanistic studies using inducers and inhibitors of vinylidene chloride metabolism, or agents

that deplete hepatic GSH levels, demonstrate the important role of biotransformation in the hepatotoxicity of vinylidene chloride ([Siegers et al., 1979, 1985](#); [Kanz et al., 1988](#); [Wijeweera et al., 1998](#)). A dose–response relationship is apparent between hepatocellular necrosis, reduced levels of GSH, and covalent binding of vinylidene chloride metabolites to liver tissue ([Gram, 1997](#)).

The toxic effects of vinylidene chloride in the lung were observed in mice exposed at 25 ppm or more by inhalation for 3 months (females) or for 2 years (males) ([NTP, 2015](#)). Lung toxicity (primarily in Clara cells) was also observed in orally exposed mice ([Forkert & Reynolds, 1982](#); [Forkert et al., 1985](#)). The severity of Clara cell injury is associated with increased covalent binding to lung tissue and reduction in GSH content ([Forkert et al., 1986a, b](#)), while decreased bioactivation of vinylidene chloride prevented Clara cell toxicity ([Dowsley et al., 1996](#); [Forkert et al., 1996a, b](#)).

Renal tubular necrosis was observed in mice and rats exposed to vinylidene chloride by inhalation ([Lee et al., 1977](#); [NTP, 2015](#)). Mechanistic studies with metabolic modulators indicated that kidney toxicity in orally exposed mice occurs independently of an anion transport system or γ -glutamyltranspeptidase activity, but does require γ -glutamylcysteine synthetase, β -lyase, and cysteine conjugate *S*-oxidase activities ([Brittebo et al., 1993](#); [Ban et al., 1995](#)).

Turbinate atrophy, hyperostosis, olfactory epithelium respiratory metaplasia, and respiratory epithelium hyperplasia occurred in all exposure groups of both sexes of rats (25, 50, and 100 ppm) and mice (6.25, 12.5, and 25 ppm) in 2-year inhalation studies ([NTP, 2015](#)). [Despite these findings, the incidence of nasal tumours was increased only in male rats.]

5. Summary of Data Reported

5.1 Exposure data

Vinylidene chloride is not naturally occurring and is not generally detected in the environment. The substance is used in the production of polyvinylidene chloride copolymers, the major application of which is in the production of flexible films for food packaging where vinylidene chloride may persist as an unintended manufacturing residue. Vinylidene chloride is a high production volume chemical; the global production capacity was estimated at 502 000 tonnes in 2012. Current and future demand have been impacted by the phasing-out of 1,1-dichloro-1-fluoroethane (HCFC-141b), which used significant quantities of vinylidene chloride as a precursor in its manufacture. The general population may be exposed from ambient air, indoor air, drinking-water, foods and beverages, and soil, with a maximum estimated total intake (in Canada) of less than 1.34 µg/kg body weight per day.

Small numbers of workers in the chemical industry are exposed to vinylidene chloride. Although exposure levels historically ranged up to about 8000 mg/m³, workers have been exposed to concentrations of less than 20 mg/m³ since the 1980s.

5.2 Human carcinogenicity data

A good-quality cohort study of mortality from cancer of the lung among workers in a plastics manufacturing plant in the USA found no association between cancer of the lung and exposure to vinylidene chloride. Two smaller occupational cohort studies had significant limitations and were considered uninformative.

5.3 Animal carcinogenicity data

Vinylidene chloride was tested for carcinogenicity in three different strains of male and/or female mice in four inhalation studies, one gavage study, one skin-application study, and one subcutaneous-injection study, and was tested as an initiator in one skin-application initiation–promotion study. Vinylidene chloride was tested in four different strains of male and female rats in six inhalation studies, three gavage studies, and one drinking-water study. The substance was also tested in male and female hamsters in one inhalation study.

In one well-conducted inhalation study conducted under good laboratory practice (GLP) conditions in mice, vinylidene chloride caused a significant increase in the incidence of, and positive trend in the incidence of, renal tubule adenoma, renal tubule carcinoma, and renal tubule adenoma or carcinoma (combined) in males, and a significant increase in the incidence of, and positive trend in the incidence of, hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) in females. In this same study in females, vinylidene chloride caused significant increases in the incidence of, and positive trend in the incidence of, liver haemangiosarcoma, and haemangioma or haemangiosarcoma (combined) of the vascular system, and a significant positive trend in the incidence of haemangiosarcoma of the vascular system. Vinylidene chloride also caused a significant increase in the incidence of, and positive trend in the incidence of, bronchioalveolar carcinoma of the lung in females.

In a second inhalation study in mice including two experiments, vinylidene chloride caused a significant increase in the incidence of pulmonary adenoma in males and females, adenocarcinoma of the kidney in males, and tumours (mostly carcinomas) of the mammary gland in females.

In one study in male and female mice exposed to vinylidene chloride by gavage, in which the maximum tolerated dose was not reached, vinylidene chloride caused a significant increase in the incidence of malignant lymphoma in females.

There was a significant increase in the incidence of skin papilloma in the initiation–promotion study in female mice. The skin-application and subcutaneous-injection studies in mice gave negative results. The two remaining inhalation studies in mice were inadequate for the evaluation of the carcinogenicity of vinylidene chloride.

In one well-conducted GLP inhalation study in rats, vinylidene chloride caused a significant increase in the incidence of, and positive trend in the incidence of, malignant mesothelioma, and a significant positive trend in the incidence of adenoma of the nasal respiratory epithelium in males. Vinylidene chloride caused a significant increase in the incidence of, and positive trend in the incidence of, thyroid C-cell adenoma and thyroid C-cell adenoma or carcinoma (combined), and a significant increase in the incidence of thyroid C-cell carcinoma in females. Vinylidene chloride also caused a significant increase in the incidence of, and positive trend in the incidence of, mononuclear cell leukaemia in females.

In another inhalation study in male and female rats, vinylidene chloride caused a significant increase in the incidence of leukaemia in females after in utero exposure followed by lifetime exposure.

Two inhalation studies, three gavage studies, and one drinking-water study in rats gave negative results. Two inhalation studies in rats were inadequate for the evaluation of the carcinogenicity of vinylidene chloride.

The inhalation study of vinylidene chloride in hamsters was inadequate for the evaluation of the carcinogenicity of vinylidene chloride.

5.4 Mechanistic and other relevant data

No data on the absorption, distribution, or excretion of vinylidene chloride were available from humans. Inhaled or ingested vinylidene chloride is rapidly and extensively absorbed from the lungs and gastrointestinal tract of rats, and is widely distributed to tissues. Vinylidene chloride is extensively oxidized in human liver microsomes and in rodents by cytochrome P450 (CYP) 2E1.

Three of the primary oxidation products (vinylidene chloride epoxide, 2-chloroacetyl chloride, and 2,2-dichloroacetaldehyde) are associated with covalent binding, GSH depletion, and cytotoxicity in the liver, kidney, and lung. Each of these metabolites is conjugated with GSH in the liver of rats. Variation in CYP2E1 expression is an important factor in tissue, species, and sex susceptibility to vinylidene chloride. Relative levels of GSH and epoxide hydrolase are also determinants of toxic response.

With regard to the key characteristics of carcinogens, there is *moderate* evidence that vinylidene chloride is genotoxic. No data were available in exposed humans. In experimental systems, vinylidene chloride was not genotoxic in the few available in vivo assays. Vinylidene chloride was mutagenic in tests conducted in vitro with an exogenous metabolic activation system in a closed environment to control for volatility. Positive responses were seen in mammalian cells for induction of gene mutations, chromosomal aberrations, sister-chromatid exchanges, and unscheduled DNA synthesis, and in assays for mutagenicity in bacteria.

Vinylidene chloride causes liver, lung (primarily in Clara cells in mice), and kidney toxicity in rats and mice after inhalation or oral exposure. The toxicity of vinylidene chloride is largely dependent on metabolism, that is, activation to electrophilic metabolites by CYP-mediated oxidation, detoxification of reactive metabolites

by GSH conjugation, and, in the kidney, activation by β -lyase.

There was inconsistency between the induction of toxicity and the occurrence of tumours in the liver, lung, and nose in the 2-year carcinogenicity bioassays. Specifically, (i) inflammation and degeneration in the liver was observed in male and female rats but liver tumour induction was detected in only female mice; and (ii) in the case of nasal respiratory epithelium toxicity, toxicity occurred in male and female rats but tumours were observed only in male rats.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

6.4 Rationale

A minority group opined that a higher classification for vinylidene chloride – *probably carcinogenic to humans* (Group 2A) – is warranted based on the similarity with vinyl chloride, which is classified as *carcinogenic to humans* (Group 1).

1. Vinyl chloride and vinylidene chloride are metabolized by CYP2E1 to electrophilic metabolites chloroethylene epoxide and vinylidene chloride epoxide, respectively.
2. There is robust evidence for the mutagenic activity for vinylidene chloride in studies *in vitro* that include exogenous metabolic activation systems. Negative *in vivo* genotoxicity studies of vinylidene chloride were primarily based on studies of rodent sperm, blood, and bone marrow cells, which are not targets of vinylidene chloride carcinogenicity. Chromosomal damage has been observed in several studies of mammalian cells incubated with vinylidene chloride. Further, vinylidene chloride was mutagenic in *Salmonella* when incubated with human S9 metabolic activation system. DNA damage by a vinylidene chloride metabolite has been observed in human lymphoblastic leukaemia cells.
3. Vinylidene chloride was carcinogenic at multiple organ sites in experimental animals, including the liver (hepatocytes and endothelial cells), kidney (renal tubule cell), lung, mammary gland, mesothelium, haematopoietic system, and thyroid (C-cell). There is similarity between two mesoderm-derived tumours of the endothelium (haemangiosarcoma) and mesoderm-derived mesothelium (malignant mesothelioma). Both the endothelium and mesothelium are single-cell layers and are derived from the mesodermal layer of the embryo, and both vinyl chloride and vinylidene chloride induce haemangiosarcoma.
4. Tumour induction by vinylidene chloride in rodents shows many similarities to that of vinyl chloride, that is, both compounds induced tumours of the lung, tumours of the mammary gland, and hepatic haemangiosarcomas in mice. The induction of hepatic haemangiosarcomas in mice has also been observed with other vinyl halides (vinyl fluoride and vinyl bromide) that are metabolized by CYP2E1 to DNA-reactive haloethylene oxide intermediates. Hepatic haemangiosarcomas are extremely rare in the general

population, but significantly elevated in workers exposed to vinyl chloride.

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LIST OF ABBREVIATIONS

8-OHdG	8-hydroxy-2'-deoxyguanosine
AAQC	Ambient Air Quality Criteria
ACGIH	American Conference of Governmental Industrial Hygienists
ADI	acceptable daily intake
BAT	biological tolerance value
BEI	biological exposure indices
bw	body weight
CalEPA	California Environmental Protection Agency
CFR	Code of Federal Regulations
CHO	Chinese hamster ovary
CI	confidence interval
CYP	cytochrome P450
DMBA	7,12-dimethylbenz[<i>a</i>]anthracene
EC	European Commission
ECD	electrolytic conductivity detection
ECHA	European Chemicals Agency
ECOD	ethoxycoumarin deethylase
ELISA	enzyme-linked immunosorbent assay
EPA	United States Environmental Protection Agency
ERPG	Emergency Response Planning Guideline
FAO	Food and Agriculture Organization of the United Nations
FTIR	Fourier transform infrared
GC	gas chromatography
GHB	gamma-hydroxybutyrate
GI	gastrointestinal
GLP	good laboratory practice
GSH	glutathione
GST	glutathione S-transferase
HCFC	hydrochlorofluorocarbon
IL-8	interleukin-8
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives

LC-MS/MS	liquid chromatography combined with tandem mass spectrometry
LOD	limit of detection
MDA	malondialdehyde
MSDI	maximized survey-derived daily intake
MTD	maximum tolerated dose
NIR	near infrared
NMR	nuclear magnetic resonance
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OSHA	Occupational Safety and Health Administration
PADI	possible average daily intake
PCNA	proliferating cell nuclear antigen
PDE	permitted daily exposure
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PTMEG	polytetramethylene ether glycol
PVC	polyvinyl chloride
PVDV	polyvinylidene chloride
ROS	reactive oxygen species
SCE	sister-chromatid exchange
SLRL	sex-linked recessive lethal
SMR	standardized mortality ratio
SOD	superoxide dismutase
STEL	short-term exposure limit
TDI	tolerable daily intake
TLV	threshold limit value
TPA	12-O-tetradecanoylphorbol-13-acetate
TWA	time-weighted average
USGS	United States Geological Survey
UV	ultraviolet
VOC	volatile organic chemical
vs	versus
WHO	World Health Organization
wt%	percentage by weight

ANNEX 1. SUPPLEMENTAL MATERIAL FOR TOXCAST/TOX21

This supplemental material (which is available online at: <http://publications.iarc.fr/575>) comprises a [spreadsheet](#) (.xlsx) analysed by the Working Group for Volume 119 of the *IARC Monographs*. The spreadsheet lists the ToxCast/Tox21 assay end-points, the associated target and/or model system (e.g. cell type, species, detection technology, etc.), their mapping to 7 of the 10 “key characteristics” of known human carcinogens, and the decision as to whether each chemical was “active” or “inactive” ([EPA, 2016a,b](#)).

References

- EPA (2016a). iCSS ToxCast Dashboard. Prod_dashboard_v2. Dashboard: v2. Available from: <http://actor.epa.gov/dashboard2/>, accessed 18 July 2018.
- EPA (2016b). Toxicity Forecaster (ToxCast) Data. Washington (DC), USA: United States Environmental Protection Agency. Available from: <http://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data>, accessed 18 July 2018.



This volume of the *IARC Monographs* provides evaluations of the carcinogenicity of: melamine, a chemical that is used to make plastic materials, including coatings, filters, adhesives, and kitchenware, and that has also been used illegally to adulterate foods and animal feeds; 1-*tert*-butoxypropan-2-ol, a solvent that is used as a substitute for other glycol ethers and in various consumer products; β -myrcene, which is found in a wide variety of plants and is used mainly as a raw material in the manufacture of chemicals such as menthol but also as a fragrance and flavouring substance; furfuryl alcohol, a chemical that is used as a solvent and in the production of furan resins and wetting agents, and that can also be formed in coffee and food during roasting, baking, or deep-frying; pyridine, a chemical that is used as a solvent or intermediate in the manufacture of pesticides, flavouring agents, vitamins, drugs, and dyes, and is also found in cigarette smoke; tetrahydrofuran, a chemical that is used as a solvent in plastics, dyes, elastomers, and glues, and is also used in the synthesis of motor fuels and in the manufacture of pharmaceuticals; and vinylidene chloride, a chemical that is used mainly in the production of copolymers for the manufacture of films for food packaging.

Exposure to all seven agents considered may occur in different occupational settings as well as in the general population.

An *IARC Monographs* Working Group reviewed epidemiological evidence, animal bioassays, and mechanistic and other relevant data to reach conclusions as to the carcinogenic hazard to humans of environmental or occupational exposure to these agents.

