ISOBUTYL NITRITE, β-PICOLINE, AND SOME ACRYLATES

VOLUME 122

IARC MONOGRAPHS
ON THE EVALUATION
OF CARCINOGENIC RISKS
TO HUMANS

ISOBUTYL NITRITE, β-PICOLINE, AND SOME ACRYLATES

VOLUME 122

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, 5–12 June 2018

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

IARC MONOGRAPHS

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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 *IARC Monographs* Group, 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 *IARC Monographs* Group, so that corrections can be reported in future volumes.

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[2] Charles William Jameson has been retained as an expert witness for plaintiffs in litigation concerning Roundup products and talc, neither of which are relevant to the agents under evaluation in *IARC Monographs* Volume 122.

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[3] Sandrine Charles attended as a Representative of the French Agency for Food, Environmental and Occupational Health and Safety (ANSES).

[4] Elodie Pasquier attended as a Representative of the French Agency for Food, Environmental and Occupational Health and Safety (ANSES).

[5] Each Observer agreed to respect the Guidelines for Observers at *IARC Monographs* meetings. Observers did not serve as Meeting Chair or Subgroup Chair, draft any part of a Monograph, or participate in the evaluations. They also agreed not to contact participants before the meeting, not to lobby them at any time, not to send them written materials, and not to offer them meals or other favours. IARC asked and reminded Working Group Members to report any contact or attempt to influence that they may have encountered, either before or during the meeting.

[6] Robert Ellis-Hutchings is currently employed by and holds stocks from the Dow Chemical Company, USA. He attended as an Observer for the Basic Acrylic Monomer Manufacturers, Inc., of which the Dow Chemical Company is a member.

[7] Lavorgie Finch is employed by Arkema Inc. He attended as an Observer for Arkema Inc.

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PREAMBLE

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

A. GENERAL PRINCIPLES AND PROCEDURES

1. Background

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

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

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

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

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

2. Objective and scope

The objective of the programme is to prepare, with the help of international Working Groups of experts, and to publish in the form of *Monographs*, critical reviews and evaluations of evidence on the carcinogenicity of a wide range of human exposures. The *Monographs* represent the first step in carcinogen risk assessment, which involves examination of all relevant information to assess the strength of the available evidence that an agent could alter the age-specific incidence of cancer in humans. The *Monographs* may also indicate where additional research efforts are needed, specifically when data immediately relevant to an evaluation are not available.

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

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

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

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

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

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

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

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

3. Selection of agents for review

Agents are selected for review on the basis of two main criteria: (a) there is evidence of human exposure and (b) there is some evidence or suspicion of carcinogenicity. Mixed exposures may occur in occupational and environmental settings and as a result of individual and cultural habits (such as tobacco smoking and dietary practices). Chemical analogues and compounds with biological or physical characteristics similar to those of suspected carcinogens may also be considered, even in the absence of data on a possible carcinogenic effect in humans or experimental animals.

The scientific literature is surveyed for published data relevant to an assessment of carcinogenicity. Ad hoc Advisory Groups convened by IARC in 1984, 1989, 1991, 1993, 1998 and 2003 made recommendations as to which agents should be evaluated in the *Monographs* series. Recent recommendations are available on the *Monographs* programme web site (http://monographs.iarc.fr). IARC may schedule other agents for review as it becomes aware of new scientific information or as national health agencies identify an urgent public health need related to cancer.

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

4.   Data for the *Monographs*

Each *Monograph* reviews all pertinent epidemiological studies and cancer bioassays in experimental animals. Those judged inadequate or irrelevant to the evaluation may be cited but not summarized. If a group of similar studies is not reviewed, the reasons are indicated.

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

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

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

Inclusion of a study does not imply acceptance of the adequacy of the study design or of the analysis and interpretation of the results, and limitations are clearly outlined in square brackets at the end of each study description (see Part B). The reasons for not giving further consideration to an individual study also are indicated in the square brackets.

5.   Meeting participants

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

(a)   The Working Group

The Working Group is responsible for the critical reviews and evaluations that are developed during the meeting. The tasks of Working Group Members are: (i) to ascertain that all appropriate data have been collected; (ii) to select the data relevant for the evaluation on the basis of scientific merit; (iii) to prepare accurate summaries of the data to enable the reader to follow the reasoning of the Working Group; (iv) to evaluate the results of epidemiological and experimental studies on cancer; (v) to evaluate data relevant to the understanding of mechanisms of carcinogenesis; and (vi) to make an overall evaluation of the carcinogenicity of the exposure to humans. Working Group Members generally have published significant research related to the carcinogenicity of the agents being reviewed, and IARC uses literature searches to identify most experts. Working Group Members are selected on the basis of (a) knowledge and experience and (b) absence of real or apparent conflicts of interests. Consideration is also given to demographic diversity and balance of scientific findings and views.

(b)   Invited Specialists

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

or subgroup chair, draft text that pertains to the description or interpretation of cancer data, or participate in the evaluations.

(c) Representatives of national and international health agencies

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

(d) Observers with relevant scientific credentials

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

(e) The IARC Secretariat

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

Before an invitation is extended, each potential participant, including the IARC Secretariat, completes the WHO Declaration of Interests to report financial interests, employment and consulting, and individual and institutional research support related to the subject of the meeting. IARC assesses these interests to determine whether there is a conflict that warrants some limitation on participation. The declarations are updated and reviewed again at the opening of the meeting. Interests related to the subject of the meeting are disclosed to the meeting participants and in the published volume (Cogliano et al., 2004).

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

All participants are listed, with their principal affiliations, at the beginning of each volume. Each participant who is a Member of a Working Group serves as an individual scientist and not as a representative of any organization, government or industry.

6. Working procedures

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

preliminary working papers for specific sections are expected to supplement the IARC literature searches with their own searches.

Industrial associations, labour unions and other knowledgeable organizations may be asked to provide input to the sections on production and use, although this involvement is not required as a general rule. Information on production and trade is obtained from governmental, trade and market research publications and, in some cases, by direct contact with industries. Separate production data on some agents may not be available for a variety of reasons (e.g. not collected or made public in all producing countries, production is small). Information on uses may be obtained from published sources but is often complemented by direct contact with manufacturers. Efforts are made to supplement this information with data from other national and international sources.

Six months before the meeting, the material obtained is sent to meeting participants to prepare preliminary working papers. The working papers are compiled by IARC staff and sent, before the meeting, to Working Group Members and Invited Specialists for review.

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

IARC Working Groups strive to achieve a consensus evaluation. Consensus reflects broad agreement among Working Group Members, but not necessarily unanimity. The chair may elect to poll Working Group Members to determine the diversity of scientific opinion on issues where consensus is not readily apparent.

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

B.   SCIENTIFIC REVIEW AND EVALUATION

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

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

Exposure data
Studies of cancer in humans

Studies of cancer in experimental animals

Mechanistic and other relevant data

Summary

Evaluation and rationale

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

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

1.   Exposure data

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

(a)   General information on the agent

For chemical agents, sections on chemical and physical data are included: the Chemical Abstracts Service Registry Number, the latest primary name and the IUPAC systematic name are recorded; other synonyms are given, but the list is not necessarily comprehensive. Information on chemical and physical properties that are relevant to identification, occurrence and biological activity is included. A description of technical products of chemicals includes trade names, relevant specifications and available information on composition and impurities. Some of the trade names given may be those of mixtures in which the agent being evaluated is only one of the ingredients.

For biological agents, taxonomy, structure and biology are described, and the degree of variability is indicated. Mode of replication, life cycle, target cells, persistence, latency, host response and clinical disease other than cancer are also presented.

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

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

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

(b)   Analysis and detection

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

(c)   Production and use

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

which may give rise to different impurities, are described.

The countries where companies report production of the agent, and the number of companies in each country, are identified. Available data on production, international trade and uses are obtained for representative regions. It should not, however, be inferred that those areas or nations are necessarily the sole or major sources or users of the agent. Some identified uses may not be current or major applications, and the coverage is not necessarily comprehensive. In the case of drugs, mention of their therapeutic uses does not necessarily represent current practice nor does it imply judgement as to their therapeutic efficacy.

(d)   Occurrence and exposure

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

Data that indicate the extent of past and present human exposure, the sources of exposure, the people most likely to be exposed and the factors that contribute to the exposure are reported. Information is presented on the range of human exposure, including occupational and environmental exposures. This includes relevant findings from both developed and developing countries. Some of these data are not distributed widely and may be available from government reports and other sources. In the case of mixtures, industries, occupations or processes, information is given about all agents known to be present. For processes, industries and occupations, a historical description is also given, noting variations in chemical composition, physical properties and levels of occupational exposure

with date and place. For biological agents, the epidemiology of infection is described.

(e)   Regulations and guidelines

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

2.   Studies of cancer in humans

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

(a)   Types of study considered

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

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

In correlation studies, the units of investigation are usually whole populations (e.g. in

particular geographical areas or at particular times), and cancer frequency is related to a summary measure of the exposure of the population to the agent under study. In correlation studies, individual exposure is not documented, which renders this kind of study more prone to confounding. In some circumstances, however, correlation studies may be more informative than analytical study designs (see, for example, the *Monograph* on arsenic in drinking-water; IARC, 2004).

In some instances, case reports and case series have provided important information about the carcinogenicity of an agent. These types of study generally arise from a suspicion, based on clinical experience, that the concurrence of two events — that is, a particular exposure and occurrence of a cancer — has happened rather more frequently than would be expected by chance. Case reports and case series usually lack complete ascertainment of cases in any population, definition or enumeration of the population at risk and estimation of the expected number of cases in the absence of exposure.

The uncertainties that surround the interpretation of case reports, case series and correlation studies make them inadequate, except in rare instances, to form the sole basis for inferring a causal relationship. When taken together with case–control and cohort studies, however, these types of study may add materially to the judgement that a causal relationship exists.

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

(b) Quality of studies considered

It is necessary to take into account the possible roles of bias, confounding and chance in the interpretation of epidemiological studies.

Bias is the effect of factors in study design or execution that lead erroneously to a stronger or weaker association than in fact exists between an agent and disease. Confounding is a form of bias that occurs when the relationship with disease is made to appear stronger or weaker than it truly is as a result of an association between the apparent causal factor and another factor that is associated with either an increase or decrease in the incidence of the disease. The role of chance is related to biological variability and the influence of sample size on the precision of estimates of effect.

In evaluating the extent to which these factors have been minimized in an individual study, consideration is given to several aspects of design and analysis as described in the report of the study. For example, when suspicion of carcinogenicity arises largely from a single small study, careful consideration is given when interpreting subsequent studies that included these data in an enlarged population. Most of these considerations apply equally to case–control, cohort and correlation studies. Lack of clarity of any of these aspects in the reporting of a study can decrease its credibility and the weight given to it in the final evaluation of the exposure.

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

Second, the authors should have taken into account — in the study design and analysis — other variables that can influence the risk of disease and may have been related to the exposure of interest. Potential confounding by such variables should have been dealt with either in the design of the study, such as by matching, or in the analysis, by statistical adjustment. In cohort studies, comparisons with local rates of disease may or may not be more appropriate than

those with national rates. Internal comparisons of frequency of disease among individuals at different levels of exposure are also desirable in cohort studies, since they minimize the potential for confounding related to the difference in risk factors between an external reference group and the study population.

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

Finally, the statistical methods used to obtain estimates of relative risk, absolute rates of cancer, confidence intervals and significance tests, and to adjust for confounding should have been clearly stated by the authors. These methods have been reviewed for case–control studies (Breslow & Day, 1980) and for cohort studies (Breslow & Day, 1987).

(c)   Meta-analyses and pooled analyses

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

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

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

(d)   Temporal effects

Detailed analyses of both relative and absolute risks in relation to temporal variables, such as age at first exposure, time since first exposure, duration of exposure, cumulative exposure, peak exposure (when appropriate) and

time since cessation of exposure, are reviewed and summarized when available. Analyses of temporal relationships may be useful in making causal inferences. In addition, such analyses may suggest whether a carcinogen acts early or late in the process of carcinogenesis, although, at best, they allow only indirect inferences about mechanisms of carcinogenesis.

(e)   Use of biomarkers in epidemiological studies

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

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

known phenotype of a genetic polymorphism can explain the carcinogenic mechanism of the agent being evaluated, data on this phenotype may be useful in making causal inferences.

(f)   Criteria for causality

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

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

Several scenarios may increase confidence in a causal relationship. On the one hand, an agent may be specific in causing tumours at one site or of one morphological type. On the other, carcinogenicity may be evident through the causation of

multiple tumour types. Temporality, precision of estimates of effect, biological plausibility and coherence of the overall database are considered. Data on biomarkers may be employed in an assessment of the biological plausibility of epidemiological observations.

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

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

3. Studies of cancer in experimental animals

All known human carcinogens that have been studied adequately for carcinogenicity in experimental animals have produced positive results in one or more animal species (Wilbourn et al., 1986; Tomatis et al., 1989). For several agents (e.g. aflatoxins, diethylstilbestrol, solar radiation, vinyl chloride), carcinogenicity in experimental animals was established or highly suspected before epidemiological studies confirmed their carcinogenicity in humans (Vainio et al., 1995). Although this association cannot establish that all agents that cause cancer in experimental animals also cause cancer in humans, it is biologically plausible that agents for which there is *sufficient evidence of carcinogenicity* in experimental animals (see Part B, Section 6b) also present a carcinogenic hazard to humans. Accordingly, in the absence of additional scientific information, these agents are considered to pose a carcinogenic hazard to humans. Examples of additional scientific information are data that demonstrate that a given agent causes cancer in animals through a species-specific mechanism that does not operate in humans or data that demonstrate that the mechanism in experimental animals also operates in humans (see Part B, Section 6).

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

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

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

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

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

(a) Qualitative aspects

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

Considerations of importance in the interpretation and evaluation of a particular study include: (i) how clearly the agent was defined and, in the case of mixtures, how adequately the sample characterization was reported; (ii) whether the dose was monitored adequately, particularly in inhalation experiments; (iii) whether the doses, duration of treatment and route of exposure were appropriate; (iv) whether the survival of treated animals was similar to that of controls; (v) whether there were adequate numbers of animals per group; (vi) whether both male and female animals were used; (vii) whether animals were allocated randomly to groups; (viii) whether the duration of observation was adequate; and (ix) whether the data were reported and analysed adequately.

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

should nevertheless be suspected of being carcinogenic and requires further investigation.

(b) Quantitative aspects

The probability that tumours will occur may depend on the species, sex, strain, genetic background and age of the animal, and on the dose, route, timing and duration of the exposure. Evidence of an increased incidence of neoplasms with increasing levels of exposure strengthens the inference of a causal association between the exposure and the development of neoplasms.

The form of the dose–response relationship can vary widely, depending on the particular agent under study and the target organ. Mechanisms such as induction of DNA damage or inhibition of repair, altered cell division and cell death rates and changes in intercellular communication are important determinants of dose–response relationships for some carcinogens. Since many chemicals require metabolic activation before being converted to their reactive intermediates, both metabolic and toxicokinetic aspects are important in determining the dose–response pattern. Saturation of steps such as absorption, activation, inactivation and elimination may produce nonlinearity in the dose–response relationship (Hoel et al., 1983; Gart et al., 1986), as could saturation of processes such as DNA repair. The dose–response relationship can also be affected by differences in survival among the treatment groups.

(c) Statistical analyses

Factors considered include the adequacy of the information given for each treatment group: (i) number of animals studied and number examined histologically, (ii) number of animals with a given tumour type and (iii) length of survival. The statistical methods used should be clearly stated and should be the generally accepted techniques refined for this purpose (Peto et al., 1980;

Gart et al., 1986; Portier & Bailer, 1989; Bieler & Williams, 1993). The choice of the most appropriate statistical method requires consideration of whether or not there are differences in survival among the treatment groups; for example, reduced survival because of non-tumour-related mortality can preclude the occurrence of tumours later in life. When detailed information on survival is not available, comparisons of the proportions of tumour-bearing animals among the effective number of animals (alive at the time the first tumour was discovered) can be useful when significant differences in survival occur before tumours appear. The lethality of the tumour also requires consideration: for rapidly fatal tumours, the time of death provides an indication of the time of tumour onset and can be assessed using life-table methods; non-fatal or incidental tumours that do not affect survival can be assessed using methods such as the Mantel-Haenzel test for changes in tumour prevalence. Because tumour lethality is often difficult to determine, methods such as the Poly-K test that do not require such information can also be used. When results are available on the number and size of tumours seen in experimental animals (e.g. papillomas on mouse skin, liver tumours observed through nuclear magnetic resonance tomography), other more complicated statistical procedures may be needed (Sherman et al., 1994; Dunson et al., 2003).

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

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

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

4. Mechanistic and other relevant data

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

These topics are not mutually exclusive; thus, the same studies may be discussed in more than one subsection. For example, a mutation in a gene that codes for an enzyme that metabolizes the agent under study could be discussed in the subsections on toxicokinetics, mechanisms and individual susceptibility if it also exists as an inherited polymorphism.

(a) Toxicokinetic data

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

(b) Data on mechanisms of carcinogenesis

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

can be divided into three non-exclusive levels as described below.

(i) Changes in physiology

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

(ii) Functional changes at the cellular level

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

(iii) Changes at the molecular level

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

The use of mechanistic data in the identification of a carcinogenic hazard is specific to the mechanism being addressed and is not readily described for every possible level and mechanism discussed above.

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

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

Positive results in tests using prokaryotes, lower eukaryotes, insects, plants and cultured mammalian cells suggest that genetic and related effects could occur in mammals. Results from such tests may also give information on the types of genetic effect produced and on the involvement of metabolic activation. Some end-points described are clearly genetic in nature (e.g. gene mutations), while others are associated with genetic effects (e.g. unscheduled DNA synthesis). In-vitro tests for tumour promotion, cell transformation and gap–junction intercellular communication may be sensitive to changes that are not necessarily the result of genetic alterations but that may have specific relevance to the process of carcinogenesis. Critical appraisals of these tests

have been published ([Montesano et al., 1986](#); [McGregor et al., 1999](#)).

Genetic or other activity manifest in humans and experimental mammals is regarded to be of greater relevance than that in other organisms. The demonstration that an agent can induce gene and chromosomal mutations in mammals in vivo indicates that it may have carcinogenic activity. Negative results in tests for mutagenicity in selected tissues from animals treated in vivo provide less weight, partly because they do not exclude the possibility of an effect in tissues other than those examined. Moreover, negative results in short-term tests with genetic end-points cannot be considered to provide evidence that rules out the carcinogenicity of agents that act through other mechanisms (e.g. receptor-mediated effects, cellular toxicity with regenerative cell division, peroxisome proliferation) ([Vainio et al., 1992](#)). Factors that may give misleading results in short-term tests have been discussed in detail elsewhere ([Montesano et al., 1986](#); [McGregor et al., 1999](#)).

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

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

For physical agents that are forms of radiation, other data relevant to carcinogenicity may include descriptions of damaging effects at the physiological, cellular and molecular level, as for chemical agents, and descriptions of how these effects occur. 'Physical agents' may also be considered to comprise foreign bodies, such as surgical implants of various kinds, and poorly soluble fibres, dusts and particles of various sizes, the pathogenic effects of which are a result of their physical presence in tissues or body cavities. Other relevant data for such materials may include characterization of cellular, tissue and physiological reactions to these materials and descriptions of pathological conditions other than neoplasia with which they may be associated.

(c) Other data relevant to mechanisms

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

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

plausible, but also have a consistent pattern of carcinogenic response across entire classes of related compounds.

(d) Susceptibility data

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

(e) Data on other adverse effects

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

5. Summary

This section is a summary of data presented in the preceding sections. Summaries can be found on the *Monographs* programme web site (http://monographs.iarc.fr).

(a) Exposure data

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

(b) Cancer in humans

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

(c) Cancer in experimental animals

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

(d) Mechanistic and other relevant data

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

6. Evaluation and rationale

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

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

These categories refer only to the strength of the evidence that an exposure is carcinogenic and not to the extent of its carcinogenic activity (potency). A classification may change as new information becomes available.

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

(a) Carcinogenicity in humans

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

Sufficient evidence of carcinogenicity:
The Working Group considers that a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence. A statement that there is *sufficient evidence* is followed by a separate sentence that identifies the target organ(s) or tissue(s) where an increased risk of cancer was observed in humans. Identification of a specific target organ or tissue does not preclude the possibility that the agent may cause cancer at other sites.

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

Inadequate evidence of carcinogenicity:
The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association between exposure and cancer, or no data on cancer in humans are available.

Evidence suggesting lack of carcinogenicity:
There are several adequate studies covering the full range of levels of exposure that humans are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent and any studied cancer at any observed level of exposure. The results from these studies alone or combined should have narrow confidence intervals with an upper limit close to the null value (e.g. a relative

risk of 1.0). Bias and confounding should be ruled out with reasonable confidence, and the studies should have an adequate length of follow-up. A conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the cancer sites, conditions and levels of exposure, and length of observation covered by the available studies. In addition, the possibility of a very small risk at the levels of exposure studied can never be excluded.

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

When the available epidemiological studies pertain to a mixture, process, occupation or industry, the Working Group seeks to identify the specific agent considered most likely to be responsible for any excess risk. The evaluation is focused as narrowly as the available data on exposure and other aspects permit.

(b) Carcinogenicity in experimental animals

Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the 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 preoplastic 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-twenty-second volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of isobutyl nitrite, β-picoline, methyl acrylate, ethyl acrylate, 2-ethylhexyl acrylate, and trimethylolpropane triacrylate.

Exposure measurements and biomonitoring studies have shown that workers and the general population are exposed to these agents. Three of these agents were evaluated previously in Volume 71 (methyl acrylate and ethyl acrylate) and in Volume 60 (2-ethylhexyl acrylate) of the *IARC Monographs* (IARC, 1994; 1999), when the Working Group evaluated methyl acrylate and 2-ethylhexyl acrylate as *not classifiable as to its carcinogenicity to humans* (Group 3) and ethyl acrylate as *possibly carcinogenic to humans* (Group 2B). Since the previous evaluations, new data have become available, primarily in experimental animals, and these data have been included and considered in the present volume. Epidemiological data were lacking for five of the agents and only one study was available for ethyl acrylate. A summary of the findings of this volume appears in *The Lancet Oncology* (Kromhout et al., 2018).

Chemicals with a high production volume

All four acrylates evaluated are "high production volume" chemicals. Sparse quantitative data were available to characterize exposure to most of these agents in the workplace or general population. Occupational exposure occurs primarily through inhalation and dermal contact during production and use as intermediates. Exposure of the general population occurs through food, consumer products (e.g. latex paints), and from materials (e.g. furniture and floor polishes) containing these agents.

Evaluation of data on the mechanisms of carcinogenesis

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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ISOBUTYL NITRITE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 542-56-3

IUPAC systematic name: 2-methylpropyl nitrite

Other names and abbreviations: IBN; iso-butyl nitrite; nitrous acid; isobutyl ester; nitrous acid; 2-methylpropyl ester

From [Royal Society of Chemistry (2018)](#).

1.1.2 Structural and molecular formulae, and relative molecular mass
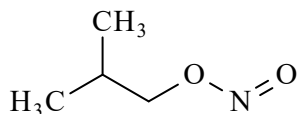
Molecular formula: $C_4H_9NO_2$
Relative molecular mass: 103.12

1.1.3 Chemical and physical properties

Description: colourless to pale yellow liquid

Stability: stable; flammable volatile liquid; gradually decomposes in water; incompatible with acids, alcohols, strong bases, and strong oxidizing agents

Boiling point: 66–67 °C (experimental)

Flash point: –21 °C (experimental)

Density: 0.87 g/mL (experimental)

Refractive index: 1.373

Relative density (water = 1): 0.87 g/cm³

Vapour pressure: 10 mm Hg [1.3 kPa] at 20 °C

Water solubility: slightly soluble and gradually decomposed by water: 935.9 mg/L, that is, < 1 mg/mL (estimated)

Conversion factor: 1 ppm = 4.22 mg/m³ (at 1 atm and 25 °C).

1.1.4 Technical products and impurities

Analysis of commercially available isobutyl nitrite revealed a purity of only 63%. The major impurity was isobutyl alcohol, formed as a result of degradation of the parent isobutyl nitrite ([Maickel, 1988](#)).

1.2 Production and use

1.2.1 Production process

Isobutyl nitrite is synthesized by reacting isobutyl alcohol with sodium nitrite in dilute sulfuric acid ([NTP, 1996](#)).

1.2.2 Production volume

No data on production volumes were available to the Working Group. Isobutyl nitrite is one of the alkyl nitrites, commonly known as

Table 1.1 Representative methods for the analysis of isobutyl nitrite

Sample matrix	Assay procedure	Limit of detection (μg/mL)	Reference
Blood and commercial liquids	GC-FID with headspace injection	0.05	Vogt et al. (2015)
Adulterated coffee drinks	GC-EI/MS	0.06	Bal et al. (1988); Seto et al. (2000)
Rat and human blood samples	GC-ECD	0.001	Kielbasa et al. (1999)
Human blood and urine	GC-FID with headspace injection, in addition to cryogenic oven trapping	0.01 for blood; 0.005 for urine	Watanabe-Suzuki et al. (2003)

ECD, electron capture detection; EI/MS, electron ionization mass spectrometry; FID, flame ionization detection; GC, gas chromatography

"poppers". The quantity of poppers ordered online from countries where they are legal, such as China, Poland, South Africa, and the United Kingdom, has recently been growing (GINAD, 2018).

1.2.3 Use

Isobutyl nitrite, like other poppers, is mainly used for its psychoactive effects; its vasodilator properties are experienced as a cerebral "rush" (Dixon et al., 1981). Poppers are illegal in many countries (e.g. Australia, Canada, and France); isobutyl nitrite and other poppers are therefore commonly marketed as air freshener or deodorizer in some clubs and head shops, and online (Jeon et al., 2016). Poppers have become popular recreational drugs among men who have sex with men since it is claimed that they prolong the sense of sexual excitement (Shesser et al., 1981).

In the 1970s in the USA, isobutyl nitrite and other poppers were widely marketed in discotheques and sex and drug paraphernalia shops under trade names such as "Rush", "Bolt", "Hardware", "Quick Silver", and "Satan's Scent". An average bottle contained 10–15 mL of liquid comprising about 90% volatile alkyl nitrites, together with small quantities of the corresponding alcohol and vegetable oil to reduce volatility (Shesser et al., 1981).

Other reported minor uses of isobutyl nitrite include as an intermediate in the synthesis of aliphatic nitrites, nail polish removers, video head cleaners, fuels, and jet propellants (NTP, 1996).

1.3 Measurement and analysis

A summary of analytical methods reported for isobutyl nitrite is provided in Table 1.1. As a volatile compound, the most reliable method for analysis of isobutyl nitrite is based on gas chromatography followed by flame ionization or electron capture detection. The limits of detection of the methods fall within the range 0.001–0.060 μg/mL.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

The Working Group did not identify any reports of involuntary population exposure caused by background environmental levels of isobutyl nitrite in outdoor air, water, dust, soil, or wildlife. This is mainly attributable to the usage profile and physicochemical properties of this chemical, especially its instability and rapid degradation in air and water (NTP, 1996; McLaughlin et al., 2007).

1.4.2 Exposure in the general population

Humans are exposed to isobutyl nitrite mainly through inhalation and, to a lesser extent, ingestion. Exposure occurs via intentional

administration of poppers liquids for recreational purposes. Upon inhalation, users experience transient euphoria, and enhanced sexual excitement and performance (Schwartz & Peary, 1986; Haverkos & Dougherty, 1988).

Poppers are popular among men who have sex with men, with 60% of this population group in Australia admitting to trying poppers (Krilis et al., 2013; Rewbury et al., 2017). Their use as a party drug is also increasing among heterosexual and younger people (Smith & Flatley, 2013), with about 1.1% of the general population in the UK reporting using poppers at least once per year; poppers are now the fourth most popular recreational drug after cannabis, cocaine, and ecstasy (Pebody, 2011). The use of poppers decreased substantially in the 1980s in the USA. For example, the proportion of high school seniors reporting ever having used nitrites declined from approximately 10% in the class of 1979 to less than 2% in the class of 1992. In the Multicenter AIDS Cohort Study, reports of popper use during the 6 months before interview in men who have sex with men decreased from approximately 66% in 1984 to approximately 35% in 1989. This reduction may be attributed to the decreased availability of poppers because of federal bans, and to increased awareness of the adverse effects of nitrites within this community (Haverkos & Drotman, 1996).

1.4.3 Occupational exposure

Occupational exposure may occur during manufacture; however, the Working Group found no information on occupational exposure to isobutyl nitrite.

1.5 Regulations and guidelines

An occupational exposure limit for isobutyl nitrite has been derived by the American Conference of Governmental Industrial Hygienists as a ceiling value of 1 ppm (ACGIH, 2017).

The same limit is in place in Belgium and Canada (Ontario) (IFA, 2018).

The use of isobutyl nitrite (in poppers) has been prohibited in the European Union since 2007 (European Union, 2006). In the UK, the Advisory Council on the Misuse of Drugs declared that alkyl nitrites (poppers) do not fall within the scope of the current definition of a "psychoactive substance" in the Psychoactive Substances Act 2016, and are therefore legal (ACMD, 2016). Poppers are illegal in the USA, but they have low priority for drug enforcement agencies (GINAD, 2018).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1

3.1 Mouse

Inhalation

Groups of 60 male and 60 female B6C3F$_1$ mice (age, 6 weeks) were exposed to isobutyl nitrite (purity, ≥ 97%; major impurity, isobutyl alcohol) by whole-body inhalation at 0 (controls), 37.5, 75, or 150 ppm, 6 hours per day (plus time to achieve 90% of the target concentration after the beginning of vapour generation, T$_{90}$, 10 minutes), 5 days per week for 103 weeks (NTP, 1996). A total of 7–10 males and 9–10 females from each group were evaluated at 15 months for alterations in haematology, histology, and clinical chemistry parameters. For the remaining rats, after 104 weeks (2 years), the survival of the exposed male mice was similar to that of controls, and body weights of exposed males were similar to those of controls. The survival rate of females at

Table 3.1 Studies of carcinogenicity with isobutyl nitrite in experimental animals

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start[a] No. of surviving animals	Incidence (%) of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6 wk 104 wk NTP (1996)	Inhalation (whole-body exposure) Isobutyl nitrite, ≥ 97% None 0 (control), 37.5, 75, 150 ppm, 6 h/d (+T$_{90}$ = 10 min), 5 d/wk, 103 wk 50, 50, 50, 53 37, 35, 35, 30	*Lung* Bronchioloalveolar adenoma 7/50* (14%), 12/50 (24%), 13/49 (27%), 17/53** (32%)	*P = 0.005 (trend), **P = 0.011; logistic regression test	Principal strengths: well-conducted GLP study Historical incidence (mean ± SD; range) for 2-yr inhalation studies with control groups for: bronchioloalveolar adenoma or carcinoma (combined), 170/773 (22.0 ± 8.7%; 10–42%); bronchioloalveolar carcinoma, 55/773 (7.1 ± 5.9%; 0–16%); thyroid follicular cell adenoma, 13/763 (1.7 ± 1.5%; 0–4%); thyroid follicular cell adenoma or carcinoma (combined), 13/763 (1.7 ± 1.5%; 0–4%); thyroid follicular cell carcinoma, 0/763
		Bronchioloalveolar adenoma (multiple) 0/50, 3/50 (6%), 3/49 (6%), 5/53* (9%)	*P ≤ 0.05, logistic regression test	
		Bronchioloalveolar carcinoma 1/50 (2%), 6/50 (12%), 5/49 (10%), 4/53 (8%)	NS	
		Bronchioloalveolar adenoma or carcinoma (combined) 8/50* (16%), 16/50 (32%), 16/49** (33%), 19/53*** (36%)	*P = 0.006 (trend), **P = 0.039, ***P = 0.008; logistic regression test	
		Alveolar epithelial hyperplasia 0/50, 4/50, 7/49*, 13/53*	*P ≤ 0.01, logistic regression test	
		Thyroid Follicular cell adenoma 1/50* (2%), 0/50, 0/50, 5/53 (9%)	*P = 0.004 (trend), logistic regression test	
		Follicular cell carcinoma 0/50, 1/50 (2%), 0/50, 0/53	NS	
		Follicular cell adenoma or carcinoma (combined) 1/50* (2%), 1/50 (2%), 0/50, 5/53 (9%)	*P = 0.011 (trend), logistic regression test	
		Follicular cell hyperplasia 8/50, 17/50*, 12/50, 20/53**	*P ≤ 0.05, **P ≤ 0.01; logistic regression test	

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[a] No. of surviving animals	Incidence (%) of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6 wk 104 wk NTP (1996)	Inhalation (whole-body exposure) Isobutyl nitrite, ≥ 97% None 0 (control), 37.5, 75, 150 ppm, 6 h/d (+T$_{90}$ = 10 min), 5 d/wk, 103 wk 51, 51, 50, 50 32, 42, 36, 37	*Lung* Bronchioloalveolar adenoma 4/51* (8%), 14/51** (27%), 7/50 (14%), 17/50*** (34%) Bronchioloalveolar carcinoma 2/51 (4%), 2/51 (4%), 2/50 (4%), 2/50 (4%) Bronchioloalveolar adenoma or carcinoma (combined) 6/51* (12%), 15/51 (29%), 9/50 (18%), 19/50** (38%) Bronchioloalveolar adenoma (multiple) 0/51, 2/51, 1/50, 2/50 Alveolar epithelial hyperplasia 0/51, 2/51, 9/50*, 8/50*	*P = 0.005 (trend), **P = 0.028, ***P = 0.002; logistic regression test NS *P = 0.005 (trend), **P = 0.003; logistic regression test NS *P ≤ 0.01, logistic regression test	Principal strengths: well-conducted GLP study Historical incidence (mean ± SD; range) for 2-yr inhalation studies with control groups for: bronchioloalveolar adenoma, 53/761 (7.0 ± 3.3%; 0–14%); bronchioloalveolar adenoma or carcinoma (combined), 75/761 (9.9 ± 3.7%; 0–16%); bronchioloalveolar carcinoma, 23/761 (3.0%; 0–6%)

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[a] No. of surviving animals	Incidence (%) of tumours	Significance	Comments
Full carcinogenicity Rat, F344 (M) 6 wk 104 wk NTP (1996)	Inhalation (whole-body exposure) Isobutyl nitrite, ≥ 97% None 0 (control), 37.5, 75, 150 ppm, 6 h/d (+T_{90} = 10 min), 5 d/wk, 103 wk 46, 46, 46, 46 17, 23, 36, 28	*Lung* Bronchioloalveolar adenoma 0/46*, 3/46 (7%), 12/46** (26%), 13/46*** (28%)	*$P < 0.001$ (trend), **$P = 0.003$, ***$P = 0.002$; logistic regression test	Principal strengths: well-conducted GLP study Historical incidence (mean ± SD; range) for 2-yr inhalation studies with control groups for bronchioloalveolar adenoma or carcinoma (combined), 22/493 (4.5 ± 3.8%; 0–10%)
		Bronchioloalveolar carcinoma 1/46* (2%), 2/46 (4%), 1/46 (2%), 6/46** (13%)	*$P = 0.015$ (trend), **$P = 0.040$; logistic regression test	
		Bronchioloalveolar adenoma or carcinoma (combined) 1/46* (2%), 5/46 (11%), 13/46** (28%), 15/46*** (33%)	*$P < 0.001$ (trend), **$P = 0.001$, ***$P < 0.001$; logistic regression test	
		Bronchioloalveolar adenoma (multiple) 0/46, 1/46 (2%), 0/46, 3/46 (7%)	NS	
		Alveolar epithelial hyperplasia 5/46, 8/46, 26/46*, 31/46*	*$P ≤ 0.01$, logistic regression test	

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[a] No. of surviving animals	Incidence (%) of tumours	Significance	Comments
Full carcinogenicity Rat, F344 (F) 6 wk 104 wk NTP (1996)	Inhalation (whole-body exposure) Isobutyl nitrite, ≥ 97% None 0 (control), 37.5, 75, 150 ppm, 6 h/d (+T_{90} = 10 min), 5 d/wk, 103 wk 46, 45, 46, 46 29, 35, 31, 33	*Lung* Bronchioloalveolar adenoma 0/46*, 2/45 (4%), 2/46 (4%), 10/46** (22%) Bronchioloalveolar carcinoma 0/46, 1/45, 0/46, 1/46 Bronchioloalveolar adenoma or carcinoma (combined) 0/46*, 3/45 (7%), 2/46 (4%), 11/46** (24%) Bronchioloalveolar adenoma (multiple) 0/46, 0/45, 0/46, 2/46 (4%) Alveolar epithelial hyperplasia 3/46, 10/45*, 11/46*, 30/46**	*$P < 0.001$ (trend), **$P = 0.001$; logistic regression test NS *$P < 0.001$ (trend), **$P < 0.001$; logistic regression test NS *$P \leq 0.05$, **$P \leq 0.01$; logistic regression test	Principal strengths: well-conducted GLP study Historical incidence (mean ± SD; range) for 2-yr inhalation studies with control groups for bronchioloalveolar adenoma or carcinoma (combined), 4/492 (0.8 ± 1.4%; 0–4%)

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

[a] ~10 animals per group were used for haematological testing

37.5 ppm was significantly greater than that of the control group, and the group exposed at the highest dose (150 ppm) had a lower body weight than controls. Necropsies were performed on all animals and all major organs were investigated by light microscopy.

A significantly increased incidence of bronchioloalveolar adenoma and of bronchioloalveolar adenoma or carcinoma (combined) of the lung was found in male and female exposed mice. In male mice, the incidence of bronchioloalveolar adenoma was increased in the group exposed at the highest dose with a significant positive trend ($P = 0.005$): the incidence was 7/50, 12/50, 13/49, and 17/53 ($P = 0.011$) for exposure at 0, 37.5, 75, and 150 ppm, respectively. There was also a significant increase in the incidence of bronchioloalveolar adenoma (multiple) in the group exposed at the highest dose (5/53 vs 0/50 for controls, $P \leq 0.05$). The incidence of bronchioloalveolar adenoma or carcinoma (combined) was also significantly increased (with a significant positive trend; $P = 0.006$) in the males exposed at the intermediate and highest doses: 8/50 (16%), 16/50 (32%), 16/49 (33%, $P = 0.039$), and 19/53 (36%, $P = 0.008$). In female mice, the incidence of bronchioloalveolar adenoma at 4/51, 14/51 ($P = 0.028$), 7/50, and 17/50 ($P = 0.002$) for exposures at 0, 37.5, 75, and 150 ppm, respectively, was significantly increased with a significant positive trend ($P = 0.005$). The incidence of bronchioloalveolar adenoma (multiple) was 0/51, 2/51, 1/50, and 2/50, respectively. The incidence of bronchioloalveolar adenoma or carcinoma (combined) was also significantly increased in the females exposed at the highest dose, with a significant positive trend ($P = 0.005$), with an incidence of 6/51 (12%), 15/51 (29%), 9/50 (18%), and 19/50 (38%, $P = 0.003$), respectively. In 2-year inhalation studies by the National Toxicology Program (NTP), the incidence of bronchioloalveolar adenoma or carcinoma (combined) in historical controls was 170/773 (22.0%; range, 10–42%) in male B6C3F$_1$ mice and 75/761 (9.9%;

range, 0–16%) in female B6C3F$_1$ mice. A significant increase in the incidence of alveolar epithelial hyperplasia was also found in male and female mice at the intermediate and highest doses.

[The Working Group noted that the incidences of lung bronchioloalveolar adenoma or carcinoma (combined) in males exposed at 75 ppm and in females exposed at 150 ppm were significantly increased compared with controls, and the incidences in all exposed groups of females exceeded the upper bound of the range for historical controls from 2-year NTP inhalation studies. In addition, alveolar epithelial hyperplasia occurred in all exposed groups of males and females (this lesion was absent in controls), and the incidence in males and females exposed at 75 and 150 ppm was significantly greater than that in the controls. The Working Group acknowledged that the increase in the incidence of bronchioloalveolar adenoma or carcinoma (combined) of the lung in female mice was mainly driven by the increase in the incidence of bronchioloalveolar adenoma: the incidence of the carcinoma was 2/51, 2/51, 2/50, and 2/50 (4%), respectively; the incidence of bronchioloalveolar carcinoma in historical controls in NTP inhalation studies was 23/761 (3.0%, range, 0–6%) in female B6C3F$_1$ mice. However, the Working Group considered the increased incidences of bronchioloalveolar adenoma and bronchioloalveolar adenoma or carcinoma (combined) in male and female mice to be related to treatment because of: (i) the strength of the statistical evidence; (ii) the increased multiplicity of bronchioloalveolar adenomas in exposed male and female mice; (iii) the comparison with the historical controls from NTP 2-year inhalation studies; and (iv) the increased incidence of alveolar epithelial hyperplasia in both sexes, supporting a continuum (the so-called adenoma–carcinoma sequence).]

In male mice, a significant positive trend ($P = 0.004$) in the incidence of follicular cell adenoma of the thyroid was found, with an

incidence of 1/50 (2%), 0/50, 0/50, and 5/53 (9%) for exposure at 0, 37.5, 75, and 150 ppm, respectively. One male mouse exposed at the lowest dose developed a follicular cell carcinoma of the thyroid. There was a significant positive trend ($P = 0.011$) in the incidence of follicular cell adenoma or carcinoma (combined) of the thyroid, with incidence of 1/50 (2%), 1/50 (2%), 0/50, and 5/53 (9%), respectively. In male mice, the incidence of follicular cell hyperplasia of the thyroid was 8/50, 17/50 ($P \leq 0.05$), 12/50, and 20/53 ($P \leq 0.01$). There was no hepatomegaly in treated male mice. In historical controls in NTP 2-year inhalation studies, the incidence of follicular cell adenoma or carcinoma (combined) of the thyroid in male mice was 13/763 (1.7%; range, 0–4%); no follicular cell carcinoma of the thyroid was observed in 763 male historical controls. [Follicular cell adenoma of the thyroid occurred with a significant positive trend in male mice, and the incidence in males exposed at 150 ppm was marginally (non-significantly) greater than that in the controls (1/50 at 0 ppm vs 5/53 at 150 ppm). Follicular cell neoplasms of the thyroid are relatively uncommon in male mice, as demonstrated by the rate in NTP historical controls. In the present study, the increase in the incidence of follicular cell adenoma or carcinoma (combined) of the thyroid was accompanied by an increase in the incidence of follicular cell hyperplasia of the thyroid. Considering the rarity of these neoplasms in male mice and the increased incidence of follicular cell hyperplasia of the thyroid in exposed males, the increased incidence of follicular cell adenoma or carcinoma (combined) of the thyroid may have been related to exposure to isobutyl nitrite. [The Working Group noted that this was a well-conducted study that complied with good laboratory practice.]

3.2 Rat

Inhalation

Groups of 56 male and 56 female Fischer 344 rats (age, 6 weeks) were exposed to isobutyl nitrite (purity, $\geq$ 97%; major impurity, isobutyl alcohol) by whole-body inhalation at 0 (controls), 37.5, 75, or 150 ppm, 6 hours per day (plus T_{90}, 10 minutes), 5 days per week for 103 weeks (NTP, 1996). A total of 10 males and 10 females from each group were evaluated at 15 months for alterations in haematology, histology, and clinical chemistry parameters. For the remaining rats, the survival rates of males exposed at 75 and 150 ppm were significantly greater than those of controls. The body weights of male and female rats exposed at 150 ppm were lower than those of the controls. Necropsies were performed on all animals and all major organs were investigated by light microscopy.

A significantly increased incidence of bronchioloalveolar adenoma, bronchioloalveolar carcinoma, and of bronchioloalveolar adenoma or carcinoma (combined) of the lung was found in exposed male rats. In male rats, the incidence of bronchioloalveolar adenoma was increased for the groups exposed at the intermediate and highest doses, with a significant positive trend ($P < 0.001$); the incidence was 0/46, 3/46 (6%), 12/46 (26%, $P = 0.003$), and 13/46 (28%, $P = 0.002$) for exposure at 0, 37.5, 75, and 150 ppm, respectively. The incidence of bronchioloalveolar carcinoma – 1/46 (2%), 2/46 (4%), 1/46 (2%), and 6/46 (13%, $P = 0.040$) – was increased in the group exposed at the highest dose, with a significant positive trend ($P = 0.015$). The respective incidence of bronchioloalveolar adenoma or carcinoma (combined) was 1/46 (2%, P for trend, < 0.001), 5/46 (11%), 13/46 (28%, $P = 0.001$), and 15/46 (33%, $P < 0.001$).

In female rats, the incidence of bronchioloalveolar adenoma – 0/46, 2/45, 2/46, and 10/46 ($P = 0.001$) – was significantly increased in the

group exposed at the highest dose, with a significant positive trend ($P < 0.001$). The incidence of bronchioloalveolar carcinoma was 0/46, 1/45, 0/46, and 1/46, respectively. The incidence of bronchioloalveolar adenoma or carcinoma (combined) was also significantly increased with a significant positive trend ($P < 0.001$); incidence was 0/46, 3/45 (7%), 2/46 (4%), and 11/46 (24%, $P < 0.001$) for exposure at 0, 37.5, 75, and 150 ppm, respectively. For historical controls in NTP 2-year inhalation studies, the incidence of bronchioloalveolar adenoma or carcinoma (combined) was 22/493 (4.5%; range, 0–10%) in males and 4/492 (0.8%; range, 0–4%) in females.

For preneoplastic lesions, there was a significant increase in the incidence of alveolar epithelial hyperplasia in male rats exposed at 75 and 150 ppm, and in female rats exposed at all concentrations.

[The Working Group concluded that the increased incidence of bronchioloalveolar adenoma and of adenoma or carcinoma (combined) in exposed male and female rats, and of bronchioloalveolar carcinoma in male rats, was related to treatment. The incidence of bronchioloalveolar adenoma or carcinoma (combined) in female rats exposed at 37.5 ppm (7%), male rats exposed at 75 ppm (28%), and male (33%) and female rats (24%) exposed at 150 ppm were clearly not within the NTP historical range for control animals. An increased incidence of alveolar epithelial hyperplasia was also observed in all exposed groups of male and female rats (except in females exposed at 75 ppm, and only significant in males exposed at 150 ppm) at the 15-month interim evaluation, and in all exposed groups of male and female rats (all significant with the exception of males exposed at 37.5 ppm) in the 2-year study. The occurrence of alveolar epithelial hyperplasia and the increased incidences of lung epithelial neoplasms in an apparent continuum (the so-called adenoma–carcinoma sequence), along with a non-significant increase in the number of rats with multiple adenomas supporting this continuum, were considered by the Working Group as evidence of carcinogenic activity in male and female rats.]

[The Working Group noted that this was a well-conducted study that complied with good laboratory practice.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Data on the absorption, distribution, and excretion of isobutyl nitrite in humans were not available to the Working Group; however, oral or inhalation exposure to the compound induces methaemoglobinaemia (see Section 4.3.1) and has vasodilating effects, indicating that absorption occurs in humans. The degradation of isobutyl nitrite in human blood at 37 °C in vitro has been reported to follow first-order kinetics, with a half-life (1.2 ± 0.2 minutes) comparable to that obtained in rat blood, but the products were not characterized in this study (Kielbasa et al., 1999).

Isobutyl nitrite is generally regarded to undergo hydrolytic decomposition in vivo (Fig. 4.1), yielding nitrite and isobutyl alcohol (NTP, 1996). Watanabe-Suzuki et al. (2003) confirmed the presence of isobutyl alcohol in the blood of three men who inhaled isobutyl nitrite for 2 minutes. Isobutyl alcohol concentrations within the range 0.35–0.75 µg/mL were observed at time zero, and declined to 0.06–0.10 µg/mL after 10 minutes. Isobutyl nitrite was not detected in any of the blood samples. The formation of isobutyl alcohol from isobutyl nitrite was rapid in vitro (< 10 minutes) in human urine and

Fig. 4.1 Proposed metabolic pathways of isobutyl nitrite, accounting for the species detected in human and/or animal models
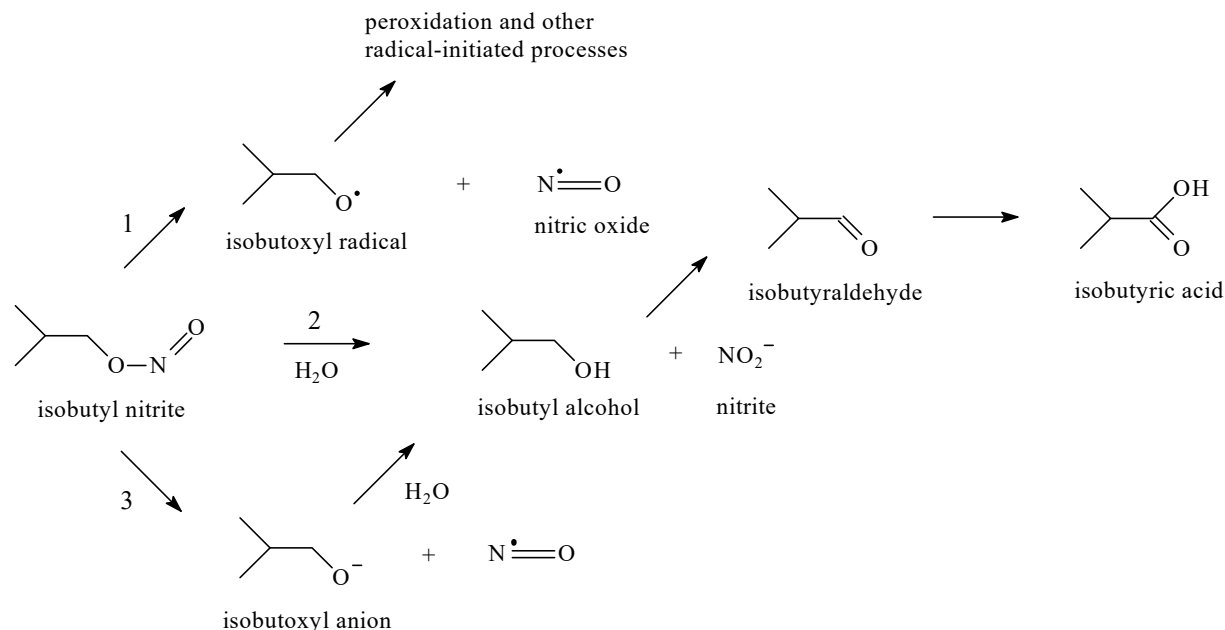
1. Spontaneous homolytic cleavage of the N–O bond; 2. Hydrolysis; 3. Enzymatic reduction
Compiled by the Working Group

whole-blood matrices spiked with isobutyl nitrite (10 nmol/mL).

A study in humans demonstrated that isobutyl alcohol is metabolized to isobutyraldehyde and isobutyric acid in vivo (Rüdell et al., 1983). This is consistent with the demonstration that alcohol and aldehyde dehydrogenase enzymes from human liver mediate the conversion of isobutyl alcohol to isobutyraldehyde and isobutyric acid in vitro (Ehrig et al., 1988).

4.1.2 Experimental systems

In male Sprague-Dawley rats exposed to isobutyl nitrite at 900 ppm by inhalation for 45 minutes, there was a rapid systemic absorption and elimination of the compound; steady-state concentrations (~290 ng/mL) were reached within 15 minutes, and declined monoexponentially with a half-life of 1.4 ± 0.2 minutes upon cessation of the exposure (Kielbasa et al., 1999).

Shorter half-lives, consistent with enzymatic degradation, were observed in biological fluids (rat whole blood and rat plasma) compared with phosphate buffer (Kielbasa et al., 1999).

The pharmacokinetics of isobutyl nitrite and its primary metabolite, isobutyl alcohol, were investigated more completely in male Sprague-Dawley rats after inhalation and intravenous infusion (Kielbasa & Fung, 2000a). The pharmacokinetic parameters of isobutyl nitrite appeared invariable over time; regardless of the rate of infusion, the half-life and volume of distribution were determined to be 1.3 ± 0.2 minutes and 5.8 ± 0.4 L/kg, respectively. After the intravenous infusion, the systemic clearance of isobutyl nitrite in rats was 3.0 ± 0.3 L/kg per minute. Isobutyl nitrite was almost completely metabolized to isobutyl alcohol (98% conversion), the concentration of which declined monoexponentially with a half-life of 5.3 minutes upon termination

of the infusion. A similar half-life was found for isobutyl alcohol when given by intravenous bolus. Urinary excretion of isobutyl alcohol was very low ($0.49 \pm 0.01\%$ of the administered dose after an intravenous bolus at 50 mg/kg), and no evidence of glucuronide or sulfate conjugates was found. [The Working Group noted that oxidation to isobutyraldehyde and isobutyric acid may have occurred faster than phase II conjugation.] The bioavailability of isobutyl nitrite upon inhalation was estimated to be 43%, suggesting that a first-pass effect may occur in the lung. The pharmacokinetics of isobutyl nitrite appeared to be independent of the route of administration; in contrast, compared with intravenous exposure, the half-life of isobutyl alcohol decreased by approximately four times after inhalation of isobutyl nitrite (from 5.3 min to 1.5 min, $P < 0.001$). The change in the disposition of isobutyl alcohol might be related to release of nitric oxide from isobutyl nitrite, with ensuing alteration of the blood flow to the lung due to relaxation of smooth muscle (Kielbasa & Fung, 2000a).

In male Sprague-Dawley rats, apparent steady-state blood levels were achieved during exposure and were proportional to exposure concentration, from 0.05 ± 0.03 µM at 23 ppm to 3.53 ± 0.35 µM at 1177 ppm (Kielbasa & Fung, 2000b).

Isobutyl nitrite was extensively metabolized to isobutyl alcohol when male Sprague-Dawley rats were exposed by inhalation or intravenous infusion (Kielbasa & Fung, 2000a). When given by intravenous infusion to New Zealand White rabbits of both sexes, isobutyl nitrite generated dose-dependent increments of nitric oxide in exhaled air that were correlated with dose-dependent decreases in systemic blood pressure (Cederqvist et al., 1994).

It is generally assumed that hydrolytic cleavage of isobutyl nitrite generates nitrite and isobutyl alcohol, whereas homolytic cleavage yields the nitric oxide and isobutoxyl radicals.

Although the reactive nitric oxide is associated with the vasodilating effect, the isobutoxyl radical may initiate peroxidation reactions (NTP, 1996). Consistent with this mechanism, it has been demonstrated that isobutyl nitrite, diluted in air at concentrations of up to 900 ppm, undergoes spontaneous decomposition under normal room light, generating nitric oxide at approximately 115 ppm (Soderberg et al., 2000).

As an alternative to homolytic cleavage, nitric oxide production from isobutyl nitrite may stem from metabolic reduction. [The Working Group noted that the reductive process will presumably also produce the isobutoxyl anion, which will be readily protonated to isobutyl alcohol.] Bovine vascular subfractions had significant catalytic activity for generation of nitric oxide, which was inhibited by heating and irradiation, consistent with enzymatic conversion to nitric oxide in vascular smooth muscle. Moreover, the major generation of nitric oxide was associated with the cytosol, and a minor and distinct activity generating nitric oxide was identified in the microsomal fraction (Kowaluk & Fung, 1991). In a later study, xanthine oxidase from bovine milk catalysed the reduction of isobutyl nitrite to nitric oxide in vitro in the presence of xanthine under anaerobic conditions; in a process following Michaelis–Menten kinetics, the production of nitric oxide compared with that of urates had a molar ratio of 2:1 (Doel et al., 2000).

4.1.3 Modulation of metabolic enzymes

After a single exposure of adult male BALB/c mice to isobutyl nitrite at 900 ppm by inhalation for 45 minutes, a significant reduction in the hepatic activities of cytochrome P450-mediated 3-cyano-7-ethoxycoumarin deethylation (81.5%), glutathione S-transferase (GST; 74.7%), and carboxylesterase (25.2%) (Turowski et al., 2007). Under the same conditions, C57BL/6 mice had corresponding, although smaller, decreases in these hepatic enzyme activities. When assessed

in C57BL/6 mice, the enzyme activities returned to control levels 24 hours after exposure. Similar decreases in hepatic enzyme activities also occurred after repeated exposure of C57BL/6 mice to isobutyl nitrite at 900 ppm for 45 minutes per day for 6 days. A follow-up mechanistic investigation in vitro, using purified rat liver GST, demonstrated that a 10-second exposure to isobutyl nitrite at 22 mM (but not to sodium nitrite at 22 mM) caused an immediate decrease in GST activity that further intensified, but not linearly, over a longer exposure (60 minutes). The addition of glutathione at 5 mM before exposure to isobutyl nitrite prevented GST inactivation, regardless of exposure time (10 seconds or 60 minutes). In contrast, GST inactivation could not be reversed by glutathione addition after exposure to isobutyl nitrite, which indicated irreversible protein oxidation. Comparative experiments investigating the exposure of GST to different nitric oxide donors indicated that GST inactivation by isobutyl nitrite was not associated with *S*-nitrosylation of the protein or disulfide formation, but rather with tyrosine nitration (Turowski et al., 2007).

In an earlier study, a rat homologue of human γ-glutamyltranspeptidase-related enzyme, which cleaves the γ-glutamyl peptide bond of glutathione, was found to be highly expressed in lung tumours during the inhalation of isobutyl nitrite at 75 or 150 ppm for 6 hours per day, 5 days per week for 2 years (NTP, 1996). Elevated expression of the human γ-glutamyltranspeptidase-related enzyme was also found in normal lung tissue from an animal exposed to isobutyl nitrite compared with a normal unexposed lung (Potdar et al., 1997).

4.2 Mechanisms of carcinogenesis

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016) in the following order: is genotoxic; induces chronic inflammation; is immunosuppressive;

alters cell proliferation, cell death, or nutrient supply; and multiple characteristics (e.g. microarrays). Insufficient data were available for evaluation of the other key characteristics of carcinogens.

4.2.1 Genetic and related effects

Isobutyl nitrite has been evaluated for genotoxicity and related potential in a variety of assays. Table 4.1, Table 4.2, and Table 4.3 summarize the studies considered the most representative of the genetic and related effects of isobutyl nitrite.

(a) Humans

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

In vitro, the ability of isobutyl nitrite to induce nuclear DNA damage was assessed in primary cultures of human lung cells using the comet assay (Robbiano et al., 2006). The cells were obtained from apparently healthy areas of lung fragments discarded during surgery for pulmonary carcinoma or adenocarcinoma. Isobutyl nitrite at 3.90–31.25 µM (purity, 95%) did not induce DNA damage (tail length and tail moment) in lung cells from two male donors (one former smoker and one smoker), whereas a dose-dependent increase was observed in one male donor (former smoker).

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.1

Male Sprague-Dawley rats given a single dose of isobutyl nitrite, corresponding to half the median lethal dose (LD_{50}), by gastric intubation had statistically significant increased DNA damage in the lung but not in the liver or kidney, as measured by the comet assay (Robbiano et al., 2006).

Isobutyl nitrite was tested using test for micronucleus formation in samples of peripheral blood from male and female B6C3F$_1$ mice exposed by

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

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Micronucleus formation	Mouse, B6C3F$_1$ (M, F)	Peripheral blood; normochromatic erythrocytes	+	150 ppm (M), 75 ppm (F)	Inhalation, 6 h/d, 5 d/wk for 13 wk	Purity, ≥ 93%	NTP (1996)
DNA strand breaks	Rat, Sprague-Dawley (M)	Lung	+	606 mg/kg	Gastric intubation, single dose in olive oil at 0.01 mL/g bw	Purity, 95%	Robbiano et al. (2006)
DNA strand breaks	Rat, Sprague-Dawley (M)	Liver and kidney	−	606 mg/kg	Gastric intubation, single dose in olive oil at 0.01 mL/g bw	Purity, 95%	Robbiano et al. (2006)

bw, body weight; d, day; F, female; h, hour; HID, highest ineffective dose; LED, lowest effective dose; M, male; ppm, parts per million; wk, week
[a] +, positive; −, negative; the level of significance was set at $P < 0.05$ in all cases

Table 4.2 Genetic and related effects of isobutyl nitrite 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	Rat, Sprague-Dawley, lung	+	NT	7.8 µM	Purity, 95%	Robbiano et al. (2006)
Mutation	Mouse, lymphoma L5178Y	+	+	75.9 µM	Purity, NR	Dunkel et al. (1989)
Sister-chromatid exchange	Chinese hamster ovary	+	+	50 µg/mL (− S9); 160 µg/mL (+ S9)	Purity, ≥ 93%	NTP (1996)
Chromosomal aberrations	Chinese hamster ovary	+	+/−	16 µg/mL (− S9); 1081 µg/mL (+ S9)	Purity, ≥ 93%	NTP (1996)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; S9, 9000 × g supernatant from rat liver
[a] +, positive; +/−, equivocal (variable response in several experiments within an adequate study); the level of significance was set at $P < 0.05$ in all cases

Table 4.3 Genetic and related effects of isobutyl nitrite in non-mammalian experimental systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Drosophila melanogaster Canton-S wildtype (M)	Sex-linked recessive lethal mutations	–	NA	25 000 ppm by injection; 100 000 ppm by feeding	Purity, ≥ 93%	Woodruff et al. (1985)
Salmonella typhimurium TA1535	Reverse mutation	+	+	1000 μg/plate	Purity, NR	Quinto (1980)
Salmonella typhimurium TA100, TA1535	Reverse mutation	+/–	+	6666 μg/plate	Purity, 92.7%	Mortelmans et al. (1986)
Salmonella typhimurium TA1537	Reverse mutation	–	–	1000 μg/plate	Purity, NR	Quinto (1980)
Salmonella typhimurium TA98, TA1537	Reverse mutation	–	–	10 000 μg/plate	Purity, 92.7%	Mortelmans et al. (1986)
Salmonella typhimurium TA100, TA1535	Reverse mutation	+	+	3333 μg/plate	Purity, NR	Dunkel et al. (1989)
Salmonella typhimurium TA98, TA1537	Reverse mutation	–	–	10 000 μg/plate	Purity, NR	Dunkel et al. (1989)
Salmonella typhimurium TA1535	Reverse mutation	+	+	Saturated vapour, 190 μg/mL	Purity, NR	Mirvish et al. (1993)
Salmonella typhimurium TA100	Reverse mutation	NT	+/–	Saturated vapour, 190 μg/mL	Purity, NR	Mirvish et al. (1993)
Salmonella typhimurium TA98	Reverse mutation	–	–	Saturated vapour, 190 μg/mL	Purity, NR	Mirvish et al. (1993)
Salmonella typhimurium TA1535	Reverse mutation	+	+	260 μg saturated solution (2.6 mg/mL)	Purity, NR	Mirvish et al. (1993)
Salmonella typhimurium TA98	Reverse mutation	–	–	10 000 μg/plate	Purity, ≥ 93%	NTP (1996)
Salmonella typhimurium TA100	Reverse mutation	+/–	+	6666 μg/plate	Purity, ≥ 93%	NTP (1996)
Salmonella typhimurium TA1535	Reverse mutation	NT	+	1000 μg/plate	Purity, ≥ 93%	NTP (1996)
Salmonella typhimurium TA98, TA1537, TA7001, TA7002, TA7003, TA7004, TA7005, TA7006, Mix TA7001–7006	Reverse mutation	–	–	1000 μg/mL	Purity, NR	Gee et al. (1998)
Salmonella typhimurium FU100	Forward mutation	+	+	28 μg/mL (without metabolic activation); 500 μg/mL (with metabolic activation)	Purity, NR	Miller et al. (2005)

HIC, highest ineffective concentration; LEC, lowest effective concentration; M, male; NA, not applicable; NR, not reported; NT, not tested; ppm, parts per million

[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

inhalation. Males and females displayed a significantly increased frequency of micronucleated normochromatic erythrocytes, with females being more sensitive (NTP, 1996).

(ii)   Non-human mammalian cells in vitro

See Table 4.2

In primary lung cells from male Sprague-Dawley rats exposed to isobutyl nitrite at concentrations of 7.8–31.2 µM for 20 hours, DNA fragmentation, as measured by tail length and tail moment using the comet assay, was significantly increased by a dose-dependent amount (Robbiano et al., 2006).

Isobutyl nitrite was tested in the L5178Y $Tk^{+/-}$ mouse lymphoma assay at concentrations of up to 1.5 mM in the absence and presence of exogenous metabolic activation. Dose-dependent increases in mutant frequency were observed, both with and without S9. The presence of S9 reduced the toxicity of the compound by approximately one order of magnitude; however, the mutation frequency remained the same at comparable toxicity levels, with and without metabolic activation (Dunkel et al., 1989).

Isobutyl nitrite was also tested for sister-chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells using standard protocols. The results were positive for sister-chromatid exchanges in the absence and presence of rat liver S9, and positive for chromosomal aberrations in the absence of exogenous metabolic activation. In contrast, mixed results were obtained for chromosomal aberrations in the presence of S9 (two trials; the results were negative in one and weakly positive in another). The lowest effective concentrations for a positive response with both end-points were higher in the presence of S9 (NTP, 1996).

(iii)   Non-mammalian experimental systems

See Table 4.3

When tested in vivo, isobutyl nitrite did not induce sex-linked recessive lethal mutations in the germ cells of Canton-S wildtype *Drosophila melanogaster* males exposed via feeding (100 000 ppm) or injection (25 000 ppm) (Woodruff et al., 1985).

Several studies have demonstrated mutagenic activity with isobutyl nitrite in the standard *Salmonella* assay when using tester strains sensitive to base-pair substitution mutations, such as TA100 and TA1535. Although the results in the absence of S9 activation were equivocal in some studies and positive in other reports, consistently positive results were obtained with the same strains in the presence of exogenous metabolic activation. Isobutyl nitrite was mutagenic in TA1535 in the absence and presence of S9 activation (Quinto, 1980). In contrast, isobutyl nitrite gave negative results in tester strains sensitive to frameshift mutations, such as TA97, TA98, and TA1537, both in the absence and presence of S9 activation (Quinto, 1980; Mortelmans et al., 1986; Dunkel et al., 1989; Mirvish et al., 1993; NTP, 1996). The saturated vapour was 11-fold more mutagenic in strain TA1535 than a saturated solution, a difference that was attributed to continuous replenishment of the hydrolysis-prone test compound by the vapour (Mirvish et al., 1993). The same study demonstrated that isobutyl nitrite in solution was more mutagenic by about 2.8-fold in TA1535 than sodium nitrite, suggesting that the compound is mutagenic per se and not as a result of hydrolytic conversion to nitrite. The other hydrolysis product, isobutyl alcohol, gave negative results in the same experiment.

A subsequent study used a modified, partially automated, liquid protocol and either individual *Salmonella his⁻* tester strains of the TA7000 series, each reverting by a specific base substitution mutation, or a mix of these strains that detected missense mutations. In contrast to the standard assays, isobutyl nitrite gave negative results in this protocol, both in the absence and presence of exogenous metabolic activation (Gee et al., 1998). More recently, isobutyl nitrite

gave positive results, both in the presence and absence of S9, in an assay for forward mutation in FU100, a *S. typhimurium* strain derived from TA100 and displaying resistance to 5-fluorouridine. The lowest effective concentration was about 18 times lower in the absence of S9 (Miller et al., 2005). [The Working Group noted that the TA7000 and the FU100 studies both used isobutyl nitrite in liquid suspension, and hydrolysis would be expected. The positive results with FU100 suggest that the assay for forward mutation may be more sensitive.]

4.2.2 Chronic inflammation

(a) Humans

No data for humans were available to the Working Group. Isobutyl nitrite is a known irritant (see Section 4.3).

(b) Experimental systems

Inhalation of isobutyl nitrite resulted in inflammatory changes in male and female Fischer 344/N rats and B6C3F$_1$ mice exposed at concentrations of up to 300 ppm for 13 weeks, 6 hours per day, 5 days per week (Gaworski et al., 1992; NTP, 1996).

Kielbasa & Fung (2000c) evaluated tissue levels and phosphorylation of nitric oxide synthase (NOS) enzymes in rat kidney, liver, lung, and spleen after a single exposure to isobutyl nitrite at either 109 or 1517 ppm by inhalation for 4 hours. Increased expression of inducible NOS, nitrotyrosine, and phosphotyrosine immunoreactive proteins were observed in the liver and kidney of rats exposed at 1517 ppm, but not in the lung or spleen. These data contrast with those of Soderberg et al. (1996a), who showed that alveolar macrophages from mice exposed to isobutyl nitrite at 900 ppm demonstrated elevated inducible NOS production after inhalation for 45 minutes per day for 14 days.

Soderberg & Ponnappan (2002) examined the formation of nitrotyrosine in the murine macrophage cell line RAW 267.4 and in peritoneal macrophages obtained from C57BL/6 mice exposed in vivo to isobutyl nitrite at 900 ppm for 45 minutes per day for 5 days. Inhibition of mitochondrial respiration was only observed in cultured RAW cells at isobutyl nitrite concentrations that induced significant cytotoxicity (> 25 mM). Reduced nitrotyrosine formation was observed in RAW cells exposed to isobutyl nitrite at 6 mM compared with unexposed controls. Similar results were obtained when inactivated peritoneal macrophages from mice exposed to isobutyl nitrite at 900 ppm as described above were used to investigate nitrotyrosine formation. When activated macrophages were used, the changes were less consistent; some proteins demonstrated reduced nitrotyrosine formation and some demonstrated increased nitrotyrosine formation compared with controls, and some proteins did not show any change in nitrotyrosine formation (Soderberg & Ponnappan, 2002). [The Working Group noted that this study in vitro indicated that peroxynitrite formation does not contribute to the observed effects.]

4.2.3 Immunosuppression

(a) Humans

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

Only a few studies described modulation of immune function after exposure in vitro to structurally related nitrites. After inhalation of amyl nitrite, a decrease in natural killer cell activity (~30%) was observed in the peripheral blood, although no significant changes in cell proliferation in response to stimulation with phytohaemaglutinin, concanavalin A, or pokeweed mitogen were noted (Dax et al., 1991). In contrast, an increased response to T-cell mitogens in peripheral blood lymphocytes was associated with self-reported use of inhaled nitrites (Ross & Drew, 1991).

In vitro, isobutyl nitrite significantly suppressed blastogenesis, natural killer cell function, antibody-dependent cell-mediated cytotoxicity, and interferon production in peripheral blood leukocytes (Hersh et al., 1983). Lymphocytes exposed to isobutyl nitrite at a concentration of 0.5% in cell culture for 72 hours demonstrated reduced cell proliferation in response to phytohaemaglutinin, concanavalin A, or pokeweed mitogen. Antibody-dependent cell-mediated cytotoxicity in lymphocytes and monocytes and adherence in monocytes were also inhibited when the cells were cultured in the presence of 0.5% isobutyl nitrite.

(b) Experimental systems

In female C57BL/6 mice, spleen cellularity was significant decreased (by 39%) after a single exposure by inhalation to isobutyl nitrite at 900 ppm for 45 minutes. Cell loss appeared to be nonspecific as the values of individual lymphocyte subpopulations were unchanged, and the numbers of leukocytes in the peripheral blood and resident peritoneal macrophages were also significantly reduced (Guo et al., 2000). In B6C3F$_1$ female mice exposed by inhalation to isobutyl nitrite at 37.5, 75, or 150 ppm for 6 hours per day, 5 days per week, for up to 15 weeks, there was a dose-related suppression of T-cell-dependent antibody responses in the spleen (Ratajczak et al., 1995). Although splenic atrophy was observed, there were no differences in the relative number of leukocyte subpopulations in the spleen. T-cell proliferation, natural killer cell activity, and infection with *Klebsiella pneumoniae* were not affected by exposure to isobutyl nitrite. A dose-related increase in interferon-induced hydrogen peroxide production in vitro by cultured alveolar macrophages isolated from female B6C3F$_1$ mice was present in the third week of exposure, but not at the termination of the study at 15 weeks (Ratajczak et al., 1995). Persistence of the immune alterations was shown in female B6C3F$_1$ mice that were allowed

to recover for 2 weeks after exposure to isobutyl nitrite at 37.5, 75, or 150 ppm for 6 hours per day, 5 days per week for up to 15 weeks. The numbers of antibody-forming cells in the spleen remained decreased, although spleen cellularity returned to control levels (Ratajczak et al., 1995).

Several studies from one laboratory demonstrated immunosuppressive effects of isobutyl nitrite in C57BL/6 mice. A single 45-minute exposure to isobutyl nitrite at 900 ppm produced transient anaemia in female C57BL/6 mice (Soderberg et al., 1996a). Erythrocyte counts, haemoglobin, and haematocrit levels (erythrocyte volume fraction) were reduced by 7%, but recovered to above normal levels 24 hours later. Blood leukocyte counts were also reduced 24 hours after exposure. In mice exposed to isobutyl nitrite at 900 ppm in an inhalation chamber for 45 minutes per day, for 14 days, the number of peripheral blood leukocytes was reduced by 32% but the number of erythrocytes was increased by 7% (Soderberg et al., 1996a, b). The numbers of bone marrow and spleen burst-forming units-erythroid were increased approximately twofold, although the numbers of colony-forming units-granulocyte/macrophage were decreased by about 50%. A reduction in the production of myeloid colony-stimulating activity was observed in bone marrow stromal cells after exposure to isobutyl nitrite. A single exposure to isobutyl nitrite depleted blood cells including erythrocytes, but single and repeated exposure to isobutyl nitrite stimulated erythropoiesis and maintained suppression of myelopoiesis (Soderberg et al., 1996b).

Soderberg & Barnett (1991) found that female C57BL/6 mice exposed to isobutyl nitrite at 900 ppm for 45 minutes per day for 14 days demonstrated consistent suppression of antibody responses after immunization with T-dependent antigen sheep erythrocytes. T-cell proliferation was also significantly inhibited. Dose-related suppression of the antigen-specific antibody response for both immunoglobulin M and G

occurred in male and female B6C3F$_1$ mice at concentrations of 750 ppm and above (Soderberg & Barnett, 1993). Exposure to isobutyl nitrite at 600 ppm increased antibody responsiveness. This biphasic response was reproducible and was not due to non-specific cell proliferation. No differences in the levels of suppression between males and females were observed, consistent with the study reported by Ratajczak et al. (1995) where normal immune responses returned 5–7 days after the final exposure. Soderberg (1994) specifically assessed end-points associated with T-cell function in female C57BL/6 mice exposed to isobutyl nitrite at 900 ppm for 45 minutes per day for 14 days. Cytotoxic T-lymphocyte activity against P815 mastocytoma cells was reduced by 36%, and T-cell proliferation after mitogenic stimulation or co-culture with allogenic leukocytes was reduced by 37% and 51%, respectively. Production of interleukin-2 (IL-2) in vitro from isolated and cultured splenic lymphocytes from exposed C57BL/6 mice was similar to that of air controls, and activated T-lymphocytes isolated from these same mice responded normally in vitro when treated with exogenous IL-2 (Soderberg, 1994). In normal T-cells co-cultured with an undefined accessory cell population from irradiated spleen cells, T-cell proliferation was inhibited in the presence of accessory cells from mice exposed to isobutyl nitrite. Exposure of female C57BL/6 mice to isobutyl nitrite at 900 ppm by inhalation reduced the number of recoverable peritoneal exudate cells, impaired the ability of peritoneal macrophages from these mice to kill P815 tumour cells in vitro, and reduced the levels of nitric oxide produced in these cells after stimulation with lipopolysaccharides. The reduction in tumoricidal activity was still observed in macrophages isolated from these mice 7 days after the cessation of treatment, but recovered to normal levels 2 weeks after treatment was stopped. The production of tumour necrosis factor-α by peritoneal macrophages and natural killer cell activity were unaffected by isobutyl nitrite exposure in these studies. Production of the proinflammatory cytokine IL-1β was significantly reduced after exposure of female C57BL/6 mice to isobutyl nitrite at 900 ppm for 5 days or 14 days (Soderberg et al., 2004). Lotzová et al. (1984) showed that inhalation exposure to isobutyl nitrite suppressed natural killer cell activity by approximately 60% in female B6D2F$_1$ mice. [The Working Group noted that it was not possible to estimate the doses achieved in this study as mice were exposed to 100% compound in an open system.]

In contrast to the studies reporting immunosuppression described in this section, isobutyl nitrite did not alter sheep erythrocyte-stimulated antibody production or T-lymphocyte mitogenesis after stimulation by phytohaemaglutinin, concanavalin A, pokeweed mitogen, and lipopolysaccharide in male and female BALB/c mice exposed via inhalation at 20, 50, or 300 ppm for 6.4 hours per day, 5 days per week, for up to 18 weeks (Lewis et al., 1985).

In mammalian cells in vitro, Hersh et al. (1983) demonstrated that isobutyl nitrite, at a concentration of 0.05% and 0.01%, significantly reduced the production of α,β-interferon in C3H/HeJ-derived mouse embryo fibroblasts stimulated with poly(I)-poly(C). [The Working Group noted that this study in vitro indicated that nitric oxide formation does not contribute to the observed effects.]

4.2.4 Altered cell proliferation, cell death, or nutrient supply

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

After exposure to isobutyl nitrite at 300 ppm for 6.5 hours per day, 5 days per week, for up to 18 weeks, BALB/cAnNCrlBR mice showed decreased thymus weight (females), decreased liver weight (males), decreased leukocyte counts

(males), and mild focal hyperplasia and vacuolization of the epithelium lining of bronchi and bronchioles of the lungs (males and females) (Lynch et al., 1985). However, changes in organ weight and haematology were not accompanied by any observed histological changes.

Hyperplasia of the bronchiolar and nasal turbinate epithelium was seen in male and female Fischer 344/N rats and B6C3F$_1$ mice after exposure to isobutyl nitrite vapours at up to 400 ppm (rats) or 600 ppm (mice) for 6 hours per day, 5 days per week, in the NTP 13-week (rats) or 14-week (mice) studies (Gaworski et al., 1992). Lymphocytic atrophy was seen in the spleen and thymus of mice. Higher concentrations resulted in mortality in rats (600 ppm or higher) and mice (800 ppm). The 13-week exposures resulted in respiratory system changes, including increased lung weights in rats and female mice exposed at 300 ppm, hyperplasia of the nasal mucosa in male rats exposed at 275 ppm and in female rats exposed at 150 ppm, and hyperplasia of the lung epithelium in male mice exposed at ≥ 150 ppm and in female mice exposed at ≥ 75 ppm (Gaworski et al., 1992).

In the 2-year NTP bioassay, hyperplasia of the alveolar epithelium was evident in rats exposed at 37.5 ppm or more, and in mice exposed at 75 ppm or more (NTP, 1996).

Exposure to isobutyl nitrite by inhalation upregulated the expression of vascular endothelial growth factor (VEGF) protein and mRNA, as well as expression of VEGF receptor 2 (VEGFR-2), VEGFR-3, Smad-5, and Smad-7 in the liver of C57BL/6 mice (Tran et al., 2003).

Exposure to isobutyl nitrite in vitro induced expression of VEGF in macrophage cells (Tran et al., 2003).

4.2.5 Multiple key characteristics

Upregulation of VEGF, VEGFR-3, Smad-5, and Smad-7 was demonstrated in a study of low-density arrays used to examine the effect of exposure to isobutyl nitrite on the expression of 23 cancer- and angiogenesis-related genes in mouse tissues (Tran et al., 2005). Various statistical methods yielded concordant results for the most significant genes, namely VEGF, VEGFR-3, Smad-5, and Smad-7. Reverse-transcription polymerase chain reaction confirmed VEGF upregulation as observed via gene arrays.

4.3 Other adverse effects

4.3.1 Humans

There are case reports showing methaemoglobinaemia in humans after ingestion or inhalation of isobutyl nitrite (Covalla et al., 1981; Shesser et al., 1981; Schwartz & Peary, 1986; O'Toole et al., 1987; Bradberry et al., 1994; Pruijm & de Meijer, 2002; Jansen et al., 2003; Lindenmann et al., 2006). In cases of intoxication with isobutyl nitrite, hypotension (Shesser et al., 1981; Lindenmann et al., 2006) as well as visual loss and maculopathy (Pece et al., 2004; Davies et al., 2012; Pahlitzsch et al., 2013) were reported.

Some cases also showed irritant contact dermatitis (Schwartz & Peary, 1986), or tracheobronchitis and/or irritation of the tracheobronchial tree (Covalla et al., 1981; Schwartz & Peary, 1986).

4.3.2 Experimental systems

Concentrations of methaemoglobin were elevated in male and female mice exposed to isobutyl nitrite at 50 and 300 ppm (Lynch et al., 1985).

Hypotension was seen after exposure of rats by inhalation and of rabbits by intravenous infusion (Kielbasa & Fung, 2000a). In rabbits exposed to isobutyl nitrite, there was an association between generation of nitric oxide and hypotension in vivo (Cederqvist et al., 1994).

4.4 Data relevant to comparisons across agents and end-points

4.4.1 High-throughput screening programmes

High-throughput screening data generated by the Toxicity Forecaster (ToxCast) and Toxicity Testing in the 21st Century (Tox21) research programmes of the government of the USA (Kavlock et al., 2012; Tice et al., 2013) were considered in the assessment of the six chemicals reviewed in *IARC Monographs* Volume 122 (isobutyl nitrite, β-picoline, methyl acrylate, ethyl acrylate, 2-ethylhexyl acrylate, and trimethylolpropane triacrylate). The United States Environmental Protection Agency (EPA) has systematically analysed more than three million concentration–response chemical assay pairs from ToxCast and Tox21. The resulting concentration–response models and activity calls were released to the public via the Interactive Chemical Safety for Sustainability ToxCast Dashboard and by downloadable files, including a data analysis pipeline (tcpl R package) and a database (invitrodb_v3) (EPA, 2017a, 2018). The underlying concentration–response data, analysis decision logic and methods, concentration–response model outputs, activity calls, and activity caution flags were also provided (Filer et al., 2017). For the six chemicals considered in the present volume, four were tested in ToxCast and in Tox21 assays and the other two solely in Tox21 assays.

Chemicals with a very low relative molecular mass (< 150) generally have only low affinity for biomolecular interactions because of limited free energy for binding (Hopkins et al., 2004). Four of the six chemicals considered in the present volume – isobutyl nitrite, β-picoline, methyl acrylate, and ethyl acrylate – have a relative molecular mass of less than 150. Screening in vitro at the concentrations used in ToxCast and Tox21 (typically 100 μM or less) may therefore be inadequate to detect receptor-type molecular interactions that do not rely on chemical reactivity. The four compounds with a low relative molecular mass also have high vapour pressures, which could lead to a loss of sample during storage and/or testing, and therefore failure to reach expected active concentrations.

The Tox21 and ToxCast in vitro assays were selected to cover a broad range of potential biological activity and are not specifically focused on carcinogenesis. The Working Group of *IARC Monographs* Volume 112 therefore mapped the 821 assay end-points available at that time to the key characteristics of known human carcinogens, yielding consensus assignments of 263 assay end-points mapped to 7 of the 10 key characteristics or to the category "other" (IARC, 2017; Chiu et al., 2018); this was later updated to 291 in *IARC Monographs* Volume 119 (IARC, 2018). New assay end-points added to Tox21 and ToxCast projects since that determination were reviewed and 57 additional assay end-points were added to the mapped key characteristics, resulting in 348 in total (including the category "other"); however, these six chemicals were only tested in 304 of these assays. The assay end-points used, the activity call, and the mapping to key characteristics are available are available as supplemental material to the present volume (Annex 1). The key characteristics, as well as number of assays included in Volume 122 and a brief description, are provided below.

1. *Is electrophilic or can be metabolically activated*: 1 assay end-point, that is, cytochrome P450 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*: 5 assay end-points including biochemical assays targeting histone deacetylases and other

enzymes modifying chromatin, as well as assays for cellular transcription factors involved in epigenetic regulation

5. *Induces oxidative stress*: 13 assay end-points, all cellular assays, targeting nuclear erythroid-related factor 2 (NRF2) and/or the antioxidant responsive element (ARE) and other stress-related transcription factors, as well as 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*: 95 assay end-points targeting nuclear receptors (including aryl hydrocarbon receptor) in cellular assays for transactivation, and 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*: 100 assay end-points measuring cytotoxicity or general development using a wide variety of assay formats in cell lines, primary human cells, and developing zebrafish larvae.

In addition, there are 35 assay end-points classified as "Other" that measure biological activity against targets not readily classifiable with respect to the 10 key characteristics.

4.4.2 Outcomes for chemicals tested

The specific assays tested, mapping to the key characteristics, and the activity calls are available as supplemental material to the present volume (Annex 1). Table 4.4 lists the number of assays

tested and the number of positive findings for each key characteristic and each chemical.

Brief summaries of potentially significant outcomes for each chemical tested are provided below (see also Table 4.4).

(a) Isobutyl nitrite

Isobutyl nitrite (CAS No. 542-56-3) was inactive in all 116 of the Tox21 programme assay end-points mapped to the key characteristics of carcinogens. Chemical quality control (QC) information was available for the Tox21 chemical library sample solution; it was graded "D" because of a purity of less than 50%, and a comment that "the sample has decomposed to the alcohol" was included (NIH, 2017). The chemical has a predicted vapour pressure of 2.13 mm Hg [1.3 kPa] and an experimental boiling point of 66.8 °C (EPA, 2017b). [The Working Group noted that there may have been limited ability to detect bioactivity in the Tox21 assays because of the low relative molecular mass of the chemical, 103.1, which may limit biomolecular interactions at the concentrations tested, and the poor analytical chemistry analysis for the tested sample.]

(b) β-Picoline

β-Picoline (CAS No. 108-99-6) was found to be bioactive in 13 of 266 ToxCast and Tox21 assay end-points mapped to the key characteristics. In two assays mapped to "induces oxidative stress" (ATG_NRF2_ARE_CIS and ATG_MRE_CIS) marginal activity was shown only at the highest concentration tested (200 μM). The result of one assay mapped to "modulates receptor-mediated effects" was called positive, but concentration–response curve-fit warning flags clearly showed this to be a bad fit and therefore a false-positive call. Five assays mapped to "alters cell proliferation, cell death, or nutrient supply" were called active. Two were transcription factor activation assays (ATG_AP_1_CIS and ATG_Xbp1_CIS) that showed marginal activity only at the highest concentration tested (200 μM). The other three

Table 4.4 Summary of activity of agents reviewed in *IARC Monographs* Volume 122 and tested in ToxCast and/or Tox21 high-throughput screening assays

Key characteristic	Isobutyl nitrite	β-Picoline	Methyl acrylate	Ethyl acrylate	2-Ethylhexyl acrylate	Trimethylolpropane triacrylate
1. Is electrophilic or can be metabolically activated	0 out of 1 assays[a]	0 out of 1 assays	0 out of 1 assays[a]	NA	0 out of 1 assays	1[b] (0) out of 1 assays
2. Is genotoxic	0 out of 9 assays	0 out of 10 assays	0 out of 10 assays	0 out of 9 assays	0 out of 10 assays	9 out of 10 assays
3. Alters DNA repair or causes genomic instability	NA	NA	NA	NA	NA	NA
4. Induces epigenetic alterations	0 out of 1 assays	0 out of 5 assays	0 out of 5 assays	0 out of 1 assays	0 out of 5 assays	2 out of 5 assays
5. Induces oxidative stress	0 out of 1 assays	2 out of 13 assays	0 out of 8 assays	0 out of 4 assays	1 out of 13 assays	5 out of 13 assays
6. Induces chronic inflammation	0 out of 1 assays	0 out of 47 assays	0 out of 2 assays	0 out of 1 assays	0 out of 47 assays	0 out of 47 assays
7. Is immunosuppressive	NA	NA	NA	NA	NA	NA
8. Modulates receptor-mediated effects	0 out of 39 assays	1[b] (0) out of 94 assays	0 out of 75 assays	0 out of 39 assays	4[b] (0) out of 95 assays	33 out of 76 assays
9. Causes immortalization	NA	NA	NA	NA	NA	NA
10. Alters cell proliferation, cell death or nutrient supply	0 out of 64 assays	5[b] (2) out of 96 assays	1[b] (0) out of 72 assays	0 out of 64 assays	8 out of 100 assays	71 out of 96 assays
Total number of assays mapped to key characteristics	116	266	173	118	271	248

NA, not applicable: no assays in ToxCast and/or Tox21 were determined to be applicable to the evaluation of the indicated key characteristic; ToxCast/Tox21, Toxicity Forecaster and Toxicity Testing in the 21st Century research programmes of the government of the USA

[a] Indicates the number of positive results out of the number of assays mapped to key characteristics of carcinogens, as listed in supplemental Table 1 (see Annex 1)

[b] Indicates an active call in an assay (i.e. "hit") which was determined to be most likely a false positive artefact upon review of the assay parameters and dose-response data by the Working Group [the number in parentheses reflects the true number of biological hits in the opinion of the Working Group]

were viability assays, each with significant curve-fitting warning flags that indicated likely false-positive results. Finally, there are five assays from the "other" category considered active, all from the Attagene (ATG) transcription factor activation assay platform; all were marginally active only at the highest concentration tested (200 μM). The analytical QC of the tested sample solution was not available. 3-Methyl pyridine has an experimental vapour pressure of 6.05 mm Hg (EPA, 2017c). [The Working Group noted that the relative molecular mass of the chemical is 93.1, which may limit biomolecular interactions at the concentrations tested, and that volatilization of the sample may have affected actual sample concentration.]

(c) Methyl acrylate

For methyl acrylate (CAS No. 96-33-3), active hit calls were made for only 1 of 173 ToxCast and Tox21 assays mapped to the key characteristics. The single active call was for a cell viability assay, but multiple curve-fit warning flags were associated with the results, indicating a false-positive finding. The chemical QC analysis of the solution used in Tox21 showed that the expected structure was not detected and no significant impurities were observed at the time of analysis. Methyl acrylate has an experimental vapour pressure of 86.6 mm Hg and a boiling point of 80.0 °C (EPA, 2017d). [The Working Group noted that the relative molecular mass of the chemical is 86.1, which may limit biomolecular interactions at the concentrations tested, and that sample volatility may have contributed to the lack of expected structure noted in the analytical QC of the Tox21 sample solution.]

(d) Ethyl acrylate

There were no active hit calls in any of the 118 Tox21 assays mapped to the key characteristics tested with ethyl acrylate (CAS No. 140-88-5). The analytical chemistry determination of the sample solution tested in Tox21 was not available. The experimental vapour pressure of ethyl acrylate was reported as 38.6 mm Hg and the boiling point as 99.5 °C (EPA, 2017e). [The Working Group noted that the relative molecular mass of the chemical is 100.1, which may limit biomolecular interactions at the concentrations tested, and that sample volatility may have limited chemical exposure in the assay.]

(e) 2-Ethylhexyl acrylate

For 2-ethylhexyl acrylate (CAS No. 103-11-7), active hit calls were made for 13 of 271 ToxCast and Tox21 assay end-points mapped to the key characteristics. One assay end-point for "induces oxidative stress" (ATG_NRF2) was active with an AC_{50} (the concentration at which the half-maximal response along a sigmoid curve is produced) of 101 μM, along with a tumour protein TP53 activation assay mapped to "is genotoxic" (ATG_p53_CIS), active at 116 μM. Orthogonal assays for NRF2 and TP53 in Tox21 were inactive. Four other active calls were mapped to key characteristic 8, "modulates receptor-mediated effects", consisting of estrogen receptor (ER) α and ERβ activation, retinoic acid receptor (RAR) activation, and progesterone receptor (PR) activation (OT_ERa_EREGFP_0120, TOX21_ERb_BLA_Antagonist, TOX21_RAR_LUC_Agonist, and TOX21_PR_BLA_Antagonist). The ERα assay curve fit was not flagged, but was not a monotonic response as would be expected for a receptor-modulated effect. In addition, the same assay but with a 4-hour incubation (OT_ERa_EREGFP_0480) rather than 2-hour incubation was completely inactive. [The Working Group noted that this was probably a false-positive result.] Both the ERβ and the PR curve fits had multiple warning flags and appeared to be false positives. The RAR response was marginal at the highest concentration tested; however, orthologous assays for RARα, RARβ, and RARγ in the ATG platform were all inactive. There were 8 active assays mapped to "alters cell proliferation, cell death, or nutrient supply". Three of these had

poor curve fits as evidenced by warning flags and visual inspection. There were cytotoxic responses seen in four primary human cell culture models that included smooth muscle cells, dermal fibroblasts, and endothelial cells (BSK_CASM3C_SRB, BSK_hDFCGF_Proliferation, BSK_hDFCGF_SRB, and BSK_3C_Proliferation). Potencies were 24–33 µM (AC_{50}). The dermal fibroblast cell cultures were shown to be particularly sensitive to oxidative stress (Kleinstreuer et al., 2014). The analytical QC analysis of the Tox21 sample solution indicated that the expected structure was present, but only at 5–30% of the expected concentration. [The Working Group noted that the low concentration may suggest volatility.]

(f) Trimethylolpropane triacrylate

For trimethylolpropane triacrylate (CAS No. 15625-89-5), there were 126 active calls for 283 ToxCast and Tox21 assay end-points mapped to the key characteristics. It was active against one assay mapped to "is electrophilic or can be metabolically activated" (TOX21_Aromatase_Inhibition); however, the corresponding cell viability assay (TOX21_Aromatase_Inhibition_viability) was active at the same concentrations, which would support the theory that the effects were due to cytotoxicity. There were nine positive assays mapped to "is genotoxic". Two of these were related to DNA repair (TOX21_DT40_100 and TOX21_DT40_657); however, activity was equivalent at the wildtype cell line (TOX21_DT40), consistent with general cytotoxicity being responsible for the activity (Nishihara et al., 2016). The assay for DNA damage (TOX21_ELG1_LUC_Agonist) was active with an AC_{50} of 5.2 µM. As this is a gain-of-signal reporter gene assay, it is less prone to cytotoxic effects that artefactually decrease the reporter signal. It was also active in an assay for TP53 activation (TOX21_p53_BLA) five times out of five tests (the TP53 assay was repeated over time to examine potential effects of chemical degradation), another gain-of-signal reporter gene assay.

The AC_{50}s fell within the range 15–101 µM. One other assay mapped to genotoxicity (TOX21_H2AX_HTRF_CHO) was positive, with an AC_{50} of 11.5 µM. Trimethylolpropane triacrylate was also considered active in five assays mapped to "induces oxidative stress", with AC_{50} values within the range 2–19 µM. It was active in 33 assays mapped to "modulates receptor-mediated effects". The most potent effect was seen for the xenobiotic pregnane X receptor (PXR) where it was active in two assays with AC_{50} values of 0.77 and 0.78 µM (ATG_PXRE_CIS and ATG_PXR_TRANS). For other receptor assays, there was a consistent pattern of partial agonist activity just before a large loss of effect at cytotoxic concentrations. Because of the confounding effects of cytotoxicity, the interpretation of receptor modulation effects, other than for PXR, is challenging. Trimethylolpropane triacrylate was active in 71 assays mapped to "alters cell proliferation, cell death, or nutrient supply". Sixty-eight of these were categorized as cytotoxicity or apoptosis with an average AC_{50} of 4.72 ± 2.73 µM for both cell lines and primary human cells. Two additional assays indicated upregulation of growth factor or growth factor receptor in primary human cells (BSK_hDFCGF_EGFR and BSK_KF3CT_TGFb1). The final assay showed upregulation of the AP1 transcription factor (TOX21_AP1_BLA_Agonist), but the curve fit was flagged because of activity at a single concentration and obvious confounding by cytotoxicity. There were two positive assay results linked to "induces epigenetic alterations" (ATG_Pax6_CIS and ATG_Sp1_CIS), but both activities were much higher than the average cytotoxicity concentrations and therefore considered not biologically significant. Finally, there were five positive assay results not mapped to any of the key characteristics but to an "other" category. Three of these (ATG_EGR_CIS, ATG_NFI_CIS, and ATG_Oct_MLP_CIS) were activated transcription factor responses for proteins characterized as being involved in cell differentiation. The ATG_SREBP_CIS assay was

also activated, an end-point associated with low cellular sterol levels for precursors of cholesterol biosynthesis. The last of these "other" activities was TOX21_TSHR_Agonist, an assay for activation of thyroid-stimulating hormone receptor that could also respond to increased levels of cyclic adenosine monophosphate. As for the majority of assays in other categories, however, all assays in the "other" category had AC_{50} values above the average cytotoxicity potency. [The Working Group noted that trimethylolpropane acrylate was highly cytotoxic and that interpretation of bioactivity in vitro in the micromolar concentration range was likely to be confounded by nonspecific effects.] The chemical QC determination of the sample solution tested with Tox21 showed the expected structure, but purity was less than 50%.

4.4.3 Overall considerations

In summary, trimethylolpropane triacrylate showed bioactivity in 126 of 283 assays, of which 248 were mapped to the key characteristics of carcinogens (119 showed bioactivity). Nine active assays were mapped to genotoxicity, although in two cases bioactivity occurred at concentrations inducing cytotoxicity in other assays. Additionally, 71 assays were mapped to "alters cell proliferation"; these were predominantly cytotoxicity assays with an average potency of 5 μM. Finally, there were 33 assays mapped to "modulates receptor-mediated effects", with the most potent effect against the xenobiotic receptor PXR; other receptor effects were at cytotoxic concentrations.

Data from high-throughput toxicity testing programmes were considered uninformative for the other compounds tested. Isobutyl nitrite was inactive in all of the 116 assays mapped to the key characteristics and had poor analytical chemistry results, probably because of chemical volatility. For β-picoline, there was weak support for oxidative and cellular stress responses based on

marginal bioactivity in a few assays, but chemical volatility may have limited the chemical exposure in these assays. For methyl acrylate, there was bioactivity in only 1 of 173 assays. Ethyl acrylate was inactive in all 118 assays, but volatility may have limited the chemical exposure in these assays. 2-Ethylhexyl acrylate showed 13 active assays out of 271 mapped to the key characteristics, but most of these results were inconsistent and not considered significant, with the exception of cytotoxicity noted in four primary human cell culture models.

5. Summary of Data Reported

5.1 Exposure data

Isobutyl nitrite is an alkyl nitrite. It is mainly used in "poppers", consumed as a recreational drug for their psychoactive effects. Poppers are illegal in many countries, and as a result are commonly sold as air fresheners or deodorizers. Other minor uses of isobutyl nitrite include as an intermediate in the synthesis of aliphatic nitrites, nail polish removers, video head cleaners, fuels, and jet propellants. There were no available data on the production volume of isobutyl nitrite. There was evidence of the increased online purchase of poppers from countries where their use is legal. Human exposure to isobutyl nitrite occurs mainly through intentional inhalation. No quantitative data on environmental concentrations or occupational exposure to isobutyl nitrite were identified.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

There was one well-conducted good laboratory practice inhalation study of isobutyl nitrite in male and female mice. In males, isobutyl nitrite significantly increased the incidence (with a significant positive trend) of bronchioloalveolar adenoma and of bronchioloalveolar adenoma or carcinoma (combined) of the lung, and of follicular cell adenoma and of follicular cell adenoma or carcinoma (combined) of the thyroid gland. In females, isobutyl nitrite significantly increased the incidence (with a significant positive trend) of bronchioloalveolar adenoma and of bronchioloalveolar adenoma or carcinoma (combined) of the lung.

There was one well-conducted good laboratory practice inhalation study of isobutyl nitrite in male and female rats. In males, isobutyl nitrite significantly increased the incidence (with a significant positive trend) of bronchioloalveolar adenoma, bronchioloalveolar carcinoma, and of bronchioloalveolar adenoma or carcinoma (combined) of the lung. In females, isobutyl nitrite significantly increased the incidence (with a significant positive trend) of bronchioloalveolar adenoma and of bronchioloalveolar adenoma or carcinoma (combined) of the lung.

5.4 Mechanistic and other relevant data

Studies on the absorption, distribution, or excretion in humans were not available, but methaemoglobinaemia and the vasodilating effects of isobutyl nitrite in humans indicate that absorption occurs. Isobutyl nitrite undergoes hydrolytic decomposition in humans, yielding nitrite and isobutyl alcohol.

Rapid systemic absorption and elimination were observed in rats exposed by inhalation, with a short half-life (~1 minute) regardless of route of administration. Isobutyl nitrite is extensively metabolized in rats and rabbits, and isobutyl alcohol was identified in rats. Nitric oxide, found in the exhaled air of exposed rabbits, can be formed by reduction of isobutyl nitrite or by homolytic cleavage of the nitric oxide bond, which also yields the isobutoxyl radical.

Regarding the key characteristics of carcinogens, there is *moderate* evidence that isobutyl nitrite is genotoxic. Results were generally positive, but there were few studies available. In one study of human lung cells in vitro, dose-dependent induction of DNA damage was detected by the comet assay in cells from one of three donors. There was increased DNA damage in rat lung but not in rat liver or kidney in one study, and a test for micronucleus formation in mice in vivo gave positive results for DNA damage. In the few studies in rodent cells in vitro, isobutyl nitrite gave positive results in tests for mutations, sister-chromatid exchanges, and chromosomal aberrations. In the Ames test, isobutyl nitrite gave positive results in strains sensitive to base substitutions, but negative results in strains sensitive to frameshift mutations.

Isobutyl nitrite is a known irritant that causes nonspecific inflammatory responses at the exposure site in humans and rodents. There is *moderate* evidence that isobutyl nitrite is immunosuppressive. No data from studies of isobutyl nitrite in exposed humans were available, and the few studies of structurally related nitrites were equivocal. A single study using human peripheral blood cells in vitro demonstrated suppressed lymphocyte blastogenesis, natural killer cell function, antibody-dependent cell-mediated cytotoxicity, and interferon production in isolated leukocytes. Dose-dependent suppression of antigen-specific antibody production occurred in most, but not all, strains of mice exposed to isobutyl nitrite via inhalation, and was shown to persist after cessation of exposure. Suppression of other indicators of immune function (including natural killer cell and cytotoxic T-lymphocyte activity) was reported in mice, but results were inconsistent.

In cases of intoxication in humans, methaemoglobinaemia, hypotension, visual effects, and irritant contact dermatitis were reported. Methaemoglobinaemia was observed in rats and rabbits, and hypotension linked to nitric oxide generation was reported in rabbits.

Hyperplasia of the lung was observed in chronically exposed rodents.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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β-PICOLINE

1. Exposure Data

1.1 Identification of the agent

See NTP (2014), HSDB (2015), Royal Society of Chemistry (2018)

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 108-99-6
Chem. Abstr. Serv. name: 3-methylpyridine
IUPAC systematic name: 3-methylpyridine
Synonyms: beta-picoline; 3-picoline; 3-mepy; pyridine; 3-methyl-; β-methylpyridine.

1.1.2 Structural and molecular formulae, and relative molecular mass
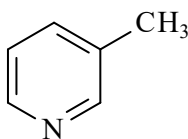
Molecular formula: C_6H_7N
Relative molecular mass: 93.13

1.1.3 Chemical and physical properties

Description: β-picoline is a colourless liquid with a sweetish odour
Boiling point: 143–144 °C (experimental)

Melting point: –18 °C (experimental)
Flash point: 36 °C
Density: 0.9566 g/mL (at 20 °C)
Vapour pressure: 6.05 mm Hg [0.80 kPa] at 25 °C
Solubility: miscible with water at 20 °C; soluble in alcohol and ether, and very soluble in acetone
Conversion factor: 1 ppm = 3.81 mg/m³ (at 1 atm and 25 °C).

1.1.4 Technical products and impurities

The industrial-scale fractionation of pyridine bases from coal tar is carried out by distillation; consequently, the β-picoline fraction may contain compounds with boiling points lower than 150 °C as principal components and small quantities of other alkylpyridines (e.g. 4-methylpyridine and 2-ethylpyridine). Commercial synthetic β-picoline is of high purity (> 90%), but may contain small quantities of other alkyl pyridines (Titon & Nardillo, 1995; NTP, 2014).

1.2 Production and use

1.2.1 Production process

β-Picoline, together with other pyridine bases, was originally isolated from pyrolysis of coal tar or coal gas. The isolation process is expensive; current production is mainly based on chemical synthesis (HSDB, 2015).

β-Picoline can be produced from the vapour-phase reaction of acetaldehyde and ammonia with formaldehyde and/or methanol in the presence of a catalyst, or from the vapour-phase reaction of acrolein with ammonia in the presence of an acid catalyst. It can also be produced from the vapour-phase reaction of 2-methylglutaronitrile over a nickel-containing catalyst in the presence of hydrogen to give 3-methylpiperidine, which then undergoes dehydrogenation over palladium-alumina to give β-picoline. Another method involves the reaction of cyclohexane and ammonia in the presence of zinc chloride (NTP, 2014; HSDB, 2015).

1.2.2 Production volume

β-Picoline is a chemical with a high production volume that is mainly produced in Asia, western Europe, and the USA (OECD, 2009). The major producers in Asia are China, including Taiwan, and India, and Japan (Scriven & Murugan, 2005). In China, total production of pyridine in thousands of metric tonnes was reported to be 14.1 in 2006, 19.3 in 2008, 50.5 in 2011, 80 in 2014, and 100 in 2016, representing an increase of sevenfold (Chinese Report, 2006, 2008, 2011, 2014, 2016). [Assuming a production ratio of 1:3 for β-picoline to total pyridines estimated by the Working Group, these would convert to about 4.7 (2006), 6.4 (2008), 16.8 (2011), 26.7 (2014), and 33.3 (2016) thousand metric tonnes of β-picoline.] In Europe, the annual production volume is estimated to be 100–1000 metric tonnes (ECHA, 2018). In the USA, reported annual production was about 21–29 million pounds (9.5–13.2 thousand metric tonnes) in 1998 (NTP, 2014) and 10–50 million pounds (4.5–22.7 thousand metric tonnes) in 2006 (HSDB, 2015).

1.2.3 Use

The major use of β-picoline is as a starting material for agrochemicals and pharmaceuticals. For example, it is used to make insecticides such as chlorpyrifos, herbicides such as fluazifop-butyl, and pharmaceuticals and/or dietary supplements such as niacin (vitamin B3) and its amide (Scriven & Murugan, 2005). It is also used as a solvent and intermediate in rubber accelerators, waterproofing agents, dyes, and resins (NTP, 2014; HSDB, 2015), as well as a flavouring substance in 31 food groups and beverages [Flavis (FL) No.: 14.135] (EFSA, 2006).

1.3 Analytical methods

β-Picoline can be determined by both gas and liquid chromatography methods (NTP, 2014). However, fewer methods based on liquid chromatography were reported for analysis of β-picoline, which may be attributed to its volatile nature. A summary of analytical methods reported for β-picoline is provided in Table 1.1.

1.4 Occurrence and exposure

1.4.1 Occurrence

β-Picoline can enter the environment through industrial wastewater due to its use as a starting material and intermediate in various industries (Scriven & Murugan, 2005). It is present in effluents from the manufacture and use of coal-derived liquid fuels and from the disposal of coal liquefaction and gasoline waste by-products (NTP, 2014). β-Picoline is also released into air as a result of cigarette smoking (Kurgat et al., 2016).

(a)   Water

β-Picoline was detected at concentrations of 1.23, 0.30, 0.20, and 0.01 mg/L at depths of 6.1, 3.3, 5.8, and 11.0 m, respectively, in groundwater samples collected from two different sites

Table 1.1 Representative methods for the analysis of β-picoline

Sample matrix	Assay procedure	Limit of detection	Reference
Water, sediment	GC-EI/MS	0.01 ng on column	Tsukioka & Murakami (1987)
Air, cigarette smoke	GC-EI/MS	0.005–0.010 ng on column	Llompart et al. (1998), Kulshreshtha & Moldoveanu (2003)
Air, exhaled breath of tobacco cigarette and electronic cigarette smokers	TD-GC/MS	5 ng/m^3, 0.16–1.60 ng per sample	Heavner et al. (1992), Vainiotalo et al. (2008), Marco & Grimalt (2015)
Cigarette smoke	RP-HPLC/UV	0.5–1.0 µg/L	Esrafili et al. (2012)
Water, urine	RP-HPLC/UV	2.5, 7.3 µg/L	Shahdousti et al. (2015)

EI, electron ionization; GC, gas chromatography; HPLC, high-performance liquid chromatography; MS, mass spectrometry; RP, reversed phase; TD, thermal desorption; UV, ultraviolet spectroscopy

in Pensacola Bay, Florida, USA. The sites were heavily contaminated with creosote, a complex distillate from coal tar used for wood preserving (Goerlitz, 1992). Middaugh et al. (1991) reported concentrations of 0.0007 and 0.1 mg/L of β-picoline in surface and groundwater samples, respectively, collected from the same contaminated area. Stuermer et al. (1982) reported combined concentrations of β- and γ-picoline (4-methylpyridine) of 0.00069–0.05100 mg/L in three groundwater samples collected near two underground coal gasification sites in north-eastern Wyoming, USA. β-Picoline was detected, but not quantified, in a survey of drinking-water samples from United States cities including Cincinnati (Ohio), Miami (Florida), New Orleans (Louisiana), Ottumwa (Iowa), Philadelphia (Pennsylvania), and Seattle (Washington) (EPA, 1984). β-Picoline was also detected at a concentration of 6.5 mg/L in oil shale condensate retort water samples collected from the Logan Wash site, Colorado, USA, in 1979 (Leenheer et al., 1982).

(b)   Air

β-Picoline is a component of tobacco smoke, and was detected in cigarette smoke with emission factors of 12–36 µg per cigarette (Singer et al., 2002). The median concentration of β-picoline in indoor air samples collected in 1991 from the homes of smokers (0.58 µg/m^3, *n* = 25) in Columbus, Ohio, USA, exceeded that

from the homes of non-smokers (0.16 µg/m^3, *n* = 24) (Heavner et al., 1995). Higher levels of β-picoline were also detected in air samples collected from the smoking areas of 10 Finnish restaurants (median, 1.4 µg/m^3) compared with the non-smoking areas (median, 0.18 µg/m^3) (Vainiotalo et al., 2008). β-Picoline was not detected in an urban air sample from Boulder, Colorado, USA, and in a rural air sample from an undeveloped area of the oil shale region (Hawthorne & Sievers, 1984).

(c)   Diet

β-Picoline was reported to occur naturally in coffee (1.3 mg/kg), beer (0.0008 mg/kg), and whisky (< 0.0006 mg/kg) (EFSA, 2006), and was detected in three types of fermented soya bean curd from Hong Kong Special Administrative Region, China, with concentrations in the range 18–55 µg/kg (Chung, 1999b). It was also found in edible crab (*Charybdis feriatus*) collected from Hong Kong Special Administrative Region, with concentrations of 14.6, 11.6, and 7.5 µg/kg in carapace, leg, and body meat, respectively (Chung, 1999a). β-Picoline was identified, but not quantified, in boiled beef (Golovnya et al., 1979) and mutton samples (Shahidi et al., 1986). It is also used as a flavouring agent in 31 food groups including dairy products, processed fruits, meat and meat products, and fish and fish products (EFSA, 2006).

1.4.2 Exposure

(a)   Exposure of the general population

Non-occupational exposure can occur via inhalation of contaminated air, ingestion of contaminated food and water, and dermal contact with products containing β-picoline (HSDB, 2015). A study by the European Food Safety Authority estimated the maximized survey-derived daily intake and the modified theoretical added maximum daily intake for β-picoline (3-methylpyridine) from its intake as a flavouring substance at 0.027 and 380 µg per person per day, respectively. Both estimates fell short of the reported threshold of concern (540 µg per person per day; EFSA, 2006).

(b)   Occupational exposure

Occupational exposure occurs primarily through inhalation or dermal contact during the production and/or use of β-picoline (HSDB, 2015). Between 1981 and 1983, the number of employees occupationally exposed to β-picoline in the USA was estimated by the United States National Institute for Occupational Safety and Health as 5202, of which 390 were women (NIOSH, 1985). Hawthorne & Sievers (1984) reported concentrations of combined β-picoline and γ-picoline (4-methylpyridine) in air samples collected in and near the shale oil wastewater treatment facility at the Logan Wash site, Colorado, in 1982. A higher concentration was measured indoors at the workbench of the operator near the activated sludge tank (35 µg/m³) compared with that measured outdoors (8 µg/m³).

1.5   Regulations and guidelines

No specific occupational exposure limits for β-picoline were available to the Working Group. The American Industrial Hygiene Association derived a workplace environmental exposure limit of 2 ppm for picolines as an 8-hour time-weighted average (TWA) and a short-term

exposure limit of 5 ppm for a 15-minute TWA (Myers, 2013). These exposure limits included a skin notation.

2.   Cancer in Humans

No data were available to the Working Group.

3.   Cancer in Experimental Animals

See Table 3.1

3.1   Mouse

Drinking-water

Groups of 50 male and 50 female B6C3F$_1$/N mice (age, 5–6 weeks) were given drinking-water containing β-picoline (purity, 96.4%) at a concentration of 0, 312.5, 625, or 1250 mg/L ad libitum for 7 days per week for 105 weeks (NTP, 2014). Average daily doses of β-picoline were approximately 0, 26, 50, and 92 mg/kg body weight (bw) for males and 0, 18, 37, and 68 mg/kg bw for females. Survival of all exposed groups was similar to that of the control groups. However, there was a small but significant positive trend in the survival of males with increasing exposure. Mean body weights of males exposed at the highest dose were at least 10% less than those of the control group after week 57, and body weights of females exposed at the highest dose were generally 10% less after week 13. Water consumption was lower in males exposed at the intermediate and highest doses and females exposed at the highest dose compared with those in the controls after the first 13 weeks of the study.

The incidence of hepatocellular carcinoma (includes multiple) (11/49, 20/50, 26/50, and 23/50) and of hepatocellular carcinoma or hepatoblastoma (combined) (12/49, 21/50, 28/50, and 24/50) was significantly increased in all exposed

Table 3.1 Studies of carcinogenicity with β-picoline in experimental animals

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, B6C3F₁/N (M) 5–6 wk 105 wk NTP (2014)	Drinking-water β-Picoline, 96.4% Tap water 0, 312.5, 625, 1250 mg/L ad libitum 50, 50, 50, 50 24, 26, 27, 33	*Lung* Bronchioloalveolar adenoma 6/50 (12%), 11/50 (22%), 16/50* (32%), 8/50 (16%)	*P = 0.037, poly-3 test	Principal strengths: well-conducted GLP study Historical incidence (mean ± SD; range): 2-yr drinking-water studies with untreated control groups, 21/100 (21.0 ± 12.7%; 12–30%); all routes, 172/1150 (15.0 ± 6.9%; 2–30%)
Full carcinogenicity Mouse, B6C3F₁/N (F) 5–6 wk 105 wk NTP (2014)	Drinking-water β-Picoline, 96.4% Tap water 0, 312.5, 625, 1250 mg/L ad libitum 50, 50, 50, 50 38, 32, 35, 33	*Liver* Hepatocellular carcinoma 11/49 (22%), 20/50* (40%), 26/50** (52%), 23/50*** (46%) Hepatoblastoma 1/49 (2%), 3/50 (6%), 4/50 (8%), 4/50 (8%) Hepatocellular carcinoma or hepatoblastoma (combined) 12/49 (24%), 21/50* (42%), 28/50** (56%), 24/50*** (48%) Hepatocellular adenoma 38/49 (78%), 46/50* (92%), 46/50 (92%), 39/50 (78%) *Lung* Bronchioloalveolar adenoma (includes multiple) 5/50 (10%), 6/50 (12%), 4/49 (8%), 11/50 (22%) Bronchioloalveolar carcinoma (includes multiple) 7/50 (14%), 8/50 (16%), 10/49 (20%), 13/50 (26%)	*P = 0.006 (trend), *P = 0.031, **P < 0.001, ***P = 0.005; poly-3 test NS *P = 0.005 (trend), *P = 0.033, **P < 0.001, ***P = 0.005; poly-3 test *P = 0.025, poly-3 test *P = 0.046 (trend), poly-3 test NS	Principal strengths: well-conducted GLP study Historical incidence (mean ± SD; range): Hepatocellular adenoma (includes multiple): drinking-water, 52/98 (53.1 ± 34.6%; 29–78%); all routes, 380/1195 (31.8 ± 21.4%; 2–78%) Hepatocellular carcinoma (includes multiple): drinking-water, 19/98 (19.4 ± 4.3%; 16–22%); all routes, 144/1195 (12.1 ± 10.8%; 0–46%) Hepatoblastoma (includes multiple): drinking-water, 1/98 (1.0 ± 1.4%; 0–2%); all routes, 4/1195 (0.3 ± 0.8%; 0–2%) Hepatocellular carcinoma or hepatoblastoma (combined): drinking-water, 20/98 (20.4 ± 5.8%; 16–24%); all routes, 148/1195 (12.4 ± 11.2%; 0–46%) Bronchioloalveolar carcinoma (includes multiple): drinking-water, 9/100 (9.0 ± 7.1%; 4–14%); all routes, 44/1196 (3.7 ± 3.3%; 0–14%) Bronchioloalveolar adenoma or carcinoma (combined): drinking-water, 13/100 (13.0 ± 12.7%; 4–22%); all routes, 100/1196 (8.4 ± 4.3%; 2–22%) Significant increase in the incidence of hyperplasia of the alveolar epithelium in females at the highest 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	Incidence (%) of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$/N (F) 5–6 wk 105 wk NTP (2014) (cont.)		Bronchioloalveolar adenoma or carcinoma (combined) 11/50 (22%), 13/50 (26%), 13/49 (27%), 21/50* (42%) Bronchioloalveolar adenoma (multiple) 0/50, 1/50 (2%), 0/49, 1/50 (2%) Bronchioloalveolar carcinoma (multiple) 0/50, 2/50 (4%), 2/49 (4%), 4/50 (8%)	$P = 0.015$ (trend), *$P = 0.022$; poly-3 test NS NS	
Full carcinogenicity Rat, F344/N (M) 6–7 wk 104 wk NTP (2014)	Drinking-water β-Picoline, 96.4% Tap water 0, 156.25, 312.5, 625 mg/L ad libitum 50, 50, 50, 50 33, 31, 32, 24	*Lung* Bronchioloalveolar carcinoma 0/50, 0/50, 4/50 (8%), 2/50 (4%) Bronchioloalveolar adenoma or carcinoma (combined) 3/50 (6%), 5/50 (10%), 5/50 (10%), 4/50 (8%) Bronchioloalveolar adenoma 3/50 (6%), 5/50 (10%), 1/50 (2%), 2/50 (4%)	NS NS NS	Principal strengths: well-conducted GLP study Historical incidence (mean ± SD; range): Bronchioloalveolar carcinoma (includes multiple): drinking-water, 0/100; all routes, 15/1249 (1.2 ± 1.4%; 0–6%) Bronchioloalveolar adenoma or carcinoma (combined): drinking-water, 7/100 (7.0 ± 1.4%; 6–8%); all routes, 45/1249 (3.6 ± 2.8%; 0–10%)
Full carcinogenicity Rat, F344/N (F) 6–7 wk 105 wk NTP (2014)	Drinking-water β-Picoline, 96.4% Tap water 0, 156.25, 312.5, 625 mg/L ad libitum 50, 50, 50, 50 30, 32, 33, 30	*Lung* Bronchioloalveolar adenoma 0/50, 3/50 (6%), 2/50 (4%), 5/50* (10%) Bronchioloalveolar carcinoma 0/50, 1/50 (2%), 0/50, 0/50 Bronchioloalveolar adenoma or carcinoma (combined) 0/50, 4/50 (8%), 2/50 (4%), 5/50* (10%)	$P = 0.029$ (trend), *$P = 0.030$; poly-3 test NS $P = 0.050$ (trend), *$P = 0.030$; poly-3 test	Principal strengths: well-conducted GLP study Historical incidence (mean ± SD; range): Bronchioloalveolar adenoma: drinking-water, 4/100 (4.0 ± 5.7%; 0–8%); all routes, 25/1200 (2.1 ± 2.9%; 0–8%) Bronchioloalveolar adenoma or carcinoma (combined): drinking-water, 4/100 (4.0 ± 5.7%; 0–8%); all routes, 27/1200 (2.3 ± 2.9%; 0–8%) Bronchioloalveolar carcinoma: drinking-water, 0/100; all routes, 3/1200 (0.3 ± 0.7%; 0–2%)

F, female; GLP, good laboratory practice; M, male; NS, not significant; SD, standard deviation; wk, week; yr, year

groups of female mice, with a significant positive trend. There was also a significant increase in the incidence of hepatocellular adenoma (includes multiple) (38/49, 46/50, 46/50, and 39/50) in female mice exposed at the lowest dose. Hepatoblastoma [a rare neoplasm in this strain of female mice] occurred in 1/49 (2%) control and 3/50 (6%), 4/50 (8%), and 4/50 (8%) exposed females; incidence in all the treated groups exceeded the upper bound of the range for historical controls for drinking-water studies (range, 0–2%) and for all routes of administration (range, 0–2%). The lung was also a target organ in female mice. The incidence of bronchioloalveolar adenoma or carcinoma (combined) (11/50, 13/50, 13/49, and 21/50) in females exposed at the highest dose was significantly increased compared with that in controls, with a significant positive trend. There was also a [non-statistically significant] dose-dependent association between exposure and the incidence of bronchioloalveolar carcinoma (includes multiple) (7/50, 8/50, 10/49, and 13/50). Additionally, multiple bronchioloalveolar adenomas and multiple bronchioloalveolar carcinomas occurred [non-statistically significant] in most of the exposed groups of females, but no multiple lung neoplasms occurred in the controls. In male mice, there was a significant increase in the incidence of bronchioloalveolar adenoma (includes multiple) (6/50, 11/50, 16/50, and 8/50) in the group exposed at the intermediate dose. [The Working Group noted that this was a well-conducted study that complied with good laboratory practice (GLP).]

3.2 Rat

Drinking-water

Groups of 50 male and 50 female Fischer 344/N rats (age, 6–7 weeks) were given drinking-water containing β-picoline (purity, 96.4%) at concentrations of 0, 156.25, 312.5, or 625 mg/L ad libitum for 7 days per week for 104 weeks (males) and 105 weeks (females) (NTP, 2014). Average daily doses of β-picoline were approximately 0, 6, 12, and 22 mg/kg bw (males), and 0, 7, 14, and 26 mg/kg bw (females). The survival of exposed groups of male and female rats was similar to that of the control groups. Mean body weights were slightly less than those of controls throughout the study for males exposed at the highest dose, and were 10% less at the end of the study. Mean body weights were slightly less than those of controls for most of the study for females exposed at the highest dose, and were 9% less for a 16-week period towards the end of the study. Decreased water consumption was evident in males and females exposed at the highest dose compared with that of the controls throughout the study.

Bronchioloalveolar adenomas were observed in all exposed groups of female rats, but not in controls, with an incidence of 0/50, 3/50 (6%), 2/50 (4%), and 5/50 (10%), respectively; there was a significant positive trend in the incidence of this neoplasm and a significant increase in the incidence in females exposed at the highest dose that exceeded the upper bound of the range for historical controls for drinking-water studies (range, 0–8%) and for all routes of administration (range, 0–8%). One bronchioloalveolar carcinoma occurred in a female exposed at the lowest dose. Bronchioloalveolar carcinoma occurred in males exposed at the intermediate dose (4/50) [non-statistically significant] and highest dose (2/50) [non-statistically significant], but not at the lowest dose or in controls. However, the incidence of bronchioloalveolar adenoma or carcinoma (combined) in males was similar between the control and exposed groups and was also consistent with the historical incidence of this combination of tumours in male Fischer 344 rats. [The Working Group noted that this was a well-conducted study that complied with GLP.]

Fig. 4.1 Proposed metabolic pathways of β-picoline
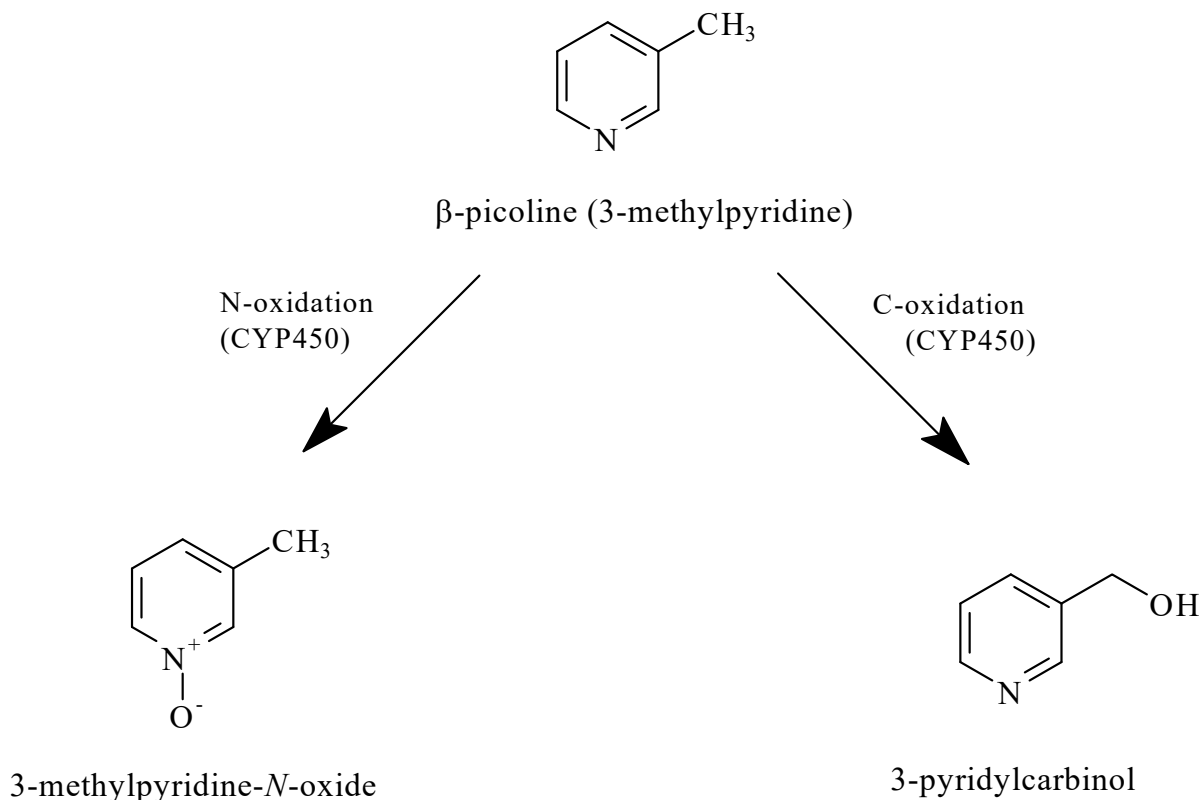
CYP450, cytochrome P450
Compiled by the Working Group

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

No data were available on the absorption, distribution, metabolism, and excretion to the Working Group.

4.1.2 Experimental systems

See Fig. 4.1

Few data were available on the absorption, distribution, metabolism, and excretion of β-picoline. It is readily absorbed from the gastrointestinal tract, intraperitoneal cavity, and the lungs, and moderately well absorbed through the skin (Trochimowicz et al., 2001).

Gorrod & Damani (1979a) investigated the metabolism of β-picoline in vitro using various organ homogenates of rabbits, guinea-pigs, and rats. β-Picoline is metabolized in mice, rats, hamsters, guinea-pigs, and rabbits through the C-oxidation and N-oxidation metabolic pathways (yielding 3-pyridylcarbinol and 3-methylpyridine-N-oxide, respectively), with the maximum activity being found in the liver and lung (Gorrod & Damani, 1979a). In a separate study, Gorrod & Damani (1979b) showed that these C-oxidation and N-oxidation reactions of β-picoline are mediated by a cytochrome P450 (CYP450) system, as shown by the reduced

Table 4.1 Genetic and related effects of β-picoline in experimental systems

Test system	End-point	Results[a]		Concentration (HIC or LEC)	Reference
		Without metabolic activation	With metabolic activation		
Mouse, B6C3F$_1$ (M, F); peripheral blood erythrocytes	Micronucleus formation	–	NA	78–1250 mg/L, drinking-water, for 3 mo	NTP (2014)
Salmonella typhimurium TA98	Reverse mutation	NT	–	1000 μg/plate	Ho et al. (1981)
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	8540 μg/plate	Haworth et al. (1983)
Salmonella typhimurium TA97, TA98, TA100, TA102	Reverse mutation	–	–	5000 μg/plate	Claxton et al. (1987)

F, female; HIC, highest ineffective concentration; LEC, lowest effective concentration; M, male; mo, month; NA, not applicable; NT, not tested
[a] –, negative; the level of significance was set at $P < 0.005$ in all cases

C-oxidation and N-oxidation in the presence of CYP450 inhibitors.

The existence of the N-oxidation pathway of β-picoline was also demonstrated in rodents in vivo. This was shown by the presence of 3-methyl-*N*-oxide at concentrations of 6.6% and 4.2% in urine of mice and rats at 72 hours after intraperitoneal injection of β-picoline at 40 mg/kg bw (Gorrod & Damani, 1980).

4.1.3 Modulation of metabolic enzymes

In female Fischer 344 rats given drinking-water containing β-picoline at concentrations of 156, 312, 625, and 1250 mg/L for 23 days, a statistically significant dose-dependent increase in the activity of hepatic 7-pentoxyresorufin-*O*-dealkylase, a marker for CYP2B1, was observed (NTP, 2014). A similar effect on 7-pentoxyresorufin-*O*-dealkylase activity was also observed in the livers of male Fischer 344 rats exposed to β-picoline at 312, 625, and 1250 mg/L (NTP, 2014).

4.2 Mechanisms of carcinogenesis

This section summarizes the available evidence for the key characteristics of carcinogens (Smith et al., 2016). Data were available only for the key characteristic "is genotoxic"; for the other key characteristics of human carcinogens, insufficient data were available for evaluation.

4.2.1 Genetic and related effects

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

See Table 4.1

In male and female B6C3F$_1$ mice given drinking-water containing β-picoline at concentrations of 78–1250 mg/L for 3 months, no increase in the frequency of micronucleus formation in peripheral blood erythrocytes was observed (NTP, 2014).

Several studies investigated the mutagenicity of β-picoline in the Ames test. β-Picoline did not induce mutations in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA102 at concentrations of up to 5000 μg per plate (Claxton et al.,

1987; NTP, 2014) or in strains TA98, TA100, TA1535, or TA1537 at concentrations of up to 8540 µg per plate (Haworth et al., 1983; NTP, 2014). Ho et al. (1981) also reported negative results for the induction of gene mutation in *S. typhimurium* strain TA98 tested with β-picoline at concentrations of up to 1000 µg per plate.

4.2.2 Other mechanistic data

No data were available to the Working Group.

4.3 Other adverse effects

In male Fischer 344 rats given drinking-water containing β-picoline at a concentration of 312, 625, or 1250 mg/L for 3 months, a significant increase in the concentration of $α_{2u}$-globulin in the kidney was observed. This increase was accompanied by progressive nephropathy in rats at 625 and 1250 mg/L, and hyaline droplet accumulation in proximal renal tubules in rats at 1250 mg/L (NTP, 2014). Neurotoxicological effects were also observed in rats (Dyer et al., 1985).

4.4 Data relevant to comparisons across agents and end-points

See the monograph on isobutyl nitrite in the present volume.

5. Summary of Data Reported

5.1 Exposure data

β-Picoline, a methylpyridine, is a "high production volume" chemical that is produced globally. A large increase in the production volume has been observed in China during the last decade. β-Picoline is widely used as a starting material for agrochemicals (e.g. chlorpyrifos) and pharmaceuticals (e.g. vitamin B3). It is also used as a solvent and intermediate in rubber accelerators, waterproofing agents, dyes, and resins, and as a flavouring substance in foods and beverages. β-Picoline is released to the environment through industrial wastewater and as a result of cigarette smoking. It also occurs naturally at very low concentrations in coffee, beer, and whisky. Occupational exposure occurs primarily through inhalation or dermal contact during the production or use of β-picoline. Exposure of the general population can occur via inhalation of tobacco smoke, ingestion of contaminated food or water, or dermal contact with products containing β-picoline.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

β-Picoline was tested for carcinogenicity in one well-conducted good laboratory practice (GLP) 2-year drinking-water study in male and female mice, and in one well-conducted GLP 2-year drinking-water study in male and female rats.

β-Picoline caused a significant increase in the incidence of hepatocellular carcinoma and of hepatocellular carcinoma or hepatoblastoma (combined) in all exposed female mice compared with controls, with a significant positive trend. There was also a significant positive trend in the incidence of hepatocellular adenoma in female mice. There was a significant positive trend in the incidence of bronchioloalveolar adenoma of the lung in female mice. The incidence of bronchioloalveolar adenoma or carcinoma (combined) in female mice exposed at the highest dose was also significantly increased compared with that in controls, with a significant positive trend. There

was a significant increase in the incidence of bronchioloalveolar adenoma in male mice.

β-Picoline caused a significant increase in the incidence of bronchioloalveolar adenoma in female rats exposed at the highest dose, with a significant positive trend. In male rats, there was no significant increase in the incidence of any tumour.

5.4 Mechanistic and other relevant data

No data on the absorption, distribution, metabolism, and excretion of β-picoline in exposed humans were available. In rodents, β-picoline is readily absorbed from the gastro-intestinal tract, intraperitoneal cavity, and the lungs, moderately well absorbed through the skin, and metabolized by cytochrome P450-mediated N-oxidation. An additional C-oxidation pathway has been demonstrated in various organ homogenates. In the Fischer 344 rat, β-picoline induced a dose-dependent increase in hepatic 7-pentoxyresorufin-O-dealkylase activity.

Regarding the key characteristics of carcinogens, β-picoline gave negative results in the mouse micronucleus test and in the Ames assay. No other relevant data were available, including from humans or human experimental systems.

In male Fischer 344 rats exposed for 3 months, β-picoline significantly increased the level of α_{2u}-globulin in the kidney.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

There is *limited evidence* in experimental animals for the carcinogenicity of β-picoline.

6.3 Overall evaluation

β-Picoline is *not classifiable as to its carcinogenicity to humans (Group 3).*

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METHYL ACRYLATE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 96-33-3

Chem. Abstr. Serv. name: 2-propenoic acid, methyl ester

IUPAC systematic name: methyl prop-2-enoate

Synonym: methyl propenoate; acrylic acid methyl ester; methyl 2-propenoate; 2-propenoic acid; methyl ester; methoxycabonylethylene.

1.1.2 Structural and molecular formulae, and relative molecular mass

Chemical formula: $C_4H_6O_2$
Relative molecular mass: 86.09
Structural formula:
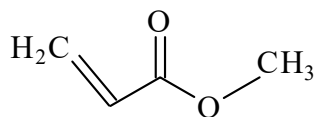
1.1.3 Chemical and physical properties

Description: colourless liquid with an acrid odour, with a low odour threshold (Budavari et al., 1996)

Boiling point: 80.7 °C at 1 atm (ACGIH, 2014)

Melting point: −76.5 °C (Budavari et al., 1996)

Solubility: slightly soluble in water; soluble in alcohol, ether, and other organic solvents (ACGIH, 2014)

Vapour pressure: 68.25 mm Hg [9.1 kPa] at 20 °C

Relative vapour density (air = 1): 2.97 (ACGIH, 2014)

Flash point: −2.8 °C, closed cup; 6.7 °C, open cup (ACGIH, 2014)

Explosive limits: upper, 25%; lower, 2.8% by volume in air (ACGIH, 2014)

Conversion factor: 1 ppm = 3.52 mg/m³ at 25 °C and 1 atm.

1.1.4 Technical products and impurities

Impurities reported in commercial-grade (technical) methyl acrylate (purity, 98.9–99.9%) include water (≤ 0.1% by weight), acrylic acid (0.01% by weight), and hydroquinone monomethyl ether (15, 200, or 1000 mg/kg) (HSDB, 2018).

1.2 Production and use

1.2.1 Production process

Methyl acrylate is produced by the oxidation of propylene to acrolein and then to acrylic acid; this is then reacted with methanol or, by a modification of the Reppe process, from acetylene and then reacted with methanol in the presence of acid and nickel carbonyl (ECETOC, 1998). Methyl acrylate can also be formed using organic carbonates as esterifying agents, isolating 2-halo-1-alkenes from hydrocarbon feedstocks, or by reacting formaldehyde with ketene to β-propiolactone, which is then reacted with methanol. To prevent spontaneous polymerization, methyl acrylate is stored with small amounts of hydroquinones (ECETOC, 1998).

1.2.2 Production volume

Methyl acrylate is a high production volume chemical (OECD, 2009), and is manufactured in and/or imported into the European Economic Area in quantities of 10–100 thousand metric tonnes per year (ECHA, 2018). The USA produced from more than 100 to 500 million pounds [> 45.4 to 227 thousand metric tonnes] in 2002 (HSDB, 2018). Production volumes for China ranged from 104 thousand metric tonnes in 2008 to 99 thousand metric tonnes in 2010 (Chinese Report, 2008, 2010). Recent figures for the first quarter of 2017 are 35.4 thousand metric tonnes (Chinese Report, 2017) [approximately 140 thousand metric tonnes in 2017, by extrapolation].

1.2.3 Use

The main uses of methyl acrylate are in the production of methyl acrylic polymers and, together with acrylonitrile, in the production of acrylic and modacrylic fibres. Methyl acrylic polymers are used in adhesives, resinous and polymeric coatings (including leather finish resins), paper, and paperboard that may come into contact with foods. Acrylic and modacrylic fibres are used in the clothing and home furnishing industries in fire-retardant fabrics, paint rollers, battery separators, and protective clothing (ECETOC, 1998; ACGIH, 2014). Methyl acrylate is also used to produce thermoplastic coatings, adhesives, sealants, amphoteric surfactants for shampoos, medical and dental prostheses, contact lenses, and speciality plastics including latex coatings, and floor and fabric finishes (ECETOC, 1998; ACGIH, 2014). It is also used in the synthesis of other organic molecules. The distribution of use in the 1990s was 38% for acrylic fibres, 15% for plastics additives, 12% for coatings and varnishes, 25% for the production of adhesives, detergents, flocculants, dispersion aids, and raw materials for organic synthesis, and 10% for other uses (ECETOC, 1998).

1.3 Analytical methods

Air sampling for methyl acrylate is conducted using charcoal adsorbent. Samples are desorbed using carbon disulfide and the extract analysed using gas chromatography with flame ionization detection by United States National Institute for Occupational Safety and Health (NIOSH) Method 1459 (NIOSH, 1994) and United States Occupational Safety and Health Administration (OSHA) Method 92 (OSHA, 2018). NIOSH Method 1459 has a detection limit of 10 μg per sample and OSHA Method 92 has a detection limit of 140 μg/m³.

Methyl acrylate can also be analysed in water; the most recently published method found by the Working Group is United States Environmental Protection Agency (EPA) Method 624.1 (EPA, 2016). This technique uses a purging chamber that transfers the volatile compounds to the vapour phase, followed by a sorbent trap. The trap is then heated and back-flushed to desorb the purgeables onto a gas chromatography column that is combined with mass spectrometry; the detection limit for methyl acrylate was

not reported. Similar purge and trap methods are also reported for other aqueous, solid (including waste and soil), and tissue samples (NEMI, 1996).

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

Methyl acrylate may be released into the environment in fugitive and stack emissions or in wastewater during its production and use. Methyl acrylate is expected to volatize from water surfaces, and is not expected to persist or to bioaccumulate in the environment. The EPA Toxics Release Inventory reported methyl acrylate emissions in fugitive and stack air, as well as wastewater emissions, from 64 facilities in the USA in 2016, with similar numbers of facilities reporting emissions between 1990 and 2016 (EPA, 2017). These facilities were mostly classified as chemical (81%), hazardous waste (6%), chemical wholesalers (5%), and non-metallic mineral product (3%) industries, as well as other industries (3%) such as petroleum, plastics, and rubber. Median reported on- and offsite releases into the air were 500, 255, 223, and 72 pounds [227, 116, 101, and 33 kg] for the years 1990, 2000, 2010, and 2016, respectively (EPA, 2017). The Canadian National Pollutant Release Inventory reported a mean annual release of 2100 kg of methyl acrylate into the air from one facility in 1994 and no releases for the years 2000, 2010, and 2016; no releases onto land or into water were reported (Government of Canada, 2017). The Working Group found no reports of measured methyl acrylate concentrations in environmental media.

1.4.2 Exposure in the general population

Methyl acrylate exposure in the general population may occur through the use of products containing this chemical, such as adhesives and sealants; however, no quantitative information on exposure was available to the Working Group.

1.4.3 Occupational exposure

Occupational exposure to methyl acrylate may occur through inhalation and dermal contact during its production and use as an intermediate in the production of fibres, resins, coatings, and other products. Average full-shift methyl acrylate concentrations in the air of 2 ppm [7 mg/m³], with peaks of 12.6–30.0 ppm [44.4–106 mg/m³] lasting 2–5 minutes and mean area concentrations of 5.4 ppm [19 mg/m³] with a range of 0.6–17.2 ppm [2.1–60.5 mg/m³], were reported for a chemical production facility in Texas, USA. The highest peak exposure was 122 ppm [429 mg/m³] (ACGIH, 2014). [These concentrations were reported in American Conference of Governmental Industrial Hygienists threshold limit value documentation from unpublished data, where the measurements were presumably made before 1996.]

Residual methyl acrylate monomer (0.05%) has been found in the polymer powder used for dental resins (Davy & Braden, 1991).

1.5 Regulations and guidelines

Occupational exposure limits for methyl acrylate are in place in numerous countries (see Table 1.1). In the majority of these countries, the 8-hour time-weighted average (TWA) limit is either 7 or 18 mg/m³, with a short-term limit of 14, 18, or 36 mg/m³. In Australia, New Zealand, Singapore, and the USA (NIOSH and OSHA), the 8-hour TWA limit is 35 mg/m³, with no short-term limit (IFA, 2018).

The United States Food and Drug Administration has established regulations for the use of monomers, polymers, and copolymers including methyl acrylate in food-contact materials. The proportion of the monomers should

Table 1.1 Occupational exposure limits for methyl acrylate

Country or region	Concentration (mg/m³)	Interpretation	Comments
Australia	35	TWA	
Austria	18	TWA	
Belgium	36	STEL	
	7.2	TWA	
Canada, Ontario	7	TWA	
Canada, Quebec	7	TWA	
China	20	TWA	
Denmark	7	TWA	
	14	STEL	
European Union	18	TWA	Indicative OEL values
	36	STEL	
Finland	7	TWA	
	18	STEL	
France	18	TWA	Restrictive statutory limit values
	36	STEL	
Germany (AGS)	7.1	TWA	
	14.2	STEL	
Germany (DFG)	7.1	TWA	
	14.2	STEL	
Hungary	18	TWA	
	18	STEL	
Ireland	18	TWA	
	36	STEL	
Italy	7	TWA	Skin notation
	35	STEL	
Japan (JSOH)	7	TWA	
Latvia	20	TWA	
Netherlands	18	TWA	
	36	STEL	
New Zealand	35	TWA	
Poland	14	TWA	
	28	STEL	
Republic of Korea	7	TWA	
Romania	18	TWA	
	36	STEL	
Singapore	35	TWA	
Spain	7.2	TWA	Skin, sensitizer notation
Sweden	18	TWA	
	36	STEL	
Switzerland	18	TWA	
	18	STEL	
Turkey	18	TWA	
	36	STEL	

Table 1.1 (continued)

Country or region	Concentration (mg/m³)	Interpretation	Comments
UK	[36]	TWA	The UK Advisory Committee on Toxic Substances has expressed concern that, for the OEL 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 UK 2002 list and its 2003 supplement, but are omitted from the published 2005 list
USA (ACGIH)	7.2	TWA	Eye, skin, upper respiratory tract irritation, eye damage
USA (NIOSH)	35	TWA	
USA (OSHA)	35	TWA	

ACGIH, American Conference of Governmental Industrial Hygienists; AGS, Ausschuss für Gefahrstoffe (Committee on Hazardous Substances); DFG, Deutsche Forschungsgemeinschaft (German Research Foundation); JSOH, Japan Society for Occupational Health; NIOSH, United States National Institute for Occupational Safety and Health; OEL, occupational exposure limit; OSHA, United States Occupational Safety and Health Administration; STEL, short-term (15-minute) exposure limit; TWA, 8-hour time-weighted average
Adapted from IFA (2018)

not exceed 5% by weight of total polymer units ([CFR, 2017](#)).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

Methyl acrylate was previously reviewed by the Working Group in Volume 39 ([IARC, 1986](#)), Supplement 7 ([IARC, 1987](#)), and Volume 71 ([IARC, 1999](#)) of the *IARC Monographs*. The Volume 71 Working Group concluded that there was *inadequate evidence* in experimental animals for the carcinogenicity of methyl acrylate. This section provides an evaluation of the studies of carcinogenicity in experimental animals reviewed in the previous Monographs and Supplement, and of all studies published since then.

See [Table 3.1](#)

3.1 Mouse

Inhalation

Groups of 50 male and 50 female B6D2F1/Crlj mice (age, 6 weeks) were exposed to methyl acrylate (purity, 99.9%) at a concentration of 0 (control), 2.5, 10, or 40 ppm [0, 9, 35, or 141 mg/m³] by whole-body inhalation for 6 hours per day, 5 days per week for 94 weeks (males) or 97 weeks (females) ([Japan Bioassay Research Center, 2017](#)). The study was originally designed for a 104-week exposure but, because the survival rates of the control groups of males and females were lower than 25% the later weeks of treatment (because of amyloidosis), the study was terminated at 94 weeks (males) and 97 weeks (females); the survival rate of males exposed at 40 ppm was significantly higher (27/50 vs 12/50 controls).

Body weights in male and female mice exposed at 40 ppm were decreased in the early exposure periods, but were similar to controls by the end of the study. No significant increase in the incidence of any neoplastic lesions was found in the exposed male or female groups compared with controls ([Japan Bioassay Research Center, 2017](#)). [The Working Group noted that this was a well-conducted study that complied with good laboratory practice.]

3.2 Rat

Inhalation

In a study by [Reininghaus et al. (1991)](#), groups of 86 male and 86 female Sprague-Dawley rats (age, 35 days) were exposed to methyl acrylate (purity, > 99.8%; main impurities, methyl propionate and ethyl acrylate) at a concentration of 0, 15, 45, or 135 ppm [0, 53, 158, or 475 mg/m³] by whole-body inhalation for 6 hours per day, 5 days per week, for 24 months. During weeks 1–13, the rats were exposed to one third of the final test substance concentration. Interim kills were carried out after 12 months (10 males and 10 females per group) and 18 months (15 male and 15 females per group). No significant sex-specific differences in mortality were observed. From week 15 to the end of the exposure period, the body weights of male and female rats exposed at the highest dose (135 ppm) were significantly lower (~4%) than those of other groups.

The incidence of sarcoma of the soft tissue (skin or subcutis) [not otherwise specified] in exposed males was increased compared with controls, with a significant positive trend [$P = 0.014$, Cochran–Armitage trend test]: 0/86, 4/86 (5%), 0/86, and 6/86 (7%), respectively [$P = 0.029$ at 135 ppm, Fisher exact test]. The incidence of "malignant leukaemic tumours" (leukaemia, lymphoma, and lymphosarcoma) in exposed males was increased compared with controls, with a significant positive trend [$P = 0.003$, Cochran–Armitage trend

Table 3.1 Studies of carcinogenicity with methyl acrylate in experimental animals

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, B6D2F$_1$/Crlj (M) 6 wk 94 wk JBRC (2017)	Inhalation (whole-body exposure) Methyl acrylate, 99.9% None 0, 2.5, 10, 40 ppm for 6 h/d, 5 d/wk 50, 50, 50, 50 12, 16, 12, 27	*Any tumour type* No significant increase in the incidence of any neoplastic lesion	NS	Principal strengths: study covered most of lifespan; well-conducted GLP study Principal limitations: survival rate of control group was < 25% in later weeks of the treatment period (due to amyloidosis); study therefore terminated at wk 94 Survival of mice exposed at 40 ppm was significantly higher
Full carcinogenicity Mouse, B6D2F$_1$/Crlj (F) 6 wk 97 wk JBRC (2017)	Inhalation (whole-body exposure) Methyl acrylate, 99.9% None 0, 2.5, 10, 40 ppm for 6 h/d, 5 d/wk 50, 50, 50, 50 12, 12, 12, 20	*Any tumour type* No significant increase in the incidence of any neoplastic lesion	NS	Principal strengths: study covered most of lifespan; well-conducted GLP study Principal limitations: survival rate of control group was < 25% in later weeks of the treatment period (due to amyloidosis); study therefore terminated at wk 97 No significant difference in survival between control and treated groups
Full carcinogenicity Rat, Sprague-Dawley (M) 35 d 24 mo Reininghaus et al. (1991)	Inhalation (whole-body exposure) Methyl acrylate, > 99.8% None 0, 15, 45, 135 ppm for 6 h/d, 5 d/wk 86, 86, 86, 86 NR, NR, NR, NR	*Soft tissues*: sarcoma [not otherwise specified] 0/86*, 4/86 (5%), 0/86, 6/86 (7%)** *Haematopoietic and lymphoid tissues*: "malignant leukaemic tumours" (leukaemia, lymphoma, and lymphosarcoma) 0/86*, 3/86 (3%), 7/86 (8%)**, 0/86	*[$P = 0.014$, Cochran–Armitage trend test]; **[$P = 0.029$, Fisher exact test] *[$P = 0.003$, Cochran–Armitage trend test]; **[$P = 0.014$, Fisher exact test]	Principal strengths: well-conducted study From week 1 to week 13, the rats were exposed to one third of the final test substance concentrations; survival similar between groups

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) 35 d 24 mo Reininghaus et al. (1991)	Inhalation (whole-body exposure) Methyl acrylate, > 99.8% None 0, 15, 45, 135 ppm for 6 h/d, 5 d/wk 86, 86, 86, 86 NR, NR, NR, NR	*Pituitary gland*: adenoma 10/86 (12%)*, 21/86 (24%)**, 23/86 (27%)***, 9/86 (10%)	*[P = 0.006, Cochran–Armitage trend test]; **[P = 0.046, Fisher exact test]; ***[P = 0.019, Fisher exact test]	Principal strengths: well-conducted study From wk 1 to wk 13, the rats were exposed to one third of the final test substance concentrations; survival similar between groups
Full carcinogenicity Rat, F344/ DuCrlCrlj (M) 6 wk 104 wk JBRC (2017)	Inhalation (whole-body exposure) Methyl acrylate, 99.9% None 0, 10, 40, 160 ppm for 6 h/d, 5 d/wk 50, 50, 50, 50 38, 42, 35, 39	*Nasal cavity*: squamous cell carcinoma 0/50*, 0/50, 1/50, 6/50**	*P ≤ 0.0002, Cochran–Armitage trend test, Peto trend test; **P = 0.0133, Fisher exact test	Principal strengths: study covered most of lifespan; well-conducted GLP study Survival in exposed groups similar to controls; historical control incidence: nasal cavity squamous cell carcinoma, 0/649
Full carcinogenicity Rat, F344/ DuCrlCrlj (F) 6 wk 104 wk JBRC (2017)	Inhalation (whole-body exposure) Methyl acrylate, 99.9% None 0, 10, 40, 160 ppm for 6 h/d, 5 d/wk 50, 50, 50, 50 40, 39, 43, 41	*Nasal cavity*: squamous cell carcinoma 0/50, 0/50, 0/50, 2/50 *Adrenal gland* Pheochromocytoma (benign or malignant, combined) 1/50*, 1/50, 1/50, 4/50 (8%) Pheochromocytoma (benign) 1/50, 0/50, 1/50, 2/50 (4%) Pheochromocytoma (malignant) 0/50, 1/50, 0/50, 2/50 (4%)	NS *P = 0.0420, Peto trend test NS NS	Principal strengths: study covered most of lifespan; well-conducted GLP study Survival in exposed groups similar to controls Historical control incidence: nasal cavity squamous cell carcinoma, 0/650; adrenal gland pheochromocytoma (benign or malignant, combined), 18/650 (range, 0–8%); adrenal gland pheochromocytoma (benign), 11/650 (range, 0–8%); adrenal gland pheochromocytoma (malignant), 7/650 (range, 0–4%)

d, day; F, female; GLP, good laboratory practice; M, male; mo, month; NR, not reported; NS, not significant; ppm, parts per million; wk, week

test]: 0/86, 3/86 (3%), 7/86 (8%), and 0/86, respectively [$P = 0.014$ at 45 ppm, Fisher exact test]. The incidence of adenoma of the pituitary gland in exposed females was increased compared with controls, with a significant positive trend [$P = 0.006$, Cochran-Armitage trend test]: 10/86 (12%), 21/86 (24%), 23/86 (27%), and 9/86 (10%), respectively [$P = 0.046$ at 15 ppm, $P = 0.019$ at 45 ppm; Fisher exact test] (Reininghaus et al., 1991). [The Working Group noted this was a well-conducted study and that the exposure schedule was unusual.]

Groups of 50 male and 50 female Fischer 344/ DuCrlCrlj rats (age, 6 weeks) were exposed to methyl acrylate (purity, 99.9%) at a concentration of 0 (control), 10, 40, or 160 ppm [0, 35, 141, or 563 mg/m^3] by whole-body inhalation for 6 hours per day, 5 days per week, for 104 weeks. No significant difference in mortality was observed between the groups. Body weights in male and female rats exposed to methyl acrylate at 160 ppm were decreased. At 104 weeks, there was a statistically significant increase in the incidence of squamous cell carcinoma of the nasal cavity in male rats at the highest dose ($P = 0.0133$, Fisher exact test) compared with controls, with a significant positive trend (0/50, 0/50, 1/50, and 6/50 (12%); $P \leq 0.0002$, Cochran–Armitage trend test); no squamous cell carcinomas of the nasal cavity were observed in 649 male historical controls from the laboratory. There were 2 cases (2/50, 4%) of squamous cell carcinoma of the nasal cavity in females exposed at 160 ppm (and none in the other groups), which was not a statistically significantly increase; however, this is a rare tumour that was not observed in 650 female historical controls from the laboratory. There was a significant positive trend in the incidence of pheochromocytoma (benign or malignant, combined) of the adrenal gland in females (1/50, 1/50, 1/50, and 4/50; $P = 0.0420$, Peto trend test) [the incidence in females exposed at 160 ppm (8%) equalled the upper limit of the range observed in female historical controls from

the laboratory (0–8%) (Japan Bioassay Research Center (2017)]. [The Working Group noted that this was a well-conducted study that complied with good laboratory practice.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Data on absorption, distribution, metabolism, and excretion of methyl acrylate in humans were not available to the Working Group.

4.1.2 Experimental systems

Methyl acrylate has been shown to be readily absorbed in rats (Sapota, 1988, 1993) and guinea-pigs (Seutter & Rijntjes, 1981) after the radiolabelled compound was given by intraperitoneal injection or orally. Dermal absorption has also been demonstrated in guinea-pigs; radiolabelled methyl acrylate had fully penetrated the dermis after 16 hours and was spread throughout the body (Seutter & Rijntjes, 1981).

Methyl acrylate was distributed to all major tissues after oral exposure or intraperitoneal injection in rats (Sapota 1988, 1993) and guinea-pigs (Seutter & Rijntjes, 1981). In rats, the highest concentration of radiolabel was detected in the liver and kidney 1 and 2 hours after intraperitoneal or oral exposure, respectively (Sapota, 1988, 1993). The highest concentrations of radiolabelled methyl acrylate detected using whole-body autoradiography of guinea-pigs were observed in the liver, bladder, and brain, or in the peritoneum and liver, 1 hour after oral exposure or intraperitoneal injection, respectively. Radiolabel quickly disappeared from all tissues, but at a slightly

slower rate after intraperitoneal injection than after oral exposure (Seutter & Rijntjes, 1981).

In rats, the major route of excretion of methyl acrylate is via expiration (as carbon dioxide, CO_2, > 50%) and urine (10–50%), and, to smaller extent, faeces (1–3%) (Sapota, 1988, 1993). The total radiolabel excreted after oral exposure or intraperitoneal injection of radiolabelled methyl acrylate within 72 hours was approximately 97% and 91% of the administered dose, respectively (Sapota, 1988). A similar excretion pattern was observed in guinea-pigs (Seutter & Rijntjes, 1981).

There are two suggested detoxification pathways for methyl acrylate (Sapota, 1993) (see Fig. 4.1): (i) hydrolysis by carboxylesterases to acrylic acid and methanol, with further hydration of the double bond of acrylic acid to form 3-hydroxypropionic acid that can then be oxidized to malonic acid and further to CO_2; and (ii) conjugation with endogenous glutathione and subsequent excretion as mercapturic acid in urine.

These two metabolic pathways are supported by several findings in the literature (Delbressine et al., 1981; Miller et al., 1981; Seutter & Rijntjes, 1981; Vodička et al., 1990; Black et al., 1993; Sapota, 1993). For instance, methyl acrylate has been shown to be hydrolysed by rat tissue carboxylesterases to acrylic acid (Miller et al., 1981). An increase in the amount of excreted mercapturic acid derivatives of methyl acrylate, more specifically thioethers, was also observed in rats and guinea-pigs after intraperitoneal injection, and in guinea-pigs after oral and dermal exposure to methyl acrylate. In rats, the thioethers were identified as N-acetyl-(2-carboxyethyl)-L-cysteine and the corresponding monomethyl ester at a ratio of 20:1 (Delbressine et al., 1981; Seutter & Rijntjes, 1981). This is consistent with the observed chemical reactivity of methyl acrylate with glutathione in vitro, with an estimated half-life of 18.4 minutes (Miller et al., 1981; Vodička et al., 1990).

4.1.3 Modulation of metabolic enzymes

At doses of up to 160 µM, methyl acrylate did not induce mRNA of the endogenous human NAD(P)H:quinone oxidoreductase (HQOR1) gene in the human hepatocarcinoma cell line (HepG2) (Winner et al., 1997). However, at 20 µM, it caused a twofold induction of quinone reductase in the mouse Hepa 1c1c7 cell line (Talalay, 1989).

4.2 Mechanisms of carcinogenesis

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016). Data were available only for the key characteristic "is genotoxic".

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

See Table 4.1

There was an increase in the frequency of micronucleated cells in the bone marrow of male BALB/c mice exposed to methyl acrylate by two intraperitoneal injections given 24 hours apart (Przybojewska et al., 1984). However, in ddY outbred mice, methyl acrylate gave negative results in assays for micronucleus formation after oral exposure (a single dose of 250 mg/kg bw) or by inhalation (2100 ppm for 3 hours) (Hachiya et al., 1982; Sofuni et al., 1984).

(ii) Non-human mammalian cells in vitro

See Table 4.2

In Chinese hamster ovary (CHO) AS52 cells, methyl acrylate was not mutagenic in the xanthine-guanine phosphoribosyl transferase (Xprt) assay (Oberly et al., 1993). In addition, no mutagenic effect was reported in the

Fig. 4.1 Proposed metabolic pathways for methyl acrylate, based on identification of acrylic acid, carbon dioxide, and mercapturic acid conjugates
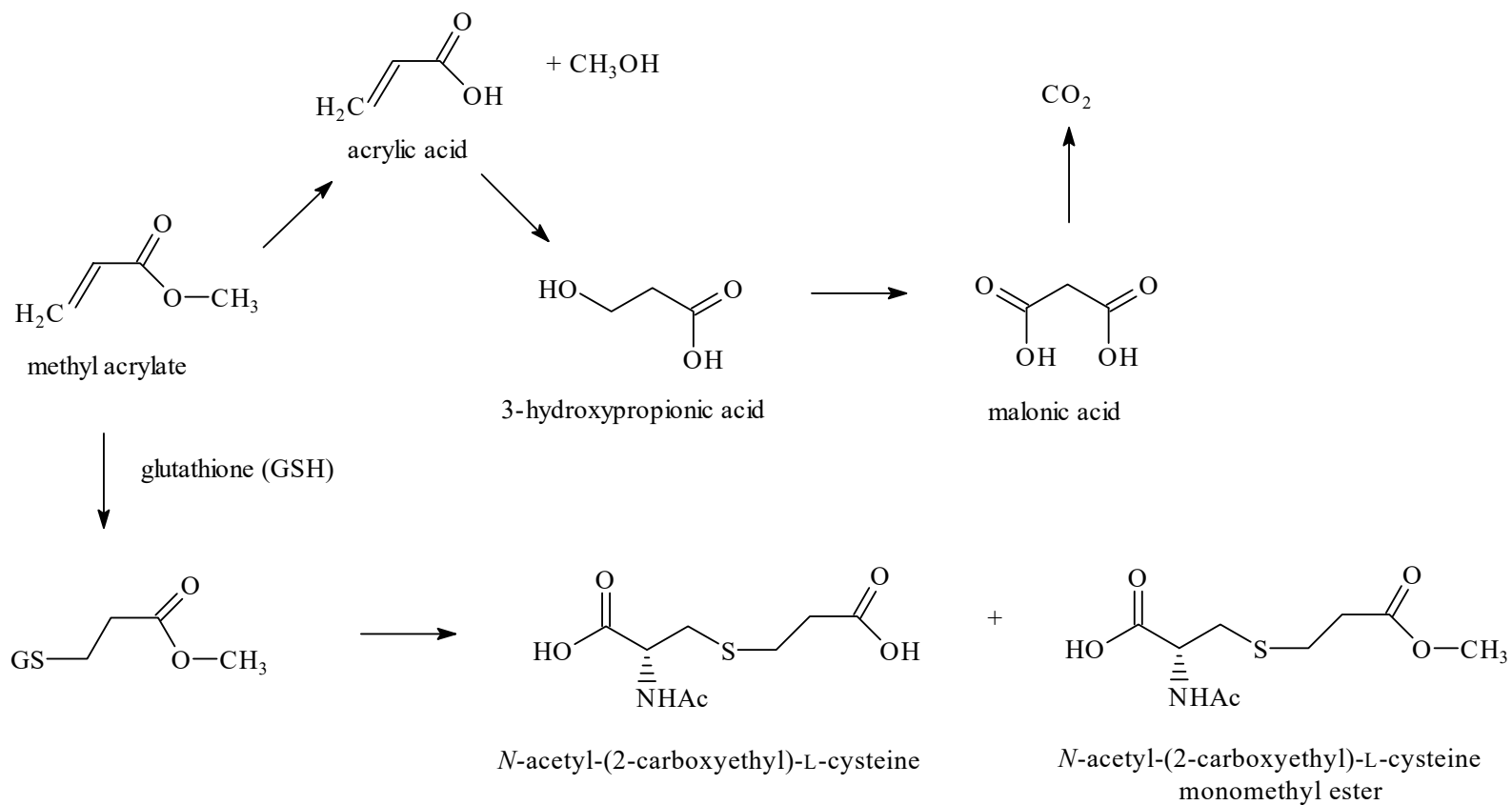
The *N*-acetyl-(2-carboxyethyl)-L-cysteine conjugate may also stem from glutathione addition to acrylic acid
Compiled by the Working Group

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

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration	Reference
Micronucleus formation	Mouse, ddY (M)	Bone marrow	–	250 mg/kg bw	Oral	Hachiya et al. 1982
Micronucleus formation	Mouse, BALB/c (M)	Bone marrow	+	37.5 mg/kg bw	Intraperitoneal injection, ×2	Przybojewska et al. (1984)
Micronucleus formation	Mouse, ddY (NR)	Bone marrow	–	2100 ppm	Inhalation, 3 h	Sofuni et al. (1984)

bw, body weight; h, hour; HID, highest ineffective dose; LED, lowest effective dose; M, male; NR, not reported; ppm, parts per million
[a]   +, positive; –, negative; the level of significance was set at $P < 0.05$ in all cases

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

End-point	Species, cell line	Results[a] Without metabolic activation	With metabolic activation	Concentration (LEC or HIC) (µg/mL)	Comments	Reference
Mutation (*Tk*)	Mouse, L5178Y lymphoma cells	(+)	NT	14	Only positive at cytotoxic concentrations	Moore et al. (1988)
Mutation (*Xprt*)	Chinese hamster ovary, CHO-AS52	–	NT	25		Oberly et al. (1993)
Mutation (*Hgprt*)	Chinese hamster ovary, CHO	–	NT	80		Moore et al. (1991)
Mutation (*Hgprt*)	Chinese hamster ovary, CHO	–	NT	18		Moore et al. (1989)
Chromosomal aberrations	Mouse, L5178Y lymphoma cells	(+)	NT	16	Only positive at cytotoxic concentrations	Moore et al. (1988)
Chromosomal aberrations	Chinese hamster ovary, CHO	(+)	NT	14	Only positive at cytotoxic concentrations	Moore et al. (1989)
Micronucleus formation	Chinese hamster ovary, CHO	–	(+)	2109	Only positive at cytotoxic concentrations	Kirpnick et al. (2005)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested
[a]   –, negative; (+), positive result in a study of limited quality; the level of significance was set at $P < 0.05$ in all cases

Table 4.3 Genetic and related effects of methyl acrylate in non-mammalian experimental systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation (Ames test)	–	–	3 μmol/plate		Florin et al. (1980)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation (Ames test)	–	–	1250 μg/plate		Waegemaekers & Bensink (1984)
Saccharomyces cerevisiae RS112	DEL recombination	(+)	–	500 μg/mL	Significant toxicity (< 5% survival)	Kirpnick et al. (2005)

DEL, deletion; HIC, highest ineffective concentration; LEC, lowest effective concentration

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

hypoxanthine-guanine phosphoribosyl transferase (*Hgprt*) assay in CHO cells exposed to methyl acrylate (Moore et al., 1989, 1991). At cytotoxic test concentrations with less than 50% cell survival, methyl acrylate induced mutations at the thymidine kinase (*Tk*$^{+/-}$) locus in L5178Y mouse lymphoma cells without metabolic activation (Moore et al., 1988), and increased the frequency of chromosomal aberrations in CHO cells and L5178Y mouse lymphoma cells in the absence of metabolic activation (Moore et al., 1988, 1989). In CHO cells, methyl acrylate increased the frequency of micronucleus formation at cytotoxic concentrations in the presence but not absence of S9 (Kirpnick et al., 2005).

(iii)   Non-mammalian systems

See Table 4.3

In *Saccharomyces cerevisiae*, methyl acrylate significantly increased the frequency of DNA deletions detected in the deletion (DEL) assay in the absence but not the presence of S9, but only at concentrations at which there was less than 5% cell viability (Kirpnick et al., 2005).

Methyl acrylate was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538, without or with metabolic activation (Florin et al., 1980; Waegemaekers & Bensink, 1984).

4.2.2 Other mechanisms

(a)   Humans

No data were available to the Working Group.

(b)   Experimental systems

Dose-related atrophy of the neurogenic epithelial cells and hyperplasia were observed in the nasal mucosa of all male and female Sprague-Dawley rats exposed to methyl acrylate by inhalation at concentrations of 0, 15, 45, and 135 ppm for 6 hours per day, 5 days per week, for 24 months (Reininghaus et al., 1991).

4.3 Other adverse effects

4.3.1 Irritancy and sensitization

(a)   Humans

Irritation and sensitization after exposure to methyl acrylate have been described, in some cases with complex exposures; positive patch-test responses to methyl acrylate have also been reported (Cavelier et al., 1981; Kanerva et al., 1994; Lammintausta et al., 2010).

(b)   Experimental systems

The immunogenicity of methyl acrylate was investigated by determining the induction of immunoglobulin G antibodies in female Hartley guinea-pigs in vivo (Bull et al., 1987). The injection of 0.25 mL of an emulsion of equal volumes of a 20 mM solution of methyl acrylate and Freund's complete adjuvant resulted in the induction of antigen-specific antibodies reactive with methyl acrylate.

Methyl acrylate was determined to be a weak sensitizer (effective concentration required to produce a threefold increase in proliferation of draining lymph node cells compared with control values), EC3, 19.6) in a local lymph node assay in female CBA/Ca mice (Dearman et al., 2007).

5.   Summary of Data Reported

5.1 Exposure data

Methyl acrylate is a chemical with a high production volume that is produced worldwide. It is used in the production of acrylic fibres, fire-retardant fabrics, resinous and polymeric coatings and varnishes, adhesives, sealants, and medical and dental prostheses, and as an intermediate in the synthesis of other compounds. Occupational exposure occurs primarily through inhalation and dermal contact during its production and use as an intermediate. One study in a chemical production facility reported concentrations at and above occupational exposure limits. Methyl acrylate may be released into the air and water during its production and use. However, information on concentrations in environmental media and exposure in the general population was not available.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

Methyl acrylate was tested for carcinogenicity in one inhalation study in male and female mice, and two inhalation studies in male and female rats.

In one well-conducted inhalation study in rats, the incidence of sarcoma of the soft tissue (of the skin or subcutis, not otherwise specified) and "malignant leukaemic tumours" (leukaemia, lymphoma, and lymphosarcoma) in males was significantly increased with a significant positive trend, and the incidence of adenoma of the pituitary gland in females was significantly increased with a significant positive trend.

In one well-conducted good laboratory practice (GLP) inhalation study in rats, a statistically significant increase in the incidence of squamous cell carcinoma of the nasal cavity in male rats (with a significant positive trend) and in the incidence of squamous cell carcinoma of the nasal cavity in female rats was observed (2/50 treated females compared with 0/650 in female historical controls). In addition, a significant positive trend in the incidence of pheochromocytoma of the adrenal gland (benign or malignant tumours combined) was observed in female rats.

In a well-conducted GLP inhalation study in mice, there was no significant increase in the incidence of any neoplastic lesions in the treated

groups of males and females compared with controls.

5.4 Mechanistic and other relevant data

No data on absorption, distribution, metabolism, or excretion in exposed humans were available. In rodents, methyl acrylate is readily absorbed via all routes of exposure, widely distributed in the body, and excreted mainly as CO_2 in expired air and as mercapturic acid conjugates in the urine. Methyl acrylate is metabolized via hydrolysis by carboxylesterases to acrylic acid and methanol, and subsequent formation of CO_2, as well as via conjugation with glutathione.

With respect to the key characteristics of human carcinogens, adequate data to evaluate methyl acrylate were only available for genetic and related effects. There is *weak* evidence that methyl acrylate is genotoxic. No data were available in exposed humans or human cells in vitro. Methyl acrylate increased the frequency of micronucleus formation in BALB/c mice after intraperitoneal exposure, but not in ddY outbred mice treated by inhalation or oral exposure. In rodent cells in vitro, methyl acrylate did not induce mutations in several studies. Some positive findings were reported for mutation, micronucleus formation, and chromosomal aberrations, but only at cytotoxic concentrations. Similarly, methyl acrylate gave positive results in the yeast DNA deletion assay at cytotoxic concentrations. Further, methyl acrylate gave negative results in the Ames test, both with and without metabolic activation.

In humans, the development of allergic contact dermatitis has been described. Immunogenicity was also shown in studies in rodents.

In the chronic bioassay, nasal toxicity was reported.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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ETHYL ACRYLATE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 140-88-5

Chem. Abstr. Serv. name: 2-propenoic acid, ethyl ester

IUPAC systematic name: ethyl prop-2-enoate

Synonyms: ethyl propenoate; acrylic acid ethyl ester; ethyl 2-propenoate; ethoxy-carbonylethylene.

1.1.2 Structural and molecular formulae, and relative molecular mass

Molecular formula: $C_5H_8O_2$
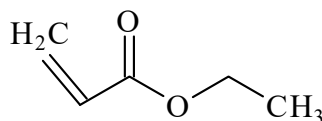
Relative molecular mass: 100.12

1.1.3 Chemical and physical properties of the pure substance

Description: colourless liquid with an acrid, penetrating odour (Budavari et al., 1996)

Boiling point: 99.4 °C (Lide, 1995)

Melting point: −71.2 °C (Lide, 1995)

Solubility: slightly soluble in water (2% w/v at 20 °C); soluble in chloroform; miscible with diethyl ether and ethanol (Lide, 1995)

Vapour pressure: 29.3 mm Hg [3.9 kPa] at 20 °C

Relative vapour density (air = 1): 3.5 (Verschueren, 1996)

Flash point: 15 °C, open cup (Budavari et al., 1996)

Explosive limits: lower explosive limit, 1.8% by volume in air (ACGIH, 2001)

Conversion factor: 1 ppm = 4.09 mg/m³ at 1 atm, 25 °C.

1.1.4 Technical products and impurities

Impurities reported in commercial-grade (technical) ethyl acrylate (purity, 99.0–99.5%) include water (0.03–0.10% by weight), acrylic acid (0.0008–0.0090% by weight), and polymerization inhibitors (15–200 mg/kg hydroquinone monomethyl ether or 1000 mg/kg hydroquinone) (HSDB, 2018).

1.2 Production and use

1.2.1 Production process

Ethyl acrylate is produced by several methods, including catalysed esterification of acrylic acid with ethanol (EPA, 2007), reaction of nickel carbonyl and acetylene with ethyl alcohol in the presence of an acid, esterification of acrylic acid

with ethyl alcohol (modified Reppe process), and vinyl chloride reacted at 270 °C at a pressure of 6895 kPa or greater with ethanol in the presence of a cobalt and palladium catalyst (HSDB, 2018). Ethyl acrylate is a monomer that polymerizes readily to a transparent, elastic substance in the presence of light, heat, or a catalyst (EPA, 2007). The monomer is stored with small amounts of hydroquinone or its methyl ether to prevent spontaneous polymerization (ACGIH, 2001).

1.2.2 Production volume

Ethyl acrylate is a chemical with a high production volume (OECD, 2009). The USA produced 160 thousand metric tonnes of ethyl acrylate in 1993, and production was from more than 100 million to 500 million pounds [> 45.4 to 227 thousand metric tonnes] in 2002 (HSDB, 2018). The production rate in the European Union was in excess of 10 thousand metric tonnes per annum (SCOEL, 2004). Production volume in China was 102 674 metric tonnes in 2008 (Chinese Report, 2008) and 108 580 metric tonnes in 2010 (Chinese Report, 2010).

1.2.3 Use

Ethyl acrylate is used primarily as a chemical intermediate during the production of polymers including water-based paints, resins, plastics, and rubber (NIOSH, 2014). It is used as a surface coating for textiles, paper, and leather (such as nubuck and suede), in food-contact materials, and in the production of acrylic fibres, adhesives, and binders (ACGIH, 2001; EPA, 2007; Arkema, 2012). It is one of the principal monomers used worldwide in the production of styrene-based polymers, which can be used for medical and dental items (SCOEL, 2004). It also has limited use as a fragrance in cosmetics and a flavouring agent in food (mostly dairy products and soft drinks) (EPA, 2007; European Commission, 2012; Silano et al., 2017).

1.3 Analytical methods

1.3.1 Detection and quantification

Air sampling for ethyl acrylate is conducted using charcoal adsorbent. Samples are desorbed using carbon disulfide and the extract analysed using gas chromatography with flame ionization detection by United States National Institute for Occupational Safety and Health (NIOSH) Method 1450 (NIOSH, 2003) or United States Occupational Safety and Health Administration (OSHA) Method 92 (OSHA, 2018). NIOSH Method 1450 has a detection limit of 2 µg per sample and OSHA Method 92 has a detection limit of 80 µg/m³.

Ethyl acrylate can also be analysed in water. The most recently published method found by the Working Group is United States Environmental Protection Agency (EPA) Method 624.1 (EPA, 2016). This method uses a purging chamber that transfers the volatile compounds to the vapour phase, followed by a sorbent trap. The trap is then heated and back-flushed to desorb the purgeables onto a gas chromatography column that is combined with mass spectrometry; the detection limit for ethyl acrylate was not reported. Similar purge and trap methods (Method 8260B) are also reported for other aqueous, solid (including waste and soil), and tissue samples (EPA, 1996).

1.3.2 Exposure assessment and biological markers

No information on biological markers of exposure to ethyl acrylate was available to the Working Group.

Historical exposure to ethyl acrylate was reconstructed for three cohorts, reported in the same study, of acrylic sheet manufacturing workers at two different facilities (Walker et al., 1991). The assessments were made separately for each cohort. For one cohort the assessment was based on monitoring data for methyl methacrylate from 1972 onwards, and on expert judgment

based on production records and interviews with plant personnel. For the other two cohorts only expert judgment was used. The scales for the three cohorts were not directly comparable. The cohort with monitoring data was the only one that had category cut points based on exposure concentrations, with categories of less than 1 ppm [< 4.09 mg/m³], 1 to less than 5 ppm [4.09 to < 20.5 mg/m³], 5–24 ppm [20.5–98.2 mg/m³], and 25 ppm or more [≥ 98.2 mg/m³]. The highest category of exposure was assigned to workers in the "boil-out" phase of acrylic sheet production and to workers performing hand operations without local exhaust ventilation.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

Ethyl acrylate can be released into the environment in fugitive and stack emissions or in wastewater during its production and use (EPA, 2000). Ethyl acrylate is expected to volatize from water surfaces and is not expected to adsorb to suspended solids and sediment (HSDB, 2018). Based on empirical data and modelling results, ethyl acrylate is not expected to be persistent or bioaccumulate in the environment (Environment Canada/Health Canada, 2011).

(a) Air

Median reported on- and offsite fugitive air releases of ethyl acrylate in the USA reported in the EPA Toxics Release Inventory were 250 pounds [113 kg], 30 pounds [14 kg], 31 pounds [14 kg], and 11 pounds [5.0 kg] for the years 1990, 2000, 2010, and 2016, respectively, with a maximum reported release by a facility of 20 913 pounds [9486.0 kg] in 1990 (EPA, 2017). In 2016, the 89 reporting facilities were primarily in the chemical (82%), hazardous waste (7%), and plastics and rubber (4%) industries. The EPA Toxics Release Inventory emissions reports and other sources of emission data are included in

the 2011 National Air Toxics Assessment database, which reported ethyl acrylate emissions of 0–5100 kg (median, 0.0004 kg) per year from 410 facilities (EPA, 2011). More than half of these facilities (236) were wastewater treatment facilities, with a reported maximum air release of 1 kg per year. The Canadian National Pollutant Release Inventory reported mean annual releases of ethyl acrylate into the air of 130, 1800, 26, and 35 kg for the years 1994, 2000, 2010, and 2016, respectively (Government of Canada, 2017).

(b) Water

The 75th percentile of the releases into water in the USA reported to the EPA Toxics Release Inventory was 0 pounds for the years 1990, 2000, 2010, and 2016, with a maximum amount of 463 pounds [210 kg] in 1990 and 14 pounds [6.4 kg] in 2016 (EPA, 2017). The Canadian National Pollutant Release Inventory had no reported releases onto land or water from the three reporting facilities (Government of Canada, 2017). Ethyl acrylate has been detected at low levels in wastewater samples (IARC, 1999; EPA, 2017).

1.4.2 Exposure in the general population

Residential exposure to ethyl acrylate may occur through exposure to compounds that contain ethyl acrylate, such as window caulking (NIOSH, 1980b) and acrylic nail compounds (Spencer et al., 2016).

Ethyl acrylate has been detected in food. Dietary exposure from naturally occurring ethyl acrylate has been estimated to be negligible compared with that from flavour additives (Silano et al., 2017). The estimated dietary intake from added ethyl acrylate was 59.1 µg/kg body weight (bw) per day for adults and 149 µg/kg bw per day for children; other dietary sources were estimated to be less than 1 µg/kg bw for both adults and children (Silano et al., 2017). Ethyl acrylate is also used in food-contact materials, and exposure

from this source was estimated to be 6000 μg per person per day or less [≤ 100 μg/kg bw per day] (Silano et al., 2017).

1.4.3 Occupational exposure

Exposure to ethyl acrylate occurs primarily through inhalation and dermal contact during its production, its use as an intermediate (e.g. in resins, coatings, and paints), and during work with products containing ethyl acrylate. Ethyl acrylate has been found in a dental composite resin in Finland (0.9% ethyl acrylate) (Aalto-Korte et al., 2007). Skin sensitization to ethyl acrylate (contact dermatitis) has been reported in nail salon workers exposed to acrylate-based nail treatments (see Section 4.3.1a) (Le et al., 2015; Spencer et al., 2016; DeKoven et al., 2017).

A few studies have quantified ethyl acrylate in the air of workplace settings (Table 1.1). Ethyl acrylate area air concentrations from paint batch mixing in a closed system ranged from less than 0.11 to 5.80 ppm [< 0.45–23.7 mg/m³] (NIOSH, 1980a). In a chemical manufacturing plant, average concentrations for full-shift samples were 0.2–2.3 ppm [0.8–9.4 mg/m³] and short-term average concentrations ranged from less than 0.1 to 30.0 ppm [0.4–123 mg/m³] (SCOEL, 2004). Time-weighted average concentrations of ethyl acrylate at four work sites of a polystyrene production plant were less than 1 to 211 ppb [< 0.004–0.863 mg/m³] (maximum, 844 ppb [3.45 mg/m³]) in the breathing zone of workers and less than 1 to 27 ppb [< 0.004–0.11 mg/m³] (maximum, 241 ppb [0.986 mg/m³]) in ambient workplace air (Samimi & Falbo, 1982). Ethyl acrylate was detected during laser cutting of plexiglass, acrylic, and lucite, with concentrations ranging from less than 0.4 to 149.0 ppm [< 2–609.4 mg/m³] in short-term samples (NIOSH, 1990). In various work areas of a chemical plant producing acrylic acid and acrylic acid esters, ethyl acrylate concentrations of 0.2 mg/m³ or greater were observed in approximately 20% of

samples collected between 1988 and 1999 (Tuček et al., 2002).

1.5 Regulations and guidelines

For ethyl acrylate, the 8-hour time-weighted (TWA) average occupational exposure limit is set at 20 mg/m³ for most countries (see Table 1.2). Only Germany and Switzerland have lower limits of 8 and 10 mg/m³, respectively. Short-term limit values vary over the range 17–62 mg/m³. The OSHA standard has a higher 8-hour TWA occupational exposure limit of 100 mg/m³ with no ceiling value (IFA, 2018; ACGIH, 2001).

The United States Food and Drug Administration has established regulations for the use of monomers, polymers, and copolymers, including ethyl acrylate, in food-contact materials. The proportion of the monomers should not exceed 5% by weight of total polymer units (CFR, 2017).

The European Food Safety Authority has set a safe limit for inclusion of (ethyl acrylate, methyl methacrylate) copolymer in food-contact materials at 2% by weight in rigid polyvinyl chloride and 5% by weight in polylactic acid and polyethylene terephthalate (EFSA, 2011).

2. Cancer in Humans

2.1 Cohort studies of occupational exposure

Only one cohort study of occupational exposure has evaluated the association between exposure to ethyl acrylate and risk of cancer (see Table 2.1).

Mortality from cancer of the colon or rectum was evaluated among workers employed at two plants manufacturing and polymerizing acrylate monomers to make acrylic sheets from 1933 to 1982, in the USA (Walker et al., 1991). Analyses

Table 1.1 Occupational exposure to ethyl acrylate

Industry Location, year	Job/process	Sampling location, duration, no. of workers	Mean	Range	Comments	Reference
Paint company Los Angeles, USA, 1980	Manufacture of polyvinyl acetate emulsion	Breathing zone of workers, full shift, 16	NR	< 0.11–5.80 ppm [< 0.45–23.7 mg/m^3]		NIOSH (1980a)
Polystyrene production plant NR, before 1982		Breathing zone of workers, 50 min–7.5 h, 50	< 1–211 ppb [< 0.004–0.863 mg/m^3]	< 1–844 ppb [< 0.004–3.45 mg/m^3]		Samimi & Falbo (1982)
Polystyrene production plant NR, before 1982		Ambient workplace air, 50 min–7.5 h, 57	< 1–27 ppb [< 0.004–0.11 mg/m^3]	< 1–241 ppb [< 0.004–0.986 mg/m^3]		Samimi & Falbo (1982)
Laser cutting plastics Longwood (Florida), USA, 1989		Ambient workplace air, short term (< 2 h), 10	34 ppm [140 mg/m^3]	< 0.4–149.0 ppm [< 2–610.0 mg/m^3]		NIOSH (1990)
Chemical plant NR, 1988–1999	Production of acrylic acid, acrylic acid esters	Ambient workplace air, NR, NR	NR	NR	Results reported as percentage of samples in concentration categories; ethyl acrylate concentrations of > 0.2 mg/m^3 were observed in ~20% of air samples	Tuček et al. (2002)
Paint company NR, before 1987		Breathing zone of workers, full shift, NR	0.2–2.3 ppm [0.8–9.4 mg/m^3]	NR	Unpublished company data submitted to SCOEL committee in 1987	SCOEL (2004)
Paint company NR, before 1987		Breathing zone of workers, short term, NR	< 0.1 to 30.0 ppm [< 0.4–123 mg/m^3]	NR	Unpublished company data submitted to SCOEL committee in 1987	SCOEL (2004)

h, hour; min, minute; NR, not reported; ppb, parts per billion; ppm, parts per million; SCOEL, Scientific Committee on Occupational Exposure Limits

Table 1.2 Occupational exposure limits for ethyl acrylate

Country or region	Concentration (mg/m³)	Interpretation	Comments
Australia	20	STEL	Ceiling limit value
Austria	20	TWA	
	40	STEL	
Belgium	21	TWA	
	42	STEL	
Canada, Ontario	20	TWA	
	61	STEL	
Canada, Quebec	20	TWA	
	61	STEL	
Denmark	20	TWA	
	40	STEL	
European Union	21	TWA	Indicative occupational exposure limit values
	42	STEL	
Finland	21	TWA	
	42	STEL	
France	21	TWA	Restrictive statutory limit values
	42	STEL	
Germany (AGS)	8.3	TWA	
	16.6	STEL	
Germany (DFG)	8.3	TWA	
	16.6	STEL	
Hungary	10	TWA	
	10	STEL	
Ireland	20	TWA	
	41	STEL	
Italy	21	TWA	Skin notation
	42	STEL	
Latvia	5	TWA	
Netherlands	21	TWA	
	42	STEL	
New Zealand	20	STEL	Ceiling limit value
Poland	20	TWA	
	40	STEL	
Republic of Korea	20	TWA	
Romania	21	TWA	
	42	STEL	
Singapore	20	TWA	
	61	STEL	
Spain	21	TWA	Sensitization notation
	62	STEL	
Sweden	20	TWA	
	40	STEL	
Switzerland	10	TWA	
	42	STEL	
Turkey	21	TWA	
	42	STEL	

Table 1.2 (continued)

Country or region	Concentration (mg/m³)	Interpretation	Comments
UK	21	TWA	
	62	STEL	
USA (OSHA)	100	TWA	
USA (ACGIH)	21	TWA	Upper respiratory tract, eye, and gastrointestinal tract irritation, central nervous system impairment, skin sensitization notations
	62	STEL	

ACGIH, American Conference of Governmental Industrial Hygienists; AGS, Ausschuss für Gefahrstoffe (Committee on Hazardous Substances); DFG, Deutsche Forschungsgemeinschaft (German Research Foundation); OSHA, United States Occupational Safety and Health Administration; STEL, short-term (15-minute) exposure limit; TWA, 8-hour time-weighted average
Compiled from IFA (2018) and ACGIH (2001)

Table 2.1 Occupational cohort studies of exposure to ethyl acrylate

Reference, location, follow-up/enrolment period	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases and/or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Walker et al. (1991) USA 1933–1986	3934 white men employed any time between 1933 and 1945 at Bristol facility Exposure assessment method: expert judgement; ordinal 0–5 scale, assessed as co-exposure with methyl methacrylate; ethyl acrylate accounted for 12% of mixture during 1939–1942, with a gradual decline from 7% in 1943 to 0% in 1956	Colon	Time (yr) since exposure at 0 to < 5 dose units			Age, calendar period	Strengths: work histories from company records Limitations: co-exposure to methyl methacrylate; no measurements for period with ethyl acrylate use
			Not exposed	11	0.96 (0.53–1.73)		
			< 5	2	4.39 (1.10–17.60)		
			5–19	5	1.41 (0.59–3.39)		
			≥ 20	31	1.45 (1.02–2.06)		
			Time (yr) since exposure at 5 to < 10 dose units				
			Exposed but at < 5 dose units	17	1.55 (0.96–2.49)		
			< 5	0	0 (0–14.20)		
			5–19	3	1.40 (0.45–4.34)		
			≥ 20	18	1.50 (0.95–2.38)		
			Time (yr) since exposure at 10 to < 15 dose units				
			Exposed but at < 10 dose units	25	1.45 (0.98–2.15)		
			< 5	0	0 (0–26.40)		
			5–19	1	0.84 (0.12–5.93)		
			≥ 20	12	1.76 (1.00–3.10)		
			Time (yr) since exposure at ≥ 15 dose units				
			Exposed but at < 15 dose units	26	1.31 (0.89–1.93)		
			< 5	0	0 (0–33.60)		
			5–19	1	1.13 (0.16–8.05)		
			≥ 20	11	2.40 (1.33–4.34)		
		Colon	Concentration of exposure (dose units) with 20-yr lag				
			Not exposed	12	1.24 (0.71–2.19)		
			0–4	13	1.39 (0.80–2.38)		
			5–9	6	1.16 (0.52–2.58)		
			10–14	1	0.45 (0.06–3.16)		
			≥ 15	11	2.40 (1.33–4.34)		

Table 2.1 (continued)

Reference, location, follow-up/enrolment period	Population size, description, exposure assessment method	Organ site	Exposure category or level		Exposed cases and/or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Walker et al. (1991) (cont.)		Rectum	Exposure concentration (dose units) with 20-yr lag				Age, calendar period	
			Not exposed	2		0.72 (0.18–2.88)		
			0–4	6		2.52 (1.13–5.60)		
			5–9	0		0 (0–2.98)		
			10–14	1		1.85 (0.26–13.10)		
			≥ 15	3		2.83 (0.91–8.76)		
Walker et al. (1991) USA 1946–1986	6548 white men hired between 1946 and 1982 at Bristol facility Exposure assessment method: expert judgement; semiquantitative scale: 0, 1–< 5, 5–24, ≥ 25 ppm, assessed as co-exposure with methyl methacrylate; ethyl acrylate accounted for 6% in 1946 and gradually declined to 0% in 1956	Colon	Exposure concentration (dose units) with 20-yr lag				Age, calendar period	Strengths: work histories from company records Limitations: co-exposure to methyl methacrylate; no measurements for period with ethyl acrylate use
			Not exposed	8		0.98 (0.49–1.95)		
			0–4	6		1.08 (0.49–2.41)		
			5–9	1		1.26 (0.18–8.92)		
Walker et al. (1991) USA 1943–1986	3381 white men employed between 1943 and 1982 at Knoxville facility Exposure assessment method: expert judgement; ordinal 0–3 scale, assessed as co-exposure with methyl methacrylate; ethyl acrylate accounted for 7% in 1943 and gradually declined to 0% in 1956	Colon	Exposure concentration (dose units) with 20-yr lag				Age, calendar period	Strengths: work histories from company records Limitations: co-exposure to methyl methacrylate; no measurements for period with ethyl acrylate use
			Not exposed	0		0 (0–4.63)		
			0–4	17		1.85 (1.15–2.98)		
			5–9	1		0[a] (0–3.66)		
			10–14	0		0 (0–5.52)		
			≥ 15	1		0.63 (0.09–4.44)		

CI, confidence interval; ppm, parts per million; yr, year

[a] The Working Group noted that the value in the original paper appeared to be erroneous; it should be 1/1.01 = 0.99

were conducted for three cohorts: (i) 3934 white men hired during 1933–1945 at the Bristol facility; (ii) 6548 white men hired during 1946–1982 at the Bristol facility; and (iii) 3381 white men employed during 1943–1982 at the Knoxville facility. Follow-up continued from the first day of employment until 1986. Semiquantitative estimates of co-exposure to vapours of ethyl acrylate and methyl methacrylate were estimated from employer work history records, production records, and interviews with plant personnel separately for each group, and were not directly comparable between groups (see Section 1.3.2). Three compounds were used for acrylic sheet manufacture in these facilities, namely, methyl methacrylate (88–100%), ethyl acrylate (0–12%), and butyl lactate (0–2%). The percentage of ethyl acrylate was 12% between 1940 and 1943, reduced to 7% in 1943, and decreased gradually to 1% between 1943 and 1955; it was eliminated in 1956. In the Bristol cohort with the earliest hire dates, excess mortality from cancer of the colon occurred 20 years or more after cumulative exposure to ethyl acrylate and methyl methacrylate combined at specified concentrations. Compared with the general population, standardized mortality ratios (SMRs) were 1.45 (95% confidence interval, CI, 1.02–2.06), 1.50 (95% CI, 0.95–2.38), 1.76 (95% CI, 1.00–3.10), and 2.40 (95% CI, 1.33–4.34) at cumulative exposures of 0 to < 5, 5 to < 10, 10 to < 15, and ≥ 15 units, respectively. A cumulative exposure of 5 units was achieved by working 3 years or more in jobs rated a score of 5 on a 0–5 scale, where a score of 5 corresponded to the "boil-out" phase of acrylic sheet production. Excess mortality from cancer of the colon was also observed in workers exposed to ethyl acrylate at low concentrations (> 0 to < 5 units). These workers may have been co-exposed to solvents such as ethylene dichloride, methylene chloride, acetone, and methyl methacrylate monomer. [The Working Group noted that these co-exposures could not be ruled out for the other cumulative exposure groups.]

Mortality from cancer of the rectum was significantly and non-significantly elevated in the same categories that showed excess rates of mortality from cancer of the colon, and was based on small numbers of cases. In the Bristol cohort with later hire dates, no excess mortality from cancer of the colon or rectum was observed. In the Knoxville cohort, an excess mortality of cancer of the colon was observed 20 years or more after accumulating 0–4 units of exposure (rate ratio, 1.95; 95% CI, 1.15–2.98). Analyses of higher-exposure categories were limited because of small numbers. No excess mortality from cancer of the rectum was observed in the Knoxville cohort.

[The Working Group noted that the Walker et al. (1991) paper was based on five internal reports that are not publicly available. Only the results of mortality from cancer of the colon and rectum were reported. Walker et al. noted in the introduction that there were no excesses of cancer of the respiratory system. The strengths of this study included a medium-sized cohort and good follow-up time; however, it has several important limitations. Ethyl acrylate exposure co-occurred with exposure to methyl methacrylate and, as a result, the observed increased risks cannot be solely attributed to ethyl acrylate. Ethyl acrylate exposure occurred over a short time period (1939–1956). Exposure metrics concerned inhalation exposure only; they did not consider dermal exposure, which may have been important. Exposure assessment for two cohorts was based on expert judgment; for one cohort (Bristol hires during 1946–1982) the exposure assessment was partly based on measurements of methyl methacrylate and not of ethyl acrylate. Finally, outcome ascertainment considered mortality from and not incidence of cancer.]

Mortality risk was also evaluated in a cohort of 4324 acrylic sheet manufacturing workers in two facilities in the UK (Tomenson et al., 2000). Decreased mortality risks in the subcohort with more than minimal exposure to methyl methacrylate were observed for all causes (SMR, 94)

and for cancer of the colon and rectum (SMR, 92) based on comparisons with the general population; the standardized mortality ratio for all cancers combined was 104. No exposure–response associations were observed with cumulative exposure to methyl methacrylate. [The Working Group noted that this cohort may have been exposed to ethyl acrylate, but this exposure was not assessed.]

2.2 Case–control studies

No results from case–control studies that evaluated cancer risk in relation to ethyl acrylate exposure were available to the Working Group.

Aliphatic esters were evaluated in a series of analyses in a general-population case–control study in Montreal, Canada, with cases and controls identified between 1979 and 1985. In analyses of 257 cases and 533 population controls, an excess risk of cancer of the rectum with substantial exposure to aliphatic esters based on expert judgment of subject-reported work histories was observed (odds ratio, OR, 3.0; 95% CI, 1.4–6.8; 10 cases) (Dumas et al., 2000). The increased risk of cancer of the colon with substantial exposure to aliphatic esters was 1.5 (90% CI, 0.8–3.0; 9 cases) (Siemiatycki, 1991). [The Working Group noted that aliphatic esters may include ethyl acrylate, in addition to thousands of aliphatic esters of other acids.]

3. Cancer in Experimental Animals

Ethyl acrylate was previously reviewed by the Working Group (*IARC Monographs* Volume 39, IARC, 1986; Supplement 7, IARC, 1987; and Volume 71, IARC, 1999. The Working Group for Volume 71 concluded that there was *sufficient evidence* in experimental animals for the carcinogenicity of ethyl acrylate. This section provides an evaluation of the studies of carcinogenicity in experimental animals reviewed previously, and of all new studies.

See Table 3.1

3.1 Mouse

3.1.1 Oral administration

In a well-conducted study, groups of 50 male and 50 female B6C3F$_1$ mice (age, 7 weeks) were given ethyl acrylate (purity, 99.0–99.5%; stabilized with 15 ppm of the monoethyl ether of hydroquinone) at a dose of 0, 100, or 200 mg/kg bw by gavage in corn oil for 5 days per week for 103 weeks (NTP, 1986). In males and females, survival was comparable between exposed groups and the control group. Mean body weights of females exposed at the lower dose were at least 10% less than those of controls during the last 22 weeks of the study. Mean body weights of exposed males and females exposed at the higher dose were comparable to controls. The incidence of squamous cell papilloma – 0/48 (*P* for trend, 0.001), 4/47 (9%), 9/50 (*P* = 0.004) (18%) – squamous cell carcinoma – 0/48 (*P* for trend, 0.017), 2/47 (4%), 5/50 (*P* = 0.040) (10%) – and squamous cell papilloma or carcinoma (combined) – 0/48 (*P* for trend, < 0.001), 5/47 (11%), 12/50 (*P* < 0.001) (24%) – of the forestomach were significantly increased in all males at the higher dose, and there was a significant positive trend in the formation of these tumours in exposed males. The incidence of squamous cell papilloma or carcinoma (combined) of the forestomach – 1/50 (2%), (*P* for trend, 0.018), 5/49 (10%), 7/48 (*P* = 0.028) (15%) – in female mice exposed at the higher dose was significantly increased, and there was a significant positive trend in exposed females. The incidence of non-neoplastic lesions of the forestomach was dose-related in male and female mice; these lesions included ulceration, inflammation, epithelial hyperplasia, and hyperkeratosis.

Table 3.1 Studies of carcinogenicity with ethyl acrylate in experimental animals

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$_1$ (M) 7 wk 104–106 wk NTP (1986)	Gavage Ethyl acrylate, 99.0–99.5% Corn oil 0, 100, 200 mg/kg bw for 5 d/wk for 103 wk 50, 50, 50 28, 36, 30	*Forestomach* Squamous cell papilloma 0/48*, 4/47 (9%), 9/50** (18%) Squamous cell carcinoma 0/48*, 2/47 (4%), 5/50** (10%) Squamous cell papilloma or carcinoma (combined) 0/48*, 5/47 (11%), 12/50** (24%)	*P = 0.001 (trend), life-table test; **P = 0.004, life-table test *P = 0.017 (trend), life-table test; **P = 0.040, life-table test *P < 0.001 (trend), life-table test; **P < 0.001, life-table test	Principal strengths: well-conducted study Several non-neoplastic lesions, including ulceration, inflammation, epithelial hyperplasia, and hyperkeratosis, were observed in the forestomach of male mice in a dose-related manner Historical incidence for gavage studies for stomach tumours: 5/881 (0.6%)
Full carcinogenicity Mouse, B6C3F$_1$ (F) 7 wk 104–106 wk NTP (1986)	Gavage Ethyl acrylate, 99.0–99.5% Corn oil 0, 100, 200 mg/kg bw for 5 d/wk for 103 wk 50, 50, 50 27, 35, 26	*Forestomach* Squamous cell papilloma 1/50 (2%), 4/49 (8%), 5/48 (10%) Squamous cell carcinoma 0/50, 1/49 (2%), 2/48 (4%) Squamous cell papilloma or carcinoma (combined) 1/50* (2%), 5/49 (10%), 7/48** (15%)	NS NS *P = 0.018 (trend), life-table test; **P = 0.028, life-table test	Principal strengths: well-conducted study Several non-neoplastic lesions, including ulceration, inflammation, epithelial hyperplasia, and hyperkeratosis, were observed in the forestomach of female mice in a dose-related manner Historical incidence for gavage studies for stomach tumours: 8/901 (0.9%)
Full carcinogenicity Mouse, B6C3F$_1$ (M) 7–9 wk 27 mo Miller et al. (1985)	Inhalation (whole-body) Ethyl acrylate, > 99.5% None 0 (control A), 0 (control B), 25, 75, 225 ppm for 6 h/d, 5 d/wk for 6 mo (then unexposed for 21 mo) 60, 61, 75, 76, 69 NR	*Thyroid*: follicular cell adenoma 1/60 (2%), 1/61 (2%), 1/75 (1%), 0/76, 7/69* (10%)	*P < 0.05 compared with control groups, Fisher exact test	Principal strengths: well-conducted study Approximately 60 mice per control group and 75 mice per exposed group at the beginning of the experiment; the number of mice at the start is the effective number of mice According to Miller et al. (1985), a historical rate for thyroid follicular cell adenoma as high as 16% has been reported in male B6C3F$_1$ control groups in other studies, but no reference was cited

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$_1$ (F) 7–9 wk 27 mo Miller et al. (1985)	Inhalation (whole-body) Ethyl acrylate, > 99.5% None 0 (control A), 0 (control B), 25, 75, 225 ppm for 6 h/d, 5 d/wk for 6 mo (then unexposed for 21 mo) 64, 61, 78, 76, 66 NR	*Any tumour type* No significant increase in the incidence of any neoplastic lesion	NS	Principal strengths: well-conducted study Approximately 60 mice per control group and 75 mice per exposed group at the beginning of the experiment; the number of mice at the start is the effective number of mice
Full carcinogenicity Rat, F344/N (M) 7 wk 104–105 wk NTP (1986)	Gavage Ethyl acrylate, 99.0–99.5% Corn oil 0, 100, 200 mg/kg bw for 5 d/wk for 103 wk 50, 50, 50 41, 32, 34	*Forestomach* Squamous cell papilloma 1/50* (2%), 15/50** (30%), 29/50** (58%) Squamous cell carcinoma 0/50*, 5/50** (10%), 12/50*** (24%) Squamous cell papilloma or carcinoma (combined) 1/50* (2%), 18/50** (36%), 36/50** (72%) *Pancreas* Acinar cell adenoma or carcinoma (combined) 0/49, 4/50* (8%), 0/49 Acinar cell adenoma 0/49, 3/50 (6%), 0/49 Acinar cell carcinoma 0/49, 1/50 (2%), 0/49	*P < 0.001 (trend), life-table test; **P < 0.001, life-table test *P < 0.001 (trend), life-table test; **P = 0.019, life-table test; ***P < 0.001, life-table test *P < 0.001 (trend), life-table test; **P < 0.001, life-table test *P = 0.041, life-table test NS by more appropriate incidental tumour test NS NS	Principal strengths: well-conducted study Several non-neoplastic lesions, including inflammation, epithelial hyperplasia, and hyperkeratosis, were observed in the forestomach of male rats in a dose-related manner Historical incidence for gavage studies for stomach tumours: 5/967 (0.5%) No pancreatic acinar cell hyperplasia in exposed rats

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 (F) 7 wk 104–105 wk NTP (1986)	Gavage Ethyl acrylate, 99.0–99.5% Corn oil 0, 100, 200 mg/kg bw for 5 d/wk for 103 wk 50, 50, 50 36, 36, 42	*Forestomach* Squamous cell papilloma 1/50* (2%), 6/50 (12%), 9/50** (18%) Squamous cell carcinoma 0/50, 0/50, 2/50 (4%) Squamous cell papilloma or carcinoma (combined) 1/50* (2%), 6/50 (12%), 11/50** (22%)	*P = 0.018 (trend), life-table test; **P = 0.021, life-table test NS *P = 0.005 (trend), life-table test; **P = 0.008, life-table test	Principal strengths: well-conducted study Several non-neoplastic lesions, including inflammation, epithelial hyperplasia, and hyperkeratosis, were observed in the forestomach of female rats in a dose-related manner Historical incidence for gavage studies for stomach tumours: 5/973 (0.5%)
Full carcinogenicity Rat, F344 (M) 3 mo 21 mo Ghanayem et al. (1994)	Gavage Ethyl acrylate, 99% Corn oil 0 (vehicle control) for 12 mo, 200 mg/kg bw for 6 mo, 200 mg/kg bw for 12 mo, 0 (vehicle control) for 12 mo + 9 mo recovery, 200 mg/kg bw for 6 mo + 15 mo recovery, and 200 mg/kg bw for 12 mo + 9 mo recovery; 5×/wk for 6 or 12 mo months and then held untreated until killed aged 24 mo NR NR	*Forestomach* Squamous cell papilloma 0/5, 0/5, 0/5, 0/16, 0/18, 1/13 (8%) Squamous cell carcinoma 0/5, 0/5, 0/5, 0/16, 0/18, 3/13 (23%) Squamous cell papilloma or carcinoma (combined) 0/5, 0/5, 0/5, 0/16, 0/18, 4/13* (13%)	[NS] [NS] *[P = 0.03, Fisher exact test]	Principal limitations: no data provided on survival, body weight, or observations on any organ except the forestomach; short durations of exposure; use of only one dose; small number of rats at each time point The study is not a true carcinogenicity study and focused on determining the time required for sustained forestomach hyperplasia to produce neoplastic transformation

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 (M) 7–9 wk 27 mo Miller et al. (1985)	Inhalation (whole-body) Ethyl acrylate, > 99.5% None 0 (control A), 0 (control B), 25, 75, 225 ppm for 6 h/d, 5 d/wk for 6 mo (then unexposed for 21 mo) 60, 60, 76, 75, 71 NR	*Thyroid*: follicular cell adenoma or carcinoma (combined) 1/60 (2%), 0/60, 5/76* (7%), 2/75 (3%), 3/71 (4%)	*P < 0.05 compared with combined control groups, Fisher exact test	Principal strengths: well-conducted study Approximately 60 rats per control group and 75 rats per exposed group at the beginning of the experiment; the number of rats at the start is the effective number of rats
Full carcinogenicity Rat, F344 (F) 7–9 wk 27 mo Miller et al. (1985)	Inhalation (whole-body) Ethyl acrylate, > 99.5% None 0 (control A), 0 (control B), 25, 75, 225 ppm for 6 h/d, 5 d/wk for 6 mo (then unexposed for 21 mo) 59, 62, 77, 78, 70 NR	*Any tumour type* No significant increase in the incidence of any neoplastic lesion	NS	Principal strengths: well-conducted study Approximately 60 rats per control group and 75 rats per exposed group at the beginning of the experiment; the number of rats at the start is the effective number of rats

bw, body weight; d, day; F, female; h, hour; M, male; mo, month; NR, not reported; NS, not significant; ppm, parts per million; wk, week

3.1.2 Skin application

(a) C3H/HeJ mice

DePass et al. (1984) tested ethyl acrylate as a complete carcinogen on mouse skin. A group of 40 male C3H/HeJ mice (age, 74–79 days) were exposed to neat ethyl acrylate (purity, 99%) at a dose of 25 μL (~23 mg) on clipped dorsal skin three times per week for their lifetime. A group of 40 male mice were given skin applications of 20 mg of acetone three times per week for their lifetime, and served as controls. Survival was comparable between the group exposed to ethyl acrylate and the acetone control group. No skin tumours or adverse effects were reported in the group exposed to ethyl acrylate or the acetone control group. [The Working Group concluded that this study was inadequate for the evaluation of the carcinogenicity of ethyl acrylate because of the use of only one sex and one dose, the lack of appropriate unexposed control group, and the lack of body-weight data.]

(b) Genetically engineered mice

Groups of 10–15 female homozygous Tg.AC mice (age, 10–12 weeks) were exposed to ethyl acrylate [purity not given] at 30 mg in 200 μL acetone by skin application three times per week for 20 weeks. A group of 10–15 female mice treated concurrently with the vehicle solvent [not reported] served as negative controls. After 20 weeks, 50% of the mice exposed to ethyl acrylate averaged 0.6 papillomas of the skin per mouse. [No information was given on the results for control mice.] Ethyl acrylate was reported to be "inactive" [not tumorigenic] in Tg.AC mice, and no gross systemic effects were observed at the end of the study (20 weeks) (Tennant et al., 1995). [The Working Group noted that the study used only one dose and one sex, there was a small number of mice in the exposed and control groups, no information on body weight or the survival of exposed mice was provided, no histopathology was performed, and no results were provided for controls. The study was judged inadequate for the evaluation of the carcinogenicity of ethyl acrylate.]

In another skin application study with homozygous Tg.AC mice (Nylander-French & French, 1998), four groups of 10 female Tg.AC mice (age, 12 weeks) were exposed to ethyl acrylate at 0 (control), 60, 300, or 600 μmol (purity, 99%) in 200 μL acetone three times per week for 20 weeks. No significant difference in survival was observed between exposed and control groups. Body weight was lower in the group exposed at the highest dose. There was no significant increase in the incidence or multiplicity of papilloma of the skin in any of the exposed groups compared with the acetone control group. [The Working Group noted that the study used only one sex, there was a small number of mice in exposed and control groups, and that no histopathology was performed on organs other than the skin. The study was judged inadequate for the evaluation of the carcinogenicity of ethyl acrylate.]

3.1.3 Inhalation

In a well-conducted study, groups of [approximately] 75 male and 75 female B6C3F$_1$ mice (age, 7–9 weeks) were exposed by whole-body inhalation to ethyl acrylate vapour (purity, > 99.5%) at concentrations of 25, 75, or 225 ppm (100, 310, or 920 mg/m^3) for 6 hours per day, 5 days per week, for 27 months (Miller et al., 1985). Two separate groups of [approximately] 60 males and 60 females served as unexposed controls. Exposure of males and females to the highest dose (225 ppm) was stopped after 6 months because of a significant decrease in body-weight gain. The mice were held without further treatment for up to 21 months. The survival of exposed groups of male and female mice was similar to or better than that of both control groups. The mean body-weight gains of males and females in the groups at 75 ppm and 225 ppm were significantly lower

than that in both control groups throughout the study. A non-significant decrease in body-weight gain was also observed in males and females at 25 ppm during the last 8 months of the study. There was a significant increase in the incidence of follicular cell adenoma of the thyroid in male mice exposed to ethyl acrylate at 225 ppm for 6 months and held for an additional 21 months (controls, combined, 2/121 (2%); lowest dose, 1/75 (1%); intermediate dose, 0/76; highest dose, 7/69 (10%), $P < 0.05$, Fisher exact test). [The authors reported that the historical rate for follicular cell adenoma of the thyroid has been as high as 16% in male $B6C3F_1$ control groups in other studies, but did not cite a reference for this.] There was no significant increase in the incidence of any tumours in females.

3.2 Rat

3.2.1 Oral administration

In a well-conducted study, groups of 50 male and 50 female Fischer 344/N rats (age, 7 weeks) were given ethyl acrylate (purity, 99–99.5%; stabilized with 15 ppm of the monoethyl ether of hydroquinone) at a dose of 0, 100, or 200 mg/kg bw, by gavage in corn oil, 5 days per week for 103 weeks (NTP, 1986). In males and females, survival was comparable between exposed groups and the control group. Mean body weights of all groups of exposed males and females were comparable to those of controls throughout the study. In male rats, the incidence of squamous cell papilloma – 1/50 (P for trend, < 0.001), 2%; 15/50 and 29/50 ($P < 0.001$), 30% – squamous cell carcinoma – 0/50 (P for trend, < 0.001), 5/50 (P = 0.019), 10%, 12/50 ($P < 0.001$), 24% – and squamous cell papilloma or carcinoma (combined) – 1/50 (P for trend, < 0.001), 2%; 18/50 (36%) and 36/50 ($P < 0.001$), 72% – of the forestomach were significantly increased in all treated groups, and there was a significant positive trend in the incidence of these tumours in exposed male rats. In female rats, the incidence

of squamous cell papilloma – 1/50 (P for trend, 0.018), 2%, 6/50 (12%), 9/50 ($P = 0.021$), 18% – and squamous cell papilloma or carcinoma (combined) – 1/50 (P for trend, 0.005), 2%, 6/50 (12%), 11/50 ($P = 0.008$), 22% – of the forestomach was significantly increased in the group at the higher dose, and there was a significant positive trend in the incidence of these tumours in exposed female rats; squamous cell carcinomas of the forestomach were only observed in two females exposed at the higher dose. The incidence of non-neoplastic lesions of the forestomach was dose-related in male and female rats; these lesions included inflammation, epithelial hyperplasia, and hyperkeratosis. The combined incidence of acinar cell adenoma (3/50) and carcinoma (1/50) of the pancreas in male rats at the lower dose (4/50) was higher (significant by the life-table test, $P = 0.041$, not significant by the more appropriate incidental tumour test) than that in the vehicle controls (0/49). There was no acinar cell hyperplasia of the pancreas in exposed males.

In a study to investigate the association between exposure to ethyl acrylate and hyperplasia of the forestomach and carcinogenicity in the forestomach in rats, two groups of [number at start unspecified] male Fischer 344 rats (age, 3 months) were given ethyl acrylate (purity, 99%; stabilized with 15–20 ppm of the monoethyl ether of hydroquinone) at a dose of 200 mg/kg bw by gavage in corn oil for 5 days per week for 6 or 12 months. A control group received corn oil only for 12 months. Five rats from each treatment group and the control group were killed 24 hours after the last dose. The remaining rats were killed at age 24 months. All rats were examined for gross lesions and the stomachs were collected and examined microscopically. No treatment-related neoplastic lesions were observed in the forestomach of rats exposed to ethyl acrylate for 6 months, with (0/18) or without (0/5) a recovery period. All rats exposed to ethyl acrylate for 12 months and then killed showed hyperplastic lesions of the forestomach (5/5 compared with

0/5 in corn oil controls), but no neoplastic lesions. However, when rats were exposed to ethyl acrylate for 12 months and killed after 9 months of recovery, they developed squamous cell carcinoma (3/13, 23%) and papilloma (1/13, 8%) – combined incidence, 4/13 (31%) [$P = 0.03$, Fisher exact test] – of the forestomach, compared with none in the controls (0/16) (Ghanayem et al., 1993, 1994). [The Working Group noted the use of only one sex and dose, the small number of animals, the lack of data on survival and body weight, and that histopathological evaluation was limited to the forestomach.]

3.2.2 Inhalation

In a well-conducted study, groups of [approximately] 75 male and 75 female Fischer 344 rats (age, 7–9 weeks) were exposed by whole-body inhalation to ethyl acrylate vapour (purity, > 99.5%) at a concentration of 25, 75, or 225 ppm (100, 310, or 920 mg/m^3) for 6 hours per day, 5 days per week, for 27 months (Miller et al., 1985). Two separate groups of [approximately] 60 males and 60 females served as unexposed controls. Exposure of males and females at the highest dose (225 ppm) was stopped after 6 months because of a significant decrease in body-weight gain. These rats were held without further treatment for up to 21 months. Survival of exposed groups of males and females was lower than, but not significantly different from, that of the control groups throughout the study. The mean body-weight gains of male and female rats in the groups at 75 ppm and 225 ppm were significantly lower than those in both control groups throughout the study. There was a significant increase in the incidence of follicular cell adenoma or carcinoma (combined) of the thyroid in male rats exposed to ethyl acrylate at 25 ppm for 27 months: control, combined, 1/120 (1%); lowest dose, 5/76 (7%), $P < 0.05$, Fisher exact test; intermediate dose, 2/75 (3%); highest dose,

3/71 (4%). There was no significant increase in the incidence of any tumours in females.

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Data on absorption, distribution, metabolism, and excretion of ethyl acrylate in humans were not available to the Working Group.

4.1.2 Experimental systems

In adult male Fischer 344 rats given single doses of 2,3-[^{14}C]-ethyl acrylate at a dose of 100, 200, or 400 mg/kg bw by oral gavage in corn oil, analysis of the stomach contents showed that more than 90% of all doses administered was absorbed within 4 hours (Ghanayem et al., 1987). Ethyl acrylate was rapidly distributed to all major organs and tissues (Ghanayem et al., 1987; Frederick et al., 1992). Ghanayem et al. (1987) demonstrated that in male Fischer 344 rats the highest concentrations of 2,3-[^{14}C]-ethyl acrylate-derived radiolabel were found in the forestomach, a target organ for carcinogenesis induced by ethyl acrylate (IARC, 1986, 1999; NTP, 1986), and in three non-target organs, the glandular stomach, small intestine, and liver, 4 hours after a single oral dose of 2,3-[^{14}C]-ethyl acrylate at 100, 200, or 400 mg/kg bw. The level of 2,3-[^{14}C]-ethyl acrylate-derived radiolabel in the rat forestomach remained greater than in other organs 24 hours after exposure to 2,3-[^{14}C]-ethyl acrylate at 200 mg/kg bw.

The major route for ethyl acrylate excretion is CO_2 exhalation (Ghanayem et al., 1987). This was demonstrated by the fact that approximately 70% of ethyl acrylate was exhaled as $^{14}CO_2$ within

24 hours of exposure to 2,3-[^{14}C]-ethyl acrylate at 200 mg/kg bw. Similar findings have been reported by deBethizy et al. (1987), who demonstrated that approximately 60% of 2,3-[^{14}C]-ethyl acrylate given by oral gavage to adult male Sprague-Dawley rats (n = 3 rats per group) at a dose of 2, 20, or 200 mg/kg bw was eliminated in 8 hours and 75% was eliminated in 24 hours by $^{14}CO_2$ exhalation. Approximately 10% of a dose of 2,3-[^{14}C]-ethyl acrylate of 200 mg/kg bw given by oral gavage was excreted in the urine, in the form of *N*-acetyl-(2-carboxyethyl)cysteine and *N*-acetyl-(2-carboxyethyl)cysteine ethyl ester, and 4% was excreted in the faeces (Ghanayem et al., 1987). In addition to *N*-acetyl-(2-carboxyethyl) cysteine and *N*-acetyl-(2-carboxyethyl)cysteine ethyl ester, two separate studies also identified the presence of 3-hydroxypropionic acid in the urine of rats exposed to ethyl acrylate (deBethizy et al., 1987; Linhart et al., 1994). In the first study, deBethizy et al. (1987) showed that 3-hydroxypropionic acid was present in the urine of adult male Sprague-Dawley rats in the 0–6-hour period after oral exposure to 2,3-[^{14}C]-ethyl acrylate at 200 mg/kg bw. In the second study, Linhart et al. (1994) reported a significant increase of 3-hydroxypropionic acid in the urine of adult female Wistar rats 24 hours after intraperitoneal injection of ethyl acrylate at 2.0 mmol/kg bw.

Several studies investigated the metabolism of ethyl acrylate in rats (see Fig. 4.1). Ethyl acrylate is rapidly metabolized, as demonstrated by its short metabolic half-life (Miller et al., 1981; Frederick et al., 1992). There are two main metabolic routes in ethyl acrylate metabolism: (i) enzymatic hydrolysis of ethyl acrylate to acrylic acid and ethanol catalysed by carboxylesterases, with a subsequent high-efficiency conversion of both metabolites to CO_2 (Miller et al., 1981; Silver & Murphy, 1981; Ghanayem et al., 1987); and (ii) binding of ethyl acrylate to glutathione (GSH) and proteins (deBethizy et al., 1987; Ghanayem et al., 1987; Frederick et al., 1992).

Three studies investigated enzymatic hydrolysis of ethyl acrylate in a reaction mediated by carboxylesterase (Miller et al., 1981; Frederick et al., 1992; McCarthy & Witz, 1997). In two studies, Miller et al. (1981) and Frederick et al. (1992) demonstrated significant carboxylesterase activity towards ethyl acrylate by tissue homogenates from Fischer 344 rats in vitro. In a separate study, McCarthy & Witz (1997) reported a high efficiency of ethyl acrylate enzymatic hydrolysis by purified porcine liver carboxylesterase.

Four studies investigated the metabolic pathways involved in the reactions binding ethyl acrylate to GSH and proteins (Ghanayem et al., 1987; Frederick et al., 1990, 1992; Potter & Tran, 1992).

Potter & Tran (1992) demonstrated a rapid and time-dependent non-enzymatic conjugation of 2,3-[^{14}C]-ethyl acrylate to GSH in Fischer 344 rats, with a second-order rate constant of 32.8 M^{-1} min^{-1}. Similarly, a second-order rate constant of 26.6 M^{-1} min^{-1} was found for the reaction of GSH conjugation with ethyl acrylate in vitro (McCarthy et al., 1994). The conjugation of ethyl acrylate with GSH is also demonstrated by the fact that the major ethyl acrylate metabolites detected in the urine of Fischer 344 rats given a single dose of ethyl acrylate at 100, 200, or 400 mg/kg bw by oral gavage were *N*-acetyl-(2-carboxyethyl)cysteine, the degradation product of an acrylic-acid–GSH adduct, and *N*-acetyl-(2-carboxyethyl)cysteine ethyl ester, a metabolite resulting from direct conjugation of ethyl acrylate with GSH (Ghanayem et al., 1987).

In addition to conjugation with GSH, ethyl acrylate exhibits a high binding efficiency for proteins (Ghanayem et al., 1987; Potter & Tran, 1992). In particular, Ghanayem et al. (1987) demonstrated that 24 hours after Fischer 344 rats were given radiolabelled ethyl acrylate at a dose of 200 mg/kg bw by oral gavage, most of the 2,3-[^{14}C]-ethyl acrylate-derived radiolabel in the forestomach was irreversibly bound to proteins, whereas in the liver most of the 2,3-[^{14}C]-ethyl

Fig. 4.1 Proposed metabolic pathways for ethyl acrylate in rats in vivo
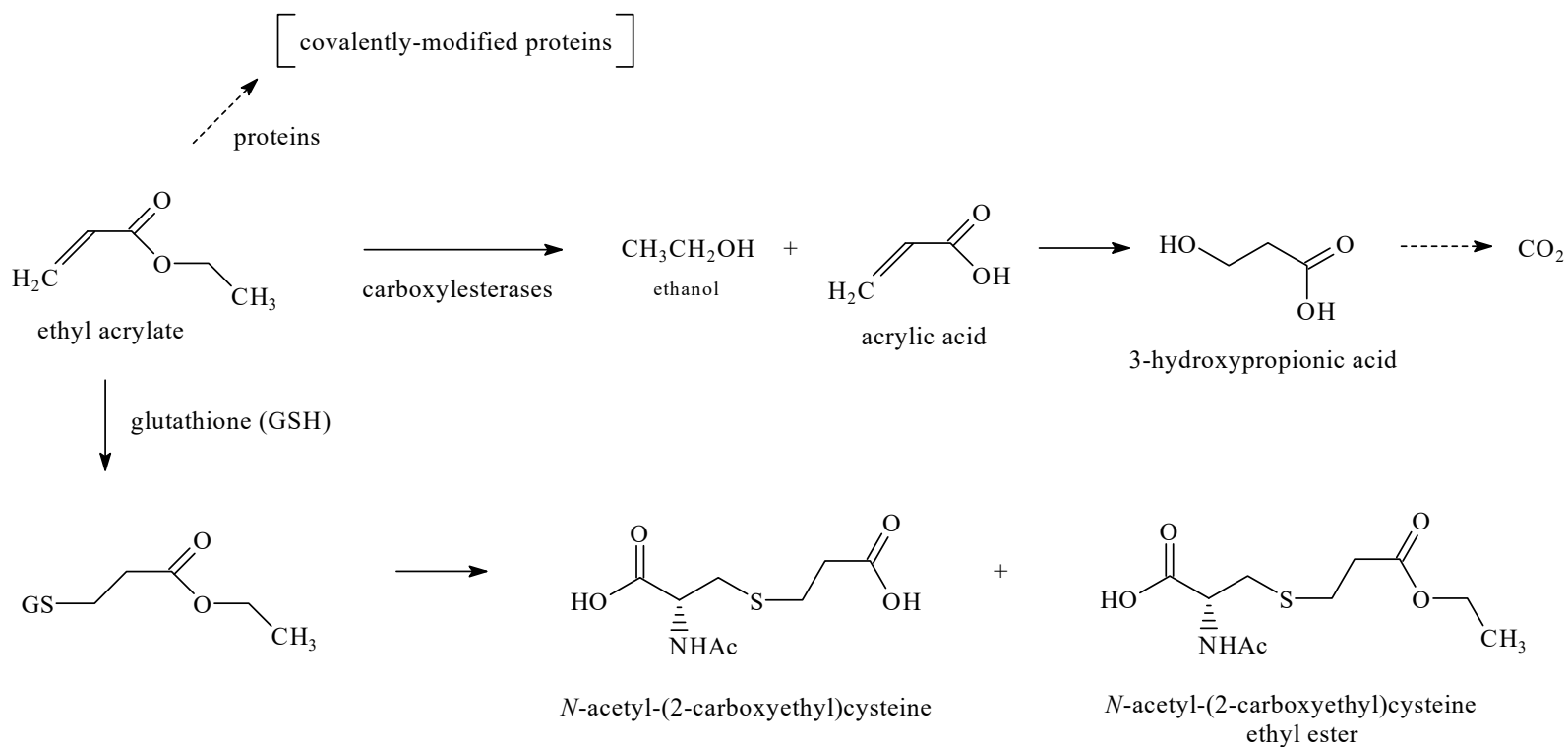
The *N*-acetyl-(2-carboxyethyl)cysteine conjugate may also stem from glutathione addition to acrylic acid. Protein binding derived from ethyl acrylate has been detected in rat forestomach, but the specific adducts have not been characterized
Compiled by the Working Group

acrylate-derived radiolabel was bound to lipids. The concentration of protein-bound 2,3-[^{14}C]-ethyl acrylate-derived radiolabel in the forestomach was fivefold that in the liver.

4.2 Mechanisms of carcinogenesis

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016) in the following order: is genotoxic; alters cell proliferation, cell death, or nutrient supply; and induces chronic inflammation. Insufficient data were available for evaluation of the other key characteristics of carcinogens.

4.2.1 Genetic and related effects

Table 4.1, Table 4.2, Table 4.3, and Table 4.4 summarize the studies evaluated and considered to be the most representative of the genetic and related effects of ethyl acrylate.

(a) Humans

See Table 4.1

In one study, cytogenetic analysis was carried out in peripheral blood lymphocytes of 60 controls and 60 workers exposed in 1987, 1992, 1993 (exposed group only), and 1997 during production of acrylic acid, acrylic acid esters, and acrylate dispersions (Tuček et al., 2002). The average exposure duration was 13 ± 5 years. The mean percentage of aberrant cells in both groups remained in normal range when analysed annually; however, in an overall analysis of all results, a borderline statistically significant ($P = 0.05$) increase in chromosomal aberrations in peripheral lymphocytes was seen in exposed workers. [The Working Group noted that the effects could not be attributed to ethyl acrylate specifically.]

In human cells in vitro, Fowler et al. (2012) analysed the effect of exposure to ethyl acrylate on micronucleus induction in human TP53-competent primary cultures of lymphocytes (HuLy), TK6 lymphoblastoid cells, and HepG2 liver cells for 3 hours followed by a 21-hour recovery period in two independent experiments. There was significant formation of micronuclei at concentrations that induced some cytotoxicity in HuLy cells, TK6 cells, and in HepG2 cells (in one of two tests). In a separate experiment involving 24-hour exposures in two independent trials (Fowler et al., 2012), there was no increase in the frequency of micronucleus formation in HuLy cells at a concentration that induced some cytotoxicity, but frequency of micronucleus formation was increased in TK6 cells and in HepG2 cells in one of the two trials.

In the human TK6 lymphoblast TP53-competent) and WIL2-NS lymphoblast (TP53-mutant) cell lines exposed to ethyl acrylate at concentrations below the predefined cytotoxicity cut-off and in the presence of cytochalasin B there was a slight induction of micronuclei that did not meet the criteria for either a positive or a negative response (Whitwell et al., 2015). In a separate experiment in the absence of cytochalasin B, the results of exposure of TK6 and WIL2-NS cells to ethyl acrylate were negative.

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.2

Several studies investigated the genotoxic effects of exposure to ethyl acrylate in experimental animals in vivo. A single dose of 1.0 mL of 4% ethyl acrylate in corn oil by gastric tube did not increase DNA damage in the forestomach squamous epithelium in male Fischer 344 rats as measured by the alkaline elution assay (Morimoto et al., 1990). In female homozygous transgenic Tg.AC (v-Ha-ras) mice, ethyl acrylate did not alter the migration of DNA isolated from peripheral blood leukocytes after up to 20 weeks of dermal topical application of ethyl acrylate at 60, 300, and 600 μmol per mouse ($n = 9$ mice per dose) three times per week, as measured by the alkaline comet assay (Tice et al., 1997). Further,

Table 4.1 Genetic and related effects of ethyl acrylate in human cells in vitro

End-point	Tissue, cell line	Results[a]		Concentration (µg/mL) (LEC or HIC)	Comments	References
		Without exogenous activation	With exogenous activation			
Micronucleus formation	Lymphocytes (HuLy)	+	NT	38, 50	Positive results observed at cytotoxic concentrations; 3 h exposure with 21 h recovery	Fowler et al. (2012)
	Lymphoblast TK6 cells	+	NT	20, 25	Positive results observed at cytotoxic concentrations; 3 h exposure with 21 h recovery	
	HepG2 hepatocarcinoma cells	+/−	NT	96	Positive in one of two experiments at the same dose; 3 h exposure with 21 h recovery	
	Lymphocytes (HuLy)	−	NT	10	24 h exposure	
	Lymphoblast TK6 cells	+/−	NT	10	Positive in one of two experiments at the same dose; 24 h exposure	
	HepG2 hepatocarcinoma cells	+/−	NT	77	Positive in one of two experiments; 24 h exposure	
Micronucleus formation	Lymphoblast TK6 cells	−	NT	6		Whitwell et al. (2015)
	Lymphoblast WIL2-NS cells	−	NT	9		
	Lymphoblast TK6 cells	+/−	NT	6	In the presence of cytochalasin B	
	Lymphoblast WIL2-NS cells	+/−	NT	9	In the presence of cytochalasin B	

h, hour; HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested

[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

Table 4.2 Genetic and related effects of ethyl acrylate in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks	Rat, Fischer 344 (M)	Forestomach	–	1.0 mL	Via gastric tube, 4% ethyl acrylate in corn oil, ×1		Morimoto et al. (1990)
DNA strand breaks	Mouse, Tg.AC transgenic (F)	Peripheral blood leukocytes	–	600 μmol	Skin application, 3×/wk for 20 wk		Tice et al. (1997)
Point mutations, deletions	Mouse, *gpt* delta transgenic (M)	Stomach, liver	–	50 mg/kg bw	Gavage, ×1/d for 28 d		Ellis-Hutchings et al. (2018)
Micronucleus formation	Mouse, BALB/c (M)	Bone marrow	+	225 mg/kg bw	Intraperitoneal injection, ×2		Przybojewska et al. (1984)
Micronucleus formation	Mouse, BALB/c (M)	Bone marrow	+/–	812 mg/kg bw	Intraperitoneal injection, ×2	Positive in one of two experiments at the same dose; observation made 30 h after second dose	Ashby et al. (1989)
Micronucleus formation	Mouse, C57BL/6J (M, F)	Bone marrow	–	738 mg/kg bw	Intraperitoneal injection, ×1	Observations made 24, 48, and 72 h after dose	
Micronucleus formation	Mouse, C57BL/6J (M)	Bone marrow	–	738 mg/kg bw	Intraperitoneal injection, ×2	Observation made 30 h after second dose	
Micronucleus formation	Mouse, Tg.AC transgenic (F)	Peripheral blood leucocytes	–	600 μmol	Skin application, ×60		Tice et al. (1997)
Micronucleus formation	Mouse, C57BL/6J (M)	Splenocytes	–	1000 mg/kg bw	Intraperitoneal injection, ×1		Kligerman et al. (1991)
Sister-chromatid exchange			–				
Chromosomal aberrations			–				

bw, body weight; d, day; F, female; h, hour; HID, highest effective dose; LED, lowest effective dose; M, male; wk, week

[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

Table 4.3 Genetic and related effects of ethyl acrylate in non-human mammalian cells in vitro

End-point	Species, cell line	Results[a] Without metabolic activation	With metabolic activation	Concentration (µg/mL) (LEC or HIC)	Comments	Reference
DNA double-strand breaks	Mouse lymphoma L5178Y	+	NT	40	Positive results observed at cytotoxic concentrations	Ciaccio et al. (1998)
Gene mutation, *Tk*	Mouse lymphoma L5178Y	+	NT	20		McGregor et al. (1988)
Gene mutation, *Tk*	Mouse lymphoma L5178Y	+	NT	20	Positive results observed at cytotoxic concentrations	Moore et al. (1988)
Gene mutation, *Tk*	Mouse lymphoma L5178Y	+	NT	20	Positive results observed at cytotoxic concentrations	Moore et al. (1989)
Gene mutation, *Tk*	Mouse lymphoma L5178Y	+	+	20	Positive results observed at cytotoxic concentrations	Dearfield et al. (1991)
Gene mutation, *Tk*	Mouse lymphoma L5178Y	+	NT	20	Positive results observed at cytotoxic concentrations	Ciaccio et al. (1998)
Gene mutation, *Hprt*	Chinese hamster ovary	−	NT	23		Moore et al. (1989)
Gene mutation, *Hprt*	Chinese hamster ovary	−	NT	80		Moore et al. (1991)
Chromosomal aberrations, *Tk*	Mouse lymphoma L5178Y	+	NT	20	Positive results observed at cytotoxic concentrations	Moore et al. (1988)
Chromosomal aberrations, *Tk*	Mouse lymphoma L5178Y	+	NT	20	Positive results observed at cytotoxic concentrations	Moore et al. (1989)
Chromosomal aberrations, *Hprt*	Chinese hamster ovary	+	NT	21	Positive results observed at cytotoxic concentrations	
Chromosomal aberrations	Chinese hamster ovary	−	+	Not clearly indicated		Loveday et al. (1990)
Micronucleus formation	Mouse leukaemia L5178Y	+/−	NT	12, 18		Whitwell et al. (2015)

Table 4.3   (continued)

End-point	Species, cell line	Results[a]		Concentration (µg/mL) (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Micronucleus formation	V79 Chinese hamster lung fibroblasts	+	NT	1, 4	Positive results observed at cytotoxic concentrations; 24 h exposure	Fowler et al. (2012)
	V79 Chinese hamster lung fibroblasts	+	NT	16, 20	Positive results observed at cytotoxic concentrations; 3 h exposure with 21 h recovery	
	Chinese hamster lung	+	NT	7, 14	Positive results observed at cytotoxic concentrations; 24 h exposure	
		+	NT	39, 40	Positive results observed at cytotoxic concentrations; 3 h exposure with 21 h recovery	
	Chinese hamster ovary	−	NT	10, 12	24 h exposure	
		+	NT	20, 32	Positive results observed at cytotoxic concentrations; 3 h exposure with 21 h recovery	
Sister-chromatid exchange	Chinese hamster ovary	−	+	Not clearly indicated		Loveday et al. (1990)

h, hour; HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested

[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

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

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without exogenous metabolic activation	With exogenous metabolic activation			
Drosophila melanogaster	Sex-linked recessive lethal mutations	–		40 000 ppm feed		Valencia et al. (1985)
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	+/–	+/–	3333 µg/plate	Inconsistent result from two different laboratories, one positive and one negative	Haworth et al. (1983)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538	Reverse mutation	–	–	2000 µg/plate		Waegemaekers & Bensink (1984)
Salmonella typhimurium TA102	Reverse mutation	–	–	15–5000 µg/plate		Kirkland et al. (2016)
Salmonella typhimurium YG7108pin3Erb5	Reverse mutation	–	–	2000 µg/plate		Emmert et al. (2006)
Saccharomyces cerevisiae D61.M	Homozygosis by mitosis Homozygosis by mitosis	– +	NT NT	733 µg/mL 733 µg/mL	In combination with propionitrile	Zimmermann & Mohr (1992)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; ppm, parts per million

[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

the frequency of micronucleated peripheral blood polychromatic or normochromatic erythrocytes was not increased after 20 weeks of treatment.

No increase in the occurrence of point mutations or deletions was seen in the stomach or liver of male *gpt* delta mice (age, 40 weeks; *n* = 6 per group) exposed to ethyl acrylate at 8, 20, or 50 mg/kg bw per day in corn oil by oral gavage for 28 days (Ellis-Hutchings et al., 2018).

Two studies (Przybojewska et al., 1984; Ashby et al., 1989) investigated micronuclei induction by ethyl acrylate in mice. Przybojewska et al. (1984) reported that in male BALB/c mice exposed to ethyl acrylate by two intraperitoneal injections at 225, 450, 900 (*n* = 4 mice per dose), or 1800 mg/kg bw (*n* = 2 mice) separated by 24 hours, significantly increased micronuclei induction in the bone marrow was observed. Ashby et al. (1989) observed a significant induction of micronuclei in male BALB/c mice (*n* = 10 mice) 30 hours after two intraperitoneal injections of ethyl acrylate at 812 mg/kg bw in one of two experiments. In contrast, in two separate experiments in male and female C57BL/6J mice, observations made 24, 48, or 72 hours after a single intraperitoneal injection, or 30 hours after two intraperitoneal injections separated by 24 hours, of ethyl acrylate at 738 mg/kg bw did not reveal induction of micronuclei in the bone marrow (Ashby et al., 1989). However, a statistically significant bone-marrow toxicity, indicated by a decreased polychromatic:normochromatic erythrocyte ratio, was observed 48 and 72 hours after exposure of male and female mice to ethyl acrylate (Ashby et al., 1989).

In male C57BL/6 mice, ethyl acrylate did not increase the frequency of chromosomal aberrations, sister-chromatid exchange, or micronucleus formation in splenocytes 24 hours after a single intraperitoneal injection of ethyl acrylate at 125, 250, 500, or 1000 mg/kg bw (Kligerman et al., 1991).

(ii)   Non-human mammalian cells in vitro

See Table 4.3

Ethyl acrylate induced DNA double-strand breaks in L5178Y *Tk*$^{+/-}$ lymphoma cells (Ciaccio et al., 1998).

In a study of mutations at the hypoxanthine-guanine phosphoribosyltransferase (*Hgprt*) gene, ethyl acrylate gave negative results in the standard and suspension protocols using Chinese hamster ovary (CHO) cells (Moore et al., 1991).

In contrast to experimental animal studies in vivo, ethyl acrylate produced a consistently positive response when tested in the mouse lymphoma assay or other non-human mammalian cell clastogenicity assays in vitro (Johannsen et al., 2008). Four studies (McGregor et al., 1988; Moore et al., 1988, 1989; Dearfield et al., 1991) that were reviewed in the previous monograph (IARC, 1999) examined the genotoxic activity of ethyl acrylate in the mouse heterozygous L5178Y *Tk*$^{+/-}$ lymphoma cell assay. The results of these studies demonstrated that exposure of mouse L5178Y lymphoblast cells to ethyl acrylate without exogenous metabolic activation by a post-mitochondrial rat S9 liver homogenate (S9 mix) increased mutation frequency. Furthermore, Moore et al. (1988) reported a dose-dependent increase in the mutation frequency after exposure of L5178Y *Tk*$^{+/-}$ lymphoma cells. Similar results were obtained in a later independent study (Ciaccio et al., 1998) that showed a concentration-dependent increase in mutation frequency in L5178Y *Tk*$^{+/-}$ lymphoma cells exposed to ethyl acrylate. It should be noted that positive genotoxic activity of ethyl acrylate in these mouse L5178Y *Tk*$^{+/-}$ lymphoma cell studies was primarily observed at concentrations that induced some cytotoxicity (McGregor et al., 1988; Moore et al., 1988, 1989; Dearfield et al., 1991; Ciaccio et al., 1998).

Loveday et al. (1990) reported that exposure of CHO cells to ethyl acrylate [concentration not clearly indicated] induced chromosomal aberrations and sister-chromatid exchange in

cells with, but not without, metabolic activation. Chromosomal aberrations were induced in L5178Y $Tk^{+/-}$ lymphoma and CHO cells exposed to ethyl acrylate, without metabolic activation (Moore et al., 1988, 1989).

Micronuclei were induced when V79, CHO, and Chinese hamster lung (CHL) cells were exposed to ethyl acrylate without metabolic S9 activation for 3 hours at concentrations that induced some cytotoxicity, followed by a 21-hour recovery (Fowler et al., 2012). In a separate experiment reported by Fowler et al. (2012), micronuclei were induced in V79 and CHL cells, but not in CHO cells, when the exposure was for 24 hours.

In the mouse Tp53-mutant lymphoma L5178Y cell line, exposure to ethyl acrylate for 24 hours induced a small dose-dependent, but statistically significant, induction of micronuclei that did not meet the criteria for either a positive or a negative response (Whitwell et al., 2015).

(iii) Non-mammalian experimental systems

See Table 4.4

Valencia et al. (1985) reported that ethyl acrylate was not mutagenic in *Drosophila melanogaster*.

Several reports showed negative results in the Ames assay (Waegemaekers & Bensink, 1984; Johannsen et al., 2008; Kirkland et al., 2016). Haworth et al. (1983) reported inconsistent results from two different laboratories, one positive and one negative.

Ethyl acrylate lacked mutagenicity in the Ames test with the metabolically competent *Salmonella typhimurium* YG7108 strain containing the plasmid pin3ERb$_5$ that encodes a complete electron transport chain, including CYP450 (CYP) reductase, cytochrome b_5, and CYP2E1 (Emmert et al., 2006).

Ethyl acrylate did not induce genetic alterations in *Saccharomyces cerevisiae* D61.M when applied alone; however, when ethyl acrylate was applied in combination with propionitrile, a strong inducer of chromosomal malsegregation, chromosome loss was observed (Zimmermann & Mohr, 1992).

4.2.2 Altered cell proliferation, cell death or nutrient supply

(a) Humans

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

In human cells in vitro, exposure to ethyl acrylate for 18 hours had a strong cytotoxic effect in normal human epidermal keratinocytes and normal human dermal fibroblasts (0.1 μmol/well), and normal human bronchial epithelium cells (1.0 μmol/well), as determined by the MTT [(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Nylander-French & French, 2000).

In the primary human gingival fibroblast and human submandibular gland adenocarcinoma cell lines, ethyl acrylate was not cytotoxic at concentrations of less than 10 μM as determined by the MTT assay. Cytotoxicity was seen at 100 μM, although no cell viability was found with ethyl acrylate at 1 mM (Fujisawa et al., 2000). Cytotoxicity was also observed in human HuLy cells, TK6 cells, and HepG2 cells (see Section 4.2.1 above).

Ethyl acrylate increased caspase3/7 activity in TK6 cells at concentrations of 6–12 μg/mL, and in WIL2-NC lymphoblast cells at concentrations of 6–16 μg/mL (Whitwell et al., 2015).

(b) Experimental systems

(i) Non-human mammals in vivo

In male and female C57BL/6J mice given a single intraperitoneal injection of ethyl acrylate at 738 mg/kg bw, statistically significant bone marrow toxicity was observed after 48 and 72 hours (Ashby et al., 1989).

Several studies examined the effect of ethyl acrylate on cell proliferation using different experimental approaches.

In a 2-year study of carcinogenicity in B6C3F$_1$ mice and Fischer 344/N rats exposed to ethyl acrylate via oral gavage (5 days per week, for 103 weeks), hyperplasia was seen in the forestomach (NTP, 1986). The incidence of hyperplasia was greater in the group exposed to ethyl acrylate at 200 mg/kg bw (26/50 male and 30/50 female B6C3F$_1$ mice, and 46/50 male and 49/50 female Fischer 344/N rats) compared with the group exposed to ethyl acrylate at 100 mg/kg bw (17/50 male and 12/50 female B6C3F$_1$ mice, and 41/50 male and 34/50 female Fischer 344/N rats). Hyperplasia of the bile duct was also seen in female Fischer 344 rats at both doses, with chronic exposure in the 2-year bioassay (NTP, 1986).

In a later study, Frederick et al. (1990) examined the forestomach and the glandular stomach of male Fischer 344 rats (n = 10 rats per dose) exposed to ethyl acrylate by oral gavage at 0.04, 0.2, 0.4, 1.0, 2.0, or 4.0% w/v (corresponding to 2, 10, 20, 50, 100, or 200 mg/kg bw) for 5 days per week for 2 weeks. At doses of 20 mg/kg bw or more, a dose-dependent increase in the incidence and severity of diffuse epithelial hyperplasia in the forestomach mucosa was seen. No treatment-related effects were observed in rats exposed to ethyl acrylate at doses of 10 mg/kg bw or less. An increased incidence and severity of diffuse epithelial hyperplasia in the forestomach was accompanied by an equal severity of hyperkeratosis. In contrast, no epithelial lesions were found in the glandular stomach in rats in any experimental group. Similarly, with exposure via drinking-water, diffuse epithelial hyperplasia in the forestomach mucosa was observed in all rats exposed to ethyl acrylate at concentrations of 1000, 2000, or 4000 ppm (99, 197, or 369 mg/kg bw), with the severity increasing in a dose-dependent manner. Hyperkeratosis, in conjunction with diffuse epithelial hyperplasia, was observed in rats exposed to ethyl acrylate at concentrations of 2000 and 4000 ppm.

Several studies from one research group investigated the role of cell proliferation in forestomach carcinogenesis induced by ethyl acrylate in rats (Ghanayem et al., 1991a,b,c, 1993, 1994). In the first report, Ghanayem et al. (1991a) showed that exposure of male Fischer 344 rats (n = 5 per group) to ethyl acrylate at a dose of 100 or 200 mg/kg bw per day by oral gavage for 14 consecutive days resulted in hyperplasia in the forestomach, the severity of which was dose-dependent. In several other studies (Ghanayem et al., 1991b,c, 1993, 1994), exposure of Fischer 344 rats to ethyl acrylate at 100 or 200 mg/kg bw by oral gavage for 5 days per week for 13 weeks induced mucosal hyperplasia in the forestomach (Ghanayem et al., 1991b). This was largely reversed after 8 weeks and 19 months of cessation of exposure for the groups exposed at 100 and 200 mg/kg bw, respectively. In two subsequent studies, the effect of exposure to ethyl acrylate at 200 mg/kg bw by oral gavage on hyperplasia in the forestomach was investigated. In the first of these studies, Ghanayem et al. (1993) reported that exposure of male Fischer 344 rats (n = 5 rats per group) at 200 mg/kg bw by oral gavage for 5 days per week for 6 and 12 months resulted in the development of mucosal hyperplasia in the forestomach in all exposed rats. This hyperplasia was reversed 15 months after cessation of treatment in all rats exposed for 6 months, but was sustained in 8 out of 13 rats (62%) 9 months after cessation of treatment in rats exposed for 12 months. This finding was confirmed in the second study (Ghanayem et al., 1994), which showed persistence of hyperplasia in the forestomach in 10 out of 13 rats (77%) 9 months after cessation of treatment in rats exposed to ethyl acrylate at 200 mg/kg bw for 12 months. Importantly, in 30% of rats exposed at 200 mg/kg bw for 12 months, the hyperplasia progressed to neoplasia.

Two articles reported the effect of ethyl acrylate on the extent of cell proliferation in the forestomach of exposed Fischer 344 rats (Gillette & Frederick, 1993; Ghanayem et al.,

1994). Gillette & Frederick (1993) reported the results of three experiments on the induction of epithelial S-phase activity in the Fischer 344 rat forestomach and glandular stomach. In the first experiment, a significant and prolonged elevation in the number of S-phase cells in the forestomach after a single gavage exposure to ethyl acrylate at 200 mg/kg bw in corn oil was evident 10 hours after treatment and remained elevated for 48 hours. In contrast to the forestomach, the glandular stomach response showed a marked increase of the S-phase activity 16 and 20 hours after treatment, which rapidly returned to normal levels 28 hours after treatment. In the second experiment, a significant induction of S-phase cells was seen in the forestomach and glandular stomach in a dose-dependent manner in rats exposed to ethyl acrylate at a concentration of 20 mg/kg bw or more. In the third experiment, in rats exposed to ethyl acrylate by oral gavage at 200 mg/kg bw in corn oil 5 days per week for 2 weeks, a significant elevation in the number of S-phase cells in the forestomach of exposed rats was detected at each post-dose time interval (6, 12, 18, and 24 hours).

In the study by Ghanayem et al. (1994), the exposure of Fischer 344 rats to ethyl acrylate by oral gavage at 200 mg/kg bw for 5 days per week for 12 months markedly increased the number of bromodeoxyuridine-stained nuclei in basal and squamous epithelial cells of the forestomach mucosa.

(ii) Non-human mammalian cells in vitro

An increase in the frequency of cell death in mouse fibroblast L929 (NCTC) cells was seen after exposure to ethyl acrylate at a concentration of 40, 70, or 100 µg/mL for 16 hours (Yang & Duerksen-Hughes, 1998). A dose-dependent increase in cytotoxicity was seen after exposure to ethyl acrylate at 0, 65, 80, 90, and 100 µg/mL for 24 hours in the Chinese hamster CHL/IU cell line when a relative population doubling index was used instead of the traditional relative cell count index (Fujita et al., 2016). Cytotoxicity was also observed in rodent V79, CHO, and CHL cells (see Section 4.2.1 above).

4.2.3 Chronic inflammation

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Several studies reported chronic inflammation in the forestomach of mice and rats exposed to ethyl acrylate. In the 2-year studies of carcinogenicity, inflammation of the forestomach was reported in male and female Fischer 344/N rats and B6C3F$_1$ mice exposed to ethyl acrylate at 100 or 200 mg/kg bw (NTP, 1986).

Exposure of Fischer 344 rats (n = 10 rats per group) to ethyl acrylate 5 days per week, for 2 weeks by oral gavage, but not by drinking-water, induced inflammation in the forestomach (Frederick et al., 1990). Concentrations of 100 and 200 mg/kg bw in corn oil resulted in submucosal inflammation in the forestomach in 6 and 10 rats, respectively, which was accompanied by a submucosal oedema in the forestomach in 2 and 9 rats, respectively. A lower incidence of inflammation was seen in the glandular stomach (1 and 6 out of 10 rats exposed at 100 and 200 mg/kg bw, respectively). In contrast, inflammation was not seen in the forestomach or the glandular stomach of Fischer 344 rats given drinking-water containing ethyl acrylate at 369 mg/kg bw per day for 2 weeks.

In rats, a single oral dose of ethyl acrylate consistently induced inflammation in the forestomach in two separate studies. deBethizy et al. (1987) reported that in male Sprague-Dawley rats (n = 3 rats per group), a single exposure to ethyl acrylate at 200 mg/kg bw by oral gavage resulted in a significant oedema and increased forestomach weight 72 hours after treatment. Ghanayem et al. (1991c) demonstrated a dose-dependent forestomach oedema in male Fischer 344 rats 4 hours

after a single exposure to ethyl acrylate at 100, 200, or 400 mg/kg bw by oral gavage in corn oil. No significant changes in the glandular stomach were observed.

Daily exposure to ethyl acrylate at 8, 20, or 50 mg/kg bw by oral gavage in corn oil for 28 days resulted in inflammatory cell infiltration in the forestomach of *gpt* delta transgenic mice (Ellis-Hutchings et al., 2018).

4.2.4 Other mechanisms

Several studies reported depletion of GSH, the principal cellular non-protein thiol, induced in human cells in vitro and in experimental systems by exposure to ethyl acrylate; these are discussed in the following sections.

(a)   Humans

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

In human cells in vitro, Nylander-French & French (2000) reported a decrease in intracellular sulfhydryl concentrations in normal human epidermal keratinocytes and normal human bronchial epithelium cells treated with ethyl acrylate at 0.01 µmol/well in 96-well plates for 18 hours.

(b)   Experimental systems

(i)   Non-human mammals in vivo

Three studies investigated the effect of ethyl acrylate on the concentration of non-protein sulfhydryl (NPSH) in tissues of exposed rats. deBethizy et al. (1987) examined the tissue concentrations of NPSH in adult male Sprague-Dawley rats (n = 3 rats per group) that were given a single dose of ethyl acrylate at 2, 20, or 200 mg/kg bw by gavage. A dose-dependent depletion of NPSH was seen in all analysed tissues (forestomach, glandular stomach, liver, and blood), with the greatest decrease in the NPSH content observed in the forestomach and glandular stomach. In male Wistar rats

exposed to ethyl acrylate by 6-hour inhalation, a dose-dependent depletion of NPSH was reported in the livers at concentrations of 20–80 mmol/m^3 and in blood at exposure concentrations of 40–80 mmol/m^3 (Vodička at al., 1990). Frederick et al. (1990) showed a rapid depletion of NPSH, primarily GSH, in the forestomach of male Fischer 344 rats exposed to ethyl acrylate at 200 mg/kg bw by oral gavage for 5 days per week for 2 weeks (Frederick et al., 1992). A less pronounced effect was seen on the NPSH content in the glandular stomach. In contrast, exposure to ethyl acrylate did not alter the NPSH concentration in the liver. Exposure at 20 mg/kg bw had a negligible effect on the NPSH content of the forestomach, and no effect on the concentrations of NPSH in the glandular stomach and liver.

Significantly decreased levels of both GSH and oxidized glutathione (GSSG) were seen in the forestomach of male C57BL/6 mice (n = 5 mice per group) 3 hours after exposure to ethyl acrylate at 0, 20, 50, or 100 mg/kg bw by oral gavage in corn oil. The relative GSH/GSSG ratio was not altered (Ellis-Hutchings et al., 2018).

(ii)   Non-human mammalian cells in vitro

In heterozygous L5178Y *Tk*$^{+/-}$ mouse lymphoma cells, exposure to ethyl acrylate at 10, 20, 30, 40, or 50 µg/mL for 4 hours resulted in time- and concentration-dependent reduction of the NPSH concentrations (Ciaccio et al., 1998).

4.3   Other adverse effects

4.3.1 Irritancy and sensitization

(a)   Humans

The major reported adverse effects of ethyl acrylate exposure in humans include sensory irritation in the nose and eyes (Hoffmeyer et al., 2016, 2017; Kleinbeck et al., 2017) and contact dermatitis (Le et al., 2015; Spencer et al., 2016; DeKoven et al., 2017).

(b)   Experimental systems

Three studies of the skin irritating effect of ethyl acrylate in mice (Hayes & Meade, 1999; Warbrick et al., 2001; Dearman et al., 2007) produced contradictory results. In the first study (Hayes & Meade, 1999), no skin irritating effect of ethyl acrylate was found in the murine local lymph node assay and in the mouse ear swelling test in B6C3F$_1$ mice. In two later studies in CBA mice (Warbrick et al., 2001; Dearman et al., 2007), the skin-irritating effect of ethyl acrylate was demonstrated in the murine local lymph node assay.

An increased incidence of retinopathy and cataracts was reported in male and female Fischer 344/N rats exposed to ethyl acrylate at 100 mg/kg bw in 2-year studies of carcinogenicity (NTP, 1986). Additionally, in studies of short-term exposure to ethyl acrylate by inhalation, leukopenia was observed in adrenalectomized male Sprague-Dawley rats (Brondeau et al., 1990) and hyperglycaemia was seen in male Wistar rats (Vodička et al., 1990).

4.4   Data relevant to comparisons across agents and end-points

See the monograph on isobutyl nitrite in the present volume.

5.   Summary of Data Reported

5.1   Exposure data

Ethyl acrylate is a high production volume chemical that is produced worldwide. It is used in the production of polymers for water-based paints, resins, plastics, and rubber, and in the production of acrylic fibres, adhesives, and binders. Ethyl acrylate is also used in surface coatings for textiles, paper, leather, and food-contact materials, and as a food flavouring agent. Occupational exposure may occur among chemical and paint manufacturing workers, nail salon workers, and dental technicians. A small number of studies have characterized occupational air exposures to ethyl acrylate in polystyrene production, paint mixing, and laser cutting of plexiglass, acrylic, and lucite materials. Exposure to the general population occurs from food flavouring additives and food-contact materials, and through materials containing ethyl acrylate, such as window caulking and acrylic nail products. Exposure concentrations in the environment and the general population have not been reported.

5.2   Cancer in humans

One cohort study found an increased risk of mortality from cancer of the colon and rectum among acrylic sheet manufacturing workers exposed to methyl methacrylate and ethyl acrylate. One cohort study found no increased risk of mortality from multiple cancer types in acrylic sheet manufacturing workers where ethyl acrylate exposure may have occurred. A general-population case–control study found an increased risk of cancer of the rectum and no increased risk of cancer of the colon for occupational exposure to aliphatic esters. However, exposure assessment in all three studies was not specific to ethyl acrylate.

5.3   Cancer in experimental animals

Ethyl acrylate was tested for carcinogenicity in one well-conducted gavage study and one well-conducted inhalation study in male and female mice. Ethyl acrylate was tested for carcinogenicity in one gavage study and one well-conducted inhalation study in male and female rats, and one gavage study in male rats.

In male mice, exposure to ethyl acrylate by gavage caused a significant increase in the

incidence and a positive trend in the incidence of squamous cell papilloma, squamous cell carcinoma, and squamous cell papilloma or carcinoma (combined) of the forestomach. In female mice, exposure to ethyl acrylate by gavage caused a significant increase in the incidence and a positive trend in the incidence of squamous cell papilloma or carcinoma (combined) of the forestomach. In male mice, exposure to ethyl acrylate by inhalation caused a significant increase in the incidence of follicular cell adenoma of the thyroid. There was no significant increase in the incidence of any tumours in female mice exposed to ethyl acrylate by inhalation.

In male rats, exposure to ethyl acrylate by gavage caused a significant increase in the incidence and a positive trend in the incidence of squamous cell papilloma, squamous cell carcinoma, and squamous cell papilloma or carcinoma (combined) of the forestomach. In female rats, exposure to ethyl acrylate by gavage caused a significant increase in the incidence and a positive trend in the incidence of squamous cell papilloma and squamous cell papilloma or carcinoma (combined) of the forestomach. In the other gavage study in male rats, ethyl acrylate caused a significant increase in the incidence of squamous cell papilloma or carcinoma (combined) of the forestomach. In male rats, exposure to ethyl acrylate by inhalation caused a significant increase in the incidence of follicular cell adenoma or carcinoma (combined) of the thyroid. There was no significant increase in the incidence of any tumours in female rats exposed to ethyl acrylate by inhalation.

5.4 Mechanistic and other relevant data

No data on absorption, distribution, metabolism, or excretion in exposed humans were available. In rats, ethyl acrylate is rapidly absorbed from the gastrointestinal tract and widely distributed. Ethyl acrylate-derived radio-label was retained to a greater extent in the rat forestomach than in other organs 24 hours after exposure by oral gavage. In rats, there are two major metabolic pathways: (i) enzymatic hydrolysis of ethyl acrylate to acrylic acid and ethanol catalysed by carboxylesterases, with a subsequent high-efficiency conversion of both metabolites to CO_2; and (ii) binding of ethyl acrylate and acrylic acid to glutathione and proteins. Ethyl acrylate is excreted primarily as CO_2 in rats exposed orally; approximately 10% is excreted as urinary mercapturates, with 4% excreted in the faeces.

Regarding the key characteristics of carcinogens, ethyl acrylate has demonstrable genotoxicity; positive results without cytotoxicity have been observed in some assays in studies conducted in vivo and in studies conducted in vitro in non-human mammalian cell lines. However, the findings are equivocal because of inconsistencies and lack of reproducibility, meaning that the evidence is not strong. In human cells in vitro, results for micronucleus formation were equivocal across multiple studies, although positive findings were reported below the predefined cytotoxicity cut-off. In rats and mice, ethyl acrylate did not induce DNA strand breaks, and mutations were not induced in *gpt* transgenic mice. In the mouse assay for micronucleus formation, ethyl acrylate gave positive results in the BALB/c strain in one study, positive results in one of two trials in another study of BALB/c mice, and negative results in the C57BL/6 strain. Results were consistently positive in mammalian cells in vitro for several end-points (including strand breaks, mutation, and chromosomal aberrations), in some cases with an increase in the frequency of micronucleus formation without cytotoxicity in a dose-dependent manner. In non-mammalian tests including the Ames assay, results were negative.

There is *strong* evidence that ethyl acrylate alters cell proliferation, cell death, or nutrient supply, based primarily on experimental animal

studies in vivo, with some evidence of cytotoxicity in various human cells in vitro. No data were available in exposed humans. Exposure to ethyl acrylate by oral gavage for 2 years resulted in hyperplasia in the forestomach of Fischer 344/N rats and B6C3F$_1$ mice, but the glandular stomach was not examined. In Fischer 344 rats given a single oral dose, cell proliferation was increased in both the forestomach and glandular stomach but did not persist in the glandular stomach. Hyperplasia was seen in the forestomach, but not in the glandular stomach, in 2-week oral gavage studies. In a 13-week study in Fischer 344 rats, hyperplasia in the forestomach was seen when ethyl acrylate was given by oral gavage or by drinking-water, but was not sustained after cessation of exposure. Reversibility was dependent on duration of treatment; rats exposed for 12 months had sustained hyperplasia in the forestomach.

There is *strong* evidence that ethyl acrylate induces chronic inflammation, based on studies in experimental animals. No data were available in exposed humans. In male and female Fischer 344/N rats and B6C3F$_1$ mice exposed to ethyl acrylate by oral gavage for 2 years, inflammation of the forestomach was induced. Exposure of Fischer 344 rats to ethyl acrylate for 2 weeks by oral gavage, but not by drinking-water, induced inflammation of the forestomach; the incidence of inflammation in the glandular stomach was lower than in the forestomach. In rats, exposure to ethyl acrylate by a single oral dose consistently induced inflammation in the forestomach in two studies. Exposure of *gpt* delta transgenic mice to ethyl acrylate by oral gavage for 28 days resulted in inflammatory cell infiltration in the forestomach.

Several studies reported depletion of glutathione, the principal cellular non-protein thiol, induced by exposure to ethyl acrylate in human cells in vitro and in rodent studies.

In humans, irritant and allergic contact dermatitis has been reported, with similar results in some studies in rodents.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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2-ETHYLHEXYL ACRYLATE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 103-11-7

Deleted Chem. Abstr. Serv. Reg. Nos: 78733-32-1; 84948-57-2; 93460-77-6

Chem. Abstr. Serv. name: 2-propenoic acid; 2-ethylhexyl ester

IUPAC systematic name: acrylic acid; 2-ethylhexyl ester

Synonyms: 2-ethylhexyl 2-propenoate; 2-ethyl hexyl acrylate; 2-ethyl-1-hexyl acrylate; 2-ethylhexanol acrylate; 2-ethylhexyl prop-2-enoate.

1.1.2 Structural and molecular formulae, and relative molecular mass

Molecular formula: $C_{11}H_{20}O_2$
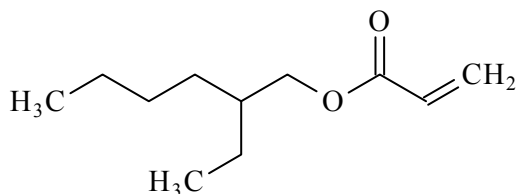
(Royal Society of Chemistry, 2018)
Relative molecular mass: 184.28

1.1.3 Chemical and physical properties

Description: colourless liquid (HSDB, 2018)

Boiling point: 214–218 °C (HSDB, 2018)

Melting point: −90 °C (HSDB, 2018)

Density: specific gravity, 0.880 g/cm³ at 25 °C (HSDB, 2018)

Solubility: slightly soluble in water (< 0.01% by weight, wt%, at 20 °C); soluble in alcohols, ethers, and many organic solvents (acetone, benzene, ethyl ether, heptane, methanol, and carbon tetrachloride) (Union Carbide Corp., 1982)

Vapour pressure: 0.14 mm Hg [19 Pa] at 20 °C

Relative vapour density (air = 1): 6.4 at 20 °C (Hoechst Celanese Corp., 1992)

Flash point: 92 °C (open cup); rapid, uncontrolled polymerization can cause explosion (Tyler, 1993)

Conversion factor: 1 ppm = 7.54 mg/m³ at 1 atm, 25 °C.

1.1.4 Technical products and impurities

2-Ethylhexyl acrylate is available as a commercial product with a purity of 99% or greater. Impurities include: water, 0.05–0.10 wt% maximum; acidity (as acrylic acid), 0.009 wt% maximum; hydroquinone (polymerization inhibitor), 90–120 ppm; and monomethyl ether of hydroquinone (polymerization inhibitor), 13–120 ppm (Union Carbide Corp., 1982;

Hoechst Celanese Corp., 1988; ECHA, 2005; HSDB, 2018).

1.2 Production and use

1.2.1 Production process

Direct, acid-catalysed esterification of acrylic acid with 2-ethylhexanol is the principal method for the manufacture of 2-ethylhexyl acrylate. The most common catalysts are sulfuric and *para*-toluenesulfonic acid, and sulfonic acid functional cation-exchange resins. The monomethyl ether of hydroquinone is added as a polymerization inhibitor, and the esters are used in this form in most industrial applications (ECHA, 2005).

1.2.2 Production volume

2-Ethylhexyl acrylate has been listed as a chemical with a high production volume (OECD, 2009). The estimated production volume of 2-ethylhexyl acrylate in the USA in 1991 was 48 thousand metric tonnes (United States International Trade Commission, 1993). By 1999, the total European Union production volume was estimated to be 70 thousand metric tonnes per year (ECHA, 2005). Accounting for imports and exports, in 1999 a total amount of 90 thousand metric tonnes per year was estimated to be available on the European market, 32 thousand metric tonnes used as internal intermediate, and 58 thousand metric tonnes sold to external processing sites (ECHA, 2005). Production volume in China was 43 thousand metric tonnes in 2008 (Chinese Report, 2008), and doubled to 85 thousand metric tonnes in 2010 (Chinese Report, 2010).

1.2.3 Use

Acrylic esters are used in the production of polymers and copolymers with a wide range of applications. Polymers containing 2-ethylhexyl acrylate are used in different types of food-packaging materials (Tyler, 1993). As a plasticizing co-monomer, 2-ethylhexyl acrylate is used in the production of resins for pressure-sensitive adhesives, latex paints, reactive diluents and/or cross-linking agents, textile and leather finishes, and coatings for paper (HSDB, 2018). 2-Ethylhexyl acrylate can also be used as a co-monomer in solution polymers for industrial metal finishing (Mannsville Chemical Products Corp., 1984; Tyler, 1993). A common use of 2-ethylhexyl acrylate is as a major component in acrylic pressure-sensitive adhesives. The typical composition of an adhesive for general-purpose tape is 75% 2-ethylhexyl acrylate (Temin, 1990).

2-Ethylhexyl acrylate is also used in ultraviolet-curable coatings without solvents, which provide a glossy, abrasion-resistant finish on book covers, for example. A typical ultraviolet-cured formulation might include 10% 2-ethylhexyl acrylate diluent monomer and small amounts of photoinitiator (Mannsville Chemical Products Corp., 1984).

More recent uses of 2-ethylhexyl acrylate include in the manufacture of plastics for transdermal drug delivery systems applied in the fields of estrogen replacement therapy, and in the delivery of anti-inflammatory drugs in eye surgery (Kotiyan & Vavia, 2001; Duarte et al., 2008).

1.3 Analytical methods

Methods for sampling and analysing air have been developed for vapours of acrylate monomers, including 2-ethylhexyl acrylate (Bosserman & Ketcham, 1980; Samimi & Falbo, 1982). The most common method used is United States Occupational Safety and Health Administration PV2026, in which the acrylate monomer vapour is adsorbed on activated silica gel or charcoal, desorbed in carbon disulfide, and analysed by gas chromatography with flame ionization detection (OSHA, 2010). The limit of quantitation is 0.01 ppm (0.08 mg/m³).

No biological markers are reported for exposure to 2-ethylhexyl acrylate.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

2-Ethylhexyl acrylate is readily biodegradable in air, water, and soil (ECHA, 2005). The atmospheric half-life is approximately 19 hours (ECHA, 2005). 2-Ethylhexyl acrylate has moderate mobility in soil (HSDB, 2018). In the effluent of an onsite waste-treatment facility, 2-ethylhexyl acrylate was detected at concentrations ranging from 0.6 to 11 ppb (μg/L) (mean, 4 ppb). The treatment facility received water from a large petrochemical plant where the influent untreated wastewater contained 2-ethylhexyl acrylate at 0.55–5.60 ppm (mg/L) (mean, 2.0 ppm) (Berglund & Whipple, 1987).

1.4.2 Exposure in the general population

2-Ethylhexyl acrylate is not known to occur as a natural product. Exposure in the general population may occur through the use of consumer products (e.g. adhesives, furniture coatings, or paints) or through inadvertent release by industry in the local environment (HSDB, 2018). No quantitative information on exposure was available to the Working Group.

1.4.3 Occupational exposure

Occupational exposure occurs in both the manufacture and use of 2-ethylhexyl acrylate. As a result of its low vapour pressure, exposure by inhalation is expected to be low. Dermal exposure may occur during spills or leaks (Björkner et al., 1980).

The exposure of workers to styrene and several acrylates (including 2-ethylhexyl acrylate) and area concentrations were monitored in a United States facility where acrylic ester-styrene copolymers were produced (Samimi & Falbo, 1982). The personal concentrations of 2-ethylhexyl acrylate at a process reactor (Reactor A) that had an opening hatch for the addition of starting products ranged from not detectable to 2 ppb [20 μg/m³] (mean, 0.4 ppb [3 μg/m³]); nine personal samples taken at a similar reactor contained no detectable concentrations. A further 13 personal samples collected from workers tending a completely closed reactor ranged from not detectable to 5 ppb [40 μg/m³] (mean, 1 ppb [8 μg/m³]). No detectable concentrations were found in six personal samples taken from workers at a closed polymer flake continuous reactor. In 11 personal samples collected at the unloading docks, concentrations ranged from not detectable to 5 ppb [40 μg/m³] (mean, 2 ppb [20 μg/m³]). Eight area samples taken at Reactor A had concentrations ranging from not detectable to 161 ppb [1.21 mg/m³] (mean, 30 ppb [230 μg/m³]); the remaining 41 area samples had no detectable concentrations (Samimi & Falbo, 1982).

Detailed data on the exposure of workers during the manufacture of 2-ethylhexyl acrylate in four plants in the USA were summarized by Tyler (1993). Workers were exposed to mean concentrations ranging from 30 to 500 ppb [0.23–3.77 mg/m³], depending upon manufacturing plant location (Tyler, 1993).

In a study from spring/summer 2016 among 13 road workers from three companies using paint containing 2-ethylhexyl acrylate, exposure to organic solvents and acrylates was measured over a 5-day working period (de Poot, 2016); 8-hour time-weighted average (TWA) concentrations of methyl methacrylate, butyl acrylate, and 2-ethylhexyl acrylate were measured. Although the highest concentrations of methyl methacrylate were measured during manual sputtering, mechanical extruding, and paint spraying, all three measurements of 2-ethylhexyl acrylate were below the limit of detection (0.4 mg/m³). For short-term task-based measurements, the highest concentrations of methyl methacrylate

resulted from filling spraying reservoirs with paint. One task-based measurement was below the limit of detection for 2-ethylhexyl acrylate (7 mg/m^3) (de Poot, 2016).

1.5 Regulations and guidelines

A small number of countries have occupational exposure limits for 2-ethylhexyl acrylate. In Germany, Poland, and Switzerland the 8-hour TWA and short-term occupational exposure limit is 38 mg/m^3, and in Austria it is 82 mg/m^3. In Latvia and the Russian Federation, there is a much lower 8-hour TWA occupational exposure limit of 1 mg/m^3 (IFA, 2018).

The United States Food and Drug Administration has established regulations for the use of monomers, polymers, and copolymers, including 2-ethylhexyl acrylate, in food-contact materials. The quantity of the monomers should not exceed 5 wt% of total polymer units (CFR, 2017).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

2-Ethylhexyl acrylate was reviewed by the Working Group in *IARC Monographs* Volume 60 (IARC, 1994). The Working Group concluded that there is *limited evidence* in experimental animals for the carcinogenicity of 2-ethylhexyl acrylate. This section provides an evaluation of the studies of carcinogenicity in experimental animals reviewed in the previous monograph.

See Table 3.1

3.1 Mouse

3.1.1 Skin application

A group of 40 male C3H/HeJ mice (age, 7–10 weeks) was exposed to a 75% (by volume) solution of 2-ethylhexyl acrylate (purity, 99%) in acetone three times per week for their lifetime (DePass, 1982; DePass et al., 1985). The fur was clipped from the back of each mouse once per week. Treated mice received "one brushful" of the dosing solution per application, a dose of approximately 20 mg per application estimated by weighing the sample bottle before and after dosing each group of 40 mice. Two groups of 40 mice were given acetone only and served as vehicle controls. Survival of the treated group at 18 months was 15/40 (38%) compared with 35/80 (44%) in the combined acetone control groups. All mice exposed to 2-ethylhexyl acrylate were dead 2 years after the start of the experiment. No information on body weights or other clinical observations were reported. A statistically significant increase in the incidence of squamous cell papilloma of the skin (4/40 (10%) vs 0/80 controls [$P = 0.0111$, Fisher exact test]) and of squamous cell papilloma or carcinoma (combined) of the skin (6/40 (15%) vs 0/80 controls [$P = 0.0011$, Fisher exact test]) was observed.

A recent publication by Murphy et al. (2018a) provided no new data on the carcinogenicity of 2-ethylhexyl acrylate, but critically evaluated the study of carcinogenicity in mice exposed dermally to 2-ethylhexyl acrylate by DePass et al. (1985). Murphy et al. (2018a) indicated that the application of contemporary evaluation criteria to the dataset on dermal carcinogenicity from DePass et al. (1985), demonstrates that 2-ethylhexyl acrylate induced skin tumours only at concentrations exceeding the maximum tolerated dose (MTD) and only in the immune-dysregulated C3H/HeJ mouse model. [The Working Group noted that the study by DePass et al. (1985) used the C3H/HeJ mouse and was designed to determine

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, C3H/HeJ (M) 7–10 wk Lifetime DePass et al. (1985)	Skin application 2-Ethylhexyl acrylate, 99% Acetone 0, 0, ~20 mg, 3×/wk 40, 40, 40 7, 5, 0	*Skin* Squamous cell papilloma 0/40, 0/40, 4/40* (10%) Squamous cell carcinoma 0/40, 0/40, 2/40 (5%) Squamous cell papilloma or carcinoma (combined) 0/40, 0/40, 6/40* (15%)	*[$P = 0.0111$ compared with combined control groups, Fisher exact test] [NS] *[$P = 0.0011$ compared with combined control groups; Fisher exact test]	Principal limitations: poor dosing method of using "one brushful" of dosing solution (75% 2-ethylhexyl acrylate in acetone), and calculating approximate dose by weighing the sample bottle before and after dosing each group of 40 mice; use of only one sex and only one dose; data and discussion of pathology findings for the skin only; limited dosing of only 3 d/wk The number of surviving mice given is at 2 yr
Full carcinogenicity Mouse, C3H/HeJ (M) 6 wk Lifetime Wenzel-Hartung et al. (1989)	Skin application 2-Ethylhexyl acrylate, ≥ 99.5% Acetone 0 (untreated), 0 (vehicle control), 2.5, 21, 43 (stop-exposure group; treatment stopped at 24 wk), 86.5% (w/w); 25 µL 3×/wk 80, 80, 80, 80, 80, 80 NR	*Skin* Papilloma 0/80, 0/80, 0/80, 4/80 (5%), 0/80, 8/80* (10%) Cornified squamous cell carcinoma 0/80, 0/80, 0/80, 20/80* (25%), 0/80, 16/80* (20%) Malignant melanoma 0/80, 0/80, 0/80, 7/80* (9%), 0/80, 9/80** (11%) Fibrosarcoma 0/80, 0/80, 0/80, 5/80* (6%), 0/80, 0/80 Haemangioma 0/80, 0/80, 0/80, 0/80, 0/80, 1/80 (1%) Basal cell carcinoma 0/80, 0/80, 0/80, 1/80 (1%), 0/80, 0/80	*[$P < 0.007$, Fisher exact test] *[$P < 0.0001$, Fisher exact test] *[$P = 0.0136$, Fisher exact test]; **[$P = 0.0031$, Fisher exact test] *[$P = 0.03$, one-tail Fisher exact test] [NS] [NS]	Principal limitations: use of only one sex and limited dosing of only 3 d/wk

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, NMRI BR (M) 48–50 d 2 yr Mellert et al. (1994)	Skin application 2-Ethylhexyl acrylate, ≥ 99.7% Acetone 0, 21.5, 43.0, 85.0% (w/w); 25 µL 3×/wk 41, 40, 39, 39 NR	*Skin* Squamous cell papilloma or squamous cell carcinoma 0/41, 0/40, 0/39, 0/39 Keratoacanthoma 0/41, 0/40, 0/39, 0/39		Principal limitations: use of only one sex and limited dosing of only 3 d/wk; data and discussion of pathology findings for the skin only; no detailed information on survival and body weight Number of mice given at start is the effective number of mice; there were ~40 mice/group at the beginning of the experiment
Initiation–promotion (tested as initiator) Mouse, NMRI BR (M) 48–50 d 2 yr Mellert et al. (1994)	Skin application 2-Ethylhexyl acrylate, ≥ 99.7% Acetone 0, 21.5, 43.0, 85.0% (w/w), treated 3×/wk with 25 µL 2-ethylhexyl acrylate for 7 mo, then no treatment for 2 mo, and finally TPA (5 µg in 0.1 mL) 2×/wk for 20 wk 37, 30, 39, 36 NR	*Skin* Squamous cell papilloma 0/37, 1/30, 1/39, 1/36 Squamous cell carcinoma 0/37, 0/30, 0/39, 0/36 Keratoacanthoma 0/37, 0/30, 0/39, 0/36	[NS]	Principal limitations: use of only one sex and limited dosing of only 3 d/wk; data and discussion of pathology findings for the skin only; no detailed information on survival and body weight Number of mice given at start is the effective number of mice; there were ~40 mice/group at the beginning of the experiment

d, day; M, male; mo, month; NR, not reported; NS, not significant; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; wk, week; w/w, weight for weight; yr, year

the carcinogenic potency of 2-ethylhexyl acrylate in rodent skin. Although this study may have used higher concentrations than recommended by current guidelines, it was conducted according to the contemporary standards of that time and in a widely used and accepted strain of mouse for skin application studies. Although the study by DePass et al. (1985) was limited because of the use of only one sex and a single dose, and a limited dosing for only 3 days per week, the Working Group considered it was still performed adequately according to the standards of that time for skin application studies for an evaluation of the carcinogenicity of 2-ethylhexyl acrylate.]

Five groups of 80 male C3H/HeJ mice (age, 6 weeks) were exposed to a 25-μL solution of 2-ethylhexyl acrylate (purity, ≥ 99.5%) in acetone at either 0% (vehicle control), 2.5% (w/w; lowest dose), 21% (intermediate dose), 43% (stop-exposure dose), or 86.5% (highest dose) three times per week for their lifetime (Wenzel-Hartung et al., 1989). The fur was clipped from the back of each mouse once per week. Treatment of the group at 43% was stopped after 24 weeks, and the mice in this group were kept for their lifetime (stop-exposure test) to determine the reversibility or persistency of the lesions. An untreated group of 80 mice served as an additional control group. There was a slight, but statistically significant, increase in body weight in all four groups of exposed mice compared with controls. Survival was similar between exposed and control mice. Scaling and scabbing were observed in all exposed groups and persisted throughout the treatment period. Regression of these skin lesions was observed within 7 weeks after stopping treatment in the stop-exposure group. Exposure to 2-ethylhexyl acrylate for life caused a statistically significant increase in the incidence of papilloma of the skin in the group exposed at the highest dose; incidences for the untreated and vehicle controls, and groups exposed at 2.5%, 21%, and 86.5%, were 0/80, 0/80, 0/80, 4/80, and 8/80 ($P < 0.007$, Fisher exact test), respectively. A

statistically significant increase in the incidence of cornified squamous cell carcinoma of the skin (0/80, 0/80, 0/80, 20/80; $P < 0.0001$, Fisher exact test), and 16/80 ($P < 0.0001$, Fisher exact test)) and of malignant melanoma (0/80, 0/80, 0/80, 7/80; $P = 0.0136$, Fisher exact test), and 9/80 ($P = 0.0031$, Fisher exact test)) was observed for groups exposed at the intermediate and highest doses. Five mice developed fibrosarcoma of the skin [significantly increased; $P = 0.03$, one-tail Fisher exact test] and one mouse developed a basal cell carcinoma of the skin in the group exposed at the intermediate dose, and one haemangioma of the skin was observed in the group exposed at the highest dose. No skin tumours were reported in the control (untreated or vehicle) groups, the group exposed at the lowest dose, or the stop-exposure group.

A recent publication by Murphy et al. (2018a) provided no new data on the carcinogenicity of 2-ethylhexyl acrylate, but critically evaluated the study of the carcinogenicity in mice exposed dermally to 2-ethylhexyl acrylate by Wenzel-Hartung et al. (1989). Murphy et al. (2018a) indicated that the application of contemporary evaluation criteria to the dataset on dermal carcinogenicity from Wenzel-Hartung et al. (1989), demonstrates that 2-ethylhexyl acrylate induced skin tumours only at concentrations exceeding the MTD and only in the immune-dysregulated C3H/HeJ mouse model. [The Working Group noted that the study by Wenzel-Hartung et al. (1989) was conducted in the C3H/HeJ mouse and was designed to determine the carcinogenic potency of 2-ethylhexyl acrylate in rodent skin. Although this study may have used higher concentrations than recommended by current guidelines, it was conducted according to the contemporary standards of that time and in a widely used and accepted strain of mouse for skin application studies. Although the study by Wenzel-Hartung et al. (1989) was limited because of the use of only one sex and limited dosing for only

3 days per week, the Working Group considered it was still performed adequately according to the standards of that time for skin application studies for an evaluation of the carcinogenicity of 2-ethylhexyl acrylate. Indeed, there exists a relationship between wound healing and cancer that has long been recognized in the literature. Chronic inflammation has been associated with malignant transformation in numerous tissues, and the biological mechanisms that regulate wound healing have been shown to promote transformation and growth of malignant cells. The Tlr4 mouse model (C3H/HeJ) reviewed by Murphy et al. (2018a) spontaneously develops tumours of the liver in males and tumours of the mammary glands in females, and not tumours of the skin. 2-Ethylhexyl acrylate induced tumours of the skin only at concentrations exceeding the MTD and in the immune-dysregulated C3H/HeJ mouse model. However, melanoma and fibrosarcoma of the skin, as well as cornified squamous cell carcinoma of the skin, are not characteristic of the immune-dysregulated C3H/HeJ mouse model in the scientific literature.]

Four groups of approximately 40 male NMRI BR mice (age, 48–50 days) were exposed to a 25-µL solution of 2-ethylhexyl acrylate (purity, ≥ 99.7%) in acetone at either 0 (vehicle control), 21.5% (w/w; lowest dose), 43.0% (intermediate dose), or 85.0% (highest dose) on their clipped dorsal skin three times per week for 2 years (Mellert et al., 1994). Body weights and survival were similar between exposed and control animals. No squamous cell papillomas, squamous cell carcinomas, or keratoacanthomas of the skin were reported in the groups exposed to 2-ethylhexyl acrylate or in the vehicle controls. A positive control group of mice exposed to benzo[a]pyrene developed squamous cell carcinomas of the skin. [The Working Group noted that the study was limited by the use of only one sex, the limited dosing of only 3 days per week, the provision of data and discussion of histopathology for the skin only, and the lack

of detailed information on survival and body weight.]

3.1.2 Initiation–promotion

Four groups of approximately 40 male NMRI BR mice (age, 48–50 days) were exposed to a 25-µL solution of 2-ethylhexyl acrylate (purity, ≥ 99.7%) in acetone at either 0% (vehicle control), 21.5% (lower dose), 43.0% (intermediate dose), or 85.0% (higher dose) on their clipped dorsal skin three times per week for 7 months (Mellert et al., 1994). Exposure to 2-ethylhexyl acrylate was discontinued at 7 months, and after 2 months mice were exposed to a solution of 12-O-tetradecanoylphorbol-13-acetate (TPA) in 0.1 mL acetone, at a dose of 5 µg per mouse twice per week for 20 weeks, and observed for up to an additional 10 months. Body weights and survival were similar between exposed and control animals. One squamous cell papilloma of the skin was seen at the application site in the groups exposed to 2-ethylhexyl acrylate (lower, intermediate, and higher doses) plus TPA; no squamous cell carcinomas or keratoacanthomas of the skin were reported in these groups. No tumours of the skin were observed in the acetone plus TPA control group. A positive control group of mice exposed to benzo[a]pyrene plus TPA developed squamous cell carcinomas or keratoacanthomas of the skin. [The Working Group noted that the study was limited by the use of only one sex, the limited dosing of only 3 days per week, the provision of data and discussion of histopathology for the skin only, and the lack of detailed information on survival and body weight.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Data on absorption, distribution, metabolism, and excretion of 2-ethylhexyl acrylate in humans were not available to the Working Group.

4.1.2 Experimental systems

2-Ethylhexyl acrylate has been shown to be readily absorbed in rats exposed via intravenous and intraperitoneal injection (Sapota, 1988); after exposure, radiolabelled 2-ethylhexyl acrylate was distributed to all major tissues in rats. One hour after exposure, the tissues with the highest percentages of 2-ethylhexyl acrylate radioactivity were kidney and liver; smaller amounts were found in brain, thymus, spleen, and blood (Gut et al., 1988; Sapota, 1988).

After the exposure of rats to 2-ethylhexyl acrylate by intraperitoneal injection, the major route of excretion was through expiration (as CO_2; > 75% within 24 hours); excretion in urine and faeces was only observed in smaller quantities (Sapota, 1988). However, after oral exposure, both expiration (50% within 24 hours) and urine (38% within 24 hours) were major routes for the elimination of radiolabel (Sapota, 1988). The total radiolabel excreted within 72 hours of the exposure of rats to radiolabelled 2-ethylhexyl acrylate, either orally or via intraperitoneal injection, was approximately 90% and 93% of the administered dose, respectively (Sapota, 1988). In another study in rats, less than 0.01% of the administered dose was excreted in the faeces. In urine, 13.5% of an intravenous dose and 7.2% of an intraperitoneal dose were excreted within 24 hours. For both routes of administration, more than 50% of the administered dose was expired, mostly as carbon dioxide (Gut et al., 1988).

2-Ethylhexyl acrylate is believed to undergo carboxylesterase-catalysed metabolism (Kopecký et al., 1985; see Fig. 4.1). After the exposure of rats to 2-ethylhexyl acrylate by intraperitoneal injection, thioether excretion in the urine was observed (Gut et al., 1988). In rats exposed by inhalation, there was a dose-related increase in the amount of excreted urinary thioethers. In addition, a decrease in the number of non-protein glutathione groups was also observed in the blood and liver of these rats (Vodička et al., 1990). In the same study, 2-ethylhexyl acrylate also showed reactivity with glutathione, with a half-life of 36.4 minutes (Vodička et al., 1990).

Two mercapturic acid metabolites have been identified in rat urine: N-acetyl-(2-carboxyethyl)cysteine and N-acetyl-2-(2-ethyl-hexyloxycarbonyl)ethylcysteine (Kopecký et al., 1985). Two unidentified metabolites were detected in the bile of rats (Cikrt et al., 1986).

4.2 Mechanisms of carcinogenesis

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016). Data were available only for the key characteristic "is genotoxic".

4.2.1 Genetic and related effects

(a) Humans

See Table 4.1

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

In human lymphocytes, 2-ethylhexyl acrylate did not increase the number of micronucleated cells after 4 hours of exposure followed by 16 hours of recovery in the absence and presence of S9, or after 20 hours of continuous exposure in the absence of S9. A statistically significant increase in the number of micronucleated cells compared with corresponding control values

Fig. 4.1 Proposed metabolic pathways for 2-ethylhexyl acrylate
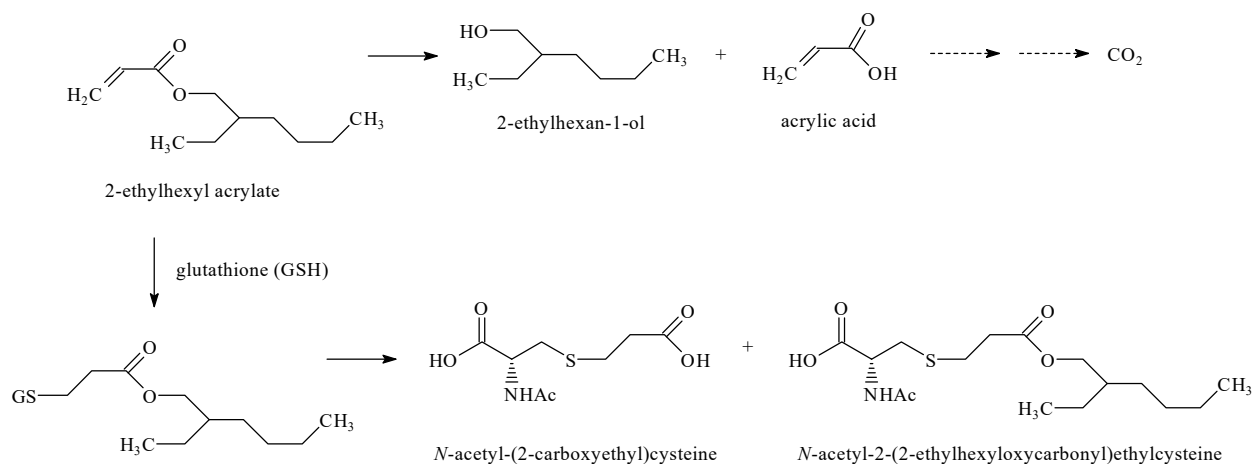
The two cysteine conjugates have been identified in rat urine. The *N*-acetyl-(2-carboxyethyl)cysteine conjugate may also stem from glutathione addition to acrylic acid
Compiled by the Working Group

was observed in the 4-hour exposure experiment in the absence of S9; however, the numbers were within the range of the 95% limit of the historical control data (Murphy et al., 2018b).

(b) Experimental systems

See Table 4.2

(i) Non-human mammalian cells in vitro

2-Ethylhexyl acrylate yielded equivocal results at the thymidine kinase *(Tk)* locus of mouse lymphoma cells without metabolic activation. The mutant frequency was increased at some test doses; however, the mutant frequency was not increased at higher concentrations and was not consistent across trials. In addition, cell survival was lower than 50% (Dearfield et al., 1989). After exposure to 2-ethylhexyl acrylate, no mutagenic effect was reported in the hypoxanthine-guanine phosphoribosyl transferase *(Hprt)* assay in Chinese hamster ovary cells without metabolic activation, and in Chinese

hamster V79 cells in the absence or presence of S9 (Moore et al., 1991, Murphy et al., 2018b).

Equivocal results were reported for the induction of chromosomal aberrations in L5178Y mouse lymphoma cells after exposure to 2-ethylhexyl acrylate; there was no clear dose–response relationship and cell survival was less than 50%. In the same cell line, 2-ethylhexyl acrylate did not increase the number of micronucleated cells (Dearfield et al., 1989).

(ii) Non-mammalian experimental systems

In *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537, 2-ethylhexyl acrylate was not mutagenic in the assay for reverse mutation in the presence or absence of metabolic activation (Zeiger et al., 1985).

Table 4.1 Genetic and related effects of 2-ethylhexyl acrylate in human cells in vitro

End-point	Tissue, cell line	Results[a]		Concentration (μg/mL) (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Micronucleus formation	Lymphocytes	–	NT	44.9	4 h exposure followed by 16 h recovery	Murphy et al. (2018b)
Micronucleus formation	Lymphocytes	NT	–	286	4 h exposure followed by 16 h recovery	Murphy et al. (2018b)
Micronucleus formation	Lymphocytes	–	NT	71.4	20 h continuous exposure	Murphy et al. (2018b)

h, hour; HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested

[a] –, negative; the level of significance was set at $P < 0.05$ in all cases

Table 4.2 Genetic and related effects of 2-ethylhexyl acrylate in experimental systems

End-point	Species, cell line	Results[a]		Concentration (μg/mL) (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
Mutation, *Tk*	Mouse L5178Y lymphoma	+/–	NT	37	Dearfield et al. (1989)
Mutation, *Hprt*	Chinese hamster ovary	–	NT	26	Moore et al. (1991)
Mutation, *Hprt*	Chinese hamster ovary	–	NT	80	Moore et al. (1991)
Mutation, *Hprt*	Chinese hamster V79	NT	–	230.4	Murphy et al. (2018b)
Mutation, *Hprt*	Chinese hamster V79	–	NT	115.2	Murphy et al. (2018b)
Chromosomal aberration	Mouse L5178Y lymphoma	+/–	NT	34	Dearfield et al. (1989)
Micronucleus formation	Mouse L5178Y lymphoma	–	NT	34	Dearfield et al. (1989)
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537	–	–	10 000 μg/plate	Zeiger et al. (1985)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested

[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

4.3 Other adverse effects

4.3.1 Irritancy and sensitization

(a) Humans

In Finland, 5 cases (all women) of occupational contact urticaria and protein contact dermatitis caused by 2-ethylhexyl acrylate were reported for the period 1990 to 1994 (Kanerva et al., 1996).

(b) Experimental systems

2-Ethylhexyl acrylate showed low potency for skin irritation in a primary irritation test in rabbits. In addition, 2-ethylhexyl acrylate showed low potency for cytotoxicity in a cultured dermis model (Tokumura et al., 2010). In male C3H/HeJ mice, dermal exposure to 2-ethylhexyl acrylate three times per week for their lifetime caused skin irritation such as scaling, scabbing, hyperkeratosis, and hyperplasia at all concentrations. In a similar study of dermal exposure to 2-ethylhexyl acrylate for 24 weeks, skin irritation was observed in all treatment groups; however, the skin damage was reversible for the two lowest doses (Wenzel-Hartung et al., 1989). The results of a 2-year study of dermal exposure to 2-ethylhexyl acrylate provide further evidence that 2-ethylhexyl acrylate is a skin irritant (Mellert et al., 1994).

2-Ethylhexyl acrylate was demonstrated to be a sensitizer in rodents (Waegemaekers & van der Walle, 1983; Dearman et al., 2007).

4.4 Data relevant to comparisons across agents and end-points

See the monograph on isobutyl nitrite in the present volume.

5. Summary of Data Reported

5.1 Exposure data

2-Ethylhexyl acrylate is a high production volume chemical that is produced worldwide. It is used as a plasticizing co-monomer in the production of resins for pressure-sensitive adhesives, latex paints, reactive diluents and/or cross-linking agents, textile and leather finishes, and coatings for paper. It is moderately volatile and has moderate mobility in soil. It is unlikely to persist in the environment. No quantitative data on exposure of the general population were identified. Workers involved in the manufacture of 2-ethylhexyl acrylate had personal concentrations well below the occupational exposure limit. Recent exposure measurements of road workers using paint containing 2-ethylhexyl acrylate were below the limit of detection.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

2-Ethylhexyl acrylate was tested for carcinogenicity in three skin application studies in male mice.

In two studies in C3H/HeJ mice, 2-ethylhexyl acrylate caused a significant increase in the incidence of squamous cell papilloma and of squamous cell papilloma or carcinoma (combined) of the skin in one study, and a significant increase in the incidence of papilloma, cornified squamous cell carcinoma, malignant melanoma, and of fibrosarcoma of the skin in the second study. In the third study, which used a different strain of mice, 2-ethylhexyl acrylate did not significantly increase the incidence of tumours of the skin either with or without subsequent application of 12-*O*-tetradecanoylphorbol-13-acetate.

5.4 Mechanistic and other relevant data

No data on the absorption, distribution, metabolism, or excretion of 2-ethylhexyl acrylate in exposed humans were available. In rats, 2-ethylhexyl acrylate is readily absorbed, distributed to all major tissues, and mainly excreted as carbon dioxide in expired air and as mercapturic acid conjugates in the urine. 2-Ethylhexyl acrylate undergoes carboxylesterase-catalysed metabolism and conjugation with glutathione.

With respect to the key characteristics of human carcinogens, there is *weak* evidence that 2-ethylhexyl acrylate is genotoxic. No data were available in exposed humans or in non-human mammals in vivo. In human cells in vitro, 2-ethylhexyl acrylate gave negative results for micronucleus formation. In a small number of studies in rodent cells in vitro, equivocal or negative results were reported for the induction of mutations, micronucleus formation, and chromosomal aberrations. Further, 2-ethylhexyl acrylate gave negative results in the Ames test, both with and without metabolic activation.

Irritant and allergic contact dermatitis have been reported in humans, with similar results in some studies in rodents.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

2-Ethylhexyl acrylate is *possibly carcinogenic to humans (Group 2B)*.

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TRIMETHYLOLPROPANE TRIACRYLATE

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 15625-89-5

Chem. Abstr. Serv. name: trimethylolpropane triacrylate

IUPAC name: 2,2-bis(prop-2-enoyloxymethyl)butyl prop-2-enoate (NIH, 2018)

Synonyms: TMPTA; 1,1,1-trimethylolpropane triacrylate; 2,2-bis[(acryloyloxy)methyl]butyl prop-2-enoate; 2-propenoic acid; 2,2-bis[[(1-oxo-2-propen-1-yl)oxy]methyl] butyl ester; acrylic acid; triester with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol

1.1.2 Structural and molecular formula, and relative molecular mass

Molecular formula: $C_{15}H_{20}O_6$
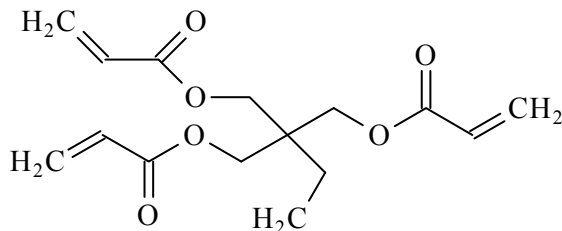
Relative molecular mass: 296.3

1.1.3 Chemical and physical properties

Description: viscous, colourless to tan liquid (NTP, 2012)

Boiling point: higher than 200 °C at 1 mm Hg (NTP, 2012)

Vapour pressure: 5.9×10^4 mm Hg at 25 °C (HSDB, 2018)

Density: 1.11 g/cm³ at 20 °C (HSDB, 2018)

Solubility: insoluble in water (NTP, 2012)

Stability: hygroscopic, light sensitive, and incompatible with strong oxidizing agents, acids, and bases; may undergo spontaneous polymerization when exposed to direct sunlight and heat, but may be stabilized with the monoethyl ester of hydroquinone (NTP, 2012)

Conversion factor: 1 ppm = 12.12 mg/m³ at 1 atm, 25 °C

1.1.4 Technical products and impurities

Technical-grade trimethylolpropane triacrylate has a purity of more than 70%, and the major impurities are acrylic acid, trimethylolpropane diacrylate, trimethylolpropane-triacrylate–trimethylolpropane-monoacrylate adduct, trimeth-ylolpropane-triacrylate–trimethylolpropane-diacrylate adduct, and water (NTP, 2012). It also contains less than 1% hydroquinone or monomethyl ether hydroquinone as polymerization inhibitor (Merck index website). [The

Working Group noted that studies with the agent with analytical-grade purity (> 90%) were not available.]

1.2 Production and use

1.2.1 Production process

Trimethylolpropane triacrylate is manufactured by esterification of trimethylolpropane (NTP, 2012).

1.2.2 Production volume

Trimethylolpropane triacrylate is a chemical with a high production volume (OECD, 2009). From 1986 to 2006, the United States Environmental Protection Agency (EPA) reported an annual national production volume of 10–50 million pounds [4500–23 000 metric tonnes] of trimethylolpropane triacrylate (HSDB, 2018). Recent production in Europe has been reported in the range of 10–100 thousand metric tonnes per year (ECHA, 2018). Production volumes in China were 3700, 4100, 8800, and 9300 metric tonnes per year for the years 2001, 2002, 2003, and 2004, respectively (Chinese Report, 2005).

1.2.3 Use

The major use of trimethylolpropane triacrylate is as a cross-linking agent in a wide range of industrial applications in adhesives and sealant chemicals, ultraviolet (UV)-curable inks, photosensitive chemicals, paint additives, coating additives, intermediates, and solvents (HSDB, 2018). Trimethylolpropane triacrylate is also used in paper and wood impregnates, wire and cable extrusion, polymer-impregnated concrete, and polymer concrete structural composites (NTP, 2012).

1.3 Analytical methods

The United States Occupational Safety and Health Administration (OSHA) has a sampling and analytical guide for trimethylolpropane triacrylate (unvalidated). Personal breathing zone air sampling is performed using XAD-7 sorbent sampling tubes, followed by solvent desorption with methanol, and analysis by high-performance liquid chromatography (HPLC) with UV spectrophotometric detection (OSHA, 2018).

A gas chromatography with mass spectrometry (MS) method has been described for the analysis of migration of trimethylolpropane triacrylate from UV ink systems (Papilloud & Baudraz, 2002). The limit of detection of this system was not reported. No methods for detection in biological media were available to the Working Group.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

Trimethylolpropane triacrylate does not occur naturally in the environment (HSDB, 2018). It readily degrades in the atmosphere by reacting with photochemically produced hydroxyl radicals; the half-life has been estimated as 11 hours. Total degradation of trimethylolpropane triacrylate in soil and water was 87% over a 4-week period with the formation of the diacrylate and monoacrylate esters plus trimethylolpropane (HSDB, 2018).

1.4.2 Exposure of the general population

Exposure in the general population may occur through dermal exposure when using products containing trimethylolpropane triacrylate, such as latex paints, and furniture and floor polishes (Voog & Jansson, 1992). No quantitative information on exposure was available to the Working Group.

1.4.3 Occupational exposure

Occupational exposure may occur through inhalation or dermal exposure in facilities manufacturing trimethylolpropane triacrylate or in industries using trimethylolpropane triacrylate. Occupational exposure to this compound has been reported primarily in printing plants, in the use of UV-curing inks, and in the adhesives and allied industries since the late 1970s. In the press area of a plastic tube department where UV-cured inks were used, air measurements of trimethylolpropane triacrylate were below the limit of detection (< 9 ppb [< 109 µg/m^3]) (NIOSH, 1994).

Studies of trimethylolpropane triacrylate have mainly investigated dermatitis and involved skin patch testing of workers or patients (Björkner et al., 1980; Dahlquist et al., 1983; Garabrant, 1985; Kanerva et al., 1998; Goon et al., 2002). Four cases of dermatitis were reported from a floor-manufacturing facility that used a varnish with an aziridine-based hardener containing 3–5% trimethylolpropane triacrylate; all four workers reacted to trimethylolpropane triacrylate in skin patch testing (Dahlquist et al., 1983). In a plant that manufactured plastic food containers, a printing process used seven acrylate oligomers, including trimethylolpropane triacrylate. One positive result of epicutaneous patch testing for trimethylolpropane triacrylate was reported among seven workers tested (Nethercott et al., 1983).

1.5   Regulations and guidelines

The American Industrial Hygiene Association derived a workplace environmental exposure level in the form of an 8-hour time-weighted average of 1 mg/m^3 for trimethylolpropane triacrylate. This limit comes with a skin notation (AIHA, 2011).

2.   Cancer in Humans

No data were available to the Working Group.

3.   Cancer in Experimental Animals

Studies of carcinogenicity in mice and rats exposed to trimethylolpropane triacrylate were limited to skin application studies conducted by the United States National Toxicology Program (NTP, 2005, 2012) and reported by Andrews & Clary (1986). Results of these studies are summarized in Table 3.1 (see also Doi et al., 2005; Surh et al., 2014).

3.1   Mouse

3.1.1 Skin application

(a)   B6C3F1/N and C3H/HeJ mice

In a study on 10 related acrylates and methacrylates (Andrews & Clary, 1986), 50 male C3H/HeJ mice [age, not reported] were exposed by skin application to trimethylolpropane triacrylate [purity, not reported] at a dose of 2.5 mg (~100 mg/kg body weight, bw, based on the assumption of a body weight of 25 g), twice per week for 80 weeks, at which point the experiment was terminated. Two groups of 50 mice each were used as negative controls; one group received no treatment and the other group was exposed to mineral oil only [whether this was a vehicle control was not stated]. The skin and body [peritoneal and thoracic] cavities were examined at necropsy and tissues were collected for histopathological examination [the specific tissues that were examined were not reported]. There were no skin tumours or systemic effects reported in treated animals. However, there were acanthoses and fibroses of the skin. [These were presumably at the site of application, although this was not stated. The specific incidence of

Table 3.1 Studies of carcinogenicity with technical-grade trimethylolpropane triacrylate in experimental animals

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) 5–6 wk 105–106 wk NTP (2012)	Skin application TMPT, > 78% Acetone 0, 0.3, 1.0, 3.0 mg/kg bw, 1×/d, 5 d/wk 50, 50, 50, 50 30, 35, 29, 38	Any tumour type: no significant increase		Principal strengths: well-conducted GLP study
Full carcinogenicity Mouse, B6C3F$_1$/N (F) 5–6 wk 105–106 wk NTP (2012)	Skin application TMPT, > 78% Acetone 0, 0.3, 1.0, 3.0 mg/kg bw, 1×/d, 5 d/wk 50, 50, 50, 50 39, 31, 30, 30	*Liver* Hepatoblastoma (includes multiple) 0/50, 4/50, 0/50, 3/50 Hepatoblastoma (multiple) 0/50, 1/50, 0/50, 3/50 Hepatocholangiocarcinoma 0/50, 0/50, 1/50, 2/50 Hepatocellular carcinoma 12/50*, 13/50, 10/50, 19/50 *Uterus* Stromal polyp or stromal sarcoma (combined) 0/50*, 1/50, 2/50, 6/50** Stromal polyp 0/50*, 1/50, 2/50, 5/50** Stromal sarcoma 0/50, 0/50, 0/50, 1/50	NS NS NS *P = 0.045 (trend), poly-3 test *P = 0.002 (trend), poly-3 test; **P = 0.014, poly-3 test *P = 0.008 (trend), poly-3 test; **P = 0.027, poly-3 test NS	Principal strengths: well-conducted GLP study See comment on purity in NTP (2012) male mouse experiment Hepatoblastoma and hepatocholangio-carcinoma are considered rare tumours in B6C3F$_1$/N female mice, with low historical control incidence Historical incidence for dermal studies (mean ± SD; range): hepatoblastoma, 2-yr, vehicle controls (all vehicles): 2/250 (0.8 ± 1.1%; 0–2%); all routes, 4/1195 (0.3 ± 0.8%; 0–2%); hepatocholangiocarcinoma: 0/250; all routes, 0/1195; hepatocellular carcinoma: 63/250 (25.2 ± 15.5%; 6–46%); all routes, 144/1195 (12.1 ± 10.8%; 0–46%); stromal polyp, vehicle controls (all vehicles): 5/250 (2.0 ± 2.5%; 0–6%); all routes, 24/1198 (2.0 ± 2.2%; 0–8%); stromal sarcoma: 0/250; all routes, 2/1198 (0.2 ± 0.6%; 0–2%); stromal polyp or stromal sarcoma (combined): 5/250 (2.0 ± 2.5%; 0–6%); all routes, 26/1198 (2.2 ± 2.2%; 0–8%)

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
Carcinogenicity with other modifying factor Mouse (transgenic), FVB/N-TgN (v-Ha-*ras*) (i.e. Tg.AC) hemizygous (M) 6 wk 28 wk NTP (2005)	Skin application TMPT, 80% Acetone 0, 0.75, 1.5, 3, 6, 12 mg/kg bw, 5×/wk 15, 15, 15, 15, 15, 15 14, 15, 12, 14, 13, 11	*Skin*: squamous cell papilloma 0/15*, 0/15, 0/15, 2/15, 12/15**, 13/15**	*P < 0.001 (trend), poly-3 test; **P < 0.001, poly-3 test	Principal strengths: well-conducted GLP study Purity: HPLC indicated a major peak and five impurities with a combined area of 22.2%. HPLC/MS indicated ten impurities including the five impurities found by HPLC, including four structurally related acrylates or adducts: trimethylolpropane diacrylate, trimethylolpropane-triacrylate–acrylic-acid adduct, trimethylolpropane-triacrylate–trimethylolpropane-monoacrylate adduct, and trimethylolpropane-triacrylate–trimethylolpropane-diacrylate adduct
Carcinogenicity with other modifying factor Mouse (transgenic), FVB/N-TgN (v-Ha-*ras*) (i.e. Tg.AC) hemizygous (F) 6 wk 28 wk NTP (2005)	Skin application TMPT, 80% Acetone 0, 0.75, 1.5, 3, 6, 12 mg/kg bw, 5×/wk 15, 15, 15, 15, 15, 15 15, 14, 12, 14, 14, 12	*Skin*: squamous cell papilloma 0/15*, 0/15, 0/15, 1/15, 11/15**, 15/15** Squamous cell carcinoma 0/15, 0/15, 1/15, 0/15, 1/15, 1/15 *Forestomach* Squamous cell papilloma 4/15*, 5/15, 4/15, 2/15, 5/15, 9/15** Squamous cell papilloma (multiple) 1/15, 1/15, 1/15, 1/15, 1/15, 3/15	*P < 0.001 (trend), poly-3 test; **P < 0.001, poly-3 test NS *P = 0.014 (trend), poly-3 test; **P = 0.040, poly-3 test NS	Principal strengths: well-conducted GLP study See comment on purity in NTP (2005) male Tg.AC mouse experiment

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) 6 wk 104–105 wk NTP (2012)	Skin application TMPT, > 78% Acetone 0, 0.3, 1.0, 3.0 mg/kg bw, 1×/d, 5 d/wk 50, 50, 50, 50 23, 18, 28, 23	*Tunica vaginalis*: malignant mesothelioma 0/50*, 2/50, 2/50, 5/50**	*P = 0.024 (trend), poly-3 test; **P = 0.031, poly-3 test	Principal strengths: well-conducted GLP study See comment on purity in NTP (2012) male mouse experiment Historical incidence of malignant mesothelioma for 2-yr dermal study vehicle controls (all vehicles) (mean ± SD; range): 8/250 (3.2 ± 3.4%; 0–8%); all routes, 40/1249 (3.2 ± 2.8%; 0–8%)
Full carcinogenicity Rat, F344/N (F) 6 wk 104–105 wk NTP (2012)	Skin application TMPT, > 78% Acetone 0, 0.3, 1.0, 3.0 mg/kg bw, 1×/d, 5 d/wk 50, 50, 50, 50 27, 31, 24, 32	Any tumour type: no significant increase		Principal strengths: well-conducted GLP study See comment on purity in NTP (2012) male mouse experiment

bw, body weight; d, day; F, female; GLP, good laboratory practice; HPLC, high-performance liquid chromatography; M, male; MS, mass spectrometry; NS, not significant; SD, standard deviation; TMPT, trimethylolpropane triacrylate; UV, ultraviolet; wk, week; yr, year

these lesions was not provided either, though the authors stated these were "frequently present".] One mouse from each control group developed a papilloma of the skin. [The Working Group considered that this study was inadequate for evaluation as it was poorly described and provided no information regarding test article purity, vehicle used, site of application, method of application, or the specific incidence of non-neoplastic lesions. Survival and body-weight data were not provided. Additionally, only one dose of trimethylolpropane triacrylate and one sex were used in the study.]

Groups of 50 male and 50 female B6C3F$_1$/N mice (age, 5–6 weeks) were exposed to technical-grade trimethylolpropane triacrylate (purity, > 78%) in acetone by skin application at a dose of 0 (control), 0.3, 1.0, or 3.0 mg/kg bw once per day, 5 days per week for 105–106 weeks. HPLC with UV detection analysis of the test agent indicated one major peak (78.2%) and four impurities, each greater than 0.1% of the total peak area (7.1, 2.3, 10.8, and 1.5%). HPLC with MS analysis tentatively identified three of the four impurities as structurally related compounds: trimethylolpropane diacrylate (7.1%), trimethylolpropane-triacrylate–trimethylolpropane-monoacrylate adduct (2.3%), and trimethylolpropane-triacrylate–trimethylolpropane-diacrylate adduct (10.8%). The impurity present at 1.5% of the total peak area was not specifically identified; however, the fragment ions were consistent with those of a trimethylolpropane triacrylate adduct. The dose levels were selected to avoid significant skin irritation (based on the severity of skin lesions in a 3-month study) and to preclude adverse effects on survival and growth of the mice, and were applied to the interscapular region of the back after clipping the hair (NTP, 2012). There were slight decreases in survival in the exposed groups of females, but the decreases were not statistically significant. In males, survival in the groups exposed at 0.3 or 3.0 mg/kg bw was slightly higher than in controls, but these increases were not statistically significant. Body weights in the exposed groups did not differ significantly from those of controls. In females, there were treatment-related increases in the incidence of hepatoblastoma and hepatocholangiocarcinoma of the liver, and of stromal polyp or stromal sarcoma of the uterus. The incidence of hepatoblastoma was 0/50, 4/50 (8%), 0/50, and 3/50 (6%) in the groups exposed at 0, 0.3, 1.0, or 3.0 mg/kg bw, respectively; the incidence in the groups exposed at the lowest and highest doses was above the upper bound of the range (0–2%) for historical controls for this tumour in female mice (historical control incidence: dermal study, 2/250; all routes, 4/1195). The respective incidence of hepatocholangiocarcinoma was 0/50, 0/50, 1/50 (2%), and 2/50 (4%); hepatocholangiocarcinoma was not observed in 250 (skin application studies) or 1195 (all routes of exposure) historical controls in female mice. [The Working Group considered hepatoblastoma and hepatocholangiocarcinoma as rare neoplasms in female mice, and considered the increased incidence to be biologically significant.] The incidence of stromal polyp or stromal sarcoma (combined) of the uterus was significantly increased (0/50 (*P* for trend, 0.002), 1/50 (2%), 2/50 (4%), and 6/50 (12%, *P* = 0.014)) in all exposed groups; one female exposed at the highest dose developed a stromal sarcoma of the uterus. There was also a small but significant (*P* = 0.045) positive trend in the incidence of hepatocellular carcinoma (12/50, 13/50, 10/50, and 19/50 (38%)) in females. There were no treatment-related increases in neoplasms of the skin in females. There were no treatment-related neoplasms in males. In males and females, there were significant increases in the incidence of epidermal hyperplasia, melanocyte hyperplasia, and chronic inflammation of the skin at the site of application (NTP, 2012). [The Working Group noted this was a well-conducted study that complied with good laboratory practice (GLP).]

(b) Transgenic mouse

Groups of 15 male and 15 female FVB/N-TgN (v-Ha-*ras*) (i.e. Tg.AC) hemizygous mice were exposed to technical-grade trimethylolpropane triacrylate (purity, ~80%) in acetone by skin application at a dose of 0 (control), 0.75, 1.5, 3, 6, or 12 mg/kg bw once per day, 5 days per week for 28 weeks (NTP, 2005). The purity of the test agent (see Table 3.1 for details) was similar to that used in the 2-year studies in B6C3F$_1$/N mice and Fischer 344/N rats conducted by NTP (2012). The doses were applied to the interscapular region of the back after clipping the hair. In males and females, there were slight decreases in survival in all except one exposed group (all males exposed at 0.75 mg/kg bw survived), but the decreases were not statistically significant. Body weights in the treated groups did not differ significantly from those of controls. In males, there was a treatment-related increase in the incidence of squamous cell papilloma of the skin (0/15, 0/15, 0/15, 2/15, 12/15, and 13/15 in groups exposed at 0, 0.75, 1.5, 3, 6, or 12 mg/kg bw, respectively, including mice with multiple papillomas of the skin in the groups exposed at 6 and 12 mg/kg bw) at the site of application. The positive trend and the increase in the incidence in the groups exposed at 6 and 12 mg/kg bw (compared with concurrent controls) were statistically significant ($P < 0.001$). In females, there was a treatment-related increase in the incidence of squamous cell papilloma of the skin at the site of application (0/15, 0/15, 0/15, 1/15, 11/15, and 15/15, including mice with multiple papillomas of the skin in the groups exposed at 6 and 12 mg/kg bw); this increased incidence in the females exposed at 6 and 12 mg/kg bw, and the positive trend, were statistically significant ($P < 0.001$). Squamous cell carcinomas of the skin (0/15, 0/15, 1/15, 0/15, 1/15, and 1/15) were also observed in some exposed groups. In females, there was also a statistically significant increase in the incidence of squamous cell papilloma of the forestomach (4/15, 5/15, 4/15, 2/15, 5/15, and 9/15) in the group exposed at 12 mg/kg bw ($P = 0.040$), with a significant positive trend ($P = 0.014$). Three females in the group exposed at 12 mg/kg bw and one female in each of the other groups (including controls) had multiple squamous cell papillomas of the forestomach. In male and female mice, there were significant increases in the incidence of epidermal hyperplasia, hyperkeratosis, and chronic inflammation of the skin at the site of application (NTP, 2005). [The Working Group noted that this was a well-conducted study that complied with GLP.]

3.2 Rat

3.2.1 Skin application

Groups of 50 male and 50 female Fischer 344/N rats (age, 6 weeks) were exposed to technical-grade trimethylolpropane triacrylate (purity, > 78%) in acetone by skin application at a dose of 0 (control), 0.3, 1.0, or 3.0 mg/kg bw once per day, 5 days per week for 104–105 weeks. The test agent was from the same batch as that used in the 2-year NTP (2012) study in mice; the types and quantities of impurities were therefore identical (see Section 3.1.1). The dose levels were selected to avoid significant skin irritation (based on the severity of skin lesions in a 3-month study) and to preclude adverse effects on survival and growth. The doses were applied to the interscapular region of the back after clipping the hair (NTP, 2012). Survival in treated groups was similar to that of controls. There were no differences in body weights in the treated groups compared with controls. There was a significant increase in the incidence of malignant mesothelioma of the tunica vaginalis in male rats (0/50, 2/50 (4%), 2/50 (4%), and 5/50 (10%) in the groups exposed at 0, 0.3, 1.0, or 3.0 mg/kg bw, respectively) in the group exposed at the highest dose ($P = 0.031$), with a significant positive trend ($P = 0.024$). The incidence in the group exposed

at the highest dose was above the upper bound of the historical control range (0–8%). There were no treatment-related neoplasms of the skin at the site of application in males or females, and no treatment-related neoplasms in other organs in females. In males and females, there were significant increases in the incidence of epidermal hyperplasia and hyperkeratosis of the skin at the site of application (NTP, 2012). [The Working Group noted that this was a well-conducted GLP study.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Data on absorption, distribution, metabolism, and excretion of the trimethylolpropane triacrylate in humans were not available to the Working Group.

4.1.2 Experimental systems

The absorption, distribution, and excretion of [^{14}C]-trimethylolpropane triacrylate were investigated in male Fischer 344/N rats and B6C3F$_1$ mice after dermal exposure, and in male Fischer 344/N rats after exposure by intravenous injection (NTP, 2005).

In rats, the percentage of trimethylolpropane triacrylate absorbed after a single dermal exposure decreased with increasing dose (55, 33, and 19% for exposure at 1.7, 15.2, and 130 mg/kg bw, respectively) after 72 hours. At 72 hours, the total radioactivity recovered in the excreta was approximately 45, 19, and 5% of the applied respective doses. The cumulative excreted radiolabel was partitioned approximately 63% in the urine, 4–6% in the faeces, and 26–30% in

exhaled carbon dioxide, regardless of the dose administered. Most of the radiolabel remaining in the rats 72 hours after dermal exposure was associated with the skin at the application site (~9% of the absorbed compound, primarily intact [^{14}C]-trimethylolpropane triacrylate). After a single dermal exposure at 124 mg/kg bw, HPLC analysis of acetone extracts from the stripped skin indicated that trimethylolpropane triacrylate (73%) was the major compound entering the systemic circulation; two additional peaks (not identified) accounted for 14% and 10% of the radiolabel. At all doses, the total radiolabel associated with collected tissues at 72 hours did not exceed 1%. Compared with other tissues, the kidney had higher tissue:blood ratios of trimethylolpropane triacrylate equivalents, which were not due to covalent binding to kidney proteins but were probably associated with the urine (NTP, 2005).

In male rats exposed to [^{14}C]-trimethylolpropane triacrylate at 9.4 mg/kg bw by intravenous bolus injection, a total of approximately 77% of the radiolabelled compound was excreted in the urine (48%), faeces (9%), and exhaled carbon dioxide (20%) 72 hours later, and the average total recovery of radiolabel was 90%. The highest concentration of radiolabel found in tissues collected 72 hours after exposure was in the blood (~5%), with other tissues (combined) accounting for approximately 2%. Contrary to that observed after dermal exposure, the tissue:blood ratio of radiolabel in the kidney was not elevated compared with other tissues; however, the systemically available radiolabelled material resulted in covalent binding to kidney macromolecules (NTP, 2005).

In male mice, the total absorbed dose 72 hours after a single dermal exposure to [^{14}C]-trimethylolpropane triacrylate at 1.2 mg/kg bw was approximately 1.4-fold the amount absorbed by rats exposed at a similar dose. The percentage of the absorbed dose remaining in the skin at the site of application (31%) was much higher in mice

than in rats. Approximately 42% of the administered dose was excreted by the mice in the urine, faeces, and exhaled carbon dioxide, which was similar to the percentage excreted by rats exposed at 1.7 mg/kg bw. However, the radiolabel in the excreta of mice at 72 hours was partitioned 39% in the urine, 13% in the faeces, and 43% in exhaled carbon dioxide, a much lower excretion in the urine and a higher excretion in the faeces and exhaled carbon dioxide compared with rats. Similarly to rats, very little radiolabel (~0.2%) was associated with mouse tissues 72 hours after exposure; compared with other tissues, the unexposed skin had a higher tissue:blood ratio of trimethylolpropane triacrylate equivalents (NTP, 2005).

No data on the specific metabolites of trimethylolpropane triacrylate were available to the Working Group. Although stability studies indicated that [^{14}C]-trimethylolpropane triacrylate is not chemically stable in the whole blood of male rats, the extent of metabolism and the identity of the metabolites have not been reported (NTP, 2005).

[The Working Group noted that the structure of trimethylolpropane triacrylate suggests susceptibility to blood esterases that may catalyse hydrolysis to acrylic acid, along with trimethylolpropane diacrylate, trimethylolpropane monoacrylate, and/or trimethylolpropane. The excretion of [^{14}C]O_2 after exposure to trimethylolpropane triacrylate by dermal application and intravenous injection in rodents (NTP, 2005) is consistent with the release of acrylic acid (IARC, 1999). Likewise, urinary metabolites of acrylic acid, including cysteine conjugates (IARC, 1999), might explain the elevated tissue:blood radiolabel ratio in the kidney found after dermal exposure of rats to radiolabelled trimethylolpropane triacrylate in the NTP study (NTP, 2005).]

4.2 Mechanisms of carcinogenesis

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016) in the following order: is genotoxic; induces chronic inflammation. Insufficient data were available for evaluation of the other key characteristics of carcinogens.

4.2.1 Genetic and related effects

Trimethylolpropane triacrylate has been evaluated for genetic and related effects in a variety of assays. Table 4.1, Table 4.2, and Table 4.3 summarize the studies that have been reported in non-human mammals in vivo, in non-human mammalian cells in vitro, and in non-mammalian experimental systems, respectively, in the primary peer-reviewed literature.

Genetic and related effects of trimethylolpropane triacrylate in human cells in vitro, and in experimental systems in vivo, were reviewed in Kirkland & Fowler (2018). [The Working Group was unable to evaluate this study independently because the data were not publicly available.]

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.1

In male and female Sprague-Dawley rats exposed to a single dose of a slurry of the trimethylolpropane triacrylate cross-linked polymer (up to 16 mL/kg bw; 5:10:25 weight proportions of polymer:ethanol:water) by oral gavage, no increase in the incidence of chromosomal aberrations in the bone marrow was observed (Thompson et al., 1991).

Technical-grade trimethylolpropane triacrylate did not induce an increase in the frequency of micronucleated normochromatic erythrocytes (NCEs) in male and female B6C3F$_1$

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

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Chromosomal aberrations	Rat, Sprague-Dawley (M, F)	Bone marrow	–	Cross-linked polymer 16 mL/kg bw (slurry: 5 g test material, 10 g ethanol, 25 g distilled water)	Oral gavage; single dose	Purity, NR Average relative molecular mass, > 1 000 000	Thompson et al. (1991)
Micronucleus formation	Mouse, B6C3F₁ (M, F)	Peripheral blood; normochromatic and polychromatic erythrocytes	–	12 mg/kg bw	Dermal; 0.75, 1.5, 3, 6, 12 mg/kg bw, 3 mo	Purity, > 78%	NTP (2005)
Micronucleus formation	Mouse, Tg.AC hemizygous (M, F)	Peripheral blood; normochromatic and polychromatic erythrocytes	–	12 mg/kg bw	Dermal; 0.75, 1.5, 3, 6, 12 mg/kg bw, 6 mo	Purity, > 78%	NTP (2005)

bw, body weight; F, female; HID, highest ineffective dose; LED, lowest effective dose; M, male; mo, month; NR, not reported

[a] –, negative; the level of significance was set at $P < 0.05$ for all cases

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

End-point	Species, tissue/cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	**With metabolic activation**			
Unscheduled DNA synthesis	Rat primary hepatocytes	–	NT	Cross-linked polymer, 1500 µg/mL	Purity, NR Average relative molecular mass, > 1 000 000	Thompson et al. (1991)
Mutation, *Tk*	Mouse L5178Y lymphoma	+	NT	0.65 µg/mL	Purity, NR	Dearfield et al. (1989)
Mutation, *Tk*	Mouse L5178Y lymphoma	–	–	Cross-linked polymer, 3300 µg/mL	Purity, NR Average relative molecular mass, > 1 000 000	Thompson et al. (1991)
Mutation, *Tk*	Mouse L5178Y lymphoma	+	–	2.5 µM	Purity, 79%	Cameron et al. (1991)
Mutation, *Hprt*	Chinese hamster ovary K1-BH4	–	NT	0.7 µg/mL	Purity, NR	Moore et al. (1989)
Mutation, *Hprt*	Chinese hamster ovary K1-BH4	–	NT	0.5 µg/mL	Purity, NR	Moore et al. (1991)
Mutation, *Hprt*	Chinese hamster ovary K1-BH4	–	NT	1.0 µg/mL	Purity, NR	Moore et al. (1991)
Chromosomal aberrations	Mouse L5178Y lymphoma	+	NT	0.7 µg/mL	Purity, NR	Dearfield et al. (1989)
Chromosomal aberrations	Chinese hamster ovary K1-BH4	+	NT	0.2 µg/mL	Purity, NR	Moore et al. (1989)
Micronuclei	Mouse L5178Y lymphoma	(+)	NT	0.7 µg/mL	Purity, NR	Dearfield et al. (1989)

HIC, highest ineffective concentration; LEC, lowest effective concentration, NR, not reported; NT, not tested

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

Table 4.3 Genetic and related effects of trimethylolpropane triacrylate in non-mammalian experimental systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA98, TA100, TA1537	Reverse mutation	–	–	10 000 μg/plate	Purity, 79%	Cameron et al. (1991)
Salmonella typhimurium TA1535	Reverse mutation	–	+/–	3333 μg/plate	Purity, 79%	Cameron et al. (1991)
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	–	–	Cross-linked polymer 6666 μg/plate	Purity, NR; average relative molecular mass, > 1 000 000	Thompson et al. (1991)
Salmonella typhimurium TA98, TA100	Reverse mutation	–	–	10 000 μg/plate	Purity, ~80%	NTP (2012)
Escherichia coli WP2uvrA/pKM101	Reverse mutation	–	–	10 000 μg/plate	Purity, ~80%	NTP (2012)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported

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

mice exposed dermally at 0.75–12 mg/kg bw for 3 months. The treatment did not affect the ratios of micronucleated polychromatic erythrocytes to NCEs in the peripheral blood, indicating that trimethylolpropane triacrylate did not induce bone marrow toxicity (NTP, 2005).

Similarly, there was no increase in the frequency of micronucleated NCEs in peripheral blood samples from male and female Tg.AC hemizygous mice exposed dermally to trimethylolpropane triacrylate at 0.75–12 mg/kg bw for 6 months. In this experiment, the percentage of circulating NCEs (in the total erythrocytes) decreased in male and female mice exposed at 12 mg/kg bw, which was an indication of erythropoiesis stimulation, with increased numbers of immature erythrocytes present in the blood (NTP, 2005).

(ii) Non-human mammalian cells in vitro

See Table 4.2

Exposure to the trimethylolpropane triacrylate cross-linked polymer at up to 1500 µg/mL did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes (Thompson et al., 1991). The trimethylolpropane triacrylate cross-linked polymer was also tested for the induction of *Tk* mutations in the L5178Y mouse lymphoma assay, both in the absence (at up to 1392 µg/mL) and presence (at up to 3300 µg/mL) of rat liver S9; the results were negative (Thompson et al., 1991).

Trimethylolpropane triacrylate at concentrations of up to 0.7 µg/mL [purity, not reported] was tested in L5178Y mouse lymphoma cells, without exogenous metabolic activation, for the induction of chromosomal aberrations, micronuclei, and forward mutations at the *Tk* locus. Concentration-related positive responses were observed for all three end-points; some cytotoxicity was observed at all concentrations. The trifluorothymidine-resistant mutants were primarily small in size (Dearfield et al., 1989). A later study confirmed the induction

of a mutagenic response by trimethylolpropane triacrylate (stated purity, 79%) in the mouse lymphoma assay in the absence of metabolic activation but, again, some cytotoxicity was observed; the addition of S9 decreased both the toxicity and the mutagenic response (Cameron et al., 1991). By contrast, an earlier review (Andrews & Clary, 1986) reported an equivocal result for trimethylolpropane triacrylate in the mouse lymphoma assay, but no details were provided regarding the experimental conditions.

When tested in K1-BH4 Chinese hamster ovary (CHO) cells using the standard monolayer protocol, trimethylolpropane triacrylate at concentrations of up to 0.5 µg/mL [purity, not reported] did not induce an increase in mutant frequency at the *Hgprt* locus of the target cells. Similarly, no mutagenicity was observed at concentrations of up to 1 µg/mL in an adapted CHO suspension assay that used cell numbers comparable to those of the L5178Y mouse lymphoma assay (Moore et al., 1991). However, the same group reported that trimethylolpropane triacrylate at concentrations of up to 0.2 µg/mL induced concentration-related increases in the frequency of chromosomal aberrations in the suspension CHO assay (Moore et al., 1989).

(iii) Non-mammalian experimental systems

See Table 4.3

Trimethylolpropane triacrylate was reported to give negative results in the Ames test, with and without exogenous metabolic activation, and in the yeast D4 assay; however, no experimental details were provided (Andrews & Clary, 1986). In a later study, trimethylolpropane triacrylate (stated purity, 79%) at up to 10 000 µg/plate was found to be weakly mutagenic in *Salmonella typhimurium* strain TA1535 in the presence of hamster (but not rat) liver S9 activation; negative results were obtained in the same strain in the absence of exogenous metabolic activation, as well as in *S. typhimurium* strains TA98, TA100, and TA1537, with and without rat or hamster

liver S9 fractions (Cameron et al., 1991). The negative results in *S. typhimurium* strains TA98 and TA100, with and without rat liver S9 mix, were confirmed in a more recent study (NTP, 2012) that used trimethylolpropane triacrylate at a concentration of up to 10 000 µg/plate (stated purity, ~80%). Negative results were similarly obtained in *Escherichia coli* strain WP2 uvrA/pKM101, considered analogous to *S. typhimurium* strain TA102 (NTP, 2012).

When tested in multiple strains of *S. typhimurium*, the trimethylolpropane cross-linked polymer was not mutagenic at concentrations of up to 6666 µg/plate, either in the absence or presence of induced rat liver S9 mix (Thompson et al., 1991).

4.2.2 Chronic inflammation

(a) Humans

No data were available to the Working Group, except for that on conjunctivitis discussed below (see Section 4.3.1).

(b) Experimental systems

Non-neoplastic inflammatory skin lesions were observed at the site of application in 14-week, 3-month, and 2-year studies of trimethylolpropane triacrylate (Doi et al., 2005; NTP, 2012). Non-neoplastic skin lesions were observed at the site of application in the 3-month studies in male and female rats and mice exposed to trimethylolpropane triacrylate at or above concentrations of 3 mg/kg bw, 5 days per week, and characterized as epidermal hyperplasia and hyperkeratosis. There was a significant increase in the incidence of non-neoplastic lesions in male and female Fischer 344/N rats after topical exposure to trimethylolpropane triacrylate at 1.0 or 3.0 mg/kg bw, 5 days per week, for 2 years. Hyperkeratosis was also increased in female rats exposed to trimethylolpropane triacrylate at 0.3 mg/kg bw. In the same studies, male and female B6C3F$_1$ mice exposed to trimethylolpropane

triacrylate at 3.0 mg/kg bw had a significantly increased incidence of epidermal hyperplasia, melanocyte hyperplasia, and chronic inflammation at the site of application. Epidermal hyperplasia was increased in female mice only after exposure to trimethylolpropane triacrylate at 1.0 mg/kg bw, although chronic inflammation was significantly increased in male mice only at the same dose (NTP, 2012). In Tg.AC mice, similar non-neoplastic lesions were observed at the site of trimethylolpropane triacrylate application and included epidermal hyperplasia, hyperkeratosis, and chronic active inflammation, which were consistently present in both males and females at doses of more than 3 mg/kg bw, 5 days per week, for 6 months (Doi et al., 2005).

4.3 Other adverse effects

4.3.1 Humans

Although much of the toxicity observed in humans exposed to trimethylolpropane triacrylate appears to be allergic in nature, there are reports of skin irritation and inflammation in the absence of sensitization (Nethercott, 1978; Cofield et al., 1985). Nethercott (1978) also reported conjunctivitis in workers exposed to a mixture of acrylic monomers in cured inks.

There are numerous case reports and studies describing the development of allergic contact dermatitis after exposure to industrial mixtures of acrylates containing trimethylolpropane triacrylate (Emmett & Kominsky, 1977; Nethercott, 1978; Björkner et al., 1980; Dahlquist et al., 1983; Nethercott et al., 1983; Garabrant, 1985; Le et al., 2015). Case reports of allergic conjunctivitis (Kanerva et al., 1998; Mancuso & Berdondini, 2008) and asthma (Kanerva et al., 1995; Sánchez-Garcia et al., 2009) have been noted for exposed individuals working with UV-cured paints and inks, with positive reactivity to trimethylolpropane triacrylate in patch tests. When patch testing was conducted, individuals frequently displayed

positive reactions to two or more acrylates (Emmett & Kominsky, 1977; Nethercott, 1978).

4.3.2 Experimental systems

Trimethylolpropane triacrylate applied directly to the skin gave positive results at non-sensitizing concentrations in a dermal irritancy study using female BALB/c mice (NTP, 1995). In a similar study using B6C3F$_1$ mice, trimethylolpropane triacrylate concentrations of 1–30% resulted in significant irritation (Hayes & Meade, 1999).

There are numerous studies in rodents describing sensitization after exposure to trimethyl-olpropane triacrylate (Nethercott et al., 1983; Parker & Turk, 1983; Hayes & Meade, 1999). Cross-reactivity to multiple acrylates has also been demonstrated in animal models (Björkner, 1984; Clemmensen, 1984; Parker et al., 1985; Hayes & Meade, 1999).

Bull et al. (1987) examined the direct immunogenicity of trimethylolpropane triacrylate after footpad injection in female Hartley guinea-pigs. Anti-trimethylolpropane triacrylate antibody levels were elevated in animals immunized with trimethylolpropane triacrylate conjugated to bovine gamma globulin in the presence of Freund's complete adjuvant, but were not detected when unconjugated trimethylolpropane triacrylate was used. The antibodies produced were cross-reactive with methyl acrylate, but not 4-vinyl pyridine (Bull et al., 1987).

4.4 Data relevant to comparisons across agents and end-points

See the monograph on isobutyl nitrite in the present volume.

5. Summary of Data Reported

5.1 Exposure data

Trimethylolpropane triacrylate is only available in technical-grade form, of purity 70–90%, and includes incomplete reaction products, inhibitors, and catalysts. Trimethylolpropane triacrylate is a high production volume chemical that is produced worldwide. It is used in ultraviolet-curable inks, photosensitive chemicals, paint additives, coating additives, and adhesive and sealant chemicals, intermediates, and solvents. Occupational exposure primarily occurs in manufacturing facilities. The concentrations of exposure to trimethylolpropane triacrylate in workers using ultraviolet inks were below the limit of detection. Dermal exposure of the general population may occur through the use of consumer products, such as latex paints and furniture and floor polishes, containing trimethylolpropane triacrylate. No quantitative information was available on environmental concentrations and exposure in the general population.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

In one 2-year good laboratory practice (GLP) skin application study in male and female mice, technical-grade trimethylolpropane triacrylate caused an increase in the incidence of hepatoblastoma and hepatocholangiocarcinoma in females; the Working Group considered hepatoblastoma and hepatocholangiocarcinoma to be rare neoplasms in female mice, and concluded that the increased incidence of these tumours was biologically significant. There was a significant increase in the incidence (with a significant

positive trend) of stromal polyp and stromal polyp or stromal sarcoma (combined) of the uterus in female mice. There was also a significant positive trend in the incidence of hepatocellular carcinoma in female mice. There was no significant increase in tumour incidence in male mice.

In one 2-year GLP skin application study in male and female rats, technical-grade trimethylolpropane triacrylate caused a significant increase in the incidence (with a significant positive trend) of malignant mesothelioma of the tunica vaginalis in males. There was no significant increase in tumour incidence in female rats.

In a 28-week GLP skin application study in male and female Tg.AC hemizygous mice, exposure to technical-grade trimethylolpropane triacrylate significantly increased the incidence (with a significant positive trend) of squamous cell papilloma of the skin at the site of application in male and female mice, and of squamous cell papilloma of the forestomach in female mice.

5.4 Mechanistic and other relevant data

No data on the absorption, distribution, and excretion of trimethylolpropane triacrylate in exposed humans were available to the Working Group. In rats, the percentage of trimethylolpropane triacrylate absorbed is inversely related to dose after dermal exposure. Regardless of the route of exposure (dermal or intravenous injection), urinary excretion is the primary elimination pathway, followed by carbon dioxide exhalation. Excretion pathways are similar in dermally exposed mice.

No data on the specific metabolites of trimethylolpropane triacrylate were available in either humans or experimental animals, although the excretion of carbon dioxide suggests the occurrence of hydrolysis to acrylic acid.

Regarding the key characteristics of carcinogens, there is *weak* evidence that trimethylolpropane triacrylate is genotoxic. No data were available in humans or in human cells in vitro. After dermal exposure, trimethylolpropane triacrylate gave negative results in the mouse peripheral blood micronucleus test. Trimethylolpropane triacrylate gave dose-dependently positive results in rodent cells in vitro, inducing chromosomal aberrations, micronucleus formation, and forward mutations at the *Tk* locus in mouse cells, and chromosomal aberrations, but not *Hgprt* mutations, in hamster cells, although some cytotoxicity was observed. Trimethylolpropane triacrylate also gave negative results in the Ames test.

There is *moderate* evidence that trimethylolpropane triacrylate induces chronic inflammation, based on observations of dermal hyperplasia in multiple cell types in chronically exposed rodents.

Irritant and allergic types of contact dermatitis were reported in humans, with similar results in studies in rodents.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of technical-grade trimethylolpropane triacrylate.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of technical-grade trimethylolpropane triacrylate.

6.3 Overall evaluation

Technical-grade trimethylolpropane triacrylate is *possibly carcinogenic to humans (Group 2B)*.

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LIST OF ABBREVIATIONS

AC_{50}	concentration that produces 50% activation
bw	body weight
CHL	Chinese hamster lung
CHO	Chinese hamster ovary
CI	confidence interval
CYP	cytochrome P450
EPA	United States Environmental Protection Agency
ER	estrogen receptor
GLP	good laboratory practice
GSH	glutathione
GSSG	oxidized glutathione
GST	glutathione S-transferase
HPLC	high-performance liquid chromatography
IL	interleukin
LD_{50}	median lethal dose
MS	mass spectrometry
MTD	maximum tolerated dose
NCE	normochromatic erythrocyte
NIOSH	United States National Institute for Occupational Safety and Health
NOS	nitric oxide synthase
NPSH	non-protein sulfhydryl
NRF2	nuclear erythroid-related factor 2
NTP	United States National Toxicology Program
OSHA	United States Occupational Safety and Health Administration
PR	progesterone receptor
PXR	pregnane X receptor
QC	quality control
RAR	retinoic acid receptor
SMR	standardized mortality ratio
Tox21	Toxicity Testing in the 21st Century
ToxCast	Toxicity Forecaster
TPA	12-O-tetradecanoylphorbol-13-acetate
TWA	time-weighted average

UV	ultraviolet
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
w/v	weight/volume

ANNEX 1. SUPPLEMENTARY MATERIAL FOR TOXCAST/TOX21

This supplemental material (which is available online at: http://publications.iarc.fr/583) comprises a spreadsheet (.xlsx) analysed by the Working Group for Volume 122 of the *IARC Monographs*. The spreadsheet lists the Toxicity Forecaster (ToxCast™) and Toxicity Testing in the 21st Century (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).

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IARC MONOGRAPHS

This volume of the *IARC Monographs* provides evaluations of the carcinogenicity of isobutyl nitrite, β-picoline, methyl acrylate, ethyl acrylate, 2-ethylhexyl acrylate, and trimethylolpropane triacrylate.

The four acrylates evaluated are chemicals with a high production volume that are produced worldwide. Methyl acrylate is used in the production of acrylic fibres and fire-retardant fabrics. Ethyl acrylate is one of the principal monomers used worldwide in the production of styrene-based polymers, which can be used for medical and dental items. Ethyl acrylate is also used in surface coatings for textiles, paper, leather, and food contact materials, and as a food flavouring agent. 2-Ethylhexyl acrylate is used as a plasticizing co-monomer in the production of resins for pressure-sensitive adhesives, latex paints, reactive diluent/cross-linking agents, textile and leather finishes, and coatings for paper. Trimethylolpropane triacrylate, available as a technical-grade product that also contains incomplete reaction products, is used primarily in production of ultraviolet-curable inks, paint additives, coatings, and adhesives.

β-Picoline, a methyl pyridine, is widely used as a starting material for pesticides (e.g. chlorpyrifos) and pharmaceuticals (e.g. vitamin B3). It is also used as a flavouring substance in foods and beverages. Isobutyl nitrite is used an intermediate in the syntheses of solvents and fuels, and exposures also occur through its use as a recreational drug.

Exposure to all six agents considered may occur in the general population as well as in various occupational settings.

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.