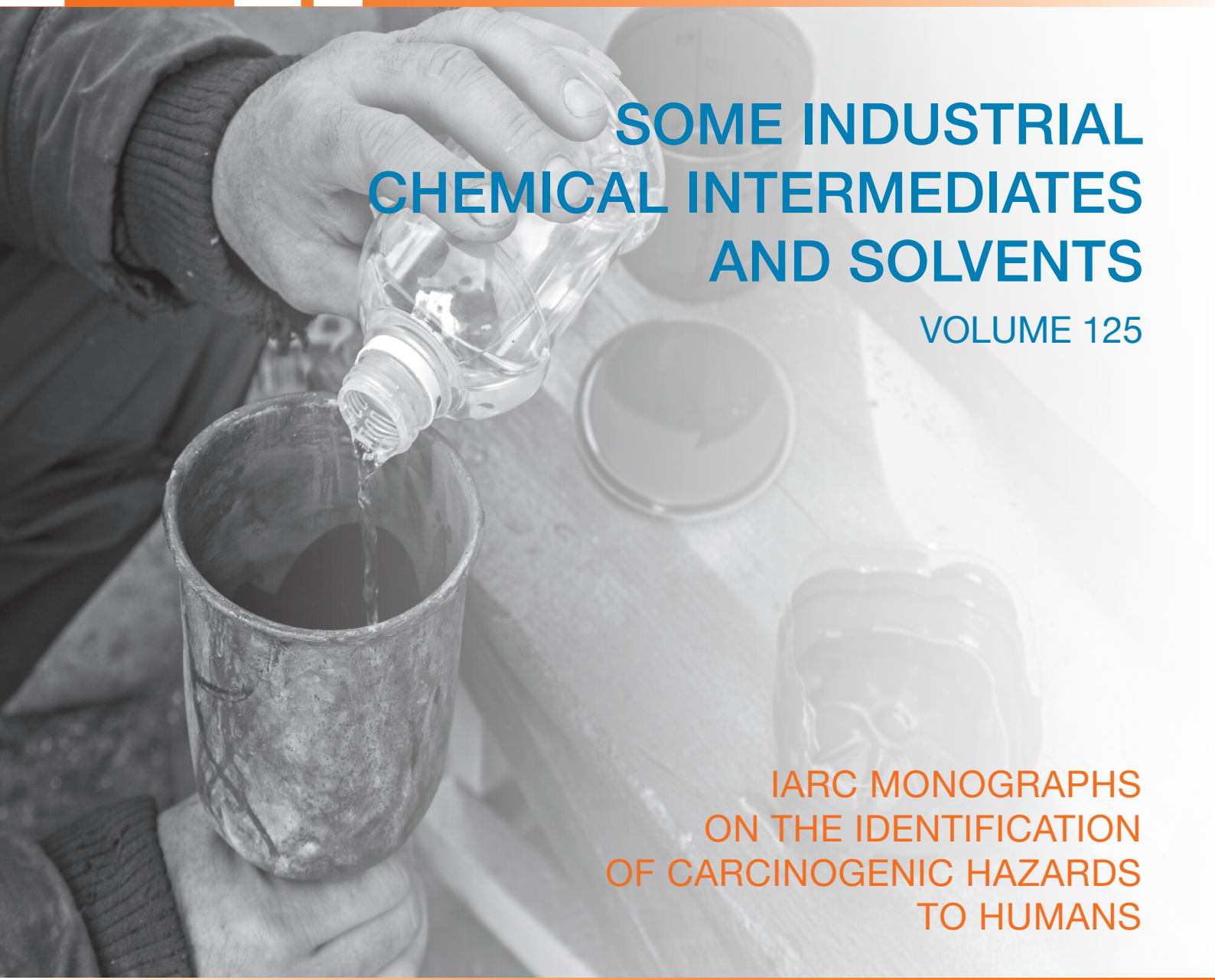
IARC MONOGRAPHS



SOME INDUSTRIAL CHEMICAL INTERMEDIATES AND SOLVENTS

VOLUME 125

IARC MONOGRAPHS
ON THE IDENTIFICATION
OF CARCINOGENIC HAZARDS
TO HUMANS

International Agency for Research on Cancer
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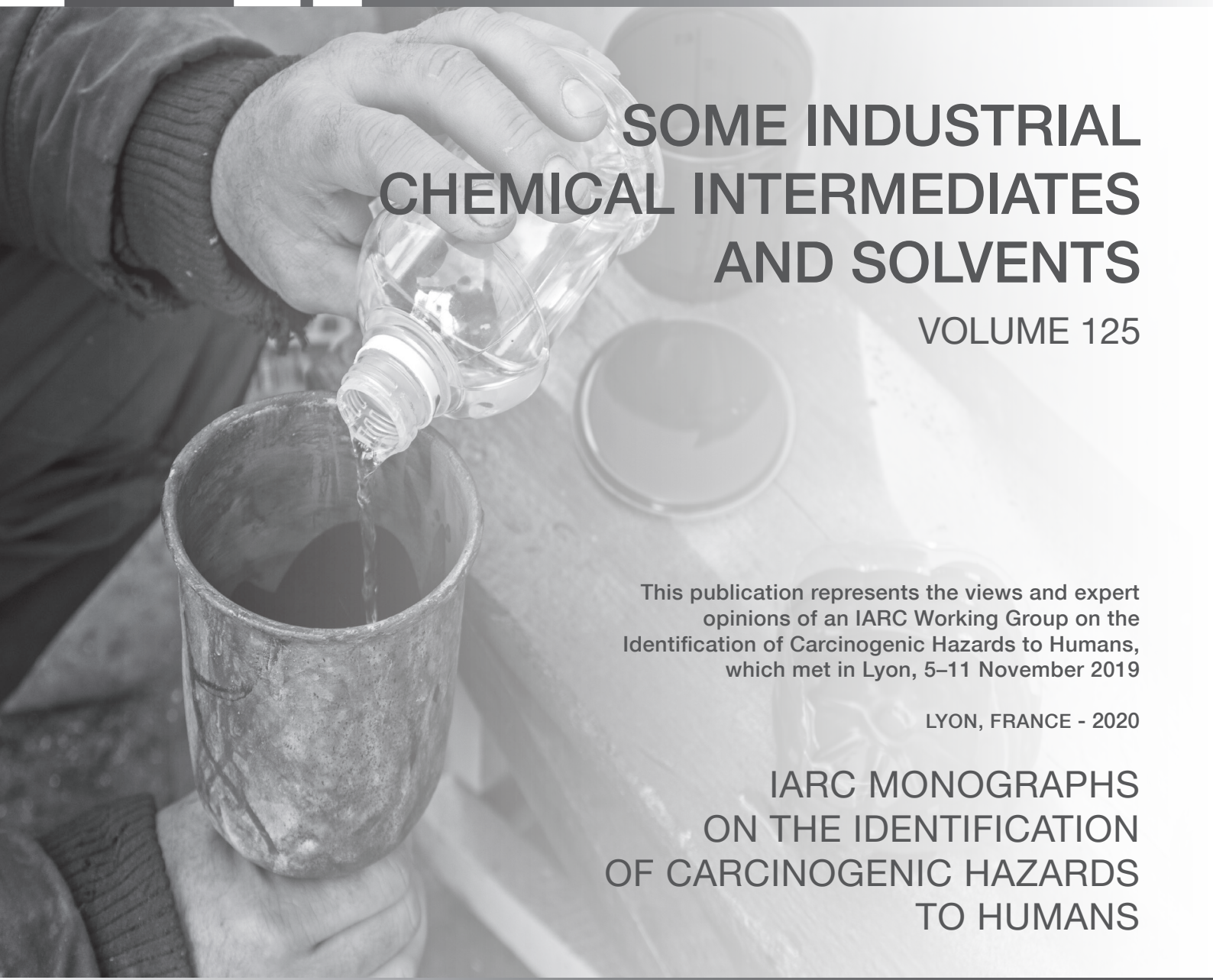

SOME INDUSTRIAL CHEMICAL INTERMEDIATES AND SOLVENTS

VOLUME 125

This publication represents the views and expert opinions of an IARC Working Group on the Identification of Carcinogenic Hazards to Humans, which met in Lyon, 5–11 November 2019

LYON, FRANCE - 2020

IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

World Health Organization

IARC MONOGRAPHS

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic hazard of chemicals to humans, involving the production of critically evaluated monographs on individual chemicals. The programme was subsequently expanded to include evaluations of carcinogenic hazards associated with exposures to complex mixtures, lifestyle factors and biological and physical agents, as well as those in specific occupations. The objective of the programme is to elaborate and publish in the form of monographs critical reviews of data on carcinogenicity for agents to which humans are known to be exposed and on specific exposure situations; to evaluate these data in terms of cancer hazard to humans with the help of international working groups of experts in carcinogenesis and related fields; and to identify gaps in evidence. The lists of IARC evaluations are regularly updated and are available on the internet at https://monographs.iarc.fr/.

This programme has been supported since 1982 by Cooperative Agreement U01 CA33193 with the United States National Cancer Institute, Department of Health and Human Services. Additional support has been provided since 1986 by the European Commission Directorate-General for Employment, Social Affairs, and Inclusion, initially by the Unit of Health, Safety and Hygiene at Work, and since 2014 by the European Union Programme for Employment and Social Innovation "EaSI" (2014–2020) (for further information please consult: https://ec.europa.eu/social/easi). Support has also been provided since 1992 by the United States National Institute of Environmental Health Sciences, Department of Health and Human Services. The contents of this volume are solely the responsibility of the Working Group and do not necessarily represent the official views of the United States National Cancer Institute, the United States National Institute of Environmental Health Sciences, the United States Department of Health and Human Services, or the European Commission.

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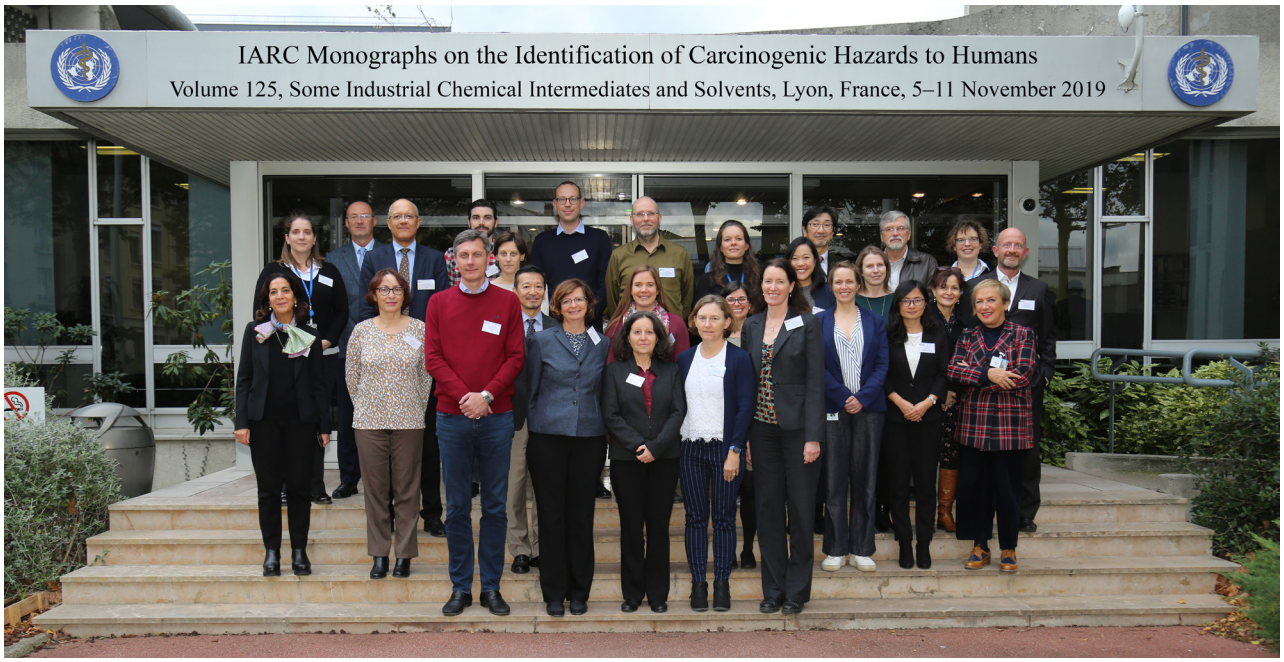

Photo above: The Working Group and other participants at the meeting for *IARC Monographs* Volume 125

The five chemicals evaluated in this volume – glycidyl methacrylate, 1-butyl glycidyl ether, 1-bromo-3-chloropropane, 4-chlorobenzotrifluoride, and allyl chloride – have a wide range of uses in industry.

About the cover: 4-Chlorobenzotrifluoride is widely used as a solvent and diluent for inks, paints, toners, and coatings, including in the car industry. Dermal or inhalation exposure may occur in various occupational and consumer settings.

Source: ©banedeki/istockphoto

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NOTE TO THE READER

The evaluations of carcinogenic hazard in the *IARC Monographs on the Identification of Carcinogenic Hazards to Humans* series are made by international working groups of independent scientists. The *IARC Monographs* classifications do not indicate the level of risk associated with a given level or circumstance of exposure. The *IARC Monographs* do not make recommendations for regulation or legislation.

Anyone who is aware of published data that may alter the evaluation of the carcinogenic hazard 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, or via email at imo@iarc.fr, 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. Corrigenda are published online on the relevant webpage for the volume concerned (IARC Publications: https://publications.iarc.fr/).

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[1] Working Group Members and Invited Specialists serve in their individual capacities as scientists and not as representatives of their government or any organization with which they are affiliated. Affiliations are provided for identification purposes only. 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. Each participant was asked to disclose pertinent research, employment, and financial interests. Current financial interests and research and employment interests during the past 4 years or anticipated in the future are identified here. Minor pertinent interests are not listed and include stock valued at no more than US$ 1000 overall, grants that provide no more than 5% of the research budget of the expert's organization and that do not support the expert's research or position, and consulting or speaking on matters not before a court or government agency that does not exceed 2% of total professional time or compensation. All grants that support the expert's research or position and all consulting or speaking on behalf of an interested party on matters before a court or government agency are listed as significant pertinent interests.

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PREAMBLE

The Preamble to the *IARC Monographs* describes the objective and scope of the programme, general principles and procedures, and scientific review and evaluations. The *IARC Monographs* embody principles of scientific rigour, impartial evaluation, transparency, and consistency. The Preamble should be consulted when reading a *Monograph* or a summary of a *Monograph*'s evaluations. Separate Instructions for Authors describe the operational procedures for the preparation and publication of a volume of the *Monographs*.

A. GENERAL PRINCIPLES AND PROCEDURES

1. Background

Soon after the International Agency for Research on Cancer (IARC) was established in 1965, it started to receive frequent requests for advice on the carcinogenicity of chemicals, including requests for lists of established and suspected human carcinogens. In 1970, an 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 next year, the IARC Governing Council adopted a resolution that IARC should prepare "monographs on the evaluation of carcinogenic risk of chemicals to man", which became the initial title of the series.

In succeeding years, the scope of the programme broadened as *Monographs* were developed for complex mixtures, occupational exposures, physical agents, biological organisms, pharmaceuticals, and other exposures. In 1988, "of chemicals" was dropped from the title, and in 2019, "evaluation of carcinogenic risks" became "identification of carcinogenic hazards", in line with the objective of the programme.

Identifying the causes of human cancer is the first step in cancer prevention. The identification of a cancer hazard may have broad and profound implications. National and international authorities and organizations can and do use information on causes of cancer in support of actions to reduce exposure to carcinogens in the workplace, in the environment, and elsewhere. Cancer prevention is needed as much today as it was when IARC was established, because the global burden of cancer is high and continues to increase as a result of population growth and ageing and upward trends in some exposures, especially in low- and middle-income countries (https://publications.iarc.fr/Non-Series-Publications/World-Cancer-Reports).

IARC's process for developing *Monographs*, which has evolved over several decades, involves

the engagement of international, interdisciplinary Working Groups of expert scientists, the transparent synthesis of different streams of evidence (exposure characterization, cancer in humans, cancer in experimental animals, and mechanisms of carcinogenesis), and the integration of these streams of evidence into an overall evaluation and classification according to criteria developed and refined by IARC. Since the *Monographs* programme was established, the understanding of carcinogenesis has greatly deepened. Scientific advances are incorporated into the evaluation methodology. In particular, strong mechanistic evidence has had an increasing role in the overall evaluations since 1991.

The Preamble is primarily a statement of the general principles and procedures used in developing a *Monograph*, to promote transparency and consistency across *Monographs* evaluations. In addition, IARC provides Instructions for Authors (https://monographs.iarc.fr/preamble-instructions-for-authors/), which specify more detailed working procedures. IARC routinely updates these Instructions for Authors to reflect advances in methods for cancer hazard identification and accumulated experience, including input from experts.

2. Objective and scope

The objective of the programme is to prepare, with the engagement of international, interdisciplinary Working Groups of experts, scientific reviews and evaluations of evidence on the carcinogenicity of a wide range of agents.

The *Monographs* assess the strength of the available evidence that an agent can cause cancer in humans, based on three streams of evidence: on cancer in humans (see Part B, Section 2), on cancer in experimental animals (see Part B, Section 3), and on mechanistic evidence (see Part B, Section 4). In addition, the exposure to each agent is characterized (see Part B, Section 1). In this Preamble, the term "agent" refers to any chemical, physical, or biological entity or exposure circumstance (e.g. occupation as a painter) for which evidence on the carcinogenicity is evaluated.

A cancer *hazard* is an agent that is capable of causing cancer, whereas a cancer *risk* is an estimate of the probability that cancer will occur given some level of exposure to a cancer hazard. The *Monographs* assess the strength of evidence that an agent is a cancer hazard. The distinction between hazard and risk is fundamental. The *Monographs* identify cancer hazards even when risks appear to be low in some exposure scenarios. This is because the exposure may be widespread at low levels, and because exposure levels in many populations are not known or documented.

Although the *Monographs* programme has focused on hazard identification, some epidemiological studies used to identify a cancer hazard are also used to estimate an exposure–response relationship within the range of the available data. However, extrapolating exposure–response relationships beyond the available data (e.g. to lower exposures, or from experimental animals to humans) is outside the scope of *Monographs* Working Groups (IARC, 2014). In addition, the *Monographs* programme does not review quantitative risk characterizations developed by other health agencies.

The identification of a cancer hazard should trigger some action to protect public health, either directly as a result of the hazard identification or through the conduct of a risk assessment. Although such actions are outside the scope of the programme, the *Monographs* are used by national and international authorities and organizations to inform risk assessments, formulate decisions about preventive measures, motivate effective cancer control programmes, and choose among options for public health decisions. *Monographs* evaluations are only one part of the body of information on which decisions to

control exposure to carcinogens may be based. Options to prevent cancer vary from one situation to another and across geographical regions and take many factors into account, including different national priorities. Therefore, no recommendations are given in the *Monographs* with regard to regulation, legislation, or other policy approaches, which are the responsibility of individual governments or organizations. The *Monographs* programme also does not make research recommendations. However, it is important to note that *Monographs* contribute significantly to the science of carcinogenesis by synthesizing and integrating streams of evidence about carcinogenicity and pointing to critical gaps in knowledge.

3. Selection of agents for review

Since 1984, about every five years IARC convenes an international, interdisciplinary Advisory Group to recommend agents for review by the *Monographs* programme. IARC selects Advisory Group members who are knowledgeable about current research on carcinogens and public health priorities. Before an Advisory Group meets, IARC solicits nominations of agents from scientists and government agencies worldwide. Since 2003, IARC also invites nominations from the public. IARC charges each Advisory Group with reviewing nominations, evaluating exposure and hazard potential, and preparing a report that documents the Advisory Group's process for these activities and its rationale for the recommendations.

For each new volume of the *Monographs*, IARC selects the agents for review from those recommended by the most recent Advisory Group, considering the availability of pertinent research studies and current public health priorities. On occasion, IARC may select other agents if there is a need to rapidly evaluate an emerging carcinogenic hazard or an urgent need to re-evaluate a previous classification. All evaluations consider the full body of available evidence, not just information published after a previous review.

A *Monograph* may review:

(a) An agent not reviewed in a previous *Monograph*, if there is potential human exposure and there is evidence for assessing its carcinogenicity. A group of related agents (e.g. metal compounds) may be reviewed together if there is evidence for assessing carcinogenicity for one or more members of the group.

(b) An agent reviewed in a previous *Monograph*, if there is new evidence of cancer in humans or in experimental animals, or mechanistic evidence to warrant re-evaluation of the classification. In the interests of efficiency, the literature searches may build on previous comprehensive searches.

(c) An agent that has been established to be carcinogenic to humans and has been reviewed in a previous *Monograph*, if there is new evidence of cancer in humans that indicates new tumour sites where there might be a causal association. In the interests of efficiency, the review may focus on these new tumour sites.

4. The Working Group and other meeting participants

Five categories of participants can be present at *Monographs* meetings:

(i) *Working Group* members are responsible for all scientific reviews and evaluations developed in the volume of the *Monographs*. The Working Group is interdisciplinary and comprises subgroups of experts in the fields of (a) exposure characterization, (b) cancer in humans, (c) cancer in experimental animals, and (d) mechanistic evidence. IARC selects Working Group members on the basis of

expertise related to the subject matter and relevant methodologies, and absence of conflicts of interest. Consideration is also given to diversity in scientific approaches and views, as well as demographic composition. Working Group members generally have published research related to the exposure or carcinogenicity of the agents being reviewed, and IARC uses literature searches to identify most experts. Since 2006, IARC also has encouraged public nominations through its Call for Experts. IARC's reliance on experts with knowledge of the subject matter and/or expertise in methodological assessment is confirmed by decades of experience documenting that there is value in specialized expertise and that the overwhelming majority of Working Group members are committed to the objective evaluation of scientific evidence and not to the narrow advancement of their own research results or a pre-determined outcome (Wild & Cogliano, 2011). Working Group members are expected to serve the public health mission of IARC, and should refrain from consulting and other activities for financial gain that are related to the agents under review, or the use of inside information from the meeting, until the full volume of the *Monographs* is published.

IARC identifies, from among Working Group members, individuals to serve as Meeting Chair and Subgroup Chairs. At the opening of the meeting, the Working Group is asked to endorse the selection of the Meeting Chair, with the opportunity to propose alternatives. The Meeting Chair and Subgroup Chairs take a leading role at all stages of the review process (see Part A, Section 7), promote open scientific discussions that involve all Working Group members in accordance with normal committee procedures, and ensure adherence to the Preamble.

(ii) *Invited Specialists* are experts who have critical knowledge and experience but who also have a conflict of interest that warrants exclusion from developing or influencing the evaluations of carcinogenicity. Invited Specialists do not draft any section of the *Monograph* that pertains to the description or interpretation of cancer data, and they do not participate in the evaluations. These experts are invited in limited numbers when necessary to assist the Working Group by contributing their unique knowledge and experience to the discussions.

(iii) *Representatives of national and international health agencies* may attend because their agencies are interested in the subject of the meeting. They do not draft any section of the *Monograph* or participate in the evaluations.

(iv) *Observers* with relevant scientific credentials may be admitted in limited numbers. Attention is given to the balance of Observers from constituencies with differing perspectives. Observers are invited to observe the meeting and should not attempt to influence it, and they agree to respect the Guidelines for Observers at *IARC Monographs* meetings. Observers do not draft any section of the *Monograph* or participate in the evaluations.

(v) The *IARC Secretariat* consists of scientists who are designated by IARC and who have relevant expertise. The IARC Secretariat coordinates and facilitates all aspects of the evaluation and ensures adherence to the Preamble throughout development of the scientific reviews and classifications (see Part A, Sections 5 and 6). The IARC Secretariat organizes and announces the meeting, identifies and recruits the Working Group members, and assesses the declared interests of all meeting participants. The IARC Secretariat supports the activities of the Working Group (see Part A, Section 7) by

Table 1 Roles of participants at *IARC Monographs* meetings

Category of participant	Role			
	Prepare text, tables, and analyses	Participate in discussions	Participate in evaluations	Eligible to serve as Chair
Working Group members	✓	✓	✓	✓
Invited Specialists	✓[a]	✓		
Representatives of health agencies		✓[b]		
Observers		✓[b]		
IARC Secretariat	✓[c]	✓	✓[d]	

[a] Only for the section on exposure characterization.
[b] Only at times designated by the Meeting Chair and Subgroup Chairs.
[c] When needed or requested by the Meeting Chair and Subgroup Chairs.
[d] Only for clarifying or interpreting the Preamble.

searching the literature and performing title and abstract screening, organizing conference calls to coordinate the development of pre-meeting drafts and discuss cross-cutting issues, and reviewing drafts before and during the meeting. Members of the IARC Secretariat serve as meeting rapporteurs, assist the Meeting Chair and Subgroup Chairs in facilitating all discussions, and may draft text or tables when designated by the Meeting Chair and Subgroup Chairs. Their participation in the evaluations is restricted to the role of clarifying or interpreting the Preamble.

All participants are listed, with their principal affiliations, in the front matter of the published volume of the *Monographs*. Working Group members and Invited Specialists serve as individual scientists and not as representatives of any organization, government, or industry (Cogliano et al., 2004).

The roles of the meeting participants are summarized in Table 1.

5. Working procedures

A separate Working Group is responsible for developing each volume of the *Monographs*. A volume contains one or more *Monographs*, which can cover either a single agent or several related agents. Approximately one year before the meeting of a Working Group, a preliminary list of agents to be reviewed, together with a Call for Data and a Call for Experts, is announced on the *Monographs* programme website (https://monographs.iarc.fr/).

Before a meeting invitation is extended, each potential participant, including the IARC Secretariat, completes the WHO Declaration of Interests form to report financial interests, employment and consulting (including remuneration for serving as an expert witness), individual and institutional research support, and non-financial interests such as public statements and positions related to the subject of the meeting. IARC assesses the declared interests to determine whether there is a conflict that warrants any limitation on participation (see Table 2).

Approximately two months before a *Monographs* meeting, IARC publishes the names and affiliations of all meeting participants together with a summary of declared interests, in the interests of transparency and to provide an opportunity for undeclared conflicts of interest to be brought to IARC's attention. 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).

Table 2 Public engagement during *Monographs* development

Approximate timeframe	Engagement
Every 5 years	IARC convenes an Advisory Group to recommend high-priority agents for future review
~1 year before a *Monographs* meeting	IARC selects agents for review in a new volume of the *Monographs* IARC posts on its website: Preliminary List of Agents to be reviewed Call for Data and Call for Experts Request for Observer Status WHO Declaration of Interests form
~8 months before a *Monographs* meeting	Call for Experts closes
~4 months before a *Monographs* meeting	Request for Observer Status closes
~2 months before a *Monographs* meeting	IARC posts the names of all meeting participants together with a summary of declared interests, and a statement discouraging contact of the Working Group by interested parties
~1 month before a *Monographs* meeting	Call for Data closes
~2–4 weeks after a *Monographs* meeting	IARC publishes a summary of evaluations and key supporting evidence
~9 months after a *Monographs* meeting	IARC Secretariat publishes the verified and edited master copy of plenary drafts as a *Monographs* volume

The Working Group meets at IARC for approximately eight days to discuss and finalize the scientific review and to develop summaries and evaluations. At the opening of the meeting, all participants update their Declaration of Interests forms, which are then reviewed by IARC. Declared interests related to the subject of the meeting are disclosed to the meeting participants during the meeting and in the published volume (Cogliano et al., 2004). The objectives of the meeting are peer review and consensus. During the first part of the meeting, subgroup sessions (covering exposure characterization, cancer in humans, cancer in experimental animals, and mechanistic evidence) review the pre-meeting drafts, develop a joint subgroup draft, and draft subgroup summaries. During the last part of the meeting, the Working Group meets in plenary session to review the subgroup drafts and summaries and to develop the consensus evaluations. As a result, the entire volume is the joint product of the Working Group, and there are no individually authored sections. After the meeting, the master copy is verified by the IARC Secretariat and is then edited and prepared for publication. The aim is to publish the volume within approximately nine months of the Working Group meeting. A summary of the evaluations and key supporting evidence is prepared for publication in a scientific journal or is made available on the *Monographs* programme website soon after the meeting.

In the interests of transparency, IARC engages with the public throughout the process, as summarized in Table 2.

6. Overview of the scientific review and evaluation process

The Working Group considers all pertinent epidemiological studies, cancer bioassays in experimental animals, and mechanistic evidence, as well as pertinent information on exposure in humans. In general, for cancer in humans, cancer in experimental animals, and mechanistic evidence, only studies that have been published or accepted for publication in the openly available scientific literature are reviewed. Under some circumstances, materials

that are publicly available and whose content is final may be reviewed if there is sufficient information to permit an evaluation of the quality of the methods and results of the studies (see Step 1, below). Such materials may include reports and databases publicly available from government agencies, as well as doctoral theses. The reliance on published and publicly available studies promotes transparency and protects against citation of premature information.

The principles of systematic review are applied to the identification, screening, synthesis, and evaluation of the evidence related to cancer in humans, cancer in experimental animals, and mechanistic evidence (as described in Part B, Sections 2–4 and as detailed in the Instructions for Authors). Each *Monograph* specifies or references information on the conduct of the literature searches, including search terms and inclusion/exclusion criteria that were used for each stream of evidence.

In brief, the steps of the review process are as follows:

Step 1. Comprehensive and transparent identification of the relevant information: The IARC Secretariat identifies relevant studies through initial comprehensive searches of literature contained in authoritative biomedical databases (e.g. PubMed, PubChem) and through a Call for Data. These literature searches, designed in consultation with a librarian and other technical experts, address whether the agent causes cancer in humans, causes cancer in experimental systems, and/or exhibits key characteristics of established human carcinogens (in humans or in experimental systems). The Working Group provides input and advice to IARC to refine the search strategies, and identifies literature through other searches (e.g. from reference lists of past *Monographs*, retrieved articles, and other authoritative reviews).

For certain types of agents (e.g. regulated pesticides and pharmaceuticals), IARC also provides an opportunity to relevant regulatory authorities, and regulated parties through such authorities, to make pertinent unpublished studies publicly available by the date specified in the Call for Data. Consideration of such studies by the Working Group is dependent on the public availability of sufficient information to permit an independent evaluation of (a) whether there has been selective reporting (e.g. on outcomes, or from a larger set of conducted studies); (b) study quality (e.g. design, methodology, and reporting of results), and (c) study results.

Step 2. Screening, selection, and organization of the studies: The IARC Secretariat screens the retrieved literature for inclusion based on title and abstract review, according to pre-defined exclusion criteria. For instance, studies may be excluded if they were not about the agent (or a metabolite of the agent), or if they reported no original data on epidemiological or toxicological end-points (e.g. review articles). The Working Group reviews the title and abstract screening done by IARC, and performs full-text review. Any reasons for exclusion are recorded, and included studies are organized according to factors pertinent to the considerations described in Part B, Sections 2–4 (e.g. design, species, and end-point). Inclusion of a study does not imply acceptance of the adequacy of the study design or of the analysis and interpretation of the results.

Step 3. Evaluation of study quality: The Working Group evaluates the quality of the included studies based on the considerations (e.g. design, methodology, and reporting of results) described in Part B, Sections 2–4. Based on these considerations, the Working Group may accord greater weight to some of the included studies. Interpretation of the

results and the strengths and limitations of a study are clearly outlined in square brackets at the end of study descriptions (see Part B).

Step 4: *Report characteristics of included studies, including assessment of study quality*: Pertinent characteristics and results of included studies are reviewed and succinctly described, as detailed in Part B, Sections 1–4. Tabulation of data may facilitate this reporting. This step may be iterative with Step 3.

Step 5: *Synthesis and evaluation of strength of evidence*: The Working Group summarizes the overall strengths and limitations of the evidence from the individual streams of evidence (cancer in humans, cancer in experimental animals, and mechanistic evidence; see Part B, Section 5). The Working Group then evaluates the strength of evidence from each stream of evidence by using the transparent methods and defined descriptive terms given in Part B, Sections 6a–c. The Working Group then develops, and describes the rationale for, the consensus classification of carcinogenicity that integrates the conclusions about the strength of evidence from studies of cancer in humans, studies of cancer in experimental animals, and mechanistic evidence (see Part B, Section 6d).

7. Responsibilities of the Working Group

The Working Group is responsible for identifying and evaluating the relevant studies and developing the scientific reviews and evaluations for a volume of the *Monographs*. The IARC Secretariat supports these activities of the Working Group (see Part A, Section 4). Briefly, the Working Group's tasks in developing the evaluation are, in sequence:

(i) Before the meeting, the Working Group ascertains that all appropriate studies have been identified and selected, and assesses the methods and quality of each individual study, as outlined above (see Part A, Section 6). The Working Group members prepare pre-meeting working drafts that present accurate tabular or textual summaries of informative studies by extracting key elements of the study design and results, and highlighting notable strengths and limitations. They participate in conference calls organized by IARC to coordinate the development of working drafts and to discuss cross-cutting issues. Pre-meeting reviews of all working drafts are generally performed by two or more subgroup members who did not participate in study identification, data extraction, or study review for the draft. Each study summary is written or reviewed by someone who is not associated with the study.

(ii) At the meeting, within subgroups, the Working Group members critically review, discuss, and revise the pre-meeting drafts and adopt the revised versions as consensus subgroup drafts. Subgroup Chairs ensure that someone who is not associated with the study leads the discussion of each study summary. A proposed classification of the strength of the evidence reviewed in the subgroup using the *IARC Monographs* criteria (see Part B, Sections 6a–c) is then developed from the consensus subgroup drafts of the evidence summaries (see Part B, Section 5).

(iii) During the plenary session, each subgroup presents its drafts for scientific review and discussion to the other Working Group members, who did not participate in study identification, data extraction, or study review for the drafts. Subgroup Chairs ensure that someone who is not associated with the study leads the discussion of each study summary.

After review, discussion, and revisions as needed, the subgroup drafts are adopted as a consensus Working Group product. The summaries and classifications of the strength of the evidence, developed in the subgroup in line with the *IARC Monographs* criteria (see Part B, Sections 6a–c), are considered, revised as needed, and adopted by the full Working Group. The Meeting Chair proposes an overall evaluation using the guidance provided in Part B, Section 6d.

The Working Group strives to achieve consensus evaluations. Consensus reflects broad agreement among the Working Group, but not necessarily unanimity. The Meeting Chair may poll the Working Group to determine the diversity of scientific opinion on issues where consensus is not apparent.

Only the final product of the plenary session represents the views and expert opinions of the Working Group. The entire *Monographs* volume is the joint product of the Working Group and represents an extensive and thorough peer review of the body of evidence (individual studies, synthesis, and evaluation) by an interdisciplinary expert group. Initial working papers and subsequent revisions are not released, because they would give an incomplete and possibly misleading impression of the consensus developed by the Working Group over a full week of deliberation.

B. SCIENTIFIC REVIEW AND EVALUATION

This part of the Preamble discusses the types of evidence that are considered and summarized in each section of a *Monograph*, followed by the scientific criteria that guide the evaluations. In addition, a section of General Remarks at the front of the volume discusses the reasons the agents were scheduled for evaluation and any key issues encountered during the meeting.

1. Exposure characterization

This section identifies the agent and describes its occurrence, main uses, and production locations and volumes, where relevant. It also summarizes the prevalence, concentrations in relevant studies, and relevant routes of exposure in humans worldwide. Methods of exposure measurement and analysis are described, and methods of exposure assessment used in key epidemiological studies reviewed by the Working Group are described and evaluated.

Over the course of the *Monographs* programme, concepts of exposure and dose have evolved substantially with deepening understanding of the interactions of agents and biological systems. The concept of exposure has broadened and become more holistic, extending beyond chemical, physical, and biological agents to stressors as construed generally, including psychosocial stressors (National Research Council, 2012; National Academies of Sciences, Engineering, and Medicine, 2017). Overall, this broader conceptualization supports greater integration between exposure characterization and other sections of the *Monographs*. Concepts of absorption, distribution, metabolism, and excretion are considered in the first subsection of mechanistic evidence (see Part B, Section 4a), whereas validated biomarkers of internal exposure or metabolites that are routinely used for exposure assessment are reported on in this section (see Part B, Section 1b).

(a) Identification of the agent

The agent being evaluated is unambiguously identified. Details will vary depending on the type of agent but will generally include physical and chemical properties relevant to the agent's identification, occurrence, and biological activity.

If the material that has been tested in experimental animals or in vitro systems is different from that to which humans are exposed, these differences are noted.

For chemical agents, the Chemical Abstracts Service Registry Number is provided, as well as the latest primary name and other names in common use, including important trade names, along with available information on the composition of common mixtures or products containing the agent, and potentially toxic and/or carcinogenic impurities. Physical properties relevant to understanding the potential for human exposure and measures of exposure used in studies in humans are summarized. These might include physical state, volatility, aqueous and fat solubility, and half-life in the environment and/or in human tissues.

For biological agents, taxonomy and structure are described. Mode of replication, life-cycle, target cells, persistence, latency, and host responses, including morbidity and mortality through pathologies other than cancer, are also presented.

For foreign bodies, fibres and particles, composition, size range, relative dimensions, and accumulation, persistence, and clearance in target organs are summarized. Physical agents that are forms of radiation are described in terms of frequency spectrum and energy transmission.

Exposures may result from, or be influenced by, a diverse range of social and environmental factors, including components of diet, sleep, and physical activity patterns. In these instances, this section will include a description of the agent, its variability across human populations, and its composition or characteristics relevant to understanding its potential carcinogenic hazard to humans and to evaluating exposure assessments in epidemiological studies.

(b) Detection and analysis

Key methods of detection and quantification of the agent are presented, with an emphasis on those used most widely in surveillance, regulation, and epidemiological studies. Measurement methods for sample matrices that are deemed important sources of human exposure (e.g. air, drinking-water, food, residential dust) and for validated exposure biomarkers (e.g. the agent or its metabolites in human blood, urine, or saliva) are described. Information on detection and quantification limits is provided when it is available and is useful for interpreting studies in humans and in experimental animals. This is not an exhaustive treatise but is meant to help readers understand the strengths and limitations of the available exposure data and of the epidemiological studies that rely on these measurements.

(c) Production and use

Historical and geographical patterns and trends in production and use are included when they are available, to help readers understand the contexts in which exposures may occur, both within key epidemiological studies reviewed by the Working Group and in human populations generally. Industries that produce, use, or dispose of the agent are described, including their global distribution, when available. National or international listing as a high-production-volume chemical or similar classification may be included. Production processes with significant potential for occupational exposure or environmental pollution are indicated. Trends in global production volumes, technologies, and other data relevant to understanding exposure potential are summarized. Minor or historical uses with significant exposure potential or with particular relevance to key epidemiological studies are included. Particular effort may be directed towards finding data on production in low- and middle-income countries, where rapid

economic development may lead to higher exposures than those in high-income countries.

(d) Exposure

A concise overview of quantitative information on sources, prevalence, and levels of exposure in humans is provided. Representative data from research studies, government reports and websites, online databases, and other citable, publicly available sources are tabulated. Data from low- and middle-income countries are sought and included to the extent feasible; information gaps for key regions are noted. Naturally occurring sources of exposure, if any, are noted. Primary exposure routes (e.g. inhalation, ingestion, skin uptake) and other considerations relevant to understanding the potential for cancer hazard from exposure to the agent are reported.

For occupational settings, information on exposure prevalence and levels (e.g. in air or human tissues) is reported by industry, occupation, region, and other characteristics (e.g. process, task) where feasible. Information on historical exposure trends, protection measures to limit exposure, and potential co-exposures to other carcinogenic agents in workplaces is provided when available.

For non-occupational settings, the occurrence of the agent is described with environmental monitoring or surveillance data. Information on exposure prevalence and levels (e.g. concentrations in human tissues) as well as exposure from and/or concentrations in food and beverages, consumer products, consumption practices, and personal microenvironments is reported by region and other relevant characteristics. Particular importance is placed on describing exposures in life stages or in states of disease or nutrition that may involve greater exposure or susceptibility.

Current exposures are of primary interest; however, information on historical exposure trends is provided when available. Historical exposures may be relevant for interpreting epidemiological studies, and when agents are persistent or have long-term effects. Information gaps for important time periods are noted. Exposure data that are not deemed to have high relevance to human exposure are generally not considered.

(e) Regulations and guidelines

Regulations or guidelines that have been established for the agent (e.g. occupational exposure limits, maximum permitted levels in foods and water, pesticide registrations) are described in brief to provide context about government efforts to limit exposure; these may be tabulated if they are informative for the interpretation of existing or historical exposure levels. Information on applicable populations, specific agents concerned, basis for regulation (e.g. human health risk, environmental considerations), and timing of implementation may be noted. National and international bans on production, use, and trade are also indicated.

This section aims to include major or illustrative regulations and may not be comprehensive, because of the complexity and range of regulatory processes worldwide. An absence of information on regulatory status should not be taken to imply that a given country or region lacks exposure to, or regulations on exposure to, the agent.

(f) Critical review of exposure assessment in key epidemiological studies

Epidemiological studies evaluate cancer hazard by comparing outcomes across differently exposed groups. Therefore, the type and quality of the exposure assessment methods used are key considerations when interpreting study findings for hazard identification. This section summarizes and critically reviews the exposure assessment methods used in the individual epidemiological studies that contribute data relevant to the *Monographs* evaluation.

Although there is no standard set of criteria for evaluating the quality of exposure assessment methods across all possible agents, some concepts are universally relevant. Regardless of the agent, all exposures have two principal dimensions: intensity (sometimes defined as concentration or dose) and time. Time considerations include duration (time from first to last exposure), pattern or frequency (whether continuous or intermittent), and windows of susceptibility. This section considers how each of the key epidemiological studies characterizes these dimensions. Interpretation of exposure information may also be informed by consideration of mechanistic evidence (e.g. as described in Part B, Section 4a), including the processes of absorption, distribution, metabolism, and excretion.

Exposure intensity and time in epidemiological studies can be characterized by using environmental or biological monitoring data, records from workplaces or other sources, expert assessments, modelled exposures, job-exposure matrices, and subject or proxy reports via questionnaires or interviews. Investigators use these data sources and methods individually or in combination to assign levels or values of an exposure metric (which may be quantitative, semi-quantitative, or qualitative) to members of the population under study.

In collaboration with the Working Group members reviewing human studies (of cancer and of mechanisms), key epidemiological studies are identified. For each selected study, the exposure assessment approach, along with its strengths and limitations, is summarized using text and tables. Working Group members identify concerns about exposure assessment methods and their impacts on overall quality for each study reviewed (see Part B, Sections 2d and 4d). In situations where the information provided in the study is inadequate to properly consider the exposure assessment, this is indicated. When adequate information is available, the likely direction of bias due to error in exposure measurement, including misclassification (overestimated effects, underestimated effects, or unknown) is discussed.

2. Studies of cancer in humans

This section includes all pertinent epidemiological studies (see Part B, Section 2b) that include cancer as an outcome. These studies encompass certain types of biomarker studies, for example, studies with biomarkers as exposure metrics (see Part B, Section 2) or those evaluating histological or tumour subtypes and molecular signatures in tumours consistent with a given exposure (Alexandrov et al., 2016). Studies that evaluate early biological effect biomarkers are reviewed in Part B, Section 4.

(a) Types of study considered

Several types of epidemiological studies contribute to the assessment of carcinogenicity in humans; they typically include cohort studies (including variants such as case–cohort and nested case–control studies), case–control studies, ecological studies, and intervention studies. Rarely, results from randomized trials may be available. Exceptionally, case reports and case series of cancer in humans may also be reviewed. In addition to these designs, innovations in epidemiology allow for many other variants that may be considered in any given *Monographs* evaluation.

Cohort and case–control studies typically have the capacity to 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. Well-conducted cohort and case–control studies provide most of the evidence of cancer in humans evaluated by Working Groups. Intervention studies are much less common, but when available can provide strong evidence for making causal inferences.

In ecological 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 in the population under study. In ecological studies, data on individual exposure and outcome are not available, which renders this type of study more prone to confounding and exposure misclassification. In some circumstances, however, ecological studies may be informative, especially when the unit of exposure is most accurately measured at the population level (see, for example, the *Monograph* on arsenic in drinking-water; IARC, 2004).

Exceptionally, case reports and case series may provide compelling evidence about the carcinogenicity of an agent. In fact, many of the early discoveries of occupational cancer hazards came about because of observations by workers and their clinicians, who noted a high frequency of cancer in workers who share a common occupation or exposure. Such observations may be the starting point for more structured investigations, but in exceptional circumstances, when the risk is high enough, the case series may in itself provide compelling evidence. This would be especially warranted in situations where the exposure circumstance is fairly unusual, as it was in the example of plants containing aristolochic acid (IARC, 2012a).

The uncertainties that surround the interpretation of case reports, case series, and ecological studies typically make them inadequate, except in rare instances as described above, to form the sole basis for inferring a causal relationship. However, when considered together with cohort and case–control studies, these types of study may support the judgement that a causal relationship exists.

Epidemiological studies of benign neoplasms, pre-neoplastic lesions, malignant precursors, and other end-points are also reviewed when they relate to the agents reviewed. On occasion they can strengthen inferences drawn from studies of cancer itself. For example, benign brain tumours may share common risk factors with those that are malignant, and benign neoplasms (or those of uncertain behaviour) may be part of the causal path to malignancies (e.g. myelodysplastic syndromes, which may progress to acute myeloid leukaemia).

(b) Identification of eligible studies of cancer in humans

Relevant studies of cancer in humans are identified by using systematic review principles as described in Part A, further elaborated in the Instructions for Authors, and as detailed below. Eligible studies include all studies in humans of exposure to the agent of interest with cancer as an outcome. Multiple publications on the same study population are identified so that the number of independent studies is accurately represented. Multiple publications may result, for example, from successive follow-ups of a single cohort, from analyses focused on different aspects of an exposure–disease association, or from inclusion of overlapping populations. Usually in such situations, only the most recent, most comprehensive, or most informative report is reviewed in detail.

(c) Assessment of study quality and informativeness

Epidemiological studies are potentially susceptible to several different sources of error, summarized briefly below. Qualities of individual studies that address these issues are also described below.

Study quality is assessed as part of the structured expert review process undertaken by the Working Group. A key aspect of quality assessment is consideration of the possible roles of chance and bias in the interpretation of epidemiological studies. Chance, which is also called

random variation, can produce misleading study results. This variability in study results is strongly influenced by the sample size: smaller studies are more likely than larger studies to have effect estimates that are imprecise. Confidence intervals around a study's point estimate of effect are used routinely to indicate the range of values of the estimate that could easily be produced by chance alone.

Bias is the effect of factors in study design or conduct that lead an association to erroneously appear stronger or weaker than the association that really exists between the agent and the disease. Biases that require consideration are varied but are usually categorized as selection bias, information bias (e.g. error in measurement of exposure and diseases), and confounding (or confounding bias), (Rothman et al., 2008). Selection bias in an epidemiological study occurs when inclusion of participants from the eligible population or their follow-up in the study is influenced by their exposure or their outcome (usually disease occurrence). Under these conditions, the measure of association found in the study will not accurately reflect the association that would otherwise have been found in the eligible population (Hernán et al., 2004). Information bias results from inaccuracy in exposure or outcome measurement. Both can cause an association between hypothesized cause and effect to appear stronger or weaker than it really is. Confounding is a mixing of extraneous effects with the effects of interest (Rothman et al., 2008). An association between the purported causal factor and another factor that is associated with an increase or decrease in incidence of disease can lead to a spurious association or absence of a real association of the presumed causal factor with the disease. When either of these occurs, confounding is present.

In assessing study quality, the Working Group consistently considers the following aspects:

- **Study description:** Clarity in describing the study design and its implementation, and the completeness of reporting of all other key information about the study and its results.
- **Study population:** Whether the study population was appropriate for evaluating the association between the agent and cancer. Whether the study was designed and carried out to minimize selection bias. Cancer cases in the study population must have been identified in a way that was independent of the exposure of interest, and exposure assessed in a way that was not related to disease (outcome) status. In these respects, completeness of recruitment into the study from the population of interest and completeness of follow-up for the outcome are essential measures.
- **Outcome measurement:** The appropriateness of the cancer outcome measure (e.g. mortality vs incidence) for the agent and cancer type under consideration, outcome ascertainment methodology, and the extent to which outcome misclassification may have led to bias in the measure(s) of association.
- **Exposure measurement:** The adequacy of the methods used to assess exposure to the agent, and the likelihood (and direction) of bias in the measure(s) of association due to error in exposure measurement, including misclassification (as described in Part B, Section 1f).
- **Assessment of potential confounding:** To what extent the authors took into account in the study design and analysis other variables (including co-exposures, as described in Part B, Section 1d) that can influence the risk of disease and may have been related to the exposure of interest. Important sources of potential confounding by such variables should have been addressed either in the design of the study, such as by matching or restriction, or in the analysis, by statistical adjustment. In some instances, where direct information on confounders is unavailable,

use of indirect methods to evaluate the potential impact of confounding on exposure–disease associations is appropriate (e.g. Axelson & Steenland, 1988; Richardson et al., 2014).

- **Other potential sources of bias:** Each epidemiological study is unique in its study population, its design, its data collection, and, consequently, its potential biases. All possible sources of bias are considered for their possible impact on the results. The possibility of reporting bias (i.e. selective reporting of some results and the suppression of others) should be explored.
- **Statistical methodology:** Adequacy of the statistical methods used and their ability to obtain unbiased estimates of exposure–outcome associations, confidence intervals, and test statistics for the significance of measures of association. Appropriateness of methods used to investigate confounding, including adjusting for matching when necessary and avoiding treatment of probable mediating variables as confounders. Detailed analyses of cancer risks in relation to summary measures of exposure such as cumulative exposure, or temporal variables such as age at first exposure or time since first exposure, are reviewed and summarized when available.

For the sake of economy and simplicity, in this Preamble the list of possible sources of error is referred to with the phrase "chance, bias, and confounding", but it should be recognized that this phrase encompasses a comprehensive set of concerns pertaining to study quality.

These sources of error do not constitute and should not be used as a formal checklist of indicators of study quality. The judgement of experienced experts is critical in determining how much weight to assign to different issues in considering how all of these potential sources of error should be integrated and how to rate the potential for error related to each of these considerations.

The informativeness of a study is its ability to show a true association, if there is one, between the agent and cancer, and the lack of an association, if no association exists. Key determinants of informativeness include: having a study population of sufficient size to obtain precise estimates of effect; sufficient elapsed time from exposure to measurement of outcome for an effect, if present, to be observable; presence of an adequate exposure contrast (intensity, frequency, and/or duration); biologically relevant definitions of exposure; and relevant and well-defined time windows for exposure and outcome.

(d) Meta-analyses and pooled analyses

Independent epidemiological studies of the same agent may lead to inconsistent results that are difficult to interpret or reconcile. Combined analyses of data from multiple studies may be conducted as a means to address this ambiguity. 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 & O'Rourke, 2008).

The strengths of combined analyses are increased precision because of increased sample size and, in the case of pooled analyses, the opportunity to better control for potential confounders and to explore in more detail interactions and modifying effects that may explain heterogeneity among studies. A disadvantage of combined analyses is the possible lack of comparability of data from various studies, because of differences in population characteristics, subject recruitment, procedures of data collection, methods of measurement, and effects of unmeasured covariates that may differ among studies. These differences in study methods and quality can influence

results of either meta-analyses or pooled analyses. If published meta-analyses are to be considered by the Working Group, their adequacy needs to be carefully evaluated, including the methods used to identify eligible studies and the accuracy of data extracted from the individual studies.

The Working Group may conduct ad hoc meta-analyses during the course of a *Monographs* meeting, when there are sufficient studies of an exposure–outcome association to contribute to the Working Group's assessment of the association. The results of such unpublished 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, the following key considerations apply: the same criteria for data quality must be applied as for individual studies; sources of heterogeneity among studies must be carefully considered; and the possibility of publication bias should be explored.

(e) Considerations in assessing the body of epidemiological evidence

The ability of the body of epidemiological evidence to inform the Working Group about the carcinogenicity of the agent is related to both the quantity and the quality of the evidence. There is no formulaic answer to the question of how many studies of cancer in humans are needed from which to draw inferences about causality, although more than a single study in a single population will almost always be needed. The number will depend on the considerations relating to evidence described below.

After the quality of individual epidemiological studies of cancer has been assessed and the informativeness of the various studies on the association between the agent and cancer has been evaluated, a judgement is made about the strength of evidence that the agent in question is carcinogenic to humans. In making its judgement, the Working Group considers several aspects of the body of evidence (e.g. Hill, 1965; Rothman et al., 2008; Vandenbroucke et al., 2016).

A strong association (e.g. a large relative risk) is more likely to indicate causality than is a weak association, because it is more difficult for confounding to falsely create a strong association. However, it is recognized that estimates of effect of small magnitude do not imply lack of causality and may have impact on public health if the disease or exposure is common. Estimates of effect of small magnitude could also contribute useful information to the assessment of causality if level of risk is commensurate with level of exposure when compared with risk estimates from populations with higher exposure (e.g. as seen in residential radon studies compared with studies of radon from uranium mining).

Associations that are consistently observed in several studies of the same design, or in studies that use different epidemiological approaches, or under different circumstances of exposure are more likely to indicate a causal relationship than are isolated observations from single studies. If there are inconsistent results among investigations, possible reasons are sought (e.g. differences in study informativeness because of latency, exposure levels, or assessment methods). Results of studies that are judged to be of high quality and informativeness are given more weight than those of studies judged to be methodologically less sound or less informative.

Temporality of the association is an essential consideration: that is, the exposure must precede the outcome.

An observation that cancer risk increases with increasing exposure is considered to be a strong indication of causality, although the absence of a graded response is not necessarily evidence against a causal relationship, and there are several reasons why the shape of the exposure–response

association may be non-monotonic (e.g. Stayner et al., 2003). 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.

Confidence in a causal interpretation of the evidence from studies of cancer in humans is enhanced if it is coherent with physiological and biological knowledge, including information about exposure to the target organ, latency and timing of the exposure, and characteristics of tumour subtypes.

The Working Group considers whether there are subpopulations with increased susceptibility to cancer from the agent. For example, molecular epidemiology studies that identify associations between genetic polymorphisms and inter-individual differences in cancer susceptibility to the agent(s) being evaluated may contribute to the identification of carcinogenic hazards to humans. Such studies may be particularly informative if polymorphisms are found to be modifiers of the exposure–response association, because evaluation of polymorphisms may increase the ability to detect an effect in susceptible subpopulations.

When, in the process of evaluating the studies of cancer in humans, the Working Group identifies several high-quality, informative epidemiological studies that clearly show either no positive association or an inverse association between an exposure and a specific type of cancer, a judgement may be made that, in the aggregate, they suggest evidence of lack of carcinogenicity for that cancer type. Such a judgement requires, first, that the studies strictly meet 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 ruled out with reasonable confidence. In addition, all studies that are judged to be methodologically sound should (a) be consistent with an estimate of relative effect of unity (or below unity) for any observed level of exposure, (b) when considered together, provide a combined estimate of relative risk that is at or below unity, and (c) have a narrow confidence interval. Moreover, neither any individual well-designed and well-conducted 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 must be noted that evidence of lack of carcinogenicity obtained from several epidemiological studies can apply only to the type(s) of cancer studied, to the exposure levels reported and the timing and route of exposure studied, to the intervals between first exposure and disease onset observed in these studies, and to the general population(s) studied (i.e. there may be susceptible subpopulations or life stages). Experience from studies of cancer in humans indicates that the period from first exposure to the development of clinical cancer is sometimes longer than 20 years; therefore, latency periods substantially shorter than about 30 years cannot provide evidence of lack of carcinogenicity. Furthermore, there may be critical windows of exposure, for example, as with diethylstilboestrol and clear cell adenocarcinoma of the cervix and vagina (IARC, 2012a).

3. Studies of cancer in experimental animals

Most human carcinogens that have been studied adequately for carcinogenicity in experimental animals have produced positive results in one or more animal species. For some agents, carcinogenicity in experimental animals was demonstrated before epidemiological studies identified their carcinogenicity in humans. Although this observation 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) present

a carcinogenic hazard to humans. Accordingly, in the absence of additional scientific information, such as strong evidence that a given agent causes cancer in experimental animals through a species-specific mechanism that does not operate in humans (see Part B, Sections 4 and 6; Capen et al., 1999; IARC, 2003), these agents are considered to pose a potential carcinogenic hazard to humans. The inference of potential carcinogenic hazard to humans does not imply tumour site concordance across species (Baan et al., 2019).

(a) Types of studies considered

Relevant studies of cancer in experimental animals are identified by using systematic review principles as described in Part A, further elaborated in the Instructions for Authors, and as detailed below. Consideration is given to all available long-term studies of cancer in experimental animals with the agent under review (or possibly metabolites or derivatives of the agent) (see Part A, Section 7) after a thorough evaluation of the study features (see Part B, Section 3b). Those studies 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, 2018).

In addition to conventional long-term bioassays, alternative studies (e.g. in genetically engineered mouse models) may be considered in assessing carcinogenicity in experimental animals, also after a critical evaluation of the study features. For studies of certain exposures, such as viruses that typically only infect humans, use of such specialized experimental animal models may be particularly important; models include genetically engineered mice with targeted expression of viral genes to tissues from which human cancers arise, as well as humanized mice implanted with the human cells usually infected by the virus.

Other types of studies can provide supportive evidence. These include: experiments in which the agent was administered in the presence of factors that modify carcinogenic effects (e.g. initiation–promotion studies); studies in which the end-point was not cancer but a defined precancerous lesion; and studies of cancer in non-laboratory animals (e.g. companion animals) exposed to the agent.

(b) Study evaluation

Considerations of importance in the interpretation and evaluation of a particular study include: (i) whether the agent was clearly characterized, including the nature and extent of impurities and contaminants and the stability of the agent, and, in the case of mixtures, whether the sample characterization was adequately reported; (ii) whether the dose was monitored adequately, particularly in inhalation experiments; (iii) whether the doses, duration and frequency of treatment, duration of observation, and route of exposure were appropriate; (iv) whether appropriate experimental animal species and strains were evaluated; (v) whether there were adequate numbers of animals per group; (vi) whether animals were allocated randomly to groups; (vii) whether the body weight, food and water consumption, and survival of treated animals were affected by any factors other than the test agent; (viii) whether the histopathology review was adequate; and (ix) whether the data were reported and analysed adequately.

(c) Outcomes and statistical analyses

An assessment of findings of carcinogenicity in experimental animals involves consideration of (i) study features such as route, doses, schedule and duration of exposure, species, strain (including genetic background where applicable), sex, age, and duration of follow-up; (ii) the spectrum of neoplastic response, from

pre-neoplastic lesions and benign tumours to malignant neoplasms; (iii) the incidence, latency, severity, and multiplicity of neoplasms and pre-neoplastic lesions; (iv) the consistency of the results for a specific target organ or organs across studies of similar design; and (v) the possible role of modifying factors (e.g. diet, infection, stress).

Key factors for statistical analysis include: (i) number of animals studied and number examined histologically, (ii) number of animals with a given tumour type or lesion, and (iii) duration of survival.

Benign tumours may be combined with malignant tumours in the assessment of tumour incidence when (a) they occur together with and originate from the same cell type as malignant tumours in an organ or tissue in a particular study and (b) they appear to represent a stage in the progression to malignancy (Huff et al., 1989). The occurrence of lesions presumed to be pre-neoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed.

Evidence of an increased incidence of neoplasms with increasing level of exposure strengthens the inference of a causal association between the exposure and the development of neoplasms. The form of the dose–response relationship can vary widely, including non-linearity, depending on the particular agent under study and the target organ. The dose–response relationship can also be affected by differences in survival among the treatment groups.

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 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 and a survival-adjusted analysis would be warranted. 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 that 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–Haenszel 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).

The concurrent control group is generally the most appropriate comparison group for statistical analysis; however, for uncommon tumours, 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, sex, 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). 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.

Meta-analyses and pooled analyses may be appropriate when the experimental protocols are sufficiently similar.

4. Mechanistic evidence

Mechanistic data may provide evidence of carcinogenicity and may also help in assessing the relevance and importance of findings of cancer in experimental animals and in humans (Guyton et al., 2009; Parkkinen et al., 2018) (see Part B, Section 6). Mechanistic studies have gained in prominence, increasing in their volume, diversity, and relevance to cancer hazard evaluation, whereas studies pertinent to other streams of evidence evaluated in the *Monographs* (i.e. studies of cancer in humans and lifetime cancer bioassays in rodents) may only be available for a fraction of agents to which humans are currently exposed (Guyton et al., 2009, 2018). Mechanistic studies and data are identified, screened, and evaluated for quality and importance to the evaluation by using systematic review principles as described in Part A, further elaborated in the Instructions for Authors, and as detailed below.

The Working Group's synthesis reflects the extent of available evidence, summarizing groups of included studies with an emphasis on characterizing consistencies or differences in results within and across experimental designs. Greater emphasis is given to informative mechanistic evidence from human-related studies than to that from other experimental test systems, and gaps are identified. Tabulation of data may facilitate this review. The specific topics addressed in the evidence synthesis are described below.

(a) Absorption, distribution, metabolism, and excretion

Studies of absorption, distribution, metabolism, and excretion in mammalian species are addressed in a summary fashion; exposure characterization is addressed in Part B, Section 1. The Working Group describes the metabolic fate of the agent in mammalian species, noting the metabolites that have been identified and their chemical reactivity. A metabolic schema may indicate the relevant metabolic pathways and products and whether supporting evidence is from studies in humans and/or studies in experimental animals. Evidence on other adverse effects that indirectly confirm absorption, distribution, and/or metabolism at tumour sites is briefly summarized when direct evidence is sparse.

(b) Evidence relevant to key characteristics of carcinogens

A review of Group 1 human carcinogens classified up to and including *IARC Monographs* Volume 100 revealed several issues relevant to improving the evaluation of mechanistic evidence for cancer hazard identification (Smith et al., 2016). First, it was noted that human carcinogens often share one or more characteristics that are related to the multiple mechanisms by which agents cause cancer. Second, different human carcinogens may exhibit a different spectrum of these key characteristics and operate through distinct mechanisms. Third, for many carcinogens evaluated before Volume 100, few data were available on some mechanisms of recognized importance in carcinogenesis, such as epigenetic alterations (Herceg et al., 2013). Fourth, there was no widely accepted method to search systematically for relevant mechanistic evidence, resulting in a lack of uniformity in the scope of mechanistic topics addressed across *IARC Monographs* evaluations.

To address these challenges, the key characteristics of human carcinogens were introduced to facilitate systematic consideration of mechanistic evidence in *IARC Monographs* evaluations (Smith et al., 2016; Guyton et al., 2018). The key characteristics described by Smith et al. (2016) (see Table 3), such as "is genotoxic", "is immunosuppressive", or "modulates receptor-mediated

Table 3 The key characteristics of carcinogens

Ten key characteristics of carcinogens	
1.	Is electrophilic or can be metabolically activated to an electrophile
2.	Is genotoxic
3.	Alters DNA repair or causes genomic instability
4.	Induces epigenetic alterations
5.	Induces oxidative stress
6.	Induces chronic inflammation
7.	Is immunosuppressive
8.	Modulates receptor-mediated effects
9.	Causes immortalization
10.	Alters cell proliferation, cell death, or nutrient supply

From Smith et al. (2016).

effects", are based on empirical observations of the chemical and biological properties associated with the human carcinogens identified by the *IARC Monographs* programme up to and including Volume 100. The list of key characteristics and associated end-points may evolve, based on the experience of their application and as new human carcinogens are identified. Key characteristics are distinct from the "hallmarks of cancer", which relate to the properties of cancer cells (Hanahan & Weinberg, 2000, 2011). Key characteristics are also distinct from hypothesized mechanistic pathways, which describe a sequence of biological events postulated to occur during carcinogenesis. As such, the evaluation approach based on key characteristics, outlined below, "avoids a narrow focus on specific pathways and hypotheses and provides for a broad, holistic consideration of the mechanistic evidence" (National Academies of Sciences, Engineering, and Medicine, 2017).

Studies in exposed humans and in human primary cells or tissues that incorporate end-points relevant to key characteristics of carcinogens are emphasized when available. For each key characteristic with adequate evidence for evaluation, studies are grouped according to whether they involve (a) humans or human primary cells or tissues or (b) experimental systems; further organization (as appropriate) is by end-point (e.g. DNA damage), duration, species, sex, strain, and target organ as well as strength of study design. Studies investigating susceptibility related to key characteristics of carcinogens (e.g. of genetic polymorphisms, or in genetically engineered animals) can be highlighted and may provide additional support for conclusions on the strength of evidence. Findings relevant to a specific tumour type may be noted.

(c) Other relevant evidence

Other informative evidence may be described when it is judged by the Working Group to be relevant to an evaluation of carcinogenicity and to be of sufficient importance to affect the overall evaluation. Quantitative structure–activity information, such as on specific chemical and/or biological features or activities (e.g. electrophilicity, molecular docking with receptors), may be informative. In addition, evidence that falls outside of the recognized key characteristics of carcinogens, reflecting emerging knowledge or important novel scientific developments on carcinogen mechanisms, may also be included. Available evidence relevant to criteria provided in authoritative publications (e.g. Capen et al., 1999; IARC, 2003) on thyroid, kidney, urinary

bladder, or other tumours in experimental animals induced by mechanisms that do not operate in humans is also described.

(d) Study quality and importance to the evaluation

Based on formal considerations of the quality of the studies (e.g. design, methodology, and reporting of results), the Working Group may give greater weight to some included studies.

For observational and other studies in humans, the quality of study design, exposure assessment, and assay accuracy and precision are considered, in collaboration with the Working Group members reviewing exposure characterization and studies of cancer in humans, as are other important factors, including those described above for evaluation of epidemiological evidence (García-Closas et al., 2006, 2011; Vermeulen et al., 2018) (Part B, Sections 1 and 2).

In general, in experimental systems, studies of repeated doses and of chronic exposures are accorded greater importance than are studies of a single dose or time-point. Consideration is also given to factors such as the suitability of the dosing range, the extent of concurrent toxicity observed, and the completeness of reporting of the study (e.g. the source and purity of the agent, the analytical methods, and the results). Route of exposure is generally considered to be a less important factor in the evaluation of experimental studies, recognizing that the exposures and target tissues may vary across experimental models and in exposed human populations. Non-mammalian studies can be synthetically summarized when they are considered to be supportive of evidence in humans or higher organisms.

In vitro test systems can provide mechanistic insights, but important considerations include the limitations of the test system (e.g. in metabolic capabilities) as well as the suitability of a particular test article (i.e. because of physical and chemical characteristics) (Hopkins et al., 2004). For studies on some end-points, such as for traditional studies of mutations in bacteria and in mammalian cells, formal guidelines, including those from the Organisation for Economic Co-operation and Development, may be informative in conducting the quality review (OECD, 1997, 2016a, b). However, existing guidelines will not generally cover all relevant assays, even for genotoxicity. Possible considerations when evaluating the quality of in vitro studies encompass the methodology and design (e.g. the end-point and test method, the number of replicate samples, the suitability of the concentration range, the inclusion of positive and negative controls, and the assessment of cytotoxicity) as well as reporting (e.g. of the source and purity of the agent, and of the analytical methods and results). High-content and high-throughput in vitro data can serve as an additional or supportive source of mechanistic evidence (Chiu et al., 2018; Guyton et al., 2018), although large-scale screening programmes measuring a variety of end-points were designed to evaluate large chemical libraries in order to prioritize chemicals for additional toxicity testing rather than to identify the hazard of a specific chemical or chemical group.

The synthesis is focused on the evidence that is most informative for the overall evaluation. In this regard, it is of note that some human carcinogens exhibit a single or primary key characteristic, evidence of which has been influential in their cancer hazard classifications. For instance, ethylene oxide is genotoxic (IARC, 1994), 2,3,7,8-tetrachlorodibenzo-*para*-dioxin modulates receptor-mediated effects (IARC, 1997), and etoposide alters DNA repair (IARC, 2012a). Similarly, oncogenic viruses cause immortalization, and certain drugs are, by design, immunosuppressive (IARC, 2012a, b). Because non-carcinogens can also induce oxidative stress, this key characteristic should be interpreted with caution unless it is found in combination

with other key characteristics (Guyton et al., 2018). Evidence for a group of key characteristics can strengthen mechanistic conclusions (e.g. "induces oxidative stress" together with "is electrophilic or can be metabolically activated to an electrophile", "induces chronic inflammation", and "is immunosuppressive"); see, for example, 1-bromopropane (IARC, 2018).

5. Summary of data reported

(a) Exposure characterization

Exposure data are summarized to identify the agent and describe its production, use, and occurrence. Information on exposure prevalence and intensity in different settings, including geographical patterns and time trends, may be included. Exposure assessment methods used in key epidemiological studies reviewed by the Working Group are described and evaluated.

(b) Cancer in humans

Results of epidemiological studies pertinent to an evaluation of carcinogenicity in humans are summarized. The overall strengths and limitations of the epidemiological evidence base are highlighted to indicate how the evaluation was reached. The target organ(s) or tissue(s) in which a positive association between the agent and cancer was observed are identified. Exposure–response and other quantitative data may be summarized when available. When the available epidemiological studies pertain to a mixed exposure, process, occupation, or industry, the Working Group seeks to identify the specific agent considered to be most likely to be responsible for any excess risk. The evaluation is focused as narrowly as the available data permit.

(c) Cancer in experimental animals

Results pertinent to an evaluation of carcinogenicity in experimental animals are summarized to indicate how the evaluation was reached. For each animal species, study design, and route of administration, there is a statement about whether an increased incidence, reduced latency, or increased severity or multiplicity of neoplasms or pre-neoplastic lesions was observed, and the tumour sites are indicated. Special conditions resulting in tumours, such as prenatal exposure or single-dose experiments, are mentioned. Negative findings, inverse relationships, dose–response patterns, and other quantitative data are also summarized.

(d) Mechanistic evidence

Results pertinent to an evaluation of the mechanistic evidence on carcinogenicity are summarized to indicate how the evaluation was reached. The summary encompasses the informative studies on absorption, distribution, metabolism, and excretion; on the key characteristics with adequate evidence for evaluation; and on any other aspects of sufficient importance to affect the overall evaluation, including on whether the agent belongs to a class of agents for which one or more members have been classified as carcinogenic or probably carcinogenic to humans, and on criteria with respect to tumours in experimental animals induced by mechanisms that do not operate in humans. For each topic addressed, the main supporting findings are highlighted from exposed humans, human cells or tissues, experimental animals, or in vitro systems. When mechanistic studies are available in exposed humans, the tumour type or target tissue studied may be specified. Gaps in the evidence are indicated (i.e. if no studies were available in exposed humans, in in vivo systems, etc.). Consistency or differences of effects across different experimental systems are emphasized.

6. Evaluation and rationale

Consensus evaluations of the strength of the evidence of cancer in humans, the evidence of cancer in experimental animals, and the mechanistic evidence are made using transparent criteria and defined descriptive terms. The Working Group then develops a consensus overall evaluation of the strength of the evidence of carcinogenicity for each agent under review.

An evaluation of the strength of the evidence is limited to the agents under review. When multiple agents being evaluated are considered by the Working Group to be sufficiently closely related, they may be grouped together for the purpose of a single and unified evaluation of the strength of the evidence.

The framework for these evaluations, described below, may not encompass all factors relevant to a particular evaluation of carcinogenicity. After considering all relevant scientific findings, the Working Group may exceptionally assign the agent to a different category than a strict application of the framework would indicate, while providing a clear rationale for the overall evaluation.

When there are substantial differences of scientific interpretation among the Working Group members, the overall evaluation will be based on the consensus of the Working Group. A summary of the alternative interpretations may be provided, together with their scientific rationale and an indication of the relative degree of support for each alternative.

The categories of the classification refer to the strength of the evidence that an exposure is carcinogenic and not to the risk of cancer from particular exposures. The terms *probably carcinogenic* and *possibly carcinogenic* have no quantitative significance and are used as descriptors of different strengths of evidence of carcinogenicity in humans; *probably carcinogenic* signifies a greater strength of evidence than *possibly carcinogenic*.

(a) Carcinogenicity in humans

Based on the principles outlined in Part B, Section 2, the evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

***Sufficient evidence of carcinogenicity*:** A causal association between exposure to the agent and human cancer has been established. That is, a positive association has been observed in the body of evidence on exposure to the agent and cancer in studies in which chance, bias, and confounding were ruled out with reasonable confidence.

***Limited evidence of carcinogenicity*:** A causal interpretation of the positive association observed in the body of evidence on exposure to the agent and cancer is credible, but chance, bias, or confounding could not be ruled out with reasonable confidence.

***Inadequate evidence regarding carcinogenicity*:** The available studies are of insufficient quality, consistency, or statistical precision to permit a conclusion to be drawn about the presence or the absence of a causal association between exposure and cancer, or no data on cancer in humans are available. Common findings that lead to a determination of inadequate evidence of carcinogenicity include: (a) there are no data available in humans; (b) there are data available in humans, but they are of poor quality or informativeness; and (c) there are studies of sufficient quality available in humans, but their results are inconsistent or otherwise inconclusive.

***Evidence suggesting lack of carcinogenicity*:** There are several high-quality 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 the studied cancers at any

observed level of exposure. The results from these studies alone or combined should have narrow confidence intervals with an upper limit below or close to the null value (e.g. a relative risk of unity). Bias and confounding were ruled out with reasonable confidence, and the studies were considered informative. A conclusion of *evidence suggesting lack of carcinogenicity* is limited to the cancer sites, populations and life stages, 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.

When there is *sufficient evidence*, a separate sentence identifies the target organ(s) or tissue(s) for which a causal interpretation has been established. When there is *limited evidence*, a separate sentence identifies the target organ(s) or tissue(s) for which a positive association between exposure to the agent and the cancer(s) was observed in humans. When there is *evidence suggesting lack of carcinogenicity*, a separate sentence identifies the target organ(s) or tissue(s) where evidence of lack of carcinogenicity was observed in humans. Identification of a specific target organ or tissue as having *sufficient evidence* or *limited evidence* or *evidence suggesting lack of carcinogenicity* does not preclude the possibility that the agent may cause cancer at other sites.

(b) Carcinogenicity in experimental animals

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

***Sufficient evidence of carcinogenicity*:** A causal relationship has been established between exposure to the agent and cancer in experimental animals based on 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 and/or under different protocols. An increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices (GLP), can also provide *sufficient evidence*.

Exceptionally, a single study in one species and sex may 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 marked 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, for example, (a) the evidence of carcinogenicity is restricted to a single experiment and does not meet the criteria for *sufficient evidence*; (b) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; (c) the agent increases tumour multiplicity or decreases tumour latency but does not increase tumour incidence; (d) the evidence of carcinogenicity is restricted to initiation–promotion studies; (e) the evidence of carcinogenicity is restricted to observational studies in non-laboratory animals (e.g. companion animals); or (f) there are unresolved questions about the adequacy of the design, conduct, or interpretation of the available studies.

***Inadequate evidence regarding carcinogenicity*:** The studies cannot be interpreted as showing either the presence or the absence

of a carcinogenic effect because of major qualitative or quantitative limitations, or no data are available on cancer in experimental animals.

***Evidence suggesting lack of carcinogenicity*:** Well-conducted studies (e.g. conducted under GLP) involving both sexes of at least two species are available showing that, within the limits of the tests used, the agent was not carcinogenic. The conclusion of *evidence suggesting lack of carcinogenicity* is limited to the species, tumour sites, age at exposure, and conditions and levels of exposure covered by the available studies.

(c) Mechanistic evidence

Based on the principles outlined in Part B, Section 4, the mechanistic evidence is classified into one of the following categories:

***Strong mechanistic evidence*:** Results in several different experimental systems are consistent, and the overall mechanistic database is coherent. Further support can be provided by studies that demonstrate experimentally that the suppression of key mechanistic processes leads to the suppression of tumour development. Typically, a substantial number of studies on a range of relevant end-points are available in one or more mammalian species. Quantitative structure–activity considerations, in vitro tests in non-human mammalian cells, and experiments in non-mammalian species may provide corroborating evidence but typically do not in themselves provide strong evidence. However, consistent findings across a number of different test systems in different species may provide strong evidence.

Of note, "strong" relates not to potency but to strength of evidence. The classification applies to three distinct topics:

(a) Strong evidence that the agent belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified as carcinogenic or probably carcinogenic to humans. The considerations can go beyond quantitative structure–activity relationships to incorporate similarities in biological activity relevant to common key characteristics across dissimilar chemicals (e.g. based on molecular docking, –omics data).

(b) Strong evidence that the agent exhibits key characteristics of carcinogens. In this case, three descriptors are possible:

1. The strong evidence is in exposed humans. Findings relevant to a specific tumour type may be informative in this determination.
2. The strong evidence is in human primary cells or tissues. Specifically, the strong findings are from biological specimens obtained from humans (e.g. ex vivo exposure), from human primary cells, and/or, in some cases, from other humanized systems (e.g. a human receptor or enzyme).
3. The strong evidence is in experimental systems. This may include one or a few studies in human primary cells and tissues.

(c) Strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans. Certain results in experimental animals (see Part B, Section 6b) would be discounted, according to relevant criteria and considerations in authoritative publications (e.g. Capen et al., 1999; IARC, 2003). Typically, this classification would not apply when there is strong mechanistic evidence that the agent exhibits key characteristics of carcinogens.

***Limited mechanistic evidence*:** The evidence is suggestive, but, for example, (a) the studies cover a narrow range of experiments, relevant end-points, and/or species; (b) there are unexplained inconsistencies in the studies of similar design; and/or (c) there is unexplained incoherence across studies of different end-points or in different experimental systems.

***Inadequate mechanistic evidence*:** Common findings that lead to a determination of inadequate mechanistic evidence include: (a) few or no data are available; (b) there are unresolved questions about the adequacy of the design, conduct, or interpretation of the studies; (c) the available results are negative.

(d) Overall evaluation

Finally, the bodies of evidence included within each stream of evidence are considered as a whole, in order to reach an overall evaluation of the carcinogenicity of the agent to humans. The three streams of evidence are integrated and the agent is classified into one of the following categories (see Table 4), indicating that the Working Group has established that:

The agent is carcinogenic to humans (Group 1)

This category applies whenever there is *sufficient evidence of carcinogenicity* in humans.

In addition, this category may apply when there is both *strong evidence in exposed humans that the agent exhibits key characteristics of carcinogens* and *sufficient evidence of carcinogenicity* in experimental animals.

The agent is probably carcinogenic to humans (Group 2A)

This category generally applies when the Working Group has made at least *two of the following* evaluations, *including at least one* that involves either exposed humans or human cells or tissues:

- *Limited evidence of carcinogenicity* in humans,
- *Sufficient evidence of carcinogenicity* in experimental animals,
- *Strong evidence that the agent exhibits key characteristics of carcinogens.*

If there is *inadequate evidence regarding carcinogenicity* in humans, there should be *strong evidence in human cells or tissues that the agent exhibits key characteristics of carcinogens*. If there is *limited evidence of carcinogenicity in humans*, then the second individual evaluation may be from experimental systems (i.e. s*ufficient evidence of carcinogenicity* in experimental animals or *strong evidence in experimental systems that the agent exhibits key characteristics of carcinogens).*

Additional considerations apply when there is *strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans* for one or more tumour sites. Specifically, the remaining tumour sites should still support an evaluation of *sufficient evidence in experimental animals* in order for this evaluation to be used to support an overall classification in Group 2A.

Separately, this category generally applies if there is *strong evidence that the agent 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*.

The agent is possibly carcinogenic to humans (Group 2B)

This category generally applies when only one of the following evaluations has been made by the Working Group:

- *Limited evidence of carcinogenicity* in humans,
- *Sufficient evidence of carcinogenicity* in experimental animals,

Table 4 Integration of streams of evidence in reaching overall classifications (the evidence in *bold italic* represents the basis of the overall evaluation)

Stream of evidence			Classification based on strength of evidence
Evidence of cancer in humans[a]	**Evidence of cancer in experimental animals**	**Mechanistic evidence**	
Sufficient	Not necessary	Not necessary	**Carcinogenic to humans (Group 1)**
Limited or Inadequate	***Sufficient***	***Strong (b)(1) (exposed humans)***	
Limited	***Sufficient***	Strong (b)(2–3), Limited, or Inadequate	**Probably carcinogenic to humans (Group 2A)**
Inadequate	***Sufficient***	***Strong (b)(2) (human cells or tissues)***	
Limited	Less than Sufficient	***Strong (b)(1–3)***	
Limited or Inadequate	Not necessary	***Strong (a) (mechanistic class)***	
Limited	Less than Sufficient	Limited or Inadequate	**Possibly carcinogenic to humans (Group 2B)**
Inadequate	***Sufficient***	Strong (b)(3), Limited, or Inadequate	
Inadequate	Less than Sufficient	***Strong b(1–3)***	
Limited	***Sufficient***	***Strong (c) (does not operate in humans)***[b]	
Inadequate	***Sufficient***	***Strong (c) (does not operate in humans)***[b]	**Not classifiable as to its carcinogenicity to humans (Group 3)**
All other situations not listed above			

[a] Human cancer(s) with highest evaluation

[b] The *strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans* must specifically be for the tumour sites supporting the classification of *sufficient evidence in experimental animals*.

• *Strong evidence that the agent exhibits key characteristics of carcinogens.*

Because this category can be based on evidence from studies in experimental animals alone, there is **no** requirement that the strong mechanistic evidence be in exposed humans or in human cells or tissues. This category may be based on *strong evidence in experimental systems that the agent exhibits key characteristics of carcinogens.*

As with Group 2A, additional considerations apply when there is *strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans* for one or more tumour sites. Specifically, the remaining tumour sites should still support an evaluation of *sufficient evidence in experimental animals* in order for this evaluation to be used to support an overall classification in Group 2B.

The agent is not classifiable as to its carcinogenicity to humans (Group 3)

Agents that do not fall into any other group are generally placed in this category.

This includes the case when there is *strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans* for one or more tumour sites in experimental animals, the remaining tumour sites do not support an evaluation of *sufficient evidence in experimental animals*, and other categories are not supported by data from studies in humans and mechanistic studies.

An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that the agent is of unknown carcinogenic potential and that there are significant gaps in research.

If the evidence suggests that the agent exhibits no carcinogenic activity, either through *evidence suggesting lack of carcinogenicity* in both humans and experimental animals, or through

evidence suggesting lack of carcinogenicity in experimental animals complemented by strong negative mechanistic evidence in assays relevant to human cancer, then the Working Group may add a sentence to the evaluation to characterize the agent as well-studied and without evidence of carcinogenic activity.

(e) Rationale

The reasoning that the Working Group used to reach its evaluation is summarized so that the basis for the evaluation offered is transparent. This section integrates the major findings from studies of cancer in humans, cancer in experimental animals, and mechanistic evidence. It includes concise statements of the principal line(s) of argument that emerged in the deliberations of the Working Group, the conclusions of the Working Group on the strength of the evidence for each stream of evidence, an indication of the body of evidence that was pivotal to these conclusions, and an explanation of the reasoning of the Working Group in making its evaluation.

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GENERAL REMARKS

This one-hundred-and-twenty-fifth volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of five High Production Volume chemicals: allyl chloride, 1-bromo-3-chloropropane, 1-butyl glycidyl ether, 4-chlorobenzotrifluoride, and glycidyl methacrylate.

Allyl chloride was considered previously by the Working Group in Volume 36, Supplement 7, and Volume 71 of the *IARC Monographs* (IARC, 1985, 1987, 1999), when it was evaluated as *not classifiable as to its carcinogenicity to humans (Group 3)* because of *inadequate evidence* in humans and in experimental animals. New data have become available, primarily good laboratory practice-compliant bioassays in experimental animals, and these data have been included and considered in the present volume. The other four agents have not been previously evaluated by the *IARC Monographs* Working Group.

A summary of the findings of this volume appears in *The Lancet Oncology* (Rusyn et al., 2020).

Occupational exposure

The extensive uses of these industrial chemicals and solvents and the fact that they are all High Production Volume chemicals suggest that there may be occupational exposure, primarily through inhalation and dermal contact during production and use as industrial intermediates, but quantitative data were very few or non-existent for all five chemicals.

4-Chlorobenzotrifluoride

Apart from its industrial applications, 4-chlorobenzotrifluoride is used as a component (at up to approximately 70%) in consumer products for cosmetic stain removal and aerosol, rust prevention, floor wax finishes, and sealers (HSDB, 2011; Lee et al., 2015). Exposure of the general population is therefore highly likely, but no exposure data are available.

Glycidyl methacrylate

Glycidyl methacrylate is used as an important component of many polymers and resins. These polymers are widely used in many industries for a variety of purposes (see Section 1, Exposure Characterization). Glycidyl methacrylate-based polymers are also increasingly used for medical applications such as hydrogel contact lenses (Musgrave & Fang, 2019), polymeric prodrug microspheres for imaging-guided diagnosis and chemotherapy (Pei et al., 2019), and for dental

material ingredients, composite resins and sealants (Vervliet et al., 2018).

Owing to its interesting chemical and physical properties, glycidyl methacrylate is currently the focus of intense research for the elaboration of new composites, especially for biomaterials (Monmaturapoj et al., 2017). Glycidyl methacrylate is not expected to be released from resins or polymers containing glycidyl methacrylate or resins, so exposure to the general population is unlikely; however, no direct data were available.

Glycidyl methacrylate is a member of a family of chemicals that possess an epoxy ring, and which includes glycidol, a structural analogue and metabolite of glycidyl methacrylate that was previously classified by the Working Group as *probably carcinogenic to humans (Group 2A)* (IARC, 2000).

Scope of systematic review

Standardized searches of the PubMed database were conducted for each agent and for each outcome (cancer in humans, cancer in experimental animals, and mechanistic evidence, including the key characteristics of carcinogens). The literature trees for each agent, including the full set of search terms for the agent name and each outcome type, are available online.[1]

For most of the agents evaluated by the Working Group at this meeting, there were very few articles in the published literature. There were no epidemiological data for four of the agents, and only one study was available for allyl chloride. Mechanistic evidence was sparse. Several technical reports made publicly available by the United States Environmental Protection Agency (US EPA) provided relevant mechanistic data that was included in the monographs on glycidyl methacrylate, 1-bromo-3-chloropropane, and 1-butyl glycidyl ether.

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[1] The literature searches for the present volume are available from: https://hawcproject.iarc.who.int/assessment/615/ (allyl chloride), https://hawcproject.iarc.who.int/assessment/616/ (1-bromo-3-chloropropane), https://hawcproject.iarc.who.int/assessment/618/ (1-butyl glycidyl ether), https://hawcproject.iarc.who.int/assessment/617/ (4-chlorobenzotrifluoride), https://hawcproject.iarc.who.int/assessment/620/ (glycidyl methacrylate).

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ALLYL CHLORIDE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

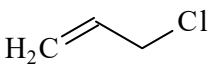
Chem. Abstr. Serv. Reg. No.: 107-05-1

Chem. Abstr. Serv. name: 3-chloro-1-propene

IUPAC systematic name: 3-chloropropene

Synonyms: allyl chloride; 3-chloro-1-propene; 3-chloropropene; 3-chloropropylene; 2-propenyl chloride; α-chloropropylene; chlorallylene; 1-chloro propene-2; 3-chloro-1-propylene; 1-chloro-2-propene; chloroallylene; 3-chloroprene; 3-chloropropene-1; propene, 3-chloro; 2-propenyl chloride.

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: C_3H_5Cl

Relative molecular mass: 76.53

1.1.3 Chemical and physical properties of the pure substance

Description: clear colourless liquid with an unpleasant pungent odour

Boiling point: 44.4 °C (HSDB, 2006)

Melting point: –134.5 °C (HSDB, 2006)

Density: 0.938 at 20 °C (O'Neil, 2013)

Vapour density: 2.64 (air = 1) (HSDB, 2006)

Solubility: slightly soluble in water, 3370 mg/L at 25 °C; miscible with alcohol, chloroform, ether, petrol ether (HSDB, 2006; O'Neil, 2013)

Volatility: 368 mm Hg at 25 °C [49.1 kPa] (HSDB, 2006)

Stability: highly flammable (CAMEO, 2019)

Reactivity: strong reducing agent and decomposes at higher temperatures (CAMEO, 2019)

Flammability: highly flammable: will be easily ignited by heat, sparks or flames; vapours may form explosive mixtures with air (HSDB, 2006)

Flash point: –27 °C (closed cup) (Krahling et al., 2011)

Auto-ignition temperature: 392 °C (Krahling et al., 2011)

*Octanol/water partition coefficient (*P*)*: log K_{ow} = 1.93 (estimated) (HSDB, 2006)

Conversion factor: 1 ppm is equivalent to 3.13 mg/m^3 at normal temperature (25 °C) and pressure (101.3 kPa).

1.1.4 Technical grade and impurities

Commercial allyl chloride used for the production of dichlorohydrin has a purity of at least 97.5%, and contains mainly 1-chloropropene, 1-chloropropane, and 1,5-hexadiene

as impurities (Krahling et al., 2011). The crude allyl chloride also contains as by-products smaller amounts of other aliphatic and cycloaliphatic hexene and hexadiene isomers, normal hexenes, methylpentenes, methylcyclopentenes and methylcyclopentadienes, and these are also present in the conventionally purified allyl chloride (De Jong & Nisbet, 1998).

1.2 Production and uses

1.2.1 Production

(a) Production process

Allyl chloride is produced on a large scale by the high-temperature (300–600 °C) chlorination of propene. At reactor temperatures higher than 500–510 °C, spontaneous pyrolysis occurs, resulting in the formation of soot and high-boiling tars. At reactor temperatures of about 600 °C, benzene can be formed (Krahling et al., 2011).

(b) Production volume

Allyl chloride is listed by the Organisation for Economic Co-operation and Development (OECD) as a High Production Volume chemical (OECD, 2009) Currently the majority of the manufacturing facilities are located in the USA, with fewer sites located in Europe and Asia (ChemSources, 2019). An estimated 800 000 tonnes were produced worldwide in 1997 (Krahling et al., 2011). An overview of historical production volumes in the USA is provided in Table 1.1, with the most recent estimate for 2016 being 113 000–227 000 tonnes. In Canada in 2006, no company reported manufacturing or importing allyl chloride in a quantity greater than or equal to the reporting threshold of 100 kg (Environment Canada, 2009). The quantity reported to be manufactured, imported or in commerce in Canada during the calendar year 1986 was 201 tonnes (Environment Canada, 2009). In 1982, production of allyl chloride in Japan was reported to range from 30 000 to 40 000 tonnes (IARC, 1985). For the European Union, the European Chemicals Agency (ECHA) provides no data on tonnage band as allyl chloride has been registered for use as a chemical intermediate only (ECHA, 2019).

1.2.2 Uses

Approximately 90% of all allyl chloride produced is used to synthesize epichlorohydrin, which is used as a basic building block for epoxy resins and in glycerol synthesis (Krahling et al., 2011). Allyl chloride is also used in the manufacture of intermediates for downstream derivatives such as other polymers, resins, and plastic materials, in processes to increase oil production, in the preparation and modification of catalysts, and in the manufacture of pesticides, adhesives, flame retardants, chelating agents, detergents, dyestuffs, flavourings, metal brighteners, perfumes, pharmaceuticals, and urethanes (Olin Corporation, 2016). Acrylic polymers synthesized using allyl chloride are used in personal-care products such as showering soaps or gels, hair conditioners, hair dyes, hair styling gels, hair shampoos, facial cleansers, facial makeup, aftershaves, shaving soaps, creams or foams, skin creams and skin peeling or scrubbing preparations (Environment Canada, 2009).

1.3 Methods of measurement and analysis

1.3.1 Detection and quantification

(a) Air monitoring

In air, allyl chloride can be measured by adsorbing on a coconut shell charcoal, desorption with benzene, and analysis by gas chromatography and flame ionization detection (GC-FID) with an absolute detection limit of 0.01 mg per sample based on National Institute for Occupational Safety and Health (NIOSH)

Table 1.1 Historical production volumes of allyl chloride in the USA

Year	Reported estimated production volume	Reference
1977	180 thousand tonnes	HSDB (2006)
1979	190 thousand tonnes	HSDB (2006)
1986	> 500 million to 1 billion pounds [~200–500 thousand tonnes]	HSDB (2006)
1990	> 500 million to 1 billion pounds [~200–500 thousand tonnes]	HSDB (2006)
1994	> 1 billion pounds [~500 thousand tonnes]	HSDB (2006)
1998	> 500 million to 1 billion pounds [~200–500 thousand tonnes]	HSDB (2006)
2002	> 500 million to 1 billion pounds [~200–500 thousand tonnes]	HSDB (2006)
2011	629 million pounds [~285 thousand tonnes]	US EPA (2016)
2012	750 million to 1 billion pounds [~340–500 thousand tonnes]	US EPA (2016)
2013–2016	250–500 million pounds [~110–200 thousand tonnes]	US EPA (2016)

method 1000 (NIOSH, 1994). The working range is 0.5 to 10 mg/m^3 for a 100 L air sample. A similar method based on the use of activated charcoal tubes and desorption using dichloromethane, separation and analysis by GC-FID has been recently described (Li et al., 2015). In a 7.5 L air sample, the minimum detectable concentration was 0.03 mg/m^3.

(b) Water analysis

In water, allyl chloride can be measured by capillary column gas chromatography and mass spectrometry with a detection limit of 0.13 µg/L based on method EPA-NERL 524.2 (NEMI, 1995).

(c) Other matrices

In ground water, aqueous sludges, caustic liquors, waste solvents, oily wastes, mousses, tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils, and sediments, allyl chloride can be measured by gas chromatography using photoionization and/or electrolytic conductivity detectors based on method EPA-OSW 8021B (NEMI, 1996a) or by gas chromatography-mass spectrometry (GC-MS) based on method EPA-OSW 8260B (NEMI, 1996b).

(d) Biomarkers

Allylmercapturic acid (ALMA) is established as a urinary biomarker of exposure to allyl chloride in humans. ALMA was identified in urine collected before and after shift from workers occupationally exposed to airborne allyl chloride; the increase in ALMA concentrations in urine during a work shift correlated well with the 8-hour time-weighted average (TWA) personal air exposure to allyl chloride. ALMA was isolated from acidified urine samples, extracted by solid-phase extraction and detected with GC-MS (de Rooij et al., 1997). ALMA can also be detected in urine after ingestion of allium vegetables such as garlic (de Rooij et al., 1997). [The Working Group noted that this may limit the usefulness of ALMA as a biomarker of allyl chloride in the general population.]

[The Working Group noted that there was also a quantitative determination for allyl chloride in rat blood samples based on gas chromatography with electron-capture detection (GC-ECD) and GC-MS (Kropscott et al., 1983). This method could be useful for exposure assessment for allyl chloride in humans.]

1.4 Occurrence and Exposure

1.4.1 Natural occurrence

Allyl chloride has not been reported to occur naturally in the environment.

1.4.2 Environmental occurrence

An overview of environmental exposure measurements of allyl chloride in indoor and outdoor air and in water is provided in Table 1.2. Reported exposures were typically below, or close to, the limit of detection.

The production and use of allyl chloride as a chemical intermediate may result in its release to the environment through various waste streams (HSDB, 2006). On the basis of its physical and chemical properties (see Section 1.1.3) and depending on the compartments to which it is released, allyl chloride is estimated to reside predominantly in air and/or water (Environment Canada, 2009). Allyl chloride is rapidly removed from the atmosphere (calculated half-life for reaction with photochemically produced hydroxyl radicals in air is less than 1 day) (OECD-SIDS, 1996). At slower rates, allyl chloride has also been reported to degrade through reaction with atmospheric ozone (Winer & Atkinson, 1987). Allyl chloride is not susceptible to direct photolysis by sunlight (HSDB, 2006). Concentrations in indoor air are assumed to be low due to its short half-life in air (Environment Canada, 2009).

Volatilization is expected to be the most significant loss process for the allyl chloride in water. Hydrolysis and biodegradation may also occur, but at slower rates (Environment Canada, 2009).

1.4.3 Occupational exposure

Allyl chloride is a highly reactive, toxic, and easily ignitable substance (see Section 1.1.3) and is therefore primarily handled in closed systems (Krahling et al., 2011). Occupational exposure to allyl chloride may occur through inhalation and dermal contact at workplaces where it is produced or used (HSDB, 2006). An overview of measurements of occupational exposure to allyl chloride is provided in Table 1.3. Exposure levels vary widely depending on type of manufacturing plant, job title, country, and year in which the measurements were taken.

1.4.4 Exposure of the general population

Exposure of the general population is possible through inhalation of ambient and indoor air, and the use of personal-care products containing the allyl chloride as a residue (including a potential for dermal exposure). However, residue levels in the personal-care products have been estimated to be very low (0.01%) (Environment Canada, 2009).

Upper-bound estimates of allyl chloride intake for each age group in the general population of Canada from environmental media range from 0.52 µg/kg body weight (bw) per day (in people aged ≥ 60 years) to 1.56 µg/kg bw per day (in children aged 0.5–4 years) and indicate that air is the most important source (comprising 99% of total exposure) (Environment Canada, 2009).

1.5 Regulations and guidelines

ECHA harmonized classification labels allyl chloride as a germ cell mutagen (Category 2) and as causing cancer (Category 2) (ECHA, 2019). Table 1.4 gives an overview of various international legally binding exposure limits for allyl chloride collated in the GESTIS database (Information system on hazardous substances of the German Social Accident Insurance; IFA, 2020). Legally binding exposure limits in the USA are consistent with the health-based exposure guidelines of the American Conference of

Table 1.2 Overview of the occurrence of allyl chloride in outdoor and indoor air and in water

Location, collection date	Sampling matrix	Number of samples	Exposure level	Exposure range	Limit of detection	Comments	Reference
Ohio, USA, 2015	Air	11	Average, 0.00 ppb	0.02–0.11 ppb [0.06–0.34 µg/m³]	NR	24-hour samples collected with a whole air sampling system The Working Group noted that the reported average exposure appeared to be inconsistent with the reported range	Ohio EPA (2016)
Olathe, Kansas, USA, 2000	Indoor air	NR	All measurements < LOD	NA	NR	Study of five homes located near an industrial site possibly acting as a point source of allyl chloride emissions	ATSDR (2001), as reported in Environment Canada (2009)
Woodland, California, USA, 1990	Indoor air	NR	All measurements < LOD	NA	0.6 µg/m³	Study of 125 homes	OEHHA (1999)
Denver, Houston, Riverside, St Louis, USA, 1980	Ambient air	NR	All measurements < LOD		0.016 µg/m³	A 24-hour around-the-clock measurement schedule for 1–2 weeks in four cities	Singh et al. (1982)
Pittsburgh, USA, 1981	Ambient air	NR	6 ppt [0.02 µg/m³]	< 1–19 ppt [< 0.003–0.059 µg/m³]	0.016 µg/m³	A 24-hour around-the-clock measurement schedule for 1–2 weeks	Singh et al. (1982)
Lima, Allen County, Ohio, USA, 1990–1991	Ambient air	21	0.16 µg/m³	Maximum, 0.32 µg/m³	NR		Kelly et al. (1991) as reported in Environment Canada (2009)
32 locations in the USA, 1988–1998	Ambient air	NR	0.266 µg/m³	< 0.156–2.57 µg/m³	NR		Rosenbaum et al. (1999), as reported in Environment Canada (2009)
Several cities and states in the USA, 2003–2005	Ambient air	NR	Median, 0.16 µg/m³	< LOD–0.19 µg/m³	NR		US EPA (2009)
Boston, Chicago, Houston, Tacoma, 1991	Ambient air	NR	NA	NA		Qualitative study: 2% of the samples contained allyl chloride	Evans et al. (1992)
Porto Allegre, Brazil, 1996–1997	Ambient air	46	All measurements < LOD		0.1 ppb [0.3 µg/m³]		Grosjean et al. (1999)

Table 1.2 (continued)

Location, collection date	Sampling matrix	Number of samples	Exposure level	Exposure range	Limit of detection	Comments	Reference
Rousse, Bulgaria, 1995–1996	Ambient air	384	5 out of 384 samples were > LOD	NR	1 µg/m^3		Islam & Stancheva (1999)
USA, 1986 or before	Whole water samples	200	< 0.5 µg/L	NR	NR		US EPA (1986)

LOD, limit of detection; NA, not applicable; NR, not reported; ppb, parts per billion; ppt, parts per trillion.

Table 1.3 Occupational exposure to allyl chloride as measured in air[a] in various industrial settings

Location, collection date	Occupation description	Number of samples	Exposure level[b]	Exposure range	Comments	Reference
Allyl chloride production factory, the Netherlands, 1991–1993	Multiple job titles	205 workshift samples collected from 136 workers	NR	< 0.1–17 mg/m³	Personal air monitoring Samples were collected during regular shut-down periods	de Rooij et al. (1997)
Sodium allyl sulfonate manufacturing plant B, China, 1982	Multiple job titles	Unknown number of samples in 27 workers	NR	0.2–25.13 mg/m³		He & Zhang (1985)
Petrochemical plant, the Netherlands, 1978	Chlorinated hydrocarbon production	Unknown number of samples in 44 workers	4 mg/m³	< 0.1–54 mg/m³	Cross-sectional study among men employed for 1–21 years	de Jong et al. (1988)
Sodium allyl sulfonate manufacturing plant A, China, 1976	Multiple job titles	68 area samples collected from 26 workers	2966 mg/m³ [c]	2.6–6.650 mg/m³	Location, timing, and duration of samples unknown	He & Zhang (1985)[c]
Allyl chloride manufacturing plant, former Soviet Union, before 1978	NR		NR	6.4–140 mg/m³	Employees occupationally exposed for > 1 year	Kasimova (1978)
Allyl chloride manufacturing plant, USA, 1976	Multiple job titles	100 personal samples	Average levels ranged from 0.47 to 1.9 ppm [1.47 to 6.0 mg/m³]	< 0.1–5.3 ppm [< 0.3–17 mg/m³]	Personal air monitoring Samples were collected for six job titles and presented in the publication	NIOSH (1976)
Chemical manufacturing plant, USA, 1975	Multiple job titles	35 personal samples	Average levels ranged from 0.05 to 3.05 ppm [0.16 to 9.55 mg/m³]	0.005–6.13 ppm [0.015–19.2 mg/m³]	Samples were collected for seven job titles and presented in the publication	NIOSH (1976)
Allyl chloride manufacturing plant, eastern Germany, 1968	Multiple job titles	60 workers	NR	1–113 ppm [~3–354 mg/m³]	Samples were collected for five job titles and presented in the publication	Häusler & Lenich (1968)

NR, not reported; ppm, parts per million.

[a] Area air monitoring unless indicated otherwise.

[b] Arithmetic mean.

[c] The Working Group noted that in subsequent communication between the United States Environmental Protection Agency and the authors, the average exposure was reported to be 138 mg/m³ as reported in Environment Canada (2009).

Table 1.4 International limit values for occupational exposure to allyl chloride

Country	Limit value, 8 hours		Limit value, short-term	
	ppm	mg/m³	ppm	mg/m³ [c]
Australia	1	3	2	6
Austria	1	3	1	3
Belgium	1	3	2[a]	6[b]
Canada, Ontario	1	3	2[c]	6[c]
Canada, Québec	1	3	2	6
Denmark	1	3	2[c]	6
Finland	1	3.2	3[c]	9.5[c]
France	1	3		
Hungary		3		3
Ireland	1	3	2[d]	6[d]
New Zealand	1	3	2	6
People's Republic of China		2		4[c]
Poland		2		
Republic of Korea	1	3	2	6
Romania	1	3[c]	2[c]	6[c]
Singapore	1	3	2	6
Spain	1	3.2	2	6.4
Sweden	1	3	3[c]	9[c]
Switzerland	1	3	1	3
USA, NIOSH	1	3	2[c]	6[c]
USA, OSHA	1	3		

NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration.
[a] Additional indication "D" means that the absorption of the agent through the skin, mucus membranes or eyes is an important part of the total exposure. It can be the result of both direct contact and its presence in the air.
[b] 15-minute limit value.
[c] 15-minute average value.
[d] 15-minute reference period.
From IFA (2020).

Governmental Industrial Hygienists (ACGIH, 2019).

2. Cancer in Humans

Olsen et al. (1994) studied 1064 male employees (12 574 person-years) of a chemical plant in the USA. Of those, 845 person-years were for employment in areas producing allyl chloride and epichlorohydrin, and 6329 person-years in the area producing glycerine where both allyl chloride and epichlorohydrin were used. No increase in cancer mortality was observed among workers exposed to allyl chloride with low co-exposure to epichlorohydrin, compared with workers not exposed to either allyl chloride or epichlorohydrin. The number of cancer deaths in the exposed groups was small (deaths for low allyl chloride/low epichlorohydrin exposure, $n = 4$; deaths for high allyl chloride/low epichlorohydrin exposure, $n = 1$). [The Working Group noted that the informativeness of this study was low due to the small number of person-years in the cohort, the high potential for co-exposure to epichlorohydrin, and the minimal adjustment for confounding.]

3. Cancer in Experimental Animals

Allyl chloride has been previously evaluated by the Working Group on two occasions (IARC, 1985, 1999). Each time, the Working Group concluded that there was *inadequate evidence* in experimental animals for the carcinogenicity of allyl chloride.

3.1 Mouse

See Table 3.1.

3.1.1 Oral administration (gavage)

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 5–7 weeks) were given allyl chloride (purity, ~98%) in corn oil by gavage (doses described below), 5 days per week, for up to 90 weeks (NCI, 1978). Groups of 20 males and 20 females were included for each vehicle and untreated control group. For males and females, there were two groups treated with allyl chloride for which the dose (mg/kg bw per day) was changed several times throughout the study. In some cases, doses were increased and then reduced, or animals were dosed in cyclic periods, alternating between no treatment and dosing by gavage (for 1 week and 4 weeks, respectively). Finally, dosing was stopped at around study week 76–77 for males at the lower dose and for both treated groups of females. In males at the lower dose, the doses ranged from 0 (during dose cycling and cessation) to 250 mg/kg bw with a TWA of 172 mg/kg bw calculated over 78 weeks. In males at the higher dose, doses ranged from 0 (during dose cycling) to 500 mg/kg bw, with a TWA of 199 mg/kg bw calculated over 78 weeks (surviving mice were removed at 56 weeks) [The Working Group noted that calculation of TWA in males at the higher dose was an underestimate; a more accurate estimate calculated over 56 weeks was 278 mg/kg bw]. In females, the doses ranged from 0 (during dose cycling and cessation) to 150 mg/kg bw and 300 mg/kg bw for the lower and higher dose, respectively, with TWAs of 129 and 258 mg/kg bw, respectively, for 78 weeks. Dosing in the vehicle control groups was stopped at weeks 76–77 to correspond with groups dosed with allyl chloride.

Survival was reduced in males at the higher dose, resulting in removal of surviving mice (10/50) at week 56. Survival in all other groups of males and females was deemed adequate for assessment of carcinogenicity. Body weights of males were similar in treated and control groups throughout the study. Body weights of females at the lower and higher dose were slightly lower than those of controls beginning at weeks 20 and 10, respectively. All mice underwent complete necropsy and full histopathological examination.

In male and female mice, there were occurrences of squamous cell papilloma (females only) and squamous cell carcinoma of the forestomach in treated groups that were not observed in control groups, and were considered rare for the testing facility. Due to high mortality in males at the higher dose, tumour analysis in males was time-adjusted to include only those mice that survived at least 52 weeks. In males at the lower dose, there was an incidence of 2/36 (5.6%) of squamous cell carcinoma of the forestomach; metastases of these tumours were observed in both males. No squamous cell carcinomas of the forestomach were observed in the 10 males at the higher dose fitting the above survival criteria. Tumour incidence in females was analysed on the basis of the full study duration. In females, the incidence of squamous cell papilloma or carcinoma (combined) of the forestomach was 3/47 (6.4%) and 3/45 (6.7%) at the lower and higher dose, respectively. While none of these tumour rates were statistically significantly increased according to the Fisher exact (pairwise) test, the Cochran–Armitage test, or the Peto (trend) test, all rates exceeded those for concurrent (0%) and historical (1/180; 0.6% for

Table 3.1 Studies of carcinogenicity with allyl chloride in mice and rats

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) Age, 5–7 wk 90 wk NCI (1978)	Oral (gavage) Purity, ~98% Corn oil 0 (untreated control), 0 (vehicle control), 129, 258 mg/kg bw (TWA) 5 days/wk 20, 20, 50, 50 16, 18, 40, 34	*Forestomach* Squamous cell papilloma or carcinoma (combined) 0/20, 0/19, 3/47, 3/45 Squamous cell papilloma 0/20, 0/19, 1/47, 3/45 Squamous cell carcinoma 0/20, 0/19, 2/47, 0/45	NS (Cochran–Armitage or Fisher exact test; see comments) NS (Cochran–Armitage or Fisher exact test; see comments) NS (Cochran–Armitage or Fisher exact test; see comments)	Principal limitations: two-dose study; study duration less than most of lifespan; problematic dosing regimen, doses were changed multiple times throughout the study in both exposed groups due to overt toxicity; small number of mice for control groups Other comments: treatment for 78 wk; no significant effect of treatment on survival; incidence of squamous cell papilloma or carcinoma (combined) of the forestomach in historical controls was 1/180 for female B6C3F$_1$ mice
Full carcinogenicity Mouse, Ha:ICR Swiss (F) Age, 6–8 wk 62–85 wk Van Duuren et al. (1979)	Skin application Purity, NR Acetone Application of 0.1 mL acetone (control), or of 31.0 or 94.0 mg allyl chloride in 0.2 mL acetone, 3×/wk for study duration 30, 30, 30 NR	*Any tumour type* No significant increase		Principal limitations: small number of mice per group; females only; chemical purity, NR; limited gross and histopathological evaluations performed Other comments: duration of study, NR specifically for allyl chloride; survival of allyl chloride-treated mice, NR
Full carcinogenicity Mouse, Ha:ICR Swiss (F) Age, 6–8 wk ≤ 631 days (90 wk) Van Duuren et al. (1979)	Subcutaneous injection Purity, NR Trioctanoin Injection in left flank of 0.05 mL trioctanoin (control) or of 1.5 mg of allyl chloride in 0.05 mL trioctanoin, 1×/wk for study duration 30, 30 NR	*Any tumour type* No significant increase		Principal limitations: small number of mice per group; females only; chemical purity, NR; limited gross and histopathological evaluations performed Other comments: duration of study for allyl chloride, ≤ 549 days (78 wk); duration of study for controls, ≤ 631 days; survival of allyl chloride-treated mice, NR

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, Crj:BDF1 (M) Age, 6 wk 104 wk JBRC (2003a)	Inhalation (whole-body exposure) Purity, > 98% Air 0, 50, 100, 200 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 35, 35, 33, 0	*Harderian gland*: adenoma 3/50, 4/50, 14/50*, 8/50 *Lung*: bronchioloalveolar adenoma 4/50, 13/50*, 11/50*, 3/50	Trend, $P < 0.01$ (Peto test); * pairwise test, $P < 0.05$ (Fisher exact test) Trend, $P < 0.01$ (Peto test); *pairwise test, $P < 0.05$ (Fisher exact test)	Principal strengths: multiple-dose study; GLP study; males and females used; study covered most of lifespan Principal limitations: decreased survival in males at 200 ppm had an impact on interpretation of tumour incidence dose–response relationship Other comments: incidence of Harderian gland adenoma exceeded the upper bound of the historical control range (all routes) at 100 and 200 ppm: males, 51/1196 (average, 4.3%; range, 0–10%); lower incidence of tumours in the group at 200 ppm may be attributed to lower survival
Full carcinogenicity Mouse, Crj:BDF1 (F) Age, 6 wk 104 wk JBRC (2003a)	Inhalation (whole-body exposure) Purity, > 98% Air 0, 50, 100, 200 ppm 6 h/day, 5 days/wk 50, 50, 49, 50 27, 26, 25, 6	*Harderian gland*: adenoma 0/50, 4/50, 8/49**, 9/50** *Lung*: bronchioloalveolar adenoma 0/50, 3/50, 6/49*, 5/50*	Trend, $P < 0.01$ (Peto and Cochran–Armitage tests); **pairwise test, $P < 0.01$ (Fisher exact test) Trend, $P < 0.01$ (Peto test) and $P < 0.05$ (Cochran–Armitage test); *pairwise test, $P < 0.05$ (Fisher exact test)	Principal strengths: multiple-dose study; GLP study; males and females used; study covered most of lifespan Principal limitations: low survival rate in all groups; treatment-related decreased survival at 200 ppm

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, A/St (M+F, combined) Age, 6–8 wk 24 wk Theiss et al. (1979)	Intraperitoneal injection Purity, NR Tricaprylin 0 (vehicle control), 15.6, 38.4, 76.8 mmol/kg bw total dose 3 injections/wk, total of 24 injections 20, 20, 20, 20 16, 20, 20, 20	*Lung* All gross tumours (mostly adenomas) Average number of tumours per animal: 0.19 ± 0.10, 0.60 ± 0.20, 0.50 ± 0.27, 0.60 ± 0.15*	*$P < 0.05$ (either *t*-test or χ^2 test)	Principal strengths: multiple-dose study; males and females used Principal limitations: short duration; small number of mice per group; sexes combined in analysis; chemical purity, NR; limited gross and histopathological evaluations performed (a few lung surface nodules were examined histologically to confirm the typical morphological appearance of pulmonary adenomas) Other comments: tumour incidence presented as average number of lung tumours per mouse, eliminating the ability to analyse incidence by mice affected or by tumour multiplicity; number of mice per group at start, 10 M + 10 F
Initiation-promotion (tested as initiator) Mouse, Ha:ICR Swiss mice (F) Age, 6–8 wk 61–82 wk Van Duuren et al. (1979)	Skin application Purity, NR Acetone Single application of 0.2 mL acetone (TPA-only control) or 94.0 mg allyl chloride in 0.2 mL acetone, followed by (after 14 days without treatment) 0.0050 mg TPA in 0.2 mL acetone 3×/wk for study duration 90, 30 NR, NR	*Skin* Squamous cell papilloma 6/90, 7/30* Squamous cell carcinoma 2/90, 0/30	*$P < 0.05$ (χ^2 test) [NS]	Principal limitations: chemical purity, NR Other comments: duration of study, NR specifically for allyl chloride; survival of allyl chloride-treated mice, NR

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Rat, F344/DuCrj (M) Age, 6 wk 104 wk JBRC (2003c)	Inhalation (whole-body exposure) Purity, > 98% Air 0, 20, 50, 100 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 38, 33, 40, 24	*Urinary bladder*: transitional cell carcinoma 0/50, 1/50, 0/50, 5/50*	Trend, $P \leq 0.01$ (Peto and Cochran–Armitage tests); *pairwise test, $P \leq 0.05$ (Fisher exact test)	Principal strengths: multiple-dose study; GLP study; males and females used; study covered most of lifespan Other comments: survival was significantly reduced in males at 100 ppm; historical control incidence of urinary bladder transitional cell carcinoma was 0/1398 [assumed to be for all routes; year range and routes not specified]; historical control range of thyroid follicular cell adenoma or adenocarcinoma (combined; all routes), 39/1393 (average, 2.8%; range, 0–8%)
		Thyroid Follicular cell adenoma or adenocarcinoma (combined) 1/50, 3/50, 4/50, 5/49	Trend: $P \leq 0.05$ (Peto test)	
		Follicular cell adenoma 1/50, 2/50, 2/50, 4/49	Trend: $P \leq 0.05$ (Peto test)	
		C-cell carcinoma 0/50, 1/50, 0/50, 3/49	Trend: $P \leq 0.05$ (Peto and Cochran–Armitage tests)	
		Peritoneum: mesothelioma 0/50, 1/50, 4/50, 4/50	Trend: $P \leq 0.01$ (Peto test); $P \leq 0.05$ (Cochran–Armitage test)	
		Lung Bronchioloalveolar adenoma 5/50, 0/50, 4/50, 8/50	Trend: $P \leq 0.05$ (Peto test)	
		Bronchioloalveolar adenoma or carcinoma (combined) 5/50, 1/50, 6/50, 9/50	Trend: $P \leq 0.05$ (Peto test)	
		Skin: keratoacanthoma 1/50, 0/50, 2/50, 4/50	Trend: $P \leq 0.05$ (Peto and Cochran–Armitage tests)	
		Mammary gland: fibroadenoma 0/50, 0/50, 3/50, 3/50	Trend: $P \leq 0.05$ (Peto and Cochran–Armitage tests)	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence or multiplicity of tumours	Significance	Comments
Full carcinogenicity Rat, F344/DuCrj (F) Age, 6 wk 104 wk JBRC (2003c)	Inhalation (whole-body exposure) Purity, > 98% Air 0, 20, 50, 100 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 40, 34, 34, 34	*Any tumour type* No significant increase	NS	Principal strengths: multiple-dose study; GLP study; males and females used; study covered most of lifespan Other comments: no significant effect of treatment on survival

bw, body weight; F, female; GLP, good laboratory practice; h, hour; M, male; NR, not reported; NS, not significant; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; ppm, parts per million; TWA, time-weighted average; wk, week.

males and for females) controls. In addition, a high incidence of non-neoplastic lesions (hyperkeratosis and acanthosis) of the forestomach was observed in groups of male and female mice treated with the lower or higher dose of allyl chloride, which was not observed in mice in the control groups (NCI, 1978). [Principal limitations included the poor survival in males at the higher dose, the inconsistent dosing regimen resulting in TWA doses with poor spacing and no dose–response relationship, the small number of mice in the control groups, the two-dose study, and less-than-lifespan exposure. The Working Group concluded that the study in male mice was inadequate for the evaluation.]

3.1.2 Skin application

Groups of 30 female Ha:ICR Swiss mice (age, 6–8 weeks) were given dorsal applications of allyl chloride [purity not reported] at a dose of 0 (in 0.1 mL of acetone), 31.0, or 94.0 mg/application per mouse (in 0.2 mL of acetone) to the shaved skin (Van Duuren et al., 1979), three times per week for the study duration. Study duration was not reported for this specific chemical, but a range of 62–85 weeks was provided for the set of nine chemicals tested in the study. Similarly, survival was not specifically reported. At termination of the experiment, routine sections of the skin, liver, stomach, and kidney were taken for histopathological examination. There were no significant or unusual histological findings in this study. Repeated skin application of allyl chloride did not induce any papillomas of the skin, and the incidence of lung papilloma and of papilloma of the forestomach was similar in control and treated mice. One adenocarcinoma of the glandular stomach was observed in a mouse at the highest dose. [Principal limitations included the limited gross and histopathological evaluations, small number of animals per group, use of females only, chemical purity not reported, study duration and number of surviving mice not specifically reported.]

3.1.3 Subcutaneous injection

Groups of 30 female Ha:ICR Swiss mice (age, 6–8 weeks) were injected subcutaneously with allyl chloride [purity not reported] at a dose of 0 (in 0.05 mL of trioctanoin) or 1.5 mg/injection per mouse (in 0.05 mL of trioctanoin) in the left flank (Van Duuren et al., 1979), weekly for the study duration. Study duration was up to 78 weeks for the treated group and up to 90 weeks for controls. Survival was not specifically reported. At termination of the experiment, routine sections of the liver and injection sites were taken for histopathological examination. The only tumour observed was a fibrosarcoma at the injection site in one mouse treated with allyl chloride. [Principal limitations included the limited gross and histopathological evaluations, small number of animals per group, use of females only, absence of reporting of chemical purity, and number of surviving animals not specifically reported.]

3.1.4 Inhalation

In a study that complied with good laboratory practice (GLP), groups of 50 male and 50 female Crj:BDF1 [B6D2F_1/Crlj] mice (age, 6 weeks) were treated by whole-body inhalation with allyl chloride (purity, > 98%; in air) at a concentration of 0, 50, 100, or 200 ppm for 6 hours per day, 5 days per week, for up to 104 weeks (JBRC, 2003a, b). Survival was significantly reduced in males and females at 200 ppm: 0/50 males survived past week 97, and 6/50 females remained at study termination. Survival in the groups of males and females at 50 and 100 ppm was similar to that in controls. Average body weights in the groups of males and females at 200 ppm were lower than in the respective controls groups. All mice

underwent complete necropsy and full histopathological examination.

In male and female mice, there was a significant increase (Fisher exact test) in the incidence of adenoma of the Harderian gland in the groups at 100 ppm (males, $P < 0.05$; females, $P < 0.01$) and 200 ppm (females, $P < 0.01$), with a significant positive trend (Peto test, $P < 0.01$; males and females). There were also occurrences of non-neoplastic lesions (Harderian gland hyperplasia) in all groups of treated males, and in one female at 100 ppm. In male and female mice, there was a significant increase (Fisher exact test) in the incidence of bronchioloalveolar adenoma in the groups at 50 ppm (males, $P < 0.05$), 100 ppm (males and females, $P < 0.05$), and 200 ppm (females, $P < 0.05$), with a significant positive trend (Peto test, $P < 0.01$; males and females). [The Working Group noted the strengths of this well-conducted study that complied with GLP: the use of males and females and multiple doses.]

3.1.5 Intraperitoneal injection

Groups of 20 (10 males and 10 females, combined) strain A/St mice (age, 6–8 weeks) were given intraperitoneal injections of allyl chloride [purity not reported] at a dose of 0 (vehicle only, tricaprylin), 0.65, 1.60, or 3.20 mmol/kg bw, three times per week for a total of 24 doses (Theiss et al., 1979). Total injected doses amounted to 0 (vehicle), 15.6, 38.4, and 76.8 mmol/kg bw. Necropsies were performed 24 weeks after the first injection. All treated mice survived until study termination; survival in the tricaprylin vehicle-control group was 16/20. [Body weights were not reported in this study.] Histopathological evaluation was limited to the lungs, and findings were reported as average number of lung tumours per mouse. A few lung surface nodules were examined histologically to confirm the typical morphological appearance of pulmonary adenoma. There was a significant increase ($P < 0.05$, either *t*-test or χ^2 test) in the average number of lung tumours per mouse in the group at 76.8 mmol/kg (0.60 ± 0.15) compared with vehicle controls (0.19 ± 0.10). The average numbers of lung tumours per mouse in the other dosed groups were similar to those in the group at 76.8 mmol/kg, but were not statistically significantly increased. [Principal limitations included the short exposure duration and small number of mice per group, that the sexes were combined in the analysis, the limited gross and histopathological evaluations, and that chemical purity and tumour incidences were not reported.]

3.1.6 Initiation–promotion

In the study described by Van Duuren et al. (1979), allyl chloride was assessed as a tumour initiator. A group of 30 female Ha:ICR Swiss mice (age, 6–8 weeks) were given a single dorsal application of allyl chloride [purity not reported] at a dose of 94.0 mg/mouse (in 0.2 mL of acetone) to the shaved skin. After application, mice remained untreated for 14 days, and then received applications of 0.0050 mg of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in 0.2 mL of acetone, three times per week for the study duration. A group of 90 females – serving as TPA-only controls – received 0.0050 mg of TPA (in 0.2 mL of acetone), three times per week for the study duration. Study duration was not specifically reported for this chemical, but a range of 61–82 weeks was provided for the set of nine chemicals tested in the study. [Survival and body weights were not reported in this study, although survival was described as being "very good" in the treated group.]

Histopathological evaluation was limited to the skin. There was a significant increase ($P < 0.05$, χ^2 test) in the incidence of skin squamous cell papilloma (7/30, 23.3%) in the group receiving allyl chloride plus TPA compared with TPA-only controls (6/90, 6.7%). There was also a reduced time to first tumour in the group receiving allyl chloride plus TPA (197 days) compared with

TPA-only controls (449 days). All tumours in the group receiving allyl chloride plus TPA were squamous cell papillomas, while two mice in the TPA-only group (2/90) also developed skin squamous cell carcinomas (Van Duuren et al., 1979). [Principal limitations included the limited gross and histopathological evaluation, and that chemical purity was not reported.]

3.2 Rat

See Table 3.1.

3.2.1 Oral administration (gavage)

Groups of 50 male and 50 female Osborne–Mendel rats (age, 6–7 weeks) were given allyl chloride (purity, ~98%) in corn oil by gavage (doses described below), 5 days per week for up to 110 weeks (NCI, 1978). Groups of 20 males and 20 females were included for each vehicle and untreated control group. In both males and females, doses in the groups treated with allyl chloride were reduced throughout the study, with cessation of dosing beginning in weeks 78–80 for all groups. The lower dose in males ranged from 55 to 70 mg/kg bw with a TWA of 57 mg/kg bw, calculated based on the number of weeks for which the rats were dosed. The higher dose in males ranged from 55 to 140 mg/kg bw, with a TWA of 77 mg/kg bw. In females, the lower dose was 55 mg/kg bw until dosing cessation, resulting in a calculated TWA of 55 mg/kg bw. In females the higher dose ranged from 55 to 110 mg/kg bw with a TWA of 73 mg/kg bw. Dosing in the vehicle-control groups was stopped at week 78 to correspond with that in groups dosed with allyl chloride. All rats underwent complete necropsy and full histopathological examination.

Survival was lower in male and female rats than in controls, with a significant association between increasing dose and mortality ($P < 0.001$, Tarone test). Survival at study termination was 14% for males at the lower dose, 0% for males at the higher dose (compared with 20–35% for male controls), 38% for females at the lower dose, and 12% for females at the higher dose (compared with 65–75% for female controls). Body weights of male rats at the higher dose were significantly reduced compared with those of controls at the end of the study, with a consistent trend of body-weight loss beginning around week 50. Treated females (lower and higher dose) had somewhat lower body weights than controls. There was no effect on tumour incidence that was attributed to exposure to allyl chloride. [Principal limitations included the poor survival in treated and control males and females, the inconsistent dosing regimen resulting in TWA doses with poor spacing and no dose–response relationship, the small number of animals in control groups, and the two-dose study. The Working Group concluded that the study in male and female rats was inadequate for the evaluation.]

3.2.2 Inhalation

In a study that complied with GLP, groups of 50 male and 50 female F344/DuCrj (Fischer) rats (age, 6 weeks) were treated by whole-body inhalation with allyl chloride (purity, > 98%; in air) at a concentration of 0, 25, 50, or 100 ppm for 6 hours per day, 5 days per week, for up to 104 weeks (JBRC, 2003c, d). Survival was reduced in the males at 100 ppm (48%) compared with controls (76%); survival in other groups of exposed males was similar to that in controls. Exposure to allyl chloride had no impact on body weight in male or female rats. All rats underwent complete necropsy and full histopathological examination.

In male rats, there was a significant increase ($P < 0.05$, Fisher exact test) in the incidence of transitional cell carcinoma of the urinary bladder in the group at 100 ppm (5/50) compared with controls (0/50), with a significant positive trend ($P < 0.01$, Peto and Cochran–Armitage tests). There were also occurrences of non-neoplastic

lesions: transitional epithelium hyperplasia, nodular hyperplasia, and squamous cell metaplasia in the urinary bladder.

In male rats, there was also a significant positive trend ($P < 0.05$, Peto trend test) in the incidence of follicular cell adenoma, and follicular cell adenoma or adenocarcinoma (combined) of the thyroid gland. There was a significant positive trend ($P < 0.01$, Peto trend test; $P < 0.05$, Cochran–Armitage trend test) in the incidence of peritoneal mesothelioma in males. There was a significant positive trend ($P < 0.05$, Peto and Cochran–Armitage trend tests) in the incidence of thyroid C-cell carcinoma, skin keratoacanthoma, and mammary gland fibroadenoma in males. There was a significant positive trend ($P < 0.05$, Peto trend test) in the incidence of bronchioloalveolar adenoma, and bronchioloalveolar adenoma or carcinoma (combined) in males.

There was no significant increase in tumour incidence in female rats. [The Working Group noted the strengths of this well-conducted GLP study covering most of the lifespan: the use of males and females and multiple doses.]

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

In workers exposed by inhalation to allyl chloride at concentrations at or below 3 mg/m^3 (the 8-hour TWA occupational exposure limit for many countries worldwide), ALMA was a major urinary metabolite, whereas traces of 3-hydroxypropylmercapturic acid (HPMA) were detected in only a few urine samples. As noted in Section 1.3.1(d), ALMA is an established urinary biomarker of exposure to allyl chloride. The calculated end-of-shift mean urinary excretion of ALMA in workers exposed to an 8-hour TWA air concentration of allyl chloride of 3 mg/m^3 was 352 µg/g creatinine. This value was proposed as a biological exposure index for human exposure to allyl chloride (de Rooij et al., 1997). ALMA was also detected in the urine of people consuming garlic (de Rooij et al., 1996a; Verhagen et al., 2001).

No data from human cells in vitro were available to the Working Group.

4.1.2 Experimental systems

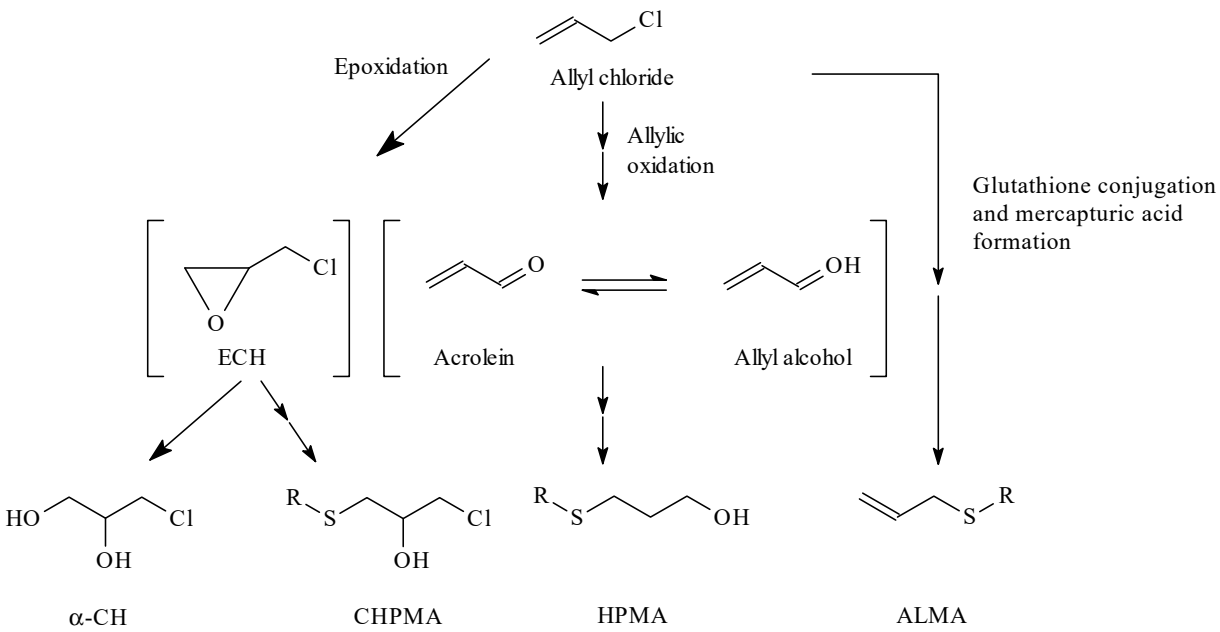
(a) Experimental systems in vivo

In male albino rats dosed subcutaneously with allyl chloride (1 mL of 10% v/v solution in oil, equivalent to 94 mg per rat), ALMA, its sulfoxide, and HPMA were identified as urinary metabolites, while *S*-allylglutathione and *S*-allyl-L-cysteine were detected in the bile. Kaye et al. (1972) proposed metabolic steps to convert allyl chloride to ALMA and its sulfoxide, as well as four possible metabolic pathways for the conversion of allyl chloride to HPMA, initiated at either C-1 or C-3 of allyl chloride.

The intraperitoneal dosing of male Wistar rats with allyl chloride (5–45 mg/kg bw) resulted in the detection of urinary ALMA (30% of the administered dose) and HPMA (< 3%) (de Rooij et al., 1996b; Fig. 4.1). In addition, two minor metabolites were identified, α-chlorohydrin (0.13%) and 3-chloro-2-hydroxypropylmercapturic acid (0.21%), indicative of the metabolic conversion of allyl chloride to epichlorohydrin. Pre-treatment of rats with pyrazole (cytochrome P450 CYP2E1 inducer), β-naphthoflavone (CYP1A1/2 inducer), and phenobarbital (CYP2B1/2 inducer) had little to no impact on the urinary excretion of ALMA. In contrast to the results of de Rooij et al. (1996b), the urinary yield of HPMA in male Sprague-Dawley rats dosed orally with allyl chloride (76 mg/kg bw) was 21.5% (Sanduja et al., 1989).

Studies of other adverse effects indirectly confirmed distribution to target tissues.

Fig. 4.1 Proposed metabolic scheme for allyl chloride in rats



ECH, epichlorohydrin; ALMA, allylmercapturic acid; HPMA, 3-hydroxypropylmercapturic acid; α-CH, α-chlorohydrin; CHPMA, 3-chloro-2-hydroxypropylmercapturic acid; R, *N*-acetyl-cysteinyl.
Reproduced with permission from de Rooij et al. (1996b). Biotransformation of allyl chloride in the rat. Influence of inducers on the urinary metabolic profile. *Drug Metab Dispos*, 24(7):765–772. [The Working Group noted that formation of allyl alcohol as a metabolic intermediate is uncertain.]

Inhalation exposure of rats, guinea-pigs, and rabbits for 5 weeks caused histological damage to the liver and kidney (Torkelson et al., 1959). Mice treated orally for 2–7 weeks developed focal kidney damage (He et al., 1981). Studies on acute and subchronic toxicity after exposure by inhalation in male rats showed dose-dependent adverse effects on the testis and suppression of the reflexes (Guseinov et al., 1981; Guseinov, 1982).

(b) Experimental systems in vitro

Emmert et al. (2006) (see Section 4.2) showed a greater mutagenicity of allyl chloride in a metabolically competent *Salmonella typhimurium* strain encoding production of CYP2E1 than in a conventional Ames test, indicating a major role of CYP2E1 in the metabolic activation of allyl chloride. On the other hand, for a series of allylic compounds including allyl chloride, good correlation was found between mutagenicity in a conventional Ames test and direct alkylating properties (Eder et al., 1980). [The Working Group noted that metabolic activation is a modifying factor, but not the principal factor in the biotransformation and mutagenicity of allyl chloride.]

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether allyl chloride is electrophilic or can be metabolically activated to electrophiles; is genotoxic; or alters cell proliferation,

cell death, or nutrient supply. Insufficient data were available for the evaluation of other key characteristics of carcinogens.

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

Allyl chloride gave positive results in the 4-(4-nitrobenzyl)pyridine (NBP) alkylation test (Eder et al., 1982). The NBP-alkylation test results correlated with direct mutagenicity potency observed in *S. typhimurium* TA100 (Henschler et al., 1983). Moreover DNA adducts were formed after exposure in isolated perfused rat liver (Eder & Zugelder, 1990) and in salmon sperm DNA in vitro (Eder et al., 1987) (see Table 4.4 and Table 4.5, respectively). The alkylated bases identified (N^3-allyladenine, N^6-allyladenine, N^2-allylguanine, N^7-allylguanine and O^6-allylguanine) contained the allyl moiety. He et al. (1995) showed that allyl chloride has the ability to covalently cross-link axonal cytoskeletal proteins. A study in rats treated by subcutaneous injection of allyl chloride 5 days per week for 3 months showed no evidence of cross-linking of neurofilament proteins from the spinal cord (Nagano et al., 1993).

4.2.2 Is genotoxic

Table 4.1, Table 4.2, Table 4.3, Table 4.4 and Table 4.5 summarize studies of the genetic and related effects of allyl chloride.

(a) Humans

(i) Exposed humans

See Table 4.1.

A cross-sectional epidemiological study of 44 men engaged in the production of various chlorinated hydrocarbons, including allyl chloride and epichlorohydrin, analysed cytogenetic end-points in blood samples (de Jong et al., 1988). The workers involved in this study had been employed in this plant for periods of between 1 and 21 years and exposures to allyl chloride were reported as 4 mg/m^3 (arithmetic mean; range, < 0.1–54). The frequencies of chromatid gaps, chromatid and chromosome breaks, and total aberrations were statistically significantly higher than those in the control group investigated during the same year. [The Working Group noted the complex nature of exposures and that no individual measurements of exposure to allyl chloride were reported.]

(ii) Human cells in vitro

See Table 4.2.

In human cells in vitro, the unscheduled DNA synthesis assay provided inconsistent results for allyl chloride: a test on human embryonic intestinal cells gave negative results with and without metabolic activation (McGregor, 1981), while a study in the HeLa S3 cell line (human cervical cancer) gave positive results without metabolic activation (not tested with metabolic activation) (Schiffmann et al., 1983).

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3.

After inhalation exposure to allyl chloride (1 or 25 ppm for 7 hours per day), rats did not show an increase in the frequency of chromosomal aberrations when treated for a single day, or of micronucleus formation, or dominant lethal mutations when treated for 5 consecutive days, and exposed mice did not demonstrate sperm abnormalities (McGregor, 1981). After oral administration, allyl chloride failed to induce micronucleus formation in exposed mice (Rim & Kim, 2015). [The Working Group noted the absence of direct evidence of target tissue exposure in these genotoxicity tests.]

(ii) Non-human mammalian cells in vitro

See Table 4.4.

Table 4.1 Genetic and related effects of allyl chloride in exposed humans

End-point	Tissue, cell type (if specified)	Location, date, setting, scenario	No. of exposed and controls	Agent, exposure level (mean, range, units)	Response (significance)	Covariates controlled	Comments	Reference
Chromosomal aberration	Peripheral blood, lymphocytes	Netherlands, 1978, plant producing various chlorinated hydrocarbons, including epichlorohydrin and allyl chloride	44, 27[a]	Allyl chloride: 4 (< 0.1–54) mg/m^3 Epichlorohydrin: 1 (< 0.1–3) mg/m^3	(+)[b] ($P < 0.05$)	Sex, age and smoking habits	Confounding exposure (epichlorohydrin)	de Jong et al. (1988)

[a] Participants engaged in manufacture of bisphenol A (diphenylolpropane) by reaction of phenol and acetone. These chemicals are not believed to be associated with the induction of chromosomal damage.
[b] (+), positive in a study of limited quality.

Table 4.2 Genetic and related effects of allyl chloride in human cells in vitro

End-point	Tissue, cell line	Results[a]		Concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
Unscheduled DNA synthesis	Human embryonic intestinal cells	–	–	9900 µg/mL	McGregor (1981)
Unscheduled DNA synthesis	HeLa S3 cell line (human cervix)	+	NT	1 mM [76.5 µg/mL]	Schiffmann et al. (1983)

HIC, highest ineffective concentration; LEC, lowest effective concentration, NT, not tested.
[a] +, positive; –, negative.

Table 4.3 Genetic and related effects of allyl chloride 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 aberration	Rat (M and F)	Bone marrow	–	1 and 25 ppm	Inhalation, 7 h per day, × 1 day, sampling after 6, 24, and 48 h		McGregor (1981)
Micronucleus formation	Rat (M and F)	Bone marrow	–	1 and 25 ppm	Inhalation, 7 h per day, × 5 days, sampling after 6, 24, and 48 h		McGregor (1981)
Dominant lethal test	Rat (M) (treated) and (F) (not treated)	Ovary and uterus	–	1 and 25 ppm	Inhalation, 7 h per day, × 5 days		McGregor (1981)
Sperm abnormality test	Mouse (M)	Testis, cauda epididymis	–	1 and 25 ppm	Inhalation, 7 h per day, × 5 days		McGregor (1981)
Micronucleus formation	Mouse, ICR (M)	Bone marrow; polychromatic erythrocytes	(–)	400 mg/kg bw	Oral, single treatment [not specified but assumed], sampling 24 h later	Single treatment followed by only one sampling time	Rim & Kim (2015)

bw, body weight; F, female; h, hour; HID, highest ineffective dose; LED, lowest effective dose (units as reported); M, male; ppm, parts per million.

[a] +, positive; –, negative; (–), negative in a study of limited quality (see OECD TG 474) (OECD, 2014).

Table 4.4 Genetic and related effects of allyl chloride in non-human mammal cells in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA-binding (adduct formation, HPLC)	Rat (perfusion of isolated liver)	+	NT	300 mg/liver[b]		Eder & Zugelder (1990)
Chromosomal aberrations	Rat liver RL1 cells	–	NT	25 µg/mL		Dean et al. (1985)
Chromosomal aberrations	Chinese hamster lung (CHL) cells	+	+	400 µg/mL	No data on cytotoxicity; the culture bottle was sealed and cultured standing after adding the test substances	JETOC (1997a)
Chromosomal aberrations	Chinese hamster lung (CHL) fibroblast cells	(–)	(–)	3 mM [~230 µg/mL]	Highest concentration is lower than that recommended by OECD TG 473 (OECD, 2016)	Rim & Kim (2014)

HIC, highest ineffective concentration; HPLC, high-performance liquid chromatography; LEC, lowest effective concentration; NT, not tested.

[a] +, positive; –, negative; (–), negative in a study of limited quality.

[b] Test substance was administered to the isolated liver in portions every 15 minutes over a period of 3–4 hours directly into the perfusion medium through the tube leading to the portal vein using a syringe.

Table 4.5 Genetic and related effects of allyl chloride in non-mammalian and acellular systems in vitro

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
Drosophila melanogaster	Sex-linked recessive lethal test	–	NA	150 ppm	McGregor (1981)
Saccharomyces cerevisiae D4	Gene conversion	+	NT	14 µg/mL	McCoy et al. (1978)
Saccharomyces cerevisiae JD1	Gene conversion	+	+	NR	Dean et al. (1985)
Aspergillus nidulans	Forward mutation	–	NT	40 µL/plate [37 600 µg/plate]	Bignami et al. (1980)
Aspergillus nidulans	Mitotic segregation	–	NT	0.6 mL/20 L desiccator[b] [0.03 µg/mL]	Crebelli et al. (1984)
Salmonella typhimurium TA1535	Reverse mutation	–	–	10 µL/plate[c] [9400 µg/plate]	McCoy et al. (1978)
Salmonella typhimurium TA1535	Reverse mutation	–	+	1 µL/plate[d] [940 µg/plate]	McCoy et al. (1978)
Salmonella typhimurium TA1535	Reverse mutation	+	+	1 µL/plate[e] (–S9) [940 µg/plate] 5 µL/plate[e] (+S9) [4700 µg/plate]	McCoy et al. (1978)
Salmonella typhimurium TA1535	Reverse mutation	–	+	10 µL/plate [9400 µg/plate]	Bignami et al. (1980)
Salmonella typhimurium TA1535	Reverse mutation	+	–	5000 µg/plate	JETOC (1997b)
Salmonella typhimurium TA100	Reverse mutation	–	–	10 µL/plate [9400 µg/plate]	Bignami et al. (1980)
Salmonella typhimurium TA100	Reverse mutation	+	–	15 µmol/mL [1150 µg/mL]	Eder et al. (1980)
Salmonella typhimurium TA100	Reverse mutation	+	NT	0.1 µL/9 L desiccator[b] [0.01 µg/mL]	Norpoth et al. (1980)
Salmonella typhimurium TA100	Reverse mutation	+	NT	0.05 µg/mL[b]	Simmon et al. (1981)
Salmonella typhimurium TA100	Reverse mutation	+ (w)	+	250 µg/plate	Neudecker & Henschler (1985)
Salmonella typhimurium TA100	Reverse mutation	NT	+	235 µg/plate	Neudecker & Henschler (1986)
Salmonella typhimurium TA100	Reverse mutation	+	–	2500 µg/plate	JETOC (1997b)
Salmonella typhimurium TA100, TA1538	Reverse mutation	–	–	10 µL/plate[d] [9400 µg/plate]	McCoy et al. (1978)
Salmonella typhimurium TA1535, TA100, TA98, TA1538	Reverse mutation	–	–	4000 µg/plate	Dean et al. (1985)
Salmonella typhimurium TA98, TA1537	Reverse mutation	–	–	5000 µg/plate	JETOC (1997b)
Salmonella typhimurium strain YG7108[f]	Reverse mutation	NT	+	500 µL/plate	Emmert et al. (2006)

Table 4.5 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
Salmonella typhimurium strain YG7108pin3ERb5[g]	Reverse mutation	+	NT	300 µg/plate	Emmert et al. (2006)
Escherichia coli WP2 and WP2*uvrA*	Reverse mutations	+	+	NR [highest dose 4000 µg/plate]	Dean et al. (1985)
Escherichia coli WP2*uvrA*/pKM101	Reverse mutation	+	+	5000 µg/plate	JETOC (1997b)
Escherichia coli pol A+/*pol* A–	Differential toxicity (spot test)	+	NT	10 µL/plate [9400 µg/plate]	McCoy et al. (1978)
Streptomyces coelicolor	Reverse mutation Forward mutation	+ +	NT NT	10 µL/plate [9400 µg/plate] 10 µL/plate [9400 µg/plate]	Bignami et al. (1980)
Salmon sperm DNA	Binding (covalent) to DNA	+ (w)	NT	9000 µg/mL	Eder et al. (1987)

HIC, highest ineffective concentration; LEC, lowest effective concentration (units as reported); NA, not applicable; NR, not reported; NT, not tested; ppm, parts per million; S9, 9000 × *g* supernatant.

[a] +, positive; –, negative; + (w), weak positive.

[b] *Salmonella typhimurium* or *Aspergillus nidulans,* on Petri dishes, were exposed to allyl chloride vapour in a 9 L or 20 L desiccator, respectively.

[c] Standard plate incorporation.

[d] Filter discs impregnated with test agent put on surface of agar.

[e] Preincubation.

[f] Strain YG7108 is similar to strain TA1535, except that it has a methyltransferase deficiency.

[g] Strain YG7108pin3ERb5 is strain YG7108 that carries plasmid *pin3ERb5*.

In several tests for chromosomal aberration, allyl chloride gave variable outcomes. Allyl chloride gave negative results in a study in rat liver cells without metabolic activation (Dean et al., 1985); the test was not carried out with metabolic activation. In Chinese hamster lung cells, allyl chloride gave positive results with and without metabolic activation (JETOC, 1997a), but negative results in another study that investigated allyl chloride at lower concentrations (Rim & Kim, 2014) [The Working Group noted that the highest dose investigated in the most recent study appeared to be too low.]

(iii) Non-mammalian and acellular systems in vitro

See Table 4.5.

Allyl chloride produced gene mutations in bacteria (McCoy et al., 1978; Bignami et al., 1980; Eder et al., 1980; Norpoth et al., 1980; Simmon et al., 1981; Dean et al., 1985; Neudecker & Henschler, 1985, 1986; JETOC, 1997b; Emmert et al., 2006) and induced gene conversion in fungi (McCoy et al., 1978; Dean et al., 1985). Allyl chloride gave negative results in the sex-linked recessive lethal test in *Drosophila melanogaster* (McGregor, 1981), and in tests for forward mutation (Bignami et al., 1980) and mitotic segregation (Crebelli et al., 1984) in *Aspergillus nidulans*.

4.2.3 Alters cell proliferation, cell death, or nutrient supply

In $B6C3F_1$ mice exposed orally to allyl chloride for 90 weeks (NCI, 1978), a high incidence of non-neoplastic lesions (hyperkeratosis and acanthosis) of the forestomach was observed in males and females.

In Crj:BDF1 mice exposed to allyl chloride by inhalation for 104 weeks (JBRC, 2003a, b), there were occurrences of non-neoplastic lesions such as hyperplasia of the Harderian gland in all groups of treated males.

In F344/DuCrj rats exposed to allyl chloride by inhalation for 104 weeks (JBRC, 2003c, d), there were occurrences of transitional epithelium hyperplasia, nodular hyperplasia, and squamous cell metaplasia in the urinary bladder in male rats at the highest dose.

4.3 Data relevant to comparisons across agents and end-points

The analysis of the bioactivity in vitro of the agents reviewed in *IARC Monographs* Volume 125 was informed by data from high-throughput screening assays generated by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the Government of the USA (Thomas et al., 2018). Allyl chloride was one of thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes as of 1 September 2019 (US EPA, 2019a). Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2019a). [The Working Group noted that the metabolic capacity of the cell-based assays is variable, and generally limited, as acknowledged in Kavlock et al. (2012).]

Allyl chloride was tested (at concentrations of up to 100 μM) in 403 assays and was found to be inactive in almost all. Active responses were observed in six assays (US EPA, 2019b). For nuclear receptors, borderline activity (potency of < 50% and/or activity observed only at the highest concentration tested) was found for the pregnane X receptor response element (PXRE), activation of human vascular endothelial growth factor receptor 1 (hVEGFR1), estrogen receptor α (ERα) agonism in two assays, estrogen receptor β (ERβ) antagonism, and TP53 activation.

5. Summary of Data Reported

5.1 Exposure characterization

Allyl chloride (CAS No. 107-05-01) is a High Production Volume chemical that is almost exclusively used in the production of epichlorohydrin, a basic building block for epoxy resins and the synthesis of glycerol. Minor uses of allyl chloride also include the synthesis of a variety of miscellaneous products including other allyl chemicals, pesticides, pharmaceuticals, adhesives, and personal-care products. A wide range of occupational exposure levels to allyl chloride have been reported in air where this chemical is produced and used, but exposures of the general population have not been reported.

5.2 Cancer in humans

The only available study of allyl chloride was conducted in a cohort of male employees from a chemical plant in the USA. The study was considered uninformative due to the small number of person-years with exposure to allyl chloride, the high potential for co-exposure to epichlorohydrin, and the minimal adjustment for confounding.

5.3 Cancer in experimental animals

Allyl chloride was tested for carcinogenicity in four different strains of male and/or female mice in one study of each of the following routes of exposure: whole-body inhalation, intraperitoneal injection, skin application, subcutaneous injection, gavage, and skin application as initiator in an initiation–promotion study. Allyl chloride was tested in two different strains of male and female rats in one study of whole-body inhalation, and in one study of administration by gavage.

The inhalation study in male and female rats was well-conducted under good laboratory practice (GLP). In male rats, exposure to allyl chloride resulted in a significant positive trend and increase in the incidence of transitional cell carcinoma of the urinary bladder. In male rats, there were also significant positive trends in the incidence of follicular cell adenoma, follicular cell adenoma or adenocarcinoma (combined), and C-cell carcinoma of the thyroid, bronchioloalveolar adenoma, and bronchioloalveolar adenoma or carcinoma (combined) of the lung, peritoneal mesothelioma, keratoacanthoma of the skin, and mammary gland fibroadenoma. There were no significant increases in tumour incidence in female rats in this inhalation study. The gavage study in male and female rats was inadequate for the evaluation of the carcinogenicity of allyl chloride.

The study of inhalation in male and female mice was well-conducted under GLP and resulted in significant positive trends and increases in the incidence of Harderian gland adenoma and lung bronchioloalveolar adenoma in males and females.

In the study of intraperitoneal injection, there was a significant increase in the average number of lung tumours by gross observation (mostly adenomas) per animal in male and female mice (combined). In the initiation–promotion study in female mice, there was a significant increase in the incidence of skin papilloma. The studies of skin application and subcutaneous injection in female mice gave negative results.

In the study of gavage in female mice, there was a non-significant increase in the incidence of forestomach tumours. The study of gavage in male mice was inadequate for the evaluation of the carcinogenicity of allyl chloride.

5.4 Mechanistic evidence

In humans exposed by inhalation to allyl chloride at low concentrations (at or below 3 mg/m^3), allylmercapturic acid (ALMA) was detected in the urine. ALMA and its sulfoxide were detected in the urine of rats exposed to allyl chloride subcutaneously or intraperitoneally. ALMA also can be detected in the urine after ingestion of allium vegetables such as garlic.

Overall, the mechanistic evidence is suggestive but incoherent across different experimental systems. Regarding the key characteristics of carcinogens, allyl chloride is electrophilic and alkylates DNA, but the evidence that it is genotoxic is incoherent across studies in different experimental systems. Allyl chloride gave generally positive results in the Ames test, but gave negative results in the two studies of genotoxicity in vivo in rodents, and yielded inconsistent results in the few studies in vitro in human and mammalian experimental systems. There is suggestive evidence that allyl chloride alters cell proliferation, cell death, or nutrient supply, based on the increased incidence of nodular hyperplasia of transitional epithelium and squamous cell metaplasia in the urinary bladder at the highest dose in male rats exposed chronically by inhalation. For other key characteristics of carcinogens, there is a paucity of available data. Allyl chloride was found to be mostly without effects in the assay battery of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes in the USA.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of allyl chloride.

6.2 Cancer in experimental animals

There is *limited evidence* in experimental animals for the carcinogenicity of allyl chloride.

6.3 Mechanistic evidence

There is *limited mechanistic evidence*.

6.4 Overall evaluation

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

6.5 Rationale

Allyl chloride was evaluated as Group 3 because the evidence of cancer in humans is *inadequate*, the evidence of cancer in experimental animals is *limited*, and the mechanistic evidence is *limited*. The evidence of cancer in humans was *inadequate* as only one, non-informative, study was available. The evidence of carcinogenicity in experimental animals was *limited* as malignant neoplasms were induced in one species (rat) and one sex (males). A small minority of the Working Group considered the evidence in experimental animals to be *sufficient* on the basis of tumour multiplicity in target tissues, and proposed a classification of *possibly carcinogenic to humans* (*Group 2B*). The mechanistic evidence was *limited* as the findings regarding key characteristics of carcinogens were suggestive, but incoherent across different experimental systems.

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1-BROMO-3-CHLOROPROPANE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 109-70-6

Chem. Abstr. Serv. name: 1-bromo-3-chloropropane

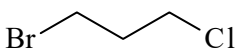
Preferred IUPAC name: 1-bromo-3-chloropropane

Synonyms: 1-chloro-3-bromopropane; 3-bromopropyl chloride; 3-chloropropyl bromide; bromochloropropane; bromochloropropane-1,3; trimethylene bromochloride; trimethylene chlorobromide; ω-chlorobromopropane.

1.1.2 Structural and molecular formulae, and relative molecular mass

Molecular formula: C_3H_6BrCl

Relative molecular mass: 157.44



1.1.3 Chemical and physical properties of the pure substance

Description: colourless liquid (NCBI, 2014)

Density (at 20 °C): 1.60 g/cm^3 (Lide, 1996)

Solubility: poorly soluble in water (2240 mg/L at 25 °C) (NCBI, 2014); soluble in oxygenated and chlorinated solvents (Ashford, 1994)

Vapour pressure: 0.85 kPa at 25 °C (ILO, 2017)

Vapour density: 5.4 (air = 1) (ILO, 2017)

Stability and reactivity: 1-bromo-3-chloropropane is flammable and considered moderately reactive to very reactive; it is also incompatible with strong oxidizing and reducing agents, and many amines, nitrides, azo/diazo compounds, alkali metals, and epoxides (NCBI, 2014)

*Octanol/water partition coefficient (*P*)*: log K_{ow} = 2.18 (ILO, 2017)

Melting point: –58.9 °C (NCBI, 2014)

Boiling point: 143.3 °C (ILO, 2017)

Flash point: 57 °C (ILO, 2017)

Henry's law constant: 2.5×10^{-4} atm m^3 mol^{-1} [25.3 Pa m^3 mol^{-1}] at 25 °C (NCBI, 2014)

Conversion factor: 1 ppm = 6.44 mg/m^3 at 25 °C and 101 kPa.

1.1.4 Technical grade and impurities

The technical product typically consists of 95% primary and 5% secondary bromochloropropanes (Gerhartz, 1985).

1.2 Production and uses

1.2.1 Production process

1-Bromo-3-chloropropane is almost always produced by the free-radical addition of anhydrous hydrogen bromide to allyl chloride (Gerhartz, 1985).

1.2.2 Production volume

1-Bromo-3-chloropropane is identified as a High Production Volume chemical by the Organisation for Economic Co-operation and Development (OECD) (OECD, 2011). Currently, the majority of registered production plants are located in Asia, but production also occurs in Europe and in the USA (Chem Sources, 2019). In the European Union, the current total manufactured and/or imported volume is between 1 and 10 tonnes per year (ECHA, 2019). Data on recent aggregated production volumes in the USA were withheld to protect company proprietary data (US EPA, 2016). In 1978, production in the USA was reported as "probably greater than" 2.27×10^6 g [2.27 tonnes] per annum (NCBI, 2014).

1.2.3 Uses

1-Bromo-3-chloropropane is mainly used as an intermediate in the manufacture of pharmaceuticals, such as antianxiety agents, antidepressants and antipsychotics, antimigraine, local anaesthetics, and antihypertensives (Ashford, 1994; Raman et al., 2017). It is also used in the production of antibacterial, antiviral and antimalarial drugs, and β2-adrenoreceptor agonists (medications to treat bronchial asthma and chronic obstructive pulmonary disease), and in the production of quinazoline derivatives that are used as drugs against cancer, inflammation, and obesity and diabetes (Krishnegowda et al., 2002; Zhang et al., 2016). Apart from pharmaceuticals, 1-bromo-3-chloropropane is also used as an intermediate in the manufacture of pesticides and other chemicals (Gerhartz, 1985; NCBI, 2014).

1.3 Methods of measurement and analysis

1.3.1 Detection and quantification

Kuznetsova & Nogina (1994) describes an analytical method for the detection of 1-bromo-3-chloropropane in air. The sample was prepared by creation of a steam and gas mixture of 1-bromo-3-chloropropane and nitrogen and then absorption of 1-bromo-3-chloropropane using carbochrome C. Final separation was carried out by gas chromatography and a detection limit was reported at 0.01 mg/m^3 (sampling volume up to 5 L). [The Working Group noted that no other information on the method, such as the detection method, could be traced.]

No methods for detection in environmental samples (e.g. water and soil) were identified. One method for the determination and quantification of 1-bromo-3-chloropropane as an impurity in pharmaceuticals could be traced in the literature (Raman et al., 2017). In this study, 1-bromo-3-chloropropane was separated using gas chromatography (stationary phase: bonded and cross-linked polyethylene glycol) and mass spectrometry. The absolute detection limit was reported as 5 ppm (Raman et al., 2017).

1.3.2 Biomarkers of exposure

No data on biomarkers of exposure were available to the Working Group.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

1-Bromo-3-chloropropane is not known to occur naturally in the environment. The production and use of 1-bromo-3-chloropropane in the manufacture of pharmaceuticals and in organic syntheses may result in its release to the environment through various waste streams (NCBI, 2014).

(a) Air

If released to the atmosphere, 1-bromo-3-chloropropane will mainly exist in the vapour phase in the ambient atmosphere (NCBI, 2014). Vapour-phase 1-bromo-3-chloropropane is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals with an estimated half-life of about 18 days (NCBI, 2014). In 1983, 1-bromo-3-chloropropane was detected in air at "source dominated" areas with a median value of 13 ng/m^3, but not in air at rural/remote or suburban/urban areas in the USA (Brodzinsky & Singh, 1983). In 1979, 1-bromo-3-chloropropane was qualitatively identified in air samples collected from a geographical area associated with the bromine industry in Arkansas, USA (DeCarlo, 1979). Pellizzari et al. (1978) reported that 1-bromo-3-chloropropane was detected in air near two production facilities in Alaska, USA. [The Working Group noted that no more recent data on environmental occurrence in air were available.]

(b) Water

If released to water, 1-bromo-3-chloropropane is not expected to adsorb to sediment or particulate matter based on its soil adsorption coefficient (K_{oc}) value (NCBI, 2014). This compound is expected to volatilize from water surfaces given its estimated Henry's law constant. Estimated volatilization half-lives for a model river and model lake are 8 hours and 6 days, respectively (NCBI, 2014). Bioconcentration in aquatic organisms are expected to be low based on an estimated bioconcentration factor value of 27 (NCBI, 2014).

(c) Soil

If released to soil, an estimated K_{oc} value of 63 suggests that 1-bromo-3-chloropropane will have high mobility (NCBI, 2014). Volatilization from dry soil surfaces is expected to be greater than from moist soil surfaces on the basis of physico-chemical properties (NCBI, 2014). 1-Bromo-3-chloropropane, inoculated with effluent from a biological waste treatment plant, reached 3% of the theoretical biochemical oxygen demand in 5 days, suggesting that biological degradation is slow (NCBI, 2014).

1.4.2 Occupational and general population exposure

Potential occupational exposure may occur through inhalation and dermal contact at workplaces where 1-bromo-3-chloropropane is produced or used. Since 1-bromo-3-chloropropane is used only as a chemical intermediate in the production of pharmaceuticals and pesticides, exposure of the general population is likely to be limited (NCBI, 2014); however, 1-bromo-3-chloropropane may occur as an impurity in drug substances (Raman et al., 2017).

1.5 Regulations and guidelines

No regulations or guidelines for this agent were available to the Working Group.

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

In a study that complied with good laboratory practice (GLP), groups of 50 male and 50 female Crj:BDF1 [B6D2F$_1$/Crlj] mice (age, 6 weeks) were treated by whole-body inhalation with 1-bromo-3-chloropropane (0, 25, 100, and 400 ppm v/v in clean air) (purity, > 99.8%) for 6 hours per day, 5 days per week, for 2 years (JBRC, 2005a, c). Survival rates were unaffected in all male and female groups exposed to 1-bromo-3-chloropropane, compared with controls. Survival to the end of 2 years for the groups at 0, 25, 100, and 400 ppm was 38/50, 33/50, 37/50, and 36/50 in males, and 30/50, 24/49, 32/50, and 33/50 in females, respectively. At cessation of treatment, body weights were significantly decreased in groups of males at 100 ppm (–10%) and 400 ppm (–15%) and in females at 400 ppm (–11%), relative to their respective control groups. All mice (except for one female at 25 ppm) underwent complete necropsy and full histopathological examination.

Inhalation of 1-bromo-3-chloropropane caused a significant dose-related increase in the incidence of bronchioloalveolar adenoma ($P < 0.01$, Peto trend test), of bronchioloalveolar carcinoma ($P < 0.05$, Peto trend test), and of bronchioloalveolar adenoma or carcinoma (combined) ($P < 0.01$, Peto trend test) in male mice. The incidence of bronchioloalveolar adenoma, of bronchioloalveolar carcinoma, and of bronchioloalveolar adenoma or carcinoma (combined) was significantly increased in male mice at 25, 100, and 400 ppm ($P < 0.01$, Fisher exact test).

Inhalation of 1-bromo-3-chloropropane caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of squamous cell papilloma of the forestomach in male mice. The incidence of forestomach squamous cell papilloma was significantly increased in male mice exposed at 400 ppm ($P < 0.05$, Fisher exact test).

Inhalation of 1-bromo-3-chloropropane caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of hepatocellular adenoma in male mice. The incidence of hepatocellular adenoma was significantly increased in male mice at 400 ppm ($P < 0.01$, Fisher exact test). Inhalation of 1-bromo-3-chloropropane did not cause any significant change in the incidence of hepatocellular carcinoma or hepatoblastoma.

Inhalation of 1-bromo-3-chloropropane caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of adenoma of the Harderian gland in male mice. The incidence of Harderian gland adenoma was significantly increased in male mice at 400 ppm ($P < 0.05$, Fisher exact test).

Exposure to 1-bromo-3-chloropropane by inhalation caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of bronchioloalveolar adenoma and of bronchioloalveolar adenoma or carcinoma (combined) in female mice. The incidence of bronchioloalveolar adenoma, of bronchioloalveolar carcinoma, and of bronchioloalveolar adenoma or carcinoma (combined) was significantly increased in female mice at 25, 100, and 400 ppm ($P < 0.01$, Fisher exact test). Inhalation of 1-bromo-3-chloropropane did not cause a statistically significant dose-related trend (Peto test or Cochran–Armitage test) in the incidence of bronchioloalveolar carcinoma in female mice.

Inhalation of 1-bromo-3-chloropropane caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of squamous cell papilloma of the forestomach in female mice. The incidence of forestomach squamous

Table 3.1 Studies of carcinogenicity with 1-bromo-3-chloropropane in mice and rats treated by inhalation (whole-body exposure)

Species, strain (sex) Age at start Duration Reference	Purity Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, Crj:BDF1 (M) Age, 6 wk 104 wk JBRC (2005a, c)	Purity, > 99.8% 0, 25, 100, 400 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 38, 33, 37, 36	*Lung* Bronchioloalveolar adenoma 5/50, 21/50**, 20/50**, 26/50**	Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); ** $P < 0.01$ (Fisher exact test)	Principal strengths: well-conducted GLP study; males and females used; study covered most of lifespan; multiple-dose study Other comments: no significant effect of treatment on survival
		Bronchioloalveolar carcinoma 3/50, 29/50**, 26/50**, 26/50**	Positive trend: $P < 0.05$ (Peto test and Cochran–Armitage test); ** $P < 0.01$ (Fisher exact test)	
		Bronchioloalveolar adenoma or carcinoma (combined) 8/50, 35/50**, 35/50**, 39/50**	Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); ** $P < 0.01$ (Fisher exact test)	
		Adenosquamous carcinoma 0/50, 0/50, 0/50, 1/50	NS	
		Squamous cell carcinoma 0/50, 0/50, 1/50, 0/50	NS	
		Bronchioloalveolar adenoma, bronchioloalveolar carcinoma, adenosquamous carcinoma, or squamous cell carcinoma (combined) 8/50, 35/50**, 35/50**, 39/50**	Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); ** $P < 0.01$ (Fisher exact test)	
		Forestomach: squamous cell papilloma 1/50, 1/50, 2/50, 8/50*	Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); * $P < 0.05$ (Fisher exact test)	
		Liver Hepatocellular adenoma 4/50, 10/50, 8/50, 14/50**	Positive trend: $P < 0.01$ (Peto test) and $P < 0.05$ (Cochran–Armitage test); ** $P < 0.01$ (Fisher exact test)	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, Crj:BDF1 (M) Age, 6 wk 104 wk JBRC (2005a, c) (cont.)		Hepatocellular carcinoma 3/50, 5/50, 3/50, 3/50	NS	
		Hepatoblastoma 0/50, 0/50, 1/50, 1/50	NS	
		Harderian gland: adenoma 4/50, 4/50, 4/50, 13/50*	Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); * $P < 0.05$ (Fisher exact test)	
Mouse, Crj:BDF1 (F) Age, 6 wk 104 wk JBRC (2005a, c)	Purity, > 99.8% 0, 25, 100, 400 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 30, 24, 32, 33	*Lung* Bronchioloalveolar adenoma 2/50, 19/49**, 25/50**, 30/50**	Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); ** $P < 0.01$ (Fisher exact test)	Principal strengths: well-conducted GLP study; males and females used; study covered most of lifespan; multiple-dose study Other comments: no significant effect of treatment on survival
		Bronchioloalveolar carcinoma 2/50, 12/49**, 20/50**, 13/50**	** $P < 0.01$ (Fisher exact test)	
		Bronchioloalveolar adenoma or carcinoma (combined) 4/50, 23/49**, 33/50**, 38/50**	Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); ** $P < 0.01$ (Fisher exact test)	
		Forestomach Squamous cell papilloma 0/50, 0/49, 1/50, 8/50**	Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); ** $P < 0.01$ by Fisher exact test	
		Squamous cell carcinoma 0/50, 1/49, 0/50, 1/50	NS	
		Harderian gland: adenoma 3/50, 0/49, 2/50, 14/50**	Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); ** $P < 0.01$ (Fisher exact test)	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/DuCrj (M) Age, 6 wk 104 wk JBRC (2005b, d)	Purity, > 99.8% 0, 25, 100, 400 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 40, 35, 38, 30	*Liver* Hepatocellular adenoma 1/50, 1/50, 2/50, 10/50**	Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); ** $P < 0.01$ (Fisher exact test)	Principal strengths: well-conducted GLP study; males and females used; study covered most of lifespan; multiple-dose study Other comments: no significant effect of treatment on survival; historical control incidence of skin trichoepithelioma: 14/1747 (with a maximum of 4% in any single control group); historical control incidence of large intestine adenoma and adenocarcinoma: 0/1749; historical control incidence of bronchioloalveolar adenoma: 62/1749 (average, 3.5%; range, 0–10%)
		Hepatocellular carcinoma 0/50, 0/50, 1/50, 6/50*	Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); * $P < 0.05$ (Fisher exact test)	
		Hepatocellular adenoma or carcinoma (combined) 1/50, 1/50, 3/50, 15/50**	Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); ** $P < 0.01$ (Fisher exact test)	
		Haemangiosarcoma 1/50, 0/50, 0/50, 2/50	NS	
		Lung Bronchioloalveolar adenoma 2/50, 1/50, 1/50, 7/50	Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test)	
		Bronchioloalveolar carcinoma 0/50, 2/50, 0/50, 0/50	NS	
		Large intestine Adenoma 0/50, 0/50, 0/50, 3/50	Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test)	
		Adenocarcinoma 0/50, 0/50, 0/50, 1/50	NS	
		Skin: trichoepithelioma 0/50, 1/50, 0/50, 3/50	Positive trend: $P < 0.05$ (Peto test and Cochran–Armitage test)	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/DuCrj (F) Age, 6 wk 104 wk JBRC (2005b, d)	Purity, > 99.8% 0, 25, 100, 400 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 38, 45, 39, 26	*Liver* Hepatocellular adenoma 1/50, 0/50, 2/50, 32/50** Hepatocellular carcinoma 0/50, 0/50, 0/50, 38/50** Hepatocellular adenoma or carcinoma (combined) 1/50, 0/50, 2/50, 43/50** Haemangioma 0/50, 0/50, 0/50, 1/50 Haemangiosarcoma 0/50, 0/50, 0/50, 6/50* Haemangioma or haemangiosarcoma (combined) 0/50, 0/50, 0/50, 7/50* *Spleen*: mononuclear cell leukaemia 5/50, 3/50, 5/50, 13/50* *Lung*: bronchioloalveolar adenoma 1/50, 0/50, 1/50, 5/50	 Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); ** $P < 0.01$ (Fisher exact test) Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); ** $P < 0.01$ (Fisher exact test) Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); ** $P < 0.01$ (Fisher exact test) NS Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); * $P < 0.05$ (Fisher exact test) Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); * $P < 0.01$ (Fisher exact test) Positive trend: $P < 0.01$ by Peto test and Cochran–Armitage test; * $P < 0.05$ by Fisher exact test Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test)	Principal strengths: well-conducted GLP study; males and females used; study covered most of lifespan; multiple-dose study Other comments: no significant effect of treatment on survival; historical control incidence of skin trichoepithelioma: 3/1597 from 32 studies at laboratory (with a maximum of 2% in any single control group); historical control incidence of large intestine adenoma: 0/1597 from 32 studies at laboratory; historical control incidence of bronchioloalveolar adenoma: 30/1597 (average, 1.9%; range, 0–10%)

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/DuCrj (F) Age, 6 wk 104 wk JBRC (2005b, d) (cont.)		*Skin*: trichoepithelioma 0/50, 0/50, 1/50, 2/50 *Large intestine*: adenoma 0/50, 0/50, 0/50, 2/50	NS NS	

F, female; GLP, good laboratory practice; M, male; NS, not significant; ppm, parts per million; wk, week.

cell papilloma was significantly increased in female mice exposed at 400 ppm ($P < 0.01$, Fisher exact test). Inhalation of 1-bromo-3-chloropropane did not cause any significant change in the incidence of squamous cell carcinoma of the forestomach.

Inhalation of 1-bromo-3-chloropropane caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of Harderian gland adenoma in female mice. The incidence of Harderian gland adenoma was significantly increased in female mice at 400 ppm ($P < 0.01$, Fisher exact test).

Inhalation of 1-bromo-3-chloropropane resulted in increased incidence and/or severity of non-neoplastic lesions in the nasal cavity in mice (respiratory metaplasia, atrophy, and eosinophilic change of the olfactory epithelium, and glandular respiratory metaplasia at 400 ppm in males and females, and exudate at 400 ppm in females only). Exposure to 1-bromo-3-chloropropane resulted in an increase in the incidence of non-neoplastic lesions of the nasopharynx (eosinophilic change at 400 ppm in males and at 100 and 400 ppm in females; exudate at 400 ppm in females). 1-Bromo-3-chloropropane resulted in increased incidence of non-neoplastic lesion of the lung (bronchioloalveolar hyperplasia at 25, 100, and 400 ppm in males and females). Exposure to 1-bromo-3-chloropropane resulted in an increase in the incidence of non-neoplastic lesions of the forestomach (squamous cell hyperplasia at 400 ppm in males and at 100 and 400 ppm in females). [The Working Group noted that this GLP study used males and females, and multiple doses.]

3.2 Rat

Inhalation

In a study that complied with GLP, groups of 50 male and 50 female F344/DuCrj rats (age, 6 weeks) were treated by whole-body inhalation with 1-bromo-3-chloropropane (0, 25, 100, and 400 ppm, v/v in clean air) (purity, > 99.8%) for 6 hours per day, 5 days per week, for 2 years (JBRC, 2005b, d). Survival rates appeared to be significantly reduced in groups of males and females at 400 ppm. Survival up to 2 years for the groups at 0, 25, 100, and 400 ppm was 40/50, 35/50, 38/50, and 30/50 in males, and 38/50, 45/50, 39/50, and 26/50 in females, respectively. At cessation of treatment, body weights were significantly decreased, relative to their respective control groups, in males (–25%) and in females (–20%) in the groups at 400 ppm. All rats underwent complete necropsy and full histopathological examination.

Inhalation of 1-bromo-3-chloropropane caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of hepatocellular adenoma, of hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined) in male rats. The incidence of hepatocellular adenoma and of hepatocellular adenoma or carcinoma (combined) was significantly increased in male rats at 400 ppm ($P < 0.01$, Fisher exact test). The incidence of hepatocellular carcinoma was significantly increased in male rats at 400 ppm ($P < 0.05$, Fisher exact test).

Inhalation of 1-bromo-3-chloropropane caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of bronchioloalveolar adenoma in male rats. The incidence of bronchioloalveolar adenoma was not significantly increased in any group of exposed male rats by pairwise comparison. Inhalation of 1-bromo-3-chloropropane did not cause any dose-related change in the incidence of bronchioloalveolar carcinoma in male rats. The incidence of bronchioloalveolar carcinoma was not significantly increased in any group of exposed male rats by pairwise comparison.

Inhalation of 1-bromo-3-chloropropane caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of adenoma of the large intestine in male rats.

The incidence of adenoma was not significantly increased in any group of exposed male rats (controls, 0/50; 25 ppm, 0/50; 100 ppm, 0/50; and 400 ppm, 3/50) by pairwise comparison. The incidence of adenocarcinoma of the large intestine was not significantly increased in any group of exposed male rats (controls, 0/50; 25 ppm, 0/50; 100 ppm, at 0/50; and 400 ppm, 1/50). [The incidence of adenoma and of adenocarcinoma of the large intestine in the group at 400 ppm exceeded the incidence (0/1749 and 0/1749, respectively) observed in the historical control group of male F344/DuCrj rats from 35 studies conducted in this laboratory.]

Inhalation of 1-bromo-3-chloropropane caused a significant dose-related increase ($P < 0.05$, Peto trend test) in the incidence of trichoepithelioma of the skin in male rats. The incidence of trichoepithelioma of the skin was not significantly increased in male rats exposed at 25, 100, and 400 ppm (controls, 0/50; 25 ppm, 1/50; 100 ppm, 0/50; and 400 ppm, 3/50). [The incidence of trichoepithelioma in the group at 400 ppm exceeded the upper bound of the range observed in the historical control group of male F344/DuCrj rats from 35 studies conducted in this laboratory (incidence, 14/1747; with a maximum of 4% in any single control group).]

Inhalation of 1-bromo-3-chloropropane caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of hepatocellular adenoma, of hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined) in female rats. The incidence of hepatocellular adenoma, of hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined) was significantly increased in female rats at 400 ppm ($P < 0.01$, Fisher exact test). Inhalation of 1-bromo-3-chloropropane caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of haemangiosarcoma of the liver in female rats. The incidence of liver haemangiosarcoma was significantly increased in female rats at 400 ppm ($P < 0.05$, Fisher exact test).

Inhalation of 1-bromo-3-chloropropane caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of mononuclear cell leukaemia of the spleen in female rats. The incidence of mononuclear cell leukaemia of the spleen was significantly increased in female rats at 400 ppm ($P < 0.05$, Fisher exact test).

Inhalation of 1-bromo-3-chloropropane caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of bronchioloalveolar adenoma in female rats. The incidence of bronchioloalveolar adenoma was not significantly increased in any group of exposed female rats by pairwise comparison.

The incidence of trichoepithelioma of the skin was not significantly increased in any group of exposed female rats (controls, 0/50; 25 ppm, 0/50; 100 ppm, 1/50; and 400 ppm, 2/50). [The incidence of trichoepithelioma in the group at 400 ppm exceeded the upper bound of the range observed in the historical control group of female F344/DuCrj rats from 32 studies conducted in this laboratory (incidence, 3/1597; with a maximum of 2% in any single control group).]

Inhalation of 1-bromo-3-chloropropane did not cause any dose-related change in the incidence of adenoma of the large intestine in female rats. The incidence of adenoma of the large intestine was not significantly increased in any group of exposed female rats (controls, 0/50; 25 ppm, 0/50; 100 ppm, 0/50; and 400 ppm, 2/50). [The incidence of adenoma in the group at 400 ppm exceeded the incidence (0/1597) observed in the historical control group of female F344/DuCrj rats from 32 studies conducted in this laboratory.]

Inhalation of 1-bromo-3-chloropropane resulted in increased incidence and/or severity of non-neoplastic lesions in the nasal cavity in male rats (inflammation of the respiratory epithelium at 25, 100, and 400 ppm; squamous metaplasia of the respiratory epithelium, respiratory

metaplasia of glands, atrophy, necrosis, and respiratory metaplasia of the olfactory epithelium at 400 ppm). Exposure to 1-bromo-3-chloropropane resulted in increased incidence and/or severity of non-neoplastic lesions in the nasal cavity in female rats (respiratory metaplasia of glands at 100 and 400 ppm; inflammation of the respiratory epithelium, squamous metaplasia of the respiratory epithelium, atrophy and necrosis of the olfactory epithelium at 400 ppm). Exposure to 1-bromo-3-chloropropane resulted in increased incidence and/or severity of non-neoplastic lesions in the liver in male rats (clear cell focus at 100 and 400 ppm; acidophilic and basophilic focus at 400 ppm). Exposure to 1-bromo-3-chloropropane resulted in an increase in the incidence and/or severity of non-neoplastic lesions in the liver in female rats (bile duct hyperplasia at 100 and 400 ppm; clear cell, acidophilic cell, and basophilic cell focus at 400 ppm). Exposure to 1-bromo-3-chloropropane resulted in an increase in the incidence of non-neoplastic lesions of the spleen (deposit of haemosiderin at 400 ppm in males). Exposure to 1-bromo-3-chloropropane resulted in an increase in the incidence of a non-neoplastic lesion of the bone marrow (increased haematopoiesis at 400 ppm in females). [The Working Group noted that this GLP study used males and females, and multiple doses.]

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

A single intraperitoneal injection of 1-bromo-3-chloropropane at a dose of 1300 µmol/kg body weight (bw) [205 mg/kg bw] resulted in concentrations of ~15 nmol/mL in plasma, ~100 nmol/g in kidney, and ~30 nmol/g in testis 1 hour after dosing in male MOL:WIST rats (Låg et al., 1991).

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether 1-bromo-3-chloropropane is genotoxic; or alters cell proliferation, cell death, or nutrient supply. Insufficient data were available for the evaluation of other key characteristics of carcinogens.

4.2.1 Is genotoxic

Table 4.1, Table 4.2, and Table 4.3 summarize the studies evaluated that report genetic and related effects of 1-bromo-3-chloropropane.

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.1.

Several studies investigated the genotoxic effects of exposure to 1-bromo-3-chloropropane in experimental animals in vivo. No increase in the frequency of *gpt* mutations was observed in the liver, bone marrow, glandular stomach, or testis of male *gpt* delta mice exposed to 1-bromo-3-chloropropane at a dose of 30, 100, or 300 mg/kg bw per day by oral gavage for 28 days (JECDB, 2000a). Unlike the positive control (mitomycin C), 1-bromo-3-chloropropane did not increase the frequency of micronucleated

Table 4.1 Genetic and related effects of 1-bromo-3-chloropropane 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 damage (alkaline elution)	Rat, MOL:WIST (M)	Kidney	–	3000 µmol/kg bw [472 mg/kg bw]	Intraperitoneal injection, 1×	Purity, NR	Låg et al. (1991)
Mutation in *gpt* delta (*gpt* and Spi-)	Mouse, C57BL/6 *gpt-delta* (M)	Liver, bone marrow, stomach, and testis	–	300 mg/kg bw	Oral, 1×/day, 7 days/week, 4 weeks		JECDB (2000a)
Chromosomal aberrations	Rat	Bone marrow	(+)	45 mg/m^3	Inhalation, chronic	Source and purity, NR Rat strain and sex, NR Duration and dosing regimen, NR	Eitingon (1971)
Micronucleus formation	Mouse, ICR (M)	Peripheral blood reticulocytes	–	645 mg/kg bw	Oral, 1×	Source and purity, NR	Kim & Ryu (2010)

bw, body weight; F, female; *gpt*, guanine phosphoribosyltransferase; HID, highest ineffective dose; LED, lowest effective dose (units as reported); M, male; NR, not reported; Spi, sensitive to prophage P2 interference.

[a] –, negative; (+), positive in a study that was poorly reported.

Table 4.2 Genetic and related effects of 1-bromo-3-chloropropane in non-human mammals in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
Gene mutation, *Tk* locus	L5178Y mouse, lymphoma cells	–	+	500 µg/mL (–S9); 200 µg/mL (+S9)	Seifried et al. (2006)
Chromosomal aberrations	Chinese hamster, lung cells	+	+	1600 µg/mL (–S9); 185 µg/mL (+S9)	Kim & Ryu (2006)
Chromosomal aberrations	Chinese hamster, lung cells	+	+	2000 µg/mL (–S9); 250 µg/mL (+S9)	JETOC (1997a)
Chromosomal aberrations	Chinese hamster, lung cells	±	+	1420 µg/mL (–S9); 250 µg/mL (+S9)	JECDB (2000b)

HIC, highest ineffective concentration; LEC, lowest effective concentration; S9, 9000 × *g* supernatant from rat liver; Tk, thymidine kinase.

[a] –, negative; +, positive; ±, equivocal.

Table 4.3 Genetic and related effects of 1-bromo-3-chloropropane in non-mammalian systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Aspergillus nidulans	Whole chromosome segregation	+	NA	5 mM [787 µg/mL]		Crebelli et al. (1995)
Salmonella typhimurium TA1535	Reverse mutation	–	+	2000 µg/plate (+/–S9 from rat liver)	Purity, NR Cytotoxicity, NR	JETOC (1997b)
Salmonella typhimurium TA1535	Reverse mutation	–	+	1250 µg/plate (–S9 from rat liver); 156 µg/plate (+S9 from rat liver)		JECDB (2000c)
Salmonella typhimurium TA100	Reverse mutation	–	– (+S9 from rat liver) + (+S9 from hamster liver)	10 000 µg/plate (–S9 from rat or hamster liver, or +S9 from rat liver); 1000 µg/plate (+S9 from hamster liver)		Seifried et al. (2006)
Salmonella typhimurium TA100	Reverse mutation	–	–	1.0 µmol/plate (157.4 µg/plate) (rat liver microsomes +/– NADPH co-factors)	Low concentration tested	Låg et al. (1994)
Salmonella typhimurium TA100	Reverse mutation	–	–	2000 µg/plate (+/–S9 from rat liver)	Purity, NR Cytotoxicity, NR	JETOC (1997b)
Salmonella typhimurium TA100	Reverse mutation	–	+	1250 µg/plate (–S9 from rat liver); 625 µg/plate (+S9 from rat liver)		JECDB (2000c)
Salmonella typhimurium TA98	Reverse mutation	–	–	10 000 µg/plate (+/–S9 from rat or hamster liver)		Seifried et al. (2006)
Salmonella typhimurium TA98	Reverse mutation	–	–	2000 µg/plate (+/–S9 from rat liver)	Purity, NR Cytotoxicity, NR	JETOC (1997b)
Salmonella typhimurium TA98	Reverse mutation	–	–	5000 µg/plate (+/–S9 from rat liver)		JECDB (2000c)
Salmonella typhimurium TA1537 and TA1538	Reverse mutation	–	–	2000 µg/plate (+/–S9 from rat liver)	Purity, NR Cytotoxicity, NR	JETOC (1997b)
Salmonella typhimurium TA1537	Reverse mutation	–	–	1250 µg/plate (+/–S9 from rat liver)		JECDB (2000c)
Escherichia coli WP2 *uvrA*	Reverse mutation	–	–	2000 µg/plate (+/–S9 from rat liver)	Purity, NR Cytotoxicity, NR	JETOC (1997b)
Escherichia coli WP2 *uvrA*	Reverse mutation	+	+	588/412 µg/plate (1st/2nd replicate; –S9 from rat liver); 1201/1715 µg/plate (1st/2nd replicate; +S9 from rat liver)		JECDB (2000c)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NA, not applicable; NADPH, nicotinamide adenine dinucleotide phosphate reduced form; NR, not reported; S9, 9000 × *g* supernatant

[a] –, negative; +, positive

peripheral blood reticulocytes in male ICR mice given a single oral dose of up to 645 mg/kg bw (Kim & Ryu, 2010). In addition, in contrast to other members of the halogenated propane family, 1-bromo-3-chloropropane did not increase renal DNA damage in male MOL:WIST rats, as assessed by alkaline elution, 48 hours after a single intraperitoneal injection of up to 3000 µmol/kg bw [470 mg/kg bw] (Låg et al., 1991). A chronic exposure to 1-bromo-3-chloropropane by inhalation at 45 mg/m^3, but not at 5.4 mg/m^3, increased the frequency of chromosomal aberrations in the bone marrow of rats (Eitingon, 1971) [the Working Group noted that the experimental details were poorly documented].

(ii) Non-human mammalian cells in vitro

See Table 4.2.

1-Bromo-3-chloropropane increased the frequency of mutations in the mouse heterozygous L5178 *Tk*$^{+/-}$ lymphoma cell assay in the presence, but not in the absence, of metabolic activation by a rat S9 liver homogenate (Seifried et al., 2006). Several studies reported that exposure of Chinese hamster lung cells to 1-bromo-3-chloropropane increased the frequency of chromosomal aberrations (JETOC, 1997a; JECDB, 2000b; Kim & Ryu, 2006). The lowest effective concentration of 1-bromo-3-chloropropane was consistent across these studies, and was decreased in the presence of metabolic activation (JETOC, 1997a; JECDB, 2000b; Kim & Ryu, 2006).

(iii) Non-mammalian experimental systems

See Table 4.3.

1-Bromo-3-chloropropane induced aberrant whole chromosome segregation in *Aspergillus nidulans* (Crebelli et al., 1995).

In the absence of metabolic activation, 1-bromo-3-chloropropane gave negative results in tests for reverse mutation in *Salmonella typhimurium* strains TA1535, TA100, TA98, and TA1537 (JETOC, 1997b; JECDB, 2000c; Seifried et al., 2006). In the presence of metabolic activation, 1-bromo-3-chloropropane was generally mutagenic in *S. typhimurium* strains that are indicators of base-substitution mutations (TA1535 and TA100), but not in *S. typhimurium* strains that are indicators of frameshift mutations (TA98, TA1537, and TA1538) (JETOC, 1997b; JECDB, 2000c; Seifried et al., 2006). Inconsistent results were reported in two tests in *Escherichia coli* WP2 *uvrA* (JETOC, 1997b; JECDB, 2000c).

4.2.2 Alters cell proliferation, cell death, and nutrient supply

In male and female Crj:BDF1 mice treated for 2 years by inhalation (JBRC, 2005a, c; see Section 3.1), an increase in the incidence and/or severity of the following non-neoplastic lesions was reported: respiratory metaplasia, atrophy, and eosinophilic change of the olfactory epithelium, and glandular respiratory metaplasia; nasopharyngeal eosinophilic change; bronchioloalveolar hyperplasia; and squamous cell hyperplasia in the forestomach.

In F344/DuCrj rats treated for 2 years by inhalation (JBRC, 2005b, d; see Section 3.2) an increase in the incidence and/or severity of the following non-neoplastic lesions was reported: respiratory epithelium squamous metaplasia, metaplasia of glands, atrophy and necrosis of the olfactory epithelium (in males and females). In the liver, foci (clear cell, acidophilic and basophilic types) were observed in males and females; while bile duct hyperplasia was observed in females. In bone marrow, increased haematopoiesis was observed in female rats.

4.2.3 Other data relevant to key characteristics

1-Bromo-3-chloropropane failed to activate both human and mouse constitutive androstane receptor (CAR) in a dual-luciferase reporter

assay using HepG2 human liver cells exposed at 1, 3, 10, and 30 μM (Imai et al., 2013).

In F344/DuCrj rats treated with 1-bromo-3-chloropropane by inhalation for 2 years (see Section 3.2), an increase in the incidence and/or severity of inflammation of the respiratory epithelium was detected in males and females (JBRC, 2005b, d).

1-Bromo-3-chloropropane was not tested in high-throughput screening assays of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the Government of the USA (Thomas et al., 2018).

5. Summary of Data Reported

5.1 Exposure characterization

1-Bromo-3-chloropropane is a High Production Volume chemical that is used as an intermediate in the manufacture of a wide range of pharmaceuticals. Minor uses include the manufacture of pesticides and other chemicals. Potential occupational exposure may occur at workplaces where 1-bromo-3-chloropropane is produced or used, whereas exposure of the general population is likely to be limited; however, actual exposure levels have not been reported in humans.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

In one well-conducted study that complied with good laboratory practice (GLP) in male and female mice treated by whole-body inhalation, 1-bromo-3-chloropropane caused a significant increase, with a significant positive trend, in the incidence of bronchioloalveolar adenoma, of bronchioloalveolar carcinoma, and of bronchioloalveolar adenoma or carcinoma (combined) in males; and a significant increase in the incidence of bronchioloalveolar adenoma (with a significant positive trend), of bronchioloalveolar adenoma or carcinoma (combined) (with a significant positive trend), and of bronchioloalveolar carcinoma in females. In male mice, there were also significant increases in the incidence, with a significant positive trend, of squamous cell papilloma of the forestomach, hepatocellular adenoma, and Harderian gland adenoma. In female mice, there were also significant increases in the incidence, with a significant positive trend, of squamous cell papilloma of the forestomach and of Harderian gland adenoma.

In one well-conducted GLP study in male and female rats treated by whole-body inhalation, 1-bromo-3-chloropropane caused a significant increase, with a significant positive trend, in the incidence of hepatocellular adenoma, of hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined) in males and females, and in the incidence of haemangiosarcoma of the liver in females. There was a significant increase, with a significant positive trend, in the incidence of mononuclear cell leukaemia of the spleen in females. There was a significant positive trend in the incidence of bronchioloalveolar adenoma in males and females, and of skin trichoepithelioma and of adenoma of the large intestine in males.

5.4 Mechanistic evidence

No informative data were available on the absorption, distribution, metabolism, or excretion of 1-bromo-3-chloropropane.

There is consistent and coherent evidence in experimental systems that 1-bromo-3-chloropropane exhibits key characteristics of carcinogens (alters cells proliferation, cell death, or nutrient supply). In male and female rats and mice, there were dose-related increases in incidence and/or

severity of various proliferative non-neoplastic lesions, including both hyperplasia and metaplasia, in point-of-contact and distal tissues after chronic exposure by inhalation. A minority of the Working Group considered this evidence suggestive, as the findings were observed in 2-year studies of carcinogenicity, by which time the induction of benign and malignant tumours had already occurred. There is suggestive evidence that 1-bromo-3-chloropropane induces chronic inflammation, on the basis of findings in male and female rats exposed chronically by inhalation of increased incidence and/or severity of inflammation of the respiratory epithelium. There is suggestive evidence that 1-bromo-3-chloropropane is genotoxic, with incoherent findings across different experimental systems (general lack of genotoxic effects in experimental models in vivo, but generally positive results in mammalian and bacterial models in vitro).

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of 1-bromo-3-chloropropane.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1-bromo-3-chloropropane.

6.3 Mechanistic evidence

There is *strong evidence* in experimental systems that 1-bromo-3-chloropropane exhibits key characteristics of carcinogens.

6.4 Overall evaluation

1-Bromo-3-chloropropane is *possibly carcinogenic to humans (Group 2B).*

6.5 Rationale

The evaluation of 1-bromo-3-chloropropane as Group 2B is based on *sufficient evidence* of cancer in experimental animals, and on *strong* mechanistic evidence. The evidence regarding cancer in humans is *inadequate*, as no data were available. The *sufficient evidence* for carcinogenicity in experimental animals is based on an increased incidence of malignant neoplasms in two species. A small minority view considered that the mechanistic evidence is *limited*. Overall, there is *strong evidence* in experimental systems that 1-bromo-3-chloropropane exhibits key characteristics of carcinogens; 1-bromo-3-chloropropane alters cell proliferation, cell death, or nutrient supply.

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1-BUTYL GLYCIDYL ETHER

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 2426-08-6

Chem. Abstr. Serv. name: butyl 2,3-epoxypropyl ether

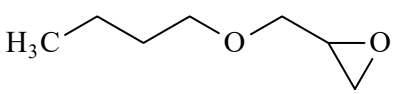
Preferred IUPAC name: 2-(butoxymethyl) oxirane

Synonyms: *n*-butyl glycidyl ether; butyl glycidyl ether; (butoxymethyl)oxirane; butyl 2,3-epoxypropyl ether; ether, butyl 2,3-epoxypropyl; oxirane, (butoxymethyl)-; 1-butoxy-2,3-epoxypropane; 3-butoxy-1,2-epoxypropane; glycidyl butyl ether; 2,3-epoxypropyl butyl ether.

1.1.2 Structural and molecular formulae, and relative molecular mass

Molecular formula: $C_7H_{14}O_2$

Relative molecular mass: 130.18 (NCBI, 2019).



1.1.3 Chemical and physical properties of the pure substance

Description: a clear, colourless to pale yellow liquid with a slightly unpleasant, irritating odour (Lewis, 2001; NTP, 2004)

Density (at 20 °C): 0.91 (NCBI, 2019)

Solubility: 20 g/L at 25 °C in water (Bingham et al., 2001)

Vapour pressure: 0.43 kPa at 25 °C (Lewis, 2001)

Vapour density: 3.78 (air = 1) (Wallace, 1979; Bingham et al., 2001)

Stability and reactivity: rapidly oxidized in air and reacts readily with acids, water and nucleophiles such as proteins and nucleic acids (Worksafe New Zealand, 2019)

*Octanol/water partition coefficient (*P):

log K_{ow} = 0.63 (Hansch et al., 1995)

Henry's law constant:

4.37×10^{-6} atm m^3 mol^{-1} [0.4 Pa m^3 mol^{-1}] at 25 °C (Environment Canada, 2010)

Melting point: 59 °C (NCBI, 2019)

Boiling point: 164 °C (Lewis, 2001)

Flash point: 64 °C (NTP, 2004)

Conversion factor: 1 ppm = 5.32 mg/m^3 at 25 °C and 101.3 kPa.

1.2 Production and uses

1.2.1 Production process

1-Butyl glycidyl ether is produced by the condensation of epichlorohydrin and *n*-butyl alcohol to form an intermediate chlorohydrin, which is then dehydrochlorinated to form an epoxide group (Bosch et al., 1985; NTP, 2004).

1.2.2 Production volume

1-Butyl glycidyl ether has been identified as a High Production Volume chemical by the Organisation for Economic Co-operation and Development (OECD) (OECD, 2009). Currently the majority of the registered manufacturing plants are located in Europe and the USA, whereas fewer sites are situated in Asia (Chem Sources, 2019). In the European Union, the total volume manufactured and/or imported is listed as between 100 and 1000 tonnes per year (ECHA, 2019). Between 1998 and 2006, aggregated production and/or import volumes in the USA were reported to be between 1 000 000 and 10 000 000 lbs [450–4500 tonnes] per year (HSDB, 2006; US EPA, 2008; OECD, 2009).

1.2.3 Uses

1-Butyl glycidyl ether is used as a viscosity-reducing agent, allowing easier handling of uncured resins (Bosch et al., 1985; Lee, 1989). It is also used as a reactive diluent for epoxy resins, as a chemical intermediate, and as an acid acceptor for stabilizing chlorinated solvents (Bingham et al., 2001; NTP, 2004; HSDB, 2006). Epoxy resins have applications as coatings, adhesives, binders, sealants, and fillers (Environment Canada, 2010). During curing, 1-butyl glycidyl ether participates in polymerization and cross-linking due to the presence of the epoxide functional group, allowing it to become covalently bound to the polymer (Bosch et al., 1985; Lee, 1989; Hamerton, 1996). Additionally, it is used as a dye-dispersing agent, a cotton or wool surface modifier, and a dye-enhancing agent (Azuma et al., 2016). 1-Butyl glycidyl ether has also been reported as an impurity in a material preservative for paint and pesticide products (Environment Canada, 2010).

1.3 Methods of measurement and analysis

1.3.1 Detection and quantification

1-Butyl glycidyl ether can be measured in air using coconut-shell charcoal solid sorbent tubes. The compound is analysed by gas chromatography (GC) with flame ionization detection (FID) using a stainless steel column, packed with 10% free fatty-acid phase (FFAP) on a 80/100 mesh Chromosorb W-AW DMCS column, the estimated limit of detection is 5 µg per sample (NIOSH Method 1616) (NIOSH, 1994, 2007). High-performance liquid chromatography (HPLC) coupled with a variable wavelength dual-beam ultraviolet-visible spectrophotometric detector can be used for the determination of 1-butyl glycidyl ether in air and other environmental media. Separation is achieved using a stainless-steel reversed-phase column. The absolute limit of detection can be as low as 1 ppb (Ramanujam et al., 1981).

1-Butyl glycidyl ether was measured in wastewater (Clark et al., 1991). The samples were extracted by liquid/liquid extraction and XAD-2 resin adsorption methodology. The extract was analysed by gas chromatography-mass spectrometry using a non-polar DB-1 fused silica column (Clark et al., 1991).

1.3.2 Biomarkers of exposure

No biomarkers of exposure in humans have been reported. [The Working Group noted that a method for the measurement of haemoglobin adducts of 1-butyl glycidyl ether has been

described in mice (Pérez et al., 1997) and concurs with the authors that this method could be useful for exposure assessment in humans.]

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

1-Butyl glycidyl ether is not known to occur naturally in the environment. Potential environmental emissions may occur from industrial facilities producing, handling, or using epoxy-based resins, coatings, and adhesives (Environment Canada, 2010).

(a) Air

In Canada, importers disclosed that 100–1000 kg were released into the air in 2006 (Environment Canada, 2010). If released to the atmosphere, 1-butyl glycidyl ether is not likely to degrade via direct photolysis. Its vapour pressure indicates that it will exist solely as a vapour in the ambient atmosphere (HSDB, 2006). The predicted atmospheric oxidation half-life for the compound is 0.54 days and it is not considered persistent in air (Environment Canada, 2010). The substance is not expected to react with photo-oxidative species such as ozone in the atmosphere (Environment Canada, 2010). Vapour-phase 1-butyl glycidyl ether will be degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals; the half-life for this reaction in air is estimated to be 19 hours (HSDB, 2006; Environment Canada, 2010).

(b) Water

Clark et al. (1991) analysed semi-volatile pollutants in effluent streams from three wastewater treatment plants in the state of New Jersey, USA. Three sampling sets were performed at each facility; 1-butyl glycidyl ether was detected at an estimated concentration of 0.5 µg/L in samples from one facility.

If released into water, 1-butyl glycidyl ether is not expected to adsorb to suspended solids and sediment based upon the estimated soil absorption coefficient (K_{oc}) (HSDB, 2006). Volatilization from water surfaces is expected to be an important fate process based upon the estimated Henry's law constant for this agent (HSDB, 2006). Estimated volatilization half-lives in a model river and model lake are 1 and 16 days, respectively (HSDB, 2006). 1-Butyl glycidyl ether contains an epoxide group, which is susceptible to hydrolysis in water; however, the rate of this reaction is estimated to be very low (half-life of 60 years at pH 7). An estimated bioconcentration factor of 3 suggests that the potential for bioconcentration in aquatic organisms is low (HSDB, 2006).

(c) Soil

If released to soil, 1-butyl glycidyl ether is expected to have high mobility based upon an estimated K_{oc} of 52 (HSDB, 2006). Volatilization from moist soil surfaces is expected to be an important fate process based upon the estimated Henry's law constant (HSDB, 2006). 1-Butyl glycidyl ether from dry soil surfaces has the potential for volatilization, based upon its vapour pressure (HSDB, 2006).

1.4.2 Occupational exposure

Studies of occupational exposure have not been identified by the Working Group. Potential human exposure to 1-butyl glycidyl ether may occur via inhalation and dermal absorption at workplaces where 1-butyl glycidyl ether is produced or used (HSDB, 2006; Worksafe New Zealand, 2019). Potential exposure to 1-butyl glycidyl ether exists until the epoxy resin is completely cured (NTP, 2004).

In New Zealand, 52 590 workers were working in industries where there are potentially exposures to 1-butyl glycidyl ether (Statistics New Zealand, 2018). These industries included

Table 1.1 International limit values for 1-butyl glycidyl ether

Country	Limit value, 8 hours		Limit value, short-term	
	ppm	mg/m³	ppm	mg/m³
Australia	25	133		
Belgium	3	16.2		
Canada, Ontario	3			
Canada, Québec	25	133		
Denmark	6	30	12	60
Finland			25[a]	140[a]
France	25	135		
Japan, JSOH	0.25	1.33		
New Zealand	25	133		
People's Republic of China		60		
Republic of Korea	10	53		
Romania	19	100	38[a]	200[a]
Singapore	25	133		
Spain	25[b]	133 [b]		
Sweden	10	50	15[a]	80[a]
Switzerland	25	135	50	270
USA, NIOSH			5.6[c]	30[b]
USA, OSHA	50	270		
United Kingdom	[25][d]	[135][d]		

JSOH, Japan Society for Occupational Health; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration.

[a] 15-minute average value.

[b] Capable of causing occupational asthma (sensitization).

[c] Ceiling limit value, 15-minute.

[d] The United Kingdom Advisory Committee on Toxic Substances has expressed concern that, for the occupational exposure limits (OELs) shown in square brackets, health may not be adequately protected because of doubts that the limit was not soundly based. These OELs were included in the published United Kingdom 2002 list and its 2003 supplement, but are omitted from the published 2005 list.

From IFA (2019).

chemical manufacturing, basic polymer manufacturing, polymer product manufacturing, electrical equipment manufacturing, residential building construction, and non-residential building construction (Statistics New Zealand, 2018; Worksafe New Zealand, 2019). The National Institute for Occupational Safety and Health (NIOSH) has estimated that 60 217 workers (14 929 of these were female) were potentially exposed to 1-butyl glycidyl ether in the USA (NOES 1981–1983; NIOSH, 1990). The main occupations exposed were in machinery except electrical, electronic and electric equipment, transportation equipment, and instruments and related products (NIOSH, 1990). In 1978, NIOSH estimated that 13 000 workers in the USA were potentially exposed (NIOSH, 1978).

1.4.3 Exposure of the general population

Studies of exposure in the general population were not available to the Working Group. Due to the reactive, covalently bound inclusion of 1-butyl glycidyl ether in epoxy resins, potential exposure of the general population from consumer products is limited (NTP, 2004).

1.5 Regulations and guidelines

Current threshold limit values by country are given in Table 1.1. The European Commission has classified 1-butyl glycidyl ether as a carcinogen (Category 2) and as a germ cell mutagen (Category 2) (ECHA, 2019). The German Research Foundation (DFG) and the Japan Society for Occupational Health (JSOH) categorized 1-butyl glycidyl ether in Group 3B and 2B of carcinogens, respectively (JSOH, 2016; DFG, 2017). The American Conference of Governmental Industrial Hygienists (ACGIH) recommended a threshold limit value–time-weighted average (TLV–TWA) of 3 ppm (16 mg/m^3) (ACGIH, 2014).

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

In a study that complied with good laboratory practice (GLP), groups of 50 male and 50 female Crj:BDF1 [B6D2F$_1$/Crlj] mice (age, 6 weeks) were exposed by whole-body inhalation to 2,3-epoxypropyl ether [1-butyl glycidyl ether] (purity, > 99.7%) at a concentration of 0, 5, 15, or 45 ppm (v/v) in clean air for 6 hours per day, 5 days per week, for 2 years (JBRC, 2005a, b). Survival rates were unaffected in all groups of males exposed to 1-butyl glycidyl ether, and decreased in groups of females at 15 and 45 ppm. Survival to the end of 2 years for the groups at 0, 5, 15, and 45 ppm was 35/50, 35/49, 32/50, and 36/49 in males, and 33/50, 31/50, 27/50, and 22/50 in females, respectively. At cessation of treatment, body weights were significantly decreased at 45 ppm in males (–24%) and in females (–11%), relative to the respective control groups. All mice (except for one male at 5 ppm and one male at 45 ppm) underwent complete necropsy and histopathological examination.

Inhalation of 1-butyl glycidyl ether caused a significant dose-related increase ($P < 0.05$, Peto trend test) in the incidence of nasal cavity haemangioma in male mice. The incidence of nasal cavity haemangioma was significantly increased in male mice at 15 and 45 ppm ($P < 0.01$, Fisher exact test). Inhalation of 1-butyl glycidyl ether did not cause any dose-related increase in the incidence of nasal cavity schwannoma, nasal cavity squamous cell carcinoma, or nasal cavity histiocytic sarcoma in male mice. The incidence of nasal cavity schwannoma (controls, 0/50; 5 ppm, 0/49; 15 ppm, 0/50; and 45 ppm, 1/49), nasal cavity histiocytic sarcoma (controls, 0/50; 5 ppm, 2/49; 15 ppm, 0/50; and 45 ppm, 0/49) and nasal cavity squamous cell carcinoma (controls, 0/50; 5 ppm, 0/49; 15 ppm, 0/50; and 45 ppm, 2/49) were not significantly increased in any group of male mice exposed to 1-butyl glycidyl ether. [The incidence of nasal cavity squamous cell carcinoma in the group at 45 ppm exceeded the incidence observed in the historical control group (0/1596) and the incidence of nasal cavity schwannoma in the group at 45 ppm exceeded the incidence observed in the same historical control group (1/1596) of male Crj:BDF1 mice from 32 studies conducted in this laboratory.]

Inhalation of 1-butyl glycidyl ether caused a significant dose-related increase ($P < 0.01$, Peto trend test) in the incidence of nasal cavity haemangioma in female mice. The incidence of nasal cavity haemangioma was significantly increased in female mice exposed at 45 ppm ($P < 0.01$, Fisher exact test). One rare nasal cavity squamous cell carcinoma occurred in the group at the highest dose, and one rare nasal cavity

Table 3.1 Studies of carcinogenicity with 1-butyl glycidyl ether in mice and rats treated by inhalation (whole-body exposure)

Species, strain (sex) Age at start Duration Reference	Purity Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, Crj: BDF1 (M) Age, 6 wk 104 wk JBRC (2005a, b)	Purity, > 99.7% 0, 5, 15, 45 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 35, 35, 32, 36	*Nasal cavity* Haemangioma 0/50, 2/49, 14/50**, 8/49**	Positive trend: $P < 0.05$ (Peto test and Cochran–Armitage test); ** $P < 0.01$ (Fisher exact test)	Principal strengths: well-conducted GLP study; males and females used; study covered most of lifespan; multiple-dose study Other comments: no significant effect of treatment on survival; incidence in historical controls from 32 studies at laboratory: nasal cavity squamous cell carcinoma, 0/1596; nasal cavity schwannoma, 1/1596; and nasal cavity histiocytic sarcoma, NR
		Schwannoma 0/50, 0/49, 0/50, 1/49	NS	
		Squamous cell carcinoma 0/50, 0/49, 0/50, 2/49	NS	
		Histiocytic sarcoma 0/50, 2/49, 0/50, 0/49	NS	
Mouse, Crj: BDF1 (F) Age, 6 wk 104 wk JBRC (2005a, b)	Purity, > 99.7% 0, 5, 15, 45 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 33, 31, 27, 22	*Nasal cavity* Haemangioma 0/50, 0/50, 2/50, 7/50**	Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); ** $P < 0.01$ (Fisher exact test)	Principal strengths: well-conducted GLP study; males and females used; study covered most of lifespan; multiple-dose study Other comments: significant decrease in survival in females at 15 ppm and 45 ppm; historical control incidence of nasal cavity squamous cell carcinoma, 0/1596 from 32 studies at laboratory; historical control incidence of histiocytic sarcoma of the uterus, 320/1595 (average, 20.1%; range, 10–32%)
		Squamous cell carcinoma 0/50, 0/50, 0/50, 1/50	NS	
		Histiocytic sarcoma 0/50, 0/50, 1/50, 0/50	NS	
		Uterus: histiocytic sarcoma 6/50, 10/50, 15/50*, 15/50*	Positive trend: $P < 0.01$ (Peto test); and NS (Cochran–Armitage test) *$P < 0.05$ (Fisher exact test)	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/DuCrlj (M) Age, 6 wk 104 wk JBRC (2005c, d)	Purity, > 99.7% 0, 10, 30, 90 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 38, 38, 38, 11	*Nasal cavity* Squamous cell carcinoma 0/50, 0/50, 0/50, 35/50* Adenoma 0/50, 0/50, 5/50*, 0/50 Squamous cell papilloma 0/50, 0/50, 0/50, 1/50 Esthesioneuroepithelioma [neuroepithelial carcinoma] 0/50, 0/50, 0/50, 1/50 *Spleen*: mononuclear cell leukaemia 10/50, 16/50, 19/50*, 7/50	Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); *$P < 0.01$ (Fisher exact test) *$P < 0.05$ (Fisher exact test) NS NS *$P < 0.05$ (Fisher exact test)	Principal strengths: study covered most of lifespan; males and females used; multiple-dose study; well-conducted GLP study Other comments: significant decrease in survival in high-dose males; historical control incidence: nasal cavity papilloma, squamous cell carcinoma or esthesioneuroepithelioma, 0/1849; nasal cavity adenoma, 2/1849 (average, 0.1%; range, 0–0.2%)
Rat, F344/DuCrlj (F) Age, 6 wk 104 wk JBRC (2005c, d)	Purity, > 99.7% 0, 10, 30, 90 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 40, 45, 41, 15	*Nasal cavity* Squamous cell carcinoma 0/50, 0/50, 0/50, 28/50* Esthesioneuroepithelioma [neuroepithelial carcinoma] 0/50, 0/50, 0/50, 2/50 Sarcoma (NOS) 0/50, 0/50, 0/50, 1/50 Adenosquamous carcinoma 0/50, 0/50, 0/50, 1/50 *Spleen*: mononuclear cell leukaemia 7/50, 8/50, 5/50, 13/50	Positive trend: $P < 0.01$ (Peto test and Cochran–Armitage test); *$P < 0.01$ (Fisher exact test) NS NS NS Positive trend: $P < 0.01$ (Peto test)	Principal strengths: study covered most of lifespan; males and females used; multiple-dose study; well-conducted GLP study Other comments: significant decrease in survival in high-dose females; incidence in historical controls: nasal cavity squamous cell carcinoma, esthesioneuroepithelioma, sarcoma (NOS) or adenosquamous carcinoma, 0/1697

F, female; GLP, good laboratory practice; h, hour; M, male; NR, not reported; NOS, not otherwise specified; NS, not significant; ppm, parts per million; wk, week.

histiocytic sarcoma occurred in the group at the intermediate dose; none of these tumours occurred in female concurrent controls from the same laboratory.

Inhalation of 1-butyl glycidyl ether caused a significant dose-related increase ($P < 0.01$, Peto test) in the incidence of histiocytic sarcoma of the uterus in female mice. The incidence of histiocytic sarcoma of the uterus was significantly increased in female mice at 15 and 45 ppm ($P < 0.05$, Fisher exact test).

Inhalation of 1-butyl glycidyl ether resulted in increased incidence and/or severity of non-neoplastic lesions in the nasal cavity of male mice (cuboidal change of the respiratory epithelium in the groups at 5, 15, and 45 ppm; angiectasis, respiratory metaplasia of glands, and respiratory metaplasia of the olfactory epithelium in the groups at 15 and 45 ppm; exudate, eosinophilic change of respiratory, and nodular hyperplasia of the transitional epithelium in the group at 45 ppm). Inhalation of 1-butyl glycidyl ether resulted in increased incidence and/or severity of non-neoplastic lesions in the nasal cavity of female mice (respiratory metaplasia of the olfactory epithelium in the groups at 5, 15, and 45 ppm; cuboidal change of the respiratory epithelium and respiratory metaplasia of glands in the groups at 15 and 45 ppm; angiectasis, and exudate and nodular hyperplasia of the transitional epithelium in the group at 45 ppm). [The strengths of this well-conducted GLP study included the use of multiple doses, the large number of animals per group, and testing in males and females.]

3.2 Rat

Inhalation

In a study that complied with GLP, groups of 50 male and 50 female F344/DuCrlj rats (age, 6 weeks) were exposed by whole-body inhalation to butyl 2,3-epoxypropylether [1-butyl glycidyl ether] (purity, > 99.7%) at a concentration of 0 (control), 10, 30, or 90 ppm (v/v) for 6 hours per day, 5 days per week, for 104 weeks (JBRC, 2005c, d). The survival rates of males and females at 90 ppm were significantly decreased compared with the respective controls; survival rates in males were 38/50 (control), 38/50 (10 ppm), 38/50 (30 ppm), and 11/50 (90 ppm); those in females were 40/50 (control), 45/50 (10 ppm), 41/50 (30 ppm), and 15/50 (90 ppm). The significantly decreased survival rates in males and females at 90 ppm were attributed to the increased number of deaths due primarily to nasal tumours. At 90 ppm, there was a significant decrease in the body weights of males (30%) and females (23%), compared with respective controls. All rats underwent complete necropsy and histopathological examination.

In the nasal cavity, there were significant increases in the incidence of squamous cell carcinoma at 90 ppm in males ($P < 0.01$, Fisher exact test) and females ($P < 0.01$, Fisher exact test), compared with respective controls, with a significant positive trend (Peto trend test, $P < 0.01$). Some cancers of the nasal cavity metastasized to the brain or Harderian gland (local invasion), and lung or lymph node. There was a significant increase in the incidence of nasal cavity adenoma in males at 30 ppm ($P < 0.05$, Fisher exact test). In the nasal cavity, there were also occurrences of esthesioneuroepithelioma [neuroepithelial carcinoma] in one male at the highest dose and two females at the highest dose, of squamous cell papilloma in one male at the highest dose, of sarcoma (not otherwise specified, NOS) in one female at the highest dose and of adenosquamous carcinoma in one female at the highest dose, which were not observed in the historical control database of the laboratory (males, 0/1849; females, 0/1697). The incidence of splenic mononuclear cell leukaemia was increased in exposed females as indicated by a significant positive trend (Peto trend test, $P < 0.01$); the incidence of splenic mononuclear cell leukaemia in males was

significantly increased only at 30 ppm ($P < 0.05$, Fisher exact test). Regarding non-neoplastic lesions, increased incidence or severity of lesions such as nasal respiratory epithelium squamous cell metaplasia, nasal squamous cell hyperplasia with atypia, nasal respiratory epithelium inflammation, olfactory epithelium atrophy, olfactory epithelium respiratory metaplasia and olfactory epithelium squamous cell metaplasia was noted in exposed males and females. [The strengths of this well-conducted GLP study included the use of multiple doses, the large number of animals per group, and testing in males and females.]

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

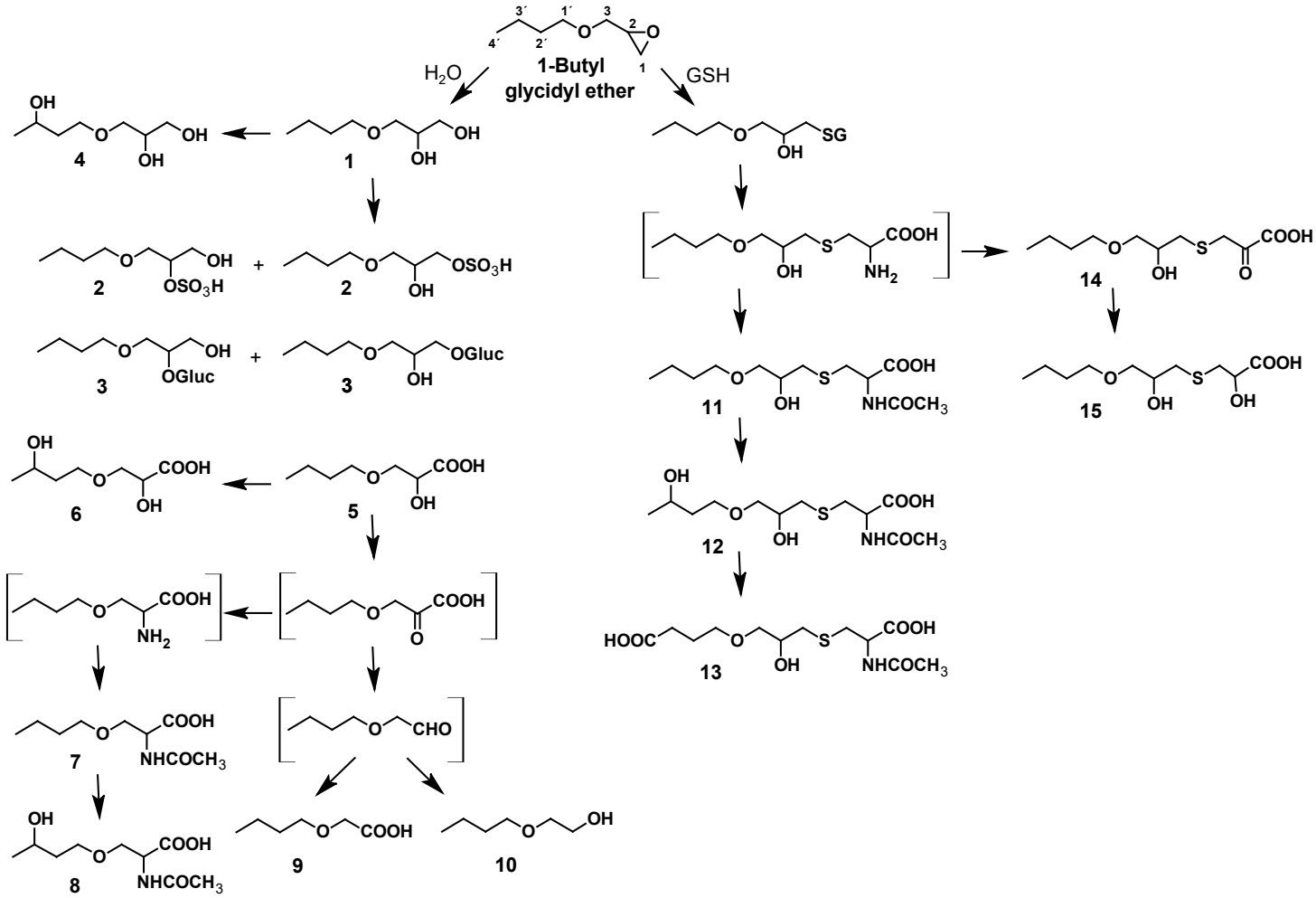
No direct data were available to the Working Group. Indirect evidence of absorption and distribution to the central nervous system was provided by Wallace (1979), who reported two clinical cases of poisoning by 1-butyl glycidyl ether.

4.1.2 Experimental systems

Radiolabelled 1-butyl glycidyl ether was administered orally to rats and mice (Chen et al., 2007). Male rats and female mice received a single dose of 2, 20, or 200 mg/kg bw by gavage, while female rats and male mice were dosed only at 200 mg/kg bw. The disposition of [1-^{14}C]butyl glycidyl ether was evaluated 24 hours after dosing. The majority of the administered dose was excreted in the urine (rats, 84–92%; mice, 64–73%), while the remainder was excreted in the faeces (rats, 2.6–7.7%; mice, 5.3–12.2%) and in expired air as carbon dioxide ($^{14}CO_2$) (rats, ≤ 1.5%; mice, 10–18%), or remained in the tissues (rats, 1.8–4.4%; mice, 1.5–1.7%). No parent compound was excreted. Detailed analysis of rat and mouse urine identified 15 metabolites in total, produced by two major metabolic pathways: (a) hydration to give a diol; and (b) conjugation with glutathione (Fig. 4.1). Hydration pathway metabolites, accounting in total for 61–76% of the administered dose, included 3-butoxy-2-hydroxypropanol (1) and its monosulfate (2) and monoglucuronic (3) conjugates and its 3′-hydroxy derivative (4), 3-butoxy-2-hydroxypropionic acid (5) and its 3′-hydroxy derivative (6), 3-butoxy-2-acetylaminopropionic acid (also known as *O*-butyl-*N*-acetylserine) (7) and its 3′-hydroxy derivative (8), butoxyacetic acid (9), and 2-butoxyethanol (10). Glutathione conjugation pathway metabolites accounted in total for 22–38% of the administered dose. Of these, 3-butoxy-1-(*N*-acetylcystein-*S*-yl)-2-propanol (also known as 3-butoxy-2-hydroxypropyl mercapturic acid) (11), its 3′-hydroxy derivative (12) and a carboxylic acid obtained by ω-oxidation of the 3-butoxy group (13) were detected both in rats and mice. Additional related products, detected in mouse urine only, were derived from the intermediate *S*-cysteine conjugate of 1-butyl glycidyl ether (3-butoxy-1-(cystein-*S*-yl)-2-propanol). Oxidative deamination of this conjugate resulted in the formation of the corresponding α-keto acid (14), followed by its reduction to the α-hydroxy acid (15).

Oral administration of [1-^{14}C]butyl glycidyl ether (20 mg/kg bw) to male Wistar rats (by gavage) or New Zealand White rabbits (by double-gelatine capsule) resulted in the rapid absorption, metabolism, and excretion of the compound (Eadsforth et al., 1985). Most of the administered [1-^{14}C]butyl glycidyl ether was eliminated within 24 hours (87% in rats, 78% in rabbit, respectively), while total elimination during 4 days accounted for 91% and 80% of the administered dose in rats and rabbits, respectively. In both species, a major route of biotransformation was via hydrolytic opening of the epoxide

Fig. 4.1 Metabolic pathways of 1-butyl glycidyl ether in rats and mice



Compounds identified in the urine: 3-butoxy-2-hydroxypropanol (1) and its monosulfate (2) and monoglucuronic (3) conjugates and a 3′-hydroxy derivative (4), 3-butoxy-2-hydroxypropionic acid (5) and its 3′-hydroxy derivative (6), 3-butoxy-2-acetylaminopropionic acid (7) and its 3′-hydroxy derivative (8), butoxyacetic acid (9), 2-butoxyethanol (10), 3-butoxy-2-hydroxypropylmercapturic acid (11) and its 3′-hydroxy derivative (12), 3-(3′-carboxy)propoxy-2-hydroxypropylmercapturic acid (13), 3-(3-butoxy-2-hydroxypropyl)thio-2-oxopropionic acid (14) and 3-(3-butoxy-2-hydroxypropyl)thio-2-hydroxypropionic acid (15).
GSH, glutathione.
Adapted with permission from Chen et al. (2007). Chen L-J, Lebetkin EH, Nwakpuda EI, Burka LT (2007). Metabolism and disposition of *n*-butyl glycidyl ether in F344 rats and B6C3F$_1$ mice. *Drug Metab Dispos*. 35(12):2218–24.

ring followed by oxidation of the resulting diol to 3-butoxy-2-hydroxypropionic acid (5) (9% in rats, 35% in rabbits) and subsequent oxidative decarboxylation to yield free butoxyacetic acid (9) (10% in rats, 5% in rabbits). In rabbit urine, another 5% of butoxyacetic acid (9) was present in a conjugated form. Additionally, 23% of the dose administered to rats was excreted in the urine as 3-butoxy-2-acetylaminopropionic acid (7), while this metabolite amounted to only 2% in rabbits. Two possible metabolic routes of the formation of 3-butoxy-2-acetylaminopropionic acid (7) from 3-butoxy-2-hydroxypropionic acid (5) were proposed of which the first one appears to be more likely: (a) dehydrogenation of the latter followed by transamination; and (b) cleavage of the epoxide ring by ammonia followed by oxidation of the terminal hydroxymethyl group and *N*-acetylation.

1-Butyl glycidyl ether is likely to be absorbed through the skin given that the median lethal dose (LD_{50}) for dermal exposures in rabbits (0.79–4.93 g/kg bw) is of similar magnitude to the LD_{50} values for oral (1.53–2.26 g/kg bw) or intraperitoneal (0.70–1.14 g/kg bw) administration in mice and rats (NTP, 2004).

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether 1-butyl glycidyl ether is electrophilic or can be metabolically activated to an electrophile; is genotoxic; alters cell proliferation, cell death, or nutrient supply; or is immunosuppressive. Insufficient data were available for the evaluation of other key characteristics of carcinogens.

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

1-Butyl glycidyl ether was a direct-acting mutagen in *Escherichia coli* WP2 *uvrA* and alkylated 4-(*p*-nitrobenzyl)-pyridine and deoxyguanosine (Hemminki et al., 1980) (see Section 4.2.2 (b)(iii) and Table 4.4). 1-Butyl glycidyl ether formed haemoglobin adducts when administered to male C3H/Hej mice in vivo (4 mg/mouse, intraperitoneal dose) (Pérez et al., 1997).

4.2.2 Is genotoxic

Table 4.1, Table 4.2, Table 4.3, and Table 4.4 summarize the studies evaluated that report genetic and related effects of 1-butyl glycidyl ether.

(a) Humans

(i) Human cells in vitro

See Table 4.1.

Several studies examined effects of 1-butyl glycidyl ether on induction of DNA repair through unscheduled DNA synthesis (UDS) assay. A linear dose–response relationship was seen in the UDS assay for 1-butyl glycidyl ether (l, 10, and 100 ppm), with significant effects at 10 and 100 ppm, in human leukocytes (US EPA, 1977). Cell viability was significantly affected at 500 ppm. Positive results were obtained in the UDS assay in human peripheral blood lymphocytes isolated from two female donors and exposed to 1-butyl glycidyl ether in vitro (Frost & Legator, 1982). Concentrations varied depending on the donor (10–1000 µg/mL and 4–1000 µg/mL), with cell viability comparable to those of control experiments, except for the highest concentration tested. [The Working Group noted that no statistical analysis was performed by the authors and insufficient information was provided to conduct statistical tests.]

Positive results in the UDS assay were also reported for 1-butyl glycidyl ether in WI38

Table 4.1 Genetic and related effects of 1-butyl glycidyl ether in human cells in vitro

End-point	Tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Unscheduled DNA synthesis	Leukocytes	+	NT	10 ppm [1300 µg/mL]	Donor information unspecified	US EPA (1977)
Unscheduled DNA synthesis	Peripheral blood lymphocytes	+	NT	100 µg/mL		Frost & Legator (1982)
Unscheduled DNA synthesis	Lung fibroblast cell line (WI38)	(–)	+	4 µg/mL	No effect at 8 µg/mL; experiments –S9 were only carried out at up to 1.2 µg/mL	Thompson et al. (1981)

HIC, highest ineffective concentration; LEC, lowest effective concentration, NT, not tested; S9, 9000 × *g* supernatant.

[a] –, negative; (–), see study quality comments.

Table 4.2 Genetic and related effects of 1-butyl glycidyl ether 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, B6D2F$_1$ (F)	Bone marrow	–	200 mg/kg bw	Oral; 5 days; 1×/day	Only one dose tested	US EPA (1977)
Micronucleus formation	Mouse, BDF (F)	Bone marrow	–	200 mg/kg bw	Oral, 5 days, 1×/day	Only one dose tested	Connor et al. (1980)
Micronucleus formation	Mouse, BDF (F)	Bone marrow	+	675 mg/kg bw	Intraperitoneal, 1 or 2 days, 1×/day		Connor et al. (1980)
Chromosomal aberrations	Rat, Sprague-Dawley CR-1 (M, F)	Bone marrow	+	104 mg/kg bw	Intraperitoneal, 5 days, 1×/day	Dose of 313 mg/kg and positive controls were without effect	US EPA (1979)
Dominant lethal mutations	Mouse, B6D2F$_1$ (M)	Fetal implants	+	1500 mg/kg bw	Skin, 8 wk, 3×/wk		US EPA (1977)
Dominant lethal mutations	Mouse, B6D2F$_1$ (M)	Fetal implants	+	1500 mg/kg bw	Skin, 16 wk, 3×/wk		Pullin (1978)
Dominant lethal mutations	Mouse, BDF (M)	Fetal implants	+	1500 mg/kg bw	Skin, 8 wk, 3×/wk		Whorton et al. (1983)
Mutagenicity of urine	Mouse, B6D2F$_1$ (F) and ICR (F)	Host-mediated assay; Ames test in *Salmonella typhimurium* strains TA1535, +/– β-glucuronidase	–	200 mg/kg bw	Oral, 4 days, 1×/day		US EPA (1977)
Mutagenicity of urine	Mice, ICR (F)	Host-mediated assay; Ames test in *S. typhimurium* strains TA1535, +/– β-glucuronidase	–	200 mg/kg bw	Oral, 4 days, 1×/day		Connor et al. (1980)
Mutagenicity of urine	Mice, BDF (M)	Host-mediated assay; Ames test in *S. typhimurium* strains TA1535 and TA98, +/– β-glucuronidase	– –	1500 mg/kg bw 3000 mg/kg bw	Dermal, 8 wk, 3×/wk Dermal, 16 wk, 3×/wk		Connor et al. (1980)

F, female; HID, highest ineffective dose; LED, lowest effective dose (units as reported); M, male; wk, week.

[a] +, positive; –, negative.

Table 4.3 Genetic and related effects of 1-butyl glycidyl ether in non-human mammals in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
Gene mutation, *Tk* locus	L5178Y mouse, lymphoma cells	+	+	100 µg/mL	Thompson et al. (1981)
Sister-chromatid exchange	Chinese hamster V79	+	NT	2.5 mM [325.5 µg/mL]	von der Hude et al. (1991)

HIC, highest ineffective concentration; LEC, lowest effective concentration, NT, not tested; *Tk*, thymidine kinase.

[a] +, positive.

Table 4.4 Genetic and related effects of 1-butyl glycidyl ether in non-mammalian systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
Salmonella typhimurium TA1535	Reverse mutation	+	–/+	0.5 µmol/plate [65 µg/plate]	US EPA (1977)
Salmonella typhimurium TA1535	Reverse mutation	+	+	260 µg/plate	Connor et al. (1980)
Salmonella typhimurium TA1535	Reverse mutation	+	+	8.2 µg/plate (–S9); 24.7 µg/plate (+S9)	Thompson et al. (1981)
Salmonella typhimurium TA1535	Reverse mutation	+	+	333 µg/plate (–S9 and + hamster S9); 1000 µg/plate (+ rat S9)	Canter et al. (1986)
Salmonella typhimurium TA100	Reverse mutation	+	+	10 000 µg/plate	Wade et al. (1979)
Salmonella typhimurium TA100	Reverse mutation	+	NT	260 µg/plate	Connor et al. (1980)
Salmonella typhimurium TA100	Reverse mutation	+	+	222.2 µg/plate	Thompson et al. (1981)
Salmonella typhimurium TA100	Reverse mutation	+	+	100 µg/plate (–S9); 333 µg/plate (+S9)	Canter et al. (1986)
Salmonella typhimurium TA98	Reverse mutation	–	–	2.0 µmol/plate [260 µg/plate]	US EPA (1977)
Salmonella typhimurium TA98	Reverse mutation	–	–	10 000 µg/plate	Wade et al. (1979)
Salmonella typhimurium TA98, TA1537, TA1538	Reverse mutation	–	NT	260 µg/plate	Connor et al. (1980)
Salmonella typhimurium TA98, TA1537, TA1538	Reverse mutation	–	–	2000 µg/plate	Thompson et al. (1981)
Escherichia coli WP2 *uvrA*	Reverse mutation	+	NT	Doses tested: 10–10 000 µM [1.3–1300 µg/mL]; LEC was not reported	Hemminki et al. (1980)
Escherichia coli PQ37	DNA damage	+	NT	1 mM [130 µg/mL]	von der Hude et al. (1990)

HIC, highest ineffective concentration; LEC, lowest effective concentration, NT, not tested; S9, 9000 × *g* supernatant.

[a] +, positive; –, negative; –/+, equivocal.

human lung fibroblast cells (Thompson et al., 1981). [The Working Group noted that this study provided no statistical analysis of the data and only qualitative comparisons are possible.]

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.2.

The potential for 1-butyl glycidyl ether to induce micronucleus formation in bone marrow cells was tested in two studies. When administered orally to B6D2F$_1$ female mice, 1-butyl glycidyl ether (200 mg/kg bw, 5 daily doses) had no significant effect on micronucleus formation in the bone marrow (US EPA, 1977). In female BDF mice, intraperitoneal (but not oral) administration of 1-butyl glycidyl ether significantly increased micronucleus formation in bone marrow (Connor et al., 1980).

The potential for 1-butyl glycidyl ether to induce chromosomal aberrations in bone marrow cells was tested in one study in Sprague-Dawley CR-1 male and female rats given 1-butyl glycidyl ether (31, 104, or 313 mg/kg bw per day) by intraperitoneal administration for five consecutive days (US EPA, 1979). Chromosomal aberrations examined included chromatid breaks, chromosome breaks, markers (dicentric, exchanges, rings or translocation), and severely damaged cells. Chromosomal aberrations were significantly elevated only in the group at the intermediate dose, but no effect was observed in the groups receiving the lowest or highest dose or the positive control. The percentage of severely damaged cells was significantly increased in the groups receiving the lowest and highest dose and in the positive controls, but not in the group receiving the intermediate dose. The percentage of aberrant cells was significantly increased in all groups. [The Working Group noted that this report did not analyse sex-specific effects, or distinguish between the types of chromosomal aberrations, and that the positive control experiments did not yield the expected results.]

Three studies investigated 1-butyl glycidyl ether for its ability to induce dominant lethal mutations in mice. In the first study in male B6D2F$_1$ mice, 1-butyl glycidyl ether (1500 mg/kg bw on the skin) significantly decreased pregnancy rates and increased the number of fetal deaths per pregnancy after mating to virgin females (US EPA, 1977). In a second study of the same design but examining 16 weeks of exposure and with an expanded dose range (750, 1500, and 3000 mg/kg bw) (Pullin, 1978), fetal deaths were increased at 3000 mg/kg bw, significant reductions in pregnancy rates were observed at 1500 and 3000 mg/kg bw, and reductions in the mean number of implants per pregnancy were also observed at 1500 and 3000 mg/kg bw. [The Working Group noted that the study by Pullin (1978) was reported as an abstract and only in summary form.] In a third study, Whorton et al. (1983) applied 1-butyl glycidyl ether (375, 750, and 1500 mg/kg bw) to the skin of male BDF hybrid mice, three times per week for 8 weeks. Each study group included 36–44 males, 96–120 pregnant females, and counted 757–1001 implants. No significant dose-related changes in pregnancy rates or in average number of implants per pregnant female were found; however, there was evidence of a significant increase in fetal death rates by the end of the first week at a dose of 1500 mg/kg bw.

Two reports did not find a mutagenic effect of urine from mice treated with 1-butyl glycidyl ether in vivo. No effects were observed when urine samples from treated female B6D2F$_1$ or female ICR mice were tested in *Salmonella typhimurium* strain TA1535 with or without the addition of β-glucuronidase (US EPA, 1977). Similarly, no effect of 1-butyl glycidyl ether was seen when urine was collected from female ICR mice treated by gavage, or from male BDF mice exposed by dermal application, and tested with *S. typhimurium* strains TA1535 and TA98 with

or without the addition of β-glucuronidase (Connor et al., 1980).

(ii) Non-human mammalian cells in vitro

See Table 4.3.

1-Butyl glycidyl ether was mutagenic but not cytotoxic in L5178Y *Tk*$^{+/-}$ mouse lymphoma cells (Thompson et al., 1981). Addition of control or Aroclor-induced rat liver S9 fraction reduced the mutagenicity of 1-butyl glycidyl ether. [The Working Group noted that no statistical analysis was performed by the authors and data included in the study were insufficient to conduct a statistical test.]

1-Butyl glycidyl ether induced a concentration-dependent increase in the frequency of sister-chromatid exchanges in Chinese hamster V79 cells in the absence of metabolic activation (von der Hude et al., 1991).

(iii) Non-mammalian experimental systems

See Table 4.4.

The potential for 1-butyl glycidyl ether to cause mutations was examined in several studies in bacterial test systems. 1-Butyl glycidyl ether was mutagenic in *S. typhimurium* TA1535 strain at all concentrations tested without metabolic activation (US EPA, 1977). Addition of either phenobarbital- or Aroclor-induced rat liver S9 fraction reduced 1-butyl glycidyl ether mutagenicity. In the same study, 1-butyl glycidyl ether was not mutagenic in *S. typhimurium* TA98 strain, with or without metabolic activation. 1-Butyl glycidyl ether was mutagenic in *S. typhimurium* strain TA100, but not in strain TA98, and addition of rat liver S9 fraction was without effect (Wade et al., 1979). 1-Butyl glycidyl ether gave positive results in *S. typhimurium* strains TA1535 and TA100, but not in strains TA1537, TA1538, or TA98 (Connor et al., 1980). 1-Butyl glycidyl ether produced a dose-dependent response (1–4 µmoles/plate), and addition of Aroclor- or phenobarbital-induced rat liver S9 slightly decreased the net frequency of revertants, with Aroclor-induced S9 producing the greatest decrease. [The Working Group noted that no significance testing was performed by the authors.] Similarly, 1-butyl glycidyl ether was mutagenic in *S. typhimurium* strains TA1535 and TA100, but not in strains TA1537, TA1538, or TA98 (Thompson et al., 1981). [The Working Group noted that no statistical significance testing was performed by the authors.] Similar results were reported by Canter et al. (1986), who found linear increases in the frequency of revertants in the TA100 strain at or above 100 µg per plate without S9, and above 333 µg/plate with either hamster or rat liver microsomes, and in the TA1535 strain at or above 333 µg/plate without S9 or with hamster S9, and at or above 1000 µg/plate with rat liver S9. [The Working Group noted that positive results in strains TA100 and TA1535 are indicative of base-pair substitution, and negative results in TA98, TA1537, and TA1538 are indicative of lack of frameshift mutations.]

In the *E. coli* WP2 *uvrA* reverse mutation assay, 1-butyl glycidyl ether was mutagenic in the absence of an exogenous metabolic system (Hemminki et al., 1980). 1-Butyl glycidyl ether induced DNA damage in the SOS chromotest with *E. coli* PQ37 (von der Hude et al., 1990).

4.2.3 Alters cell proliferation, cell death, or nutrient supply

No effect of 1-butyl glycidyl ether on the mitotic index in bone marrow was found in Sprague-Dawley CR-1 male and female rats given 1-butyl glycidyl ether (up to 313 mg/kg bw per day) by intraperitoneal administration for five consecutive days (US EPA, 1979).

In a 13-week study in mice (Crj:BDF1), there were lesions in the respiratory epithelium and olfactory epithelium of the nasal cavity attributable to treatment with 1-butyl glycidyl ether (JBRC, 2003b). These lesions included squamous metaplasia in respiratory epithelium (at 200 ppm), as well as respiratory metaplasia (at 25 ppm or greater) and atrophy (at 50 ppm or

greater) in olfactory epithelium. In F344/DuCrj rats, there were lesions in the respiratory epithelium and olfactory epithelium of the nasal cavity attributable to treatment with 1-butyl glycidyl ether (JBRC, 2003a). In respiratory epithelium, these lesions included hyperplasia (at 50 ppm or greater), inflammation and necrosis (both lesions at 100 ppm or greater), and squamous metaplasia (at 200 ppm). In olfactory epithelium, these lesions included atrophy (at 100 ppm or greater) and inflammation, respiratory metaplasia, and necrosis (all three lesions at 200 ppm).

In Crj:BDF1 mice treated with 1-butyl glycidyl ether by inhalation for 2 years (JBRC, 2005a, b), exposures resulted in increased incidence and/or severity of the following non-neoplastic lesions in the nasal cavity in both males and females (see Section 3.1 for greater detail): cuboidal change of the respiratory epithelium, respiratory metaplasia of glands, and respiratory metaplasia of the olfactory epithelium; and nodular hyperplasia of the transitional epithelium. In male mice, eosinophilic change in the respiratory epithelium was also observed.

In F344/DuCrlj rats treated with 1-butyl glycidyl ether by inhalation for 104 weeks (JBRC, 2005c, d), exposures resulted in increased incidence and/or severity of the following non-neoplastic lesions in the nasal cavity in both males and females (see Section 3.2 for greater detail): nasal respiratory epithelium squamous cell metaplasia, nasal squamous cell hyperplasia with atypia, olfactory epithelium atrophy, olfactory respiratory epithelium metaplasia and olfactory epithelium squamous cell metaplasia.

4.2.4 Is immunosuppressive

1-Butyl glycidyl ether decreased relative thymus weight in males at doses above 100 ppm and in females at a dose of 200 ppm in a 13-week study in F344/DuCrj rats treated by inhalation (JBRC, 2003a). A reduction in thymus weight and T-cell count in the peripheral blood was observed in mice (strain unspecified) exposed to 1-butyl glycidyl ether by gavage (at 450 but not at 225 mg/kg bw for 14 days) (Xue & Lei, 1988). Concavalin A-stimulated lymphocyte proliferation was reduced in both dose groups; however, no effect of 1-butyl glycidyl ether was observed in the plaque-forming cell assay. [The Working Group noted that these results were reported as an abstract and only in summary form.]

4.2.5 Evidence on other key characteristics of carcinogens

In F344/DuCrlj rats treated with 1-butyl glycidyl ether by inhalation for 104 weeks (JBRC, 2005c, d), exposures resulted in increased incidence and/or severity of nasal respiratory epithelium inflammation in both males and females (see Section 3.2 for greater detail).

One study investigated the ability of 1-butyl glycidyl ether to transform Balb/3T3 mouse embryo fibroblast cells in vitro (Connor et al., 1980). 1-Butyl glycidyl ether at non-cytotoxic concentrations (between 10 and 670 µg/mL) had no effect on cell transformation.

No significant lesions were seen in a study of dermal exposure to 1-butyl glycidyl ether (0.75, 1.5 and 3 g/kg bw) for 16 weeks in B6D2F_1 male and female mice (Pullin, 1978) that examined histopathological changes in the lung, liver, and testes. [The Working Group noted that these results were reported as an abstract and only in summary form.]

4.3 Data relevant to comparisons across agents and end-points

The analysis of the bioactivity in vitro of the agents reviewed in *IARC Monographs* Volume 125 was informed by data from high-throughput screening assays generated by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the Government of the USA (Thomas et al., 2018).

1-Butyl glycidyl ether was one of thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes as of 1 September 2019 (US EPA, 2019). Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is also publicly available (US EPA, 2019). [The Working Group noted that the metabolic capacity of the cell-based assays is variable, and generally limited, as acknowledged in Kavlock et al. (2012).]

Among the 432 assays in which 1-butyl glycidyl ether (at concentrations up to 100 µM) was tested, it was found to be inactive in almost all assays. Active responses were observed in several assays for nuclear receptor activity and cell viability (US EPA, 2019). For nuclear receptors, borderline activity (potency of less than 50% or non-monotone dose–response fits) was found for only three assays: nuclear receptor subfamily 1, group I, member 2 (pregnane X receptor, PXR); thyrotropin-releasing hormone receptor; and nuclear factor, erythroid 2-like 2 (antioxidant response element). For cell viability, 1-butyl glycidyl ether was shown to be cytotoxic in human embryonic kidney HEK 293 cells at a half-maximal activity concentration (AC_{50}) of 22.9–24.3 µM, and in human hepatoma HEPG2 cells at an AC_{50} of 29.9–32.3 µM.

5. Summary of Data Reported

5.1 Exposure characterization

1-Butyl glycidyl ether is a High Production Volume chemical that is used as a reactive intermediate and viscosity-reducing solvent in the manufacture of epoxy resins. Additionally, it is used for stabilizing chlorinated compounds and as a surface modifier in the dyeing of cotton and wool. Potential occupational exposure may occur at workplaces where 1-butyl glycidyl ether is produced or used, whereas exposure of the general population is likely to be limited due to its participation in the polymerizing process. However, published studies documenting actual exposure levels were not identified.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

In one well-conducted study that complied with good laboratory practice (GLP) in male and female mice exposed by whole-body inhalation, 1-butyl glycidyl ether caused a significant increase, with a significant positive trend, in the incidence of haemangioma of the nasal cavity in males and females, and histiocytic sarcoma of the uterus in females. In the same study, inhalation of 1-butyl glycidyl ether caused occurrence of squamous cell carcinoma of the nasal cavity in males and females that was never reported in a large number of historical controls of similar sex and strain in the same laboratory.

In one well-conducted study that complied with GLP in male and female rats exposed by whole-body inhalation, 1-butyl glycidyl ether significantly increased the incidence of nasal cavity squamous cell carcinoma in males and females with a significant positive trend, and nasal cavity adenoma in males. Other rare tumours of the nasal cavity such as esthesioneuroepithelioma [neuroepithelial carcinoma], adenosquamous carcinoma, sarcoma (not otherwise specified), or squamous cell papilloma were also found in the treated groups of male and female rats. In males, there was a significant increase in the incidence of splenic mononuclear cell leukaemia. In females, there was a significant positive trend in the incidence of splenic mononuclear cell leukaemia.

5.4 Mechanistic evidence

No direct data on absorption, distribution, metabolism, or excretion in humans were available; however, two clinical cases of poisoning by inhalation of 1-butyl glycidyl ether provide indirect evidence of absorption and distribution to the central nervous system in humans. Studies of oral administration of 1-butyl glycidyl ether in rats, mice, and rabbits showed nearly complete absorption and rapid (within 24 hours of dosing) elimination, primarily in the urine. Skin absorption of 1-butyl glycidyl ether is likely to be as efficient as oral absorption on the basis of the similarity in median lethal dose (LD_{50}) between these routes of exposure and species. There are two metabolic pathways for 1-butyl glycidyl ether: hydration to the diol intermediates (accounting for about 75% of metabolites produced) and conjugation with glutathione.

Overall, there is consistent and coherent evidence in experimental systems that 1-butyl glycidyl ether exhibits key characteristics of carcinogens (alters cell proliferation, cell death, or nutrient supply). There is consistent evidence for increases in the incidence of various proliferative non-neoplastic lesions in the respiratory system of rodents exposed chronically by inhalation. Effects included respiratory and olfactory epithelium metaplasia in both species, squamous epithelium hyperplasia with atypia in male and female rats, and nodular hyperplasia of the transitional epithelium in male and female mice. There is suggestive evidence that 1-butyl glycidyl ether is electrophilic. 1-Butyl glycidyl ether is an epoxide that forms haemoglobin adducts in mice, and is a direct-acting mutagen and an alkylating compound in studies in bacteria and with deoxyguanosine, but adducts have not been characterized. There is suggestive evidence that 1-butyl glycidyl ether is genotoxic, as the studies cover a narrow range of experiments. In primary human cells, 1-butyl glycidyl ether induced unscheduled DNA synthesis in the few available studies. 1-Butyl glycidyl ether caused micronucleus formation in mice and chromosomal aberrations in rats when administered intraperitoneally, but not orally. 1-Butyl glycidyl ether induced dominant lethal mutations after repeated dermal exposure in mice. 1-Butyl glycidyl ether was mutagenic in one in vitro test each of gene mutation and of sister-chromatid exchanges in rodent cells. In bacteria, 1-butyl glycidyl ether showed consistent effects indicative of induction of base-pair substitution, but was without effects on frameshift mutations. Metabolic activation was not required for these effects; in fact, most studies showed that metabolic activation decreased the mutagenicity of 1-butyl glycidyl ether. 1-Butyl glycidyl ether was without effect in studies of the mutagenicity of urine after oral or dermal administration to mice. There is suggestive evidence that 1-butyl glycidyl ether is immunosuppressive; reduced thymus weight was seen in two studies in rodents. For other key characteristics of carcinogens, there is a paucity of available data. 1-Butyl glycidyl ether was found to be mostly without effects in the assay battery of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of 1-butyl glycidyl ether.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1-butyl glycidyl ether.

6.3 Mechanistic evidence

There is *strong evidence* in experimental systems that 1-butyl glycidyl ether exhibits key characteristics of carcinogens.

6.4 Overall evaluation

1-Butyl glycidyl ether is *possibly carcinogenic to humans (Group 2B)*.

6.5 Rationale

The evaluation of 1-butyl glycidyl ether as Group 2B is based on *sufficient evidence* of cancer in experimental animals, and on *strong* mechanistic evidence. The evidence for cancer in humans is *inadequate*, as no data were available. The *sufficient evidence* of carcinogenicity in experimental animals is based on the induction of malignant neoplasms in two species. There is also *strong evidence* in experimental systems that 1-butyl glycidyl ether exhibits key characteristics of carcinogens; 1-butyl glycidyl ether alters cell proliferation, cell death, or nutrient supply.

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4-CHLOROBENZOTRIFLUORIDE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

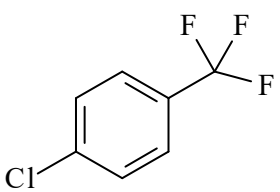
Chem. Abstr. Serv. Reg. No.: 98-56-6

Chem. Abstr. Serv. name: 1-chloro-4-(trifluoromethyl)benzene

Preferred IUPAC name: 1-chloro-4-(trifluoromethyl)benzene

Synonyms: 4-chlorobenzotrifluoride; 4-chloro-α,α,α-trifluorotoluene; *p*-chloro-α,α,α-trifluorotoluene; *p*-chlorobenzotri-fluoride; (*p*-chlorophenyl)trifluoromethane; *p*-chlorotrifluoromethylbenzene; *p*-chloro-(trifluoromethyl)benzene; *p*-trifluoromethylphenyl chloride; *p*-(trifluoromethyl)-chlorobenzene; 4-trifluoromethylchloro-benzene; 1-(trifluoro-methyl)-4-chlorobenzene; 4-chloro trifluoro-toluene.

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_7H_4ClF_3$

Relative molecular mass: 180.55

1.1.3 Chemical and physical properties of the pure substance

Description: clear, colourless liquid with a strong, aromatic, sweet pleasant odour (O'Neil, 2006; NTP, 2018)

Solubility (in water): 84.5 mg/L at 25 °C (predicted; NCBI, 2019)

Density (at 20 °C): 1.34 (NTP, 1992)

Vapour pressure: 7.63 mm Hg [1.02 kPa] at 25 °C (NCBI, 2019)

Vapour density: 6.24 (air = 1) (NTP, 1992)

Stability and reactivity: highly flammable (NCBI, 2019)

*Octanol/water partition coefficient (*P*):* log K_{ow} = 3.60 at 25 °C (NCBI, 2019)

Henry's law constant: 0.035 atm m^3 mol^{-1} [3.5 kPa m^3 mol^{-1}] at 25 °C (NCBI, 2019)

Melting point: –33 °C (NTP, 1992)

Boiling point: 139.3 °C (Lewis, 2007)

Flash point: 43 °C (O'Neil, 2006), 47 °C (NTP, 1992)

Conversion factor: 1 ppm = 7.38 mg/m^3 at 25 °C and 101.3 kPa.

1.2 Production and uses

1.2.1 Production process

4-Chlorobenzotrifluoride is synthesized from the reaction of 4-chlorotoluene and anhydrous hydrogen fluoride under atmospheric or high-pressure conditions (Boudakian, 1999). Alternatively, it can be produced by chlorination of benzotrifluoride and subsequent distillation of the isomer mixture (Albers & Kooyman, 1964; Maul et al., 1999).

1.2.2 Production volume

4-Chlorobenzotrifluoride is identified as a High Production Volume chemical by the Organisation for Economic Co-operation and Development (OECD) (OECD, 2009). Currently, there are several registered manufacturing plants in Europe, USA, and Asia (Chem Sources, 2019). In the European Union, the total volume manufactured and/or imported is listed as between 100 and 1000 tonnes per year (ECHA, 2019). The reported production/import in the USA was 10 000 000–50 000 000 lbs [4500–23 000 tonnes] in 2012–2015, of which most was imported (US EPA, 2016). In 2011, domestic production and import in the USA was reported to be approximately 29 000 000 lbs [~13 200 tonnes] (Lee et al., 2015; US EPA, 2016). In 1977, production of 4-chlorobenzotrifluoride in the USA was estimated to be between 4300 and 23 000 tonnes (NTP, 1992).

1.2.3 Uses

4-Chlorobenzotrifluoride was originally used as an industrial intermediate in the production of selected pesticides (Siegemund et al., 2008). Since the mid-1990s, 4-chlorobenzotrifluoride has been widely used as a solvent in many inks, paints, toners, and coatings due to its loading capacity for dissolving high volumes of ink (Wolf & Morris, 2006; NTP, 2018, ECHA, 2019). 4-Chlorobenzotrifluoride is extensively used in multiple dispersive applications in the automotive industry throughout the vehicle-manufacturing process, such as autobody coating formulations, thinners for coatings, repair and maintenance cleaning solvents, cosmetic stain removal, and aerosol rust inhibition (Wolf & Morris, 2006; Lee et al., 2015). Use as a dielectric fluid has also been reported (Lewis, 2007). 4-Chlorobenzotrifluoride is used as a component (at up to approximately 70%) in consumer products for cosmetic stain removal and aerosol, rust prevention, floor wax finishes, and sealers (HSDB, 2011; Lee et al., 2015).

1.3 Methods of measurement and analysis

1.3.1 Detection and quantification

Various methods for the determination and quantification of 4-chlorobenzotrifluoride in environmental samples are detailed in the literature (Table 1.1) (Kozlova & Kocherovskaia, 1986; Yost & Harper, 2000; NIOSH, 2003; Lava et al., 2013; Lee et al., 2015).

(a) Air

Active and passive sampling methods were evaluated by Lee et al. (2015). The passive sampling method used diffusive charcoal badges, while the active sampling method used coconut shell charcoal samplers. Active sampling has been carried out at a flow rate of 0.01–0.2 L/minute (NIOSH 1026 method). The extracts from both active and passive sampling were analysed by gas chromatography with flame ionization detection (NIOSH, 2003; Lee et al., 2015).

(b) Water

4-Chlorobenzotrifluoride was also extracted from water samples using a purge-and-trap extraction and concentration methodology. The extracts were analysed by gas chromatography

Table 1.1 Selected methods of analysis of 4-chlorobenzotrifluoride in various matrices

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Water	10 mL water sample was placed in 30 mL threaded bottles, 10 min in thermostat at 90 °C, injection of vapour phase into chromatograph	Vapour phase analysis method, GC-FID	4-Chlorobenzotrifluoride: 0.011 mg/L Toluene: 0.5 mg/L	Kozlova & Kocherovskaia (1986)
Water	20 mL of water was placed in 40 mL screw-top vials Purge and trap system with direct thermal desorption from the concentrator.	Vapour phase analysis method, GC-MS	0.002 µg/L	Lava et al. (2013)
Air	The samplers were desorbed by the introduction of 2 mL of carbon disulfide into the body of the sampler. The samplers were then shaken on a specially designed desorption shaker (SKC 226d-03) for 30 min	GC[a]	NA	Yost & Harper (2000)
Air	Coconut-shell charcoal sampler desorbed using 1.0 mL of carbon disulfide:methanol (99:1) solvent and allowed to stand for 30 min with occasional agitation	GC-FID	NR	NIOSH (2003)
Air	Coconut-shell charcoal sampler and diffusive charcoal badges were desorbed using 1.0 mL of carbon disulfide:methanol (99:1) solvent and allowed to stand for 30 min with occasional agitation	GC-FID	0.1–0.7 µg	Lee et al. (2015)

GC, gas chromatography; GC-FID, gas chromatography-flame ionization detection; GC-MS, gas chromatography-mass spectrometry; min, minute; NA, not applicable; NR, not reported.
[a] Detection method not reported.

and mass spectrometry, or gas chromatography with flame ionization detection, using low- to mid-polarity stationary phases (Kozlova & Kocherovskaia, 1986; Lava et al., 2013).

(c) Other matrices

No other specific methods for the detection of 4-chlorobenzotrifluoride in environmental matrices (e.g. dietary products, and soil) were identified in the literature.

1.3.2 Biomarkers of exposure

No data on biomarkers of exposure were available to the Working Group.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

4-Chlorobenzotrifluoride is not known to occur naturally in the environment; however, the substance may be released to the environment through various waste streams and from both indoor and outdoor use of products containing this chemical (ECHA, 2019). Environmental exposure to 4-chlorobenzotrifluoride may result from spillage or improper disposal in industrial settings (NTP, 2018).

(a) Air

If released to the atmosphere, 4-chlorobenzotrifluoride will exist solely as a vapour and it is expected to volatilize rapidly from water surfaces (Lyman et al., 1990; HSDB, 2011). Vapour-phase 4-chlorobenzotrifluoride will be

degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals (Atkinson et al., 1985); the half-life for this reaction in air is estimated to be 67 days (HSDB, 2011). 4-Chlorobenzotrifluoride is considered to have negligible photochemical reactivity (Atkinson et al., 1985; Young et al., 2008). 4-Chlorobenzotrifluoride does not contain chromophores that absorb at wavelengths > 290 nm and therefore is not expected to be susceptible to direct photolysis by sunlight (HSDB, 2011).

(b) Water

In a geographical area close to a major manufacturer in Holley, NY, USA, groundwater levels of 4-chlorobenzotrifluoride were reported to be 49 mg/L (US EPA, 2005). Industrial releases of 4-chlorobenzotrifluoride by a major manufacturer of the chemical in the early 1990s in Niagara Falls, NY, USA, resulted in detection of 4-chlorobenzotrifluoride in groundwater at up to 4.6 mg/L (US EPA, 2001). 4-Chlorobenzotrifluoride was detected in groundwater samples in Vicenza, Italy, at concentrations up to 1 mg/L, as a result of industrial contamination (Cacco & Ferrari, 1982). Water samples from Love Canal, NY, USA, have qualitatively tested positive for 4-chlorobenzotrifluoride (US EPA, 1982).

If released into water, 4-chlorobenzotrifluoride is expected to adsorb to suspended solids and sediment based upon the estimated soil absorption coefficient (K_{oc}). Volatilization from water surfaces is expected to be an important fate process based upon this compound's estimated Henry's law constant. Estimated volatilization half-lives for a model river and model lake are 4 hours and 5 days, respectively. An estimated bioconcentration factor of 110 suggests that the potential for bioconcentration in aquatic organisms is high. Hydrolysis is not expected to be an important environmental fate process since 4-chlorobenzotrifluoride lacks functional groups that hydrolyse under environmental conditions (HSDB, 2011).

(c) Soil and other matrices

4-Chlorobenzotrifluoride was detected in various media in a geographical area close to a major manufacturer in the 1980s, in sediment (up to 2 ppm) from the Bloody Run Creek, Niagara River watershed, and in fish (0.17–2.0 ppm) (Yurawecz, 1979; Elder et al., 1981).

If released to soil, 4-chlorobenzotrifluoride is expected to have low mobility based upon an estimated K_{oc} of 1600 (Swann et al., 1983). Volatilization from moist soil surfaces is expected to be an important fate process based upon the estimated Henry's law constant. 4-Chlorobenzotrifluoride may volatilize from dry soil surfaces based upon its vapour pressure. In an anaerobic screening test using digester sludge, 64% of the originally applied 4-chlorobenzotrifluoride was degraded in 59 days (HSDB, 2011).

1.4.2 Occupational and general population exposure

Exposure of humans to 4-chlorobenzotrifluoride can occur via inhalation, ingestion, and dermal absorption.

(a) Occupational exposure

Lee et al. (2015) reported collection of 28 personal and 8 area sample pairs at four vehicle-manufacturing sites, and 64 personal and 26 area sample pairs at three paint-manufacturing sites. The vehicle-manufacturing plants build helicopters, automobiles, or aircraft, and use 4-chlorobenzotrifluoride as a cleaning solvent, a primer, or a plastic-adhesion promoter. The workers at the paint-manufacturing plants use 4-chlorobenzotrifluoride in transferring to other containers, mixing or adding materials, and in the quality assurance laboratories. Overall, the geometric mean of personal exposures was reported to be 2.1 ppm (range, 0.1–12.2 ppm)

[~16 mg/m^3 (range, 0.7–90.1 mg/m^3)] at the vehicle-manufacturing plants and 0.7 ppm (range, 0.1–7.7 ppm) [~5.2 mg/m^3 (range, 0.7–57 mg/m^3)] at the paint-manufacturing plants (Lee et al., 2015).

(b) General population

No studies of exposure of the general population were identified by the Working Group. [The Working Group noted there is a high likelihood of exposure in consumers due to widespread use as a solvent in many formulations.]

1.5 Regulations and guidelines

The United States Environmental Protection Agency (US EPA) set 4-chlorobenzotrifluoride preliminary remediation goals for non-cancer end-points for air (73 µg/m^3), drinking-water (7.3×10^2 µg/L), and residential soil (1.2×10^3 mg/kg), and industrial soil (1.2×10^4 mg/kg) contamination related to superfund sites (US EPA, 2004, as cited in NTP, 2018). 4-Chlorobenzotrifluoride is considered by the state of New York, USA, as a "principal organic contaminant" and a maximum contaminant level has been established for drinking-water, ground water, and surface water at 5 µg/L (New York State Department of Health, 1998). No occupational or environmental air threshold limit values were identified by the Working Group.

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

In a study that complied with good laboratory practice (GLP), groups of 50 male and 50 female B6C3F$_1$/N mice (age, 5–6 weeks) were exposed to 4-chlorobenzotrifluoride (purity, > 99.5%) by whole-body inhalation for 6 hours per day, 5 days per week, for 104–105 weeks (NTP, 2018). The concentration in the exposure chamber was 0 (control), 100, 200, or 400 ppm. The survival rate for males at 400 ppm was significantly lower than for controls, and the survival rate of females at 400 ppm was non-significantly lower than for controls. Survival rates in males were 40/50 (control), 40/50 (100 ppm), 35/50 (200 ppm), and 28/50 (400 ppm); those in females were 38/50 (control), 33/50 (100 ppm), 37/50 (200 ppm), and 27/50 (400 ppm). The decreased survival rates in males and females at 400 ppm were attributed to increased number of deaths caused primarily by hepatocellular tumours. No significant difference in body-weight gain was observed in males. A significant increase in body-weight gain was observed in females for all exposed groups (> 10% increase at the end of the exposure period). All mice underwent complete necropsy and histopathological examination.

In males, the incidence of hepatocellular carcinoma was 8/50 (control), 19/50 (100 ppm), 16/50 (200 ppm), and 35/50 (400 ppm, with a significantly higher incidence of multiple tumours), and significantly increased in all exposed groups ($P < 0.05$, Poly-3 test). The incidence of hepatoblastoma was 1/50 (control), 1/50 (100 ppm), 1/50 (200 ppm), and 15/50 (400 ppm, with a significantly higher incidence of multiple tumours) and significantly increased at 400 ppm ($P < 0.001$, Poly-3 test). There were significant positive trends ($P < 0.001$, Poly-3 trend test) in the incidence of hepatocellular carcinoma and of hepatoblastoma. The incidence of hepatocellular adenoma was 25/50 (control), 24/50 (100 ppm),

Table 3.1 Studies of carcinogenicity with 4-chlorobenzotrifluoride in mice and rats treated by inhalation (whole-body exposure)

Species, strain (sex) Age at start Duration Reference	Purity (vehicle) Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$/N (M) Age, 5–6 wk 104–105 wk NTP (2018)	Purity, > 99.5% (clean air) 0, 100, 200, 400 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 40, 40, 35, 28	*Liver* Hepatocellular carcinoma 8/50, 19/50*, 16/50*, 35/50**	Trend: $P < 0.001$ (Poly-3 test) *$P < 0.05$ (Poly-3 test) **$P < 0.001$ (Poly-3 test)	Principal strengths: GLP study; study covered most of lifespan; males and females used; multiple-dose study Other comments: survival rate of males at 400 ppm was significantly decreased
		Hepatoblastoma 1/50, 1/50, 1/50, 15/50*	Trend: $P < 0.001$ (Poly-3 test) *$P < 0.001$ (Poly-3 test)	
		Hepatocellular adenoma 25/50, 24/50, 31/50, 29/50	NS	
		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) 31/50, 37/50, 40/50*, 48/50**	Trend: $P < 0.001$ (Poly-3 test) *$P < 0.05$ (Poly-3 test) **$P < 0.001$ (Poly-3 test)	
Mouse, B6C3F$_1$/N (F) Age, 5–6 wk 104–105 wk NTP (2018)	Purity, > 99.5% (clean air) 0, 100, 200, 400 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 38, 33, 37, 27	*Liver* Hepatocellular adenoma 12/50, 14/50, 24/50*, 34/50**	Trend: $P < 0.001$ (Poly-3 test) *$P = 0.004$ (Poly-3 test) **$P < 0.001$ (Poly-3 test)	Principal strengths: GLP study; study covered most of lifespan; males and females used; multiple-dose study Other comments: no significant effect of treatment on survival; historical incidence of Harderian gland adenocarcinoma for inhalation studies: 8/300 (2.7% ± 3.5%); range, 0–8%; all routes: 12/550 (2.2% ± 2.8%); range, 0–8%
		Hepatocellular carcinoma 7/50, 8/50, 12/50, 34/50*	Trend: $P < 0.001$ (Poly-3 test) *$P < 0.001$ (Poly-3 test)	
		Hepatoblastoma 0/50, 0/50, 1/50, 8/50*	Trend: $P < 0.001$ (Poly-3 test) *$P = 0.003$ (Poly-3 test)	
		Hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) 18/50, 18/50, 29/50*, 46/50**	Trend: $P < 0.001$ (Poly-3 test) *$P = 0.008$ (Poly-3 test) **$P < 0.001$ (Poly-3 test)	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity (vehicle) Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$/N (F) Age, 5–6 wk 104–105 wk NTP (2018) (cont.)		*Harderian gland* Adenoma 2/50, 6/50, 6/50, 8/50* Adenocarcinoma 0/50, 0/50, 3/50, 0/50 Adenoma or adenocarcinoma (combined) 2/50, 6/50, 9/50*, 8/50**	 Trend: $P = 0.049$ (Poly-3 test) *$P = 0.041$ (Poly-3 test) NS Trend: $P = 0.046$ (Poly-3 test) *$P = 0.026$ (Poly-3 test), **$P = 0.041$ (Poly-3 test)	
Rat, Hsd:Sprague-Dawley (M) Age, 6 wk 104–105 wk NTP (2018)	Purity, > 99.5% (clean air) 0, 100, 300, 1000 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 25, 21, 15, 5	*Thyroid*: C-cell adenoma 2/50, 5/49, 3/49, 12/50* *Lung* Bronchioloalveolar adenoma 0/50, 2/50, 0/50, 1/50 Bronchioloalveolar carcinoma 0/50, 0/50, 0/50, 2/50 Bronchioloalveolar adenoma or carcinoma (combined) 0/50, 2/50, 0/50, 3/50	 Trend: $P < 0.001$ (Poly-3 test) *$P < 0.001$ (Poly-3 test) NS Trend: $P = 0.032$ (Poly-3 test) NS	Principal strengths: GLP study; study covered most of lifespan; males and females used; multiple-dose study Other comments: survival rate significantly decreased in males at 1000 ppm; incidence of bronchioloalveolar adenoma or carcinoma (combined) in historical controls for all routes in 2-year studies (incidence per study), 0/99 (0/50, 0/49)
Rat, Hsd:Sprague-Dawley (F) Age, 6 wk 104–105 wk NTP (2018)	Purity, > 99.5% (clean air) 0, 100, 300, 1000 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 23, 21, 25, 30	*Thyroid* C-cell adenoma 2/50, 8/50, 8/50, 14/50* C-cell carcinoma 0/50, 2/50, 0/50, 1/50 C-cell adenoma or carcinoma (combined) 2/50, 10/50*, 8/50, 15/50**	 Trend: $P = 0.008$ (Poly-3 test) *$P = 0.003$ (Poly-3 test) NS Trend: $P = 0.009$ (Poly-3 test) *$P = 0.017$ (Poly-3 test), **$P = 0.002$ (Poly-3 test)	Principal strengths: GLP study; study covered most of lifespan; males and females used; multiple-dose study Other comments: no significant effect of treatment on survival; incidence of thyroid C-cell carcinoma in historical controls for all routes in 2-year studies (incidence per study), 0/99 (0/49, 0/50)

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity (vehicle) Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, Hsd:Sprague-Dawley (F) Age, 6 wk 104–105 wk NTP (2018) (cont.)		*Adrenal medulla*: pheochromocytoma (benign) 0/49, 3/50, 4/50, 6/50* *Uterus* Adenocarcinoma 1/50, 1/50, 0/50, 5/50 Stromal polyp 7/50, 9/50, 16/50*, 12/50	*P = 0.035 (Poly-3 test) Trend P = 0.017 (Poly-3 test) *P = 0.047 (Poly-3 test)	

F, female; GLP, good laboratory practice; h, hour; M, male; NS, not significant; ppm, parts per million; wk, week.

31/50 (200 ppm), and 29/50 (400 ppm), respectively (not significant by Poly-3 pairwise or Poly-3 trend tests, but with a significantly higher incidence of multiple tumours at 200 ppm and 400 ppm). The incidence of hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined) showed a significant positive trend ($P < 0.001$, Poly-3 trend test), and was significantly increased at 200 ppm ($P < 0.05$, Poly-3 test) and 400 ppm ($P < 0.001$, Poly-3 test).

In females, the incidence of hepatocellular adenoma was 12/50 (control), 14/50 (100 ppm), 24/50 (200 ppm), and 34/50 (400 ppm, with a significantly higher incidence of multiple tumours) and significantly increased at 200 and 400 ppm ($P \leq 0.004$, Poly-3 test). The incidence of hepatocellular carcinoma was 7/50 (control), 8/50 (100 ppm), 12/50 (200 ppm), and 34/50 (400 ppm, with a significantly higher incidence of multiple tumours) and significantly increased at 400 ppm ($P < 0.001$, Poly-3 test). The incidence of hepatoblastoma was 0/50 (control), 0/50 (100 ppm), 1/50 (200 ppm), and 8/50 (400 ppm) and significantly increased at 400 ppm ($P = 0.003$, Poly-3 test). The incidence of hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined) was significantly increased at 200 and 400 ppm ($P \leq 0.008$, Poly-3 test). There were significant positive trends ($P < 0.001$, Poly-3 trend test) in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma, and of hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma (combined).

In females, the incidence of Harderian gland adenoma was 2/50 (control), 6/50 (100 ppm), 6/50 (200 ppm), and 8/50 (400 ppm) and significantly increased at 400 ppm ($P = 0.041$, Poly-3 test) with a significant positive trend ($P = 0.049$, Poly-3 trend test). The incidence of Harderian gland adenoma or adenocarcinoma (combined) was 2/50 (control), 6/50 (100 ppm), 9/50 (200 ppm), and 8/50 (400 ppm) and significantly increased at 200 and 400 ppm ($P \leq 0.041$, Poly-3 test) with a significant positive trend ($P = 0.046$, Poly-3 trend test).

Regarding non-neoplastic lesions, there were significant increases in the incidence of centrilobular hepatocyte hypertrophy, eosinophilic foci, multinucleated hepatocyte, and hepatocyte necrosis in the liver, in groups of exposed males and females (NTP, 2018). [The Working Group noted this was a well-conducted GLP study with multiple doses, a high number of animals per group, and use of males and females.]

3.2 Rat

Inhalation

In a study that complied with GLP, groups of 50 male and 50 female Hsd:Sprague-Dawley rats (age, 6 weeks) were exposed to 4-chlorobenzotrifluoride (purity, > 99.5%) by whole-body inhalation for 6 hours per day, 5 days per week, for 104–105 weeks (NTP, 2018). The concentration in the exposure chamber was: 0 (control), 100, 300, or 1000 ppm. The survival rate was significantly decreased in males at 1000 ppm. Survival rates in males were 25/50 (control), 21/50 (100 ppm), 15/50 (300 ppm), and 5/50 (1000 ppm); those in females were 23/50 (control), 21/50 (100 ppm), 25/50 (300 ppm), and 30/50 (1000 ppm). The decreased survival rate in males at 1000 ppm was attributed to the increased number of deaths caused primarily by nephropathy. A decrease in body weight was observed in males at the highest dose (6% lower at the end of the exposure period) and females (10% lower at the end of the exposure period). All rats underwent complete necropsy and histopathological examination.

In males, the incidence of thyroid C-cell adenoma was 2/50 (control), 5/49 (100 ppm), 3/49 (300 ppm), and 12/50 (1000 ppm), and significantly increased at 1000 ppm ($P < 0.001$, Poly-3 test). There was a significant positive trend in the incidence of thyroid C-cell adenoma ($P < 0.001$, by Poly-3 trend test). The incidence

of bronchioloalveolar carcinoma occurred with a significant positive trend (P = 0.032, by Poly-3 trend test) and was 0/50 (control), 0/50 (100 ppm), 0/50 (300 ppm), and 2/50 (1000 ppm), respectively. The incidence of bronchioloalveolar adenoma or carcinoma (combined) was 0/50 (control), 2/50 (100 ppm), 0/50 (300 ppm), and 3/50 (1000 ppm), and not statistically different (by Poly-3 pairwise test or Poly-3 trend test). In historical controls, the incidence of bronchioloalveolar adenoma or carcinoma (combined) in males was 0/99.

In females, the incidence of thyroid C-cell adenoma was 2/50 (control), 8/50 (100 ppm), 8/50 (300 ppm), and 14/50 (1000 ppm) and significantly increased at 1000 ppm (P = 0.003, Poly-3 test). There was a significant positive trend in the incidence of thyroid C-cell adenoma (P = 0.008, Poly-3 trend test). The incidence of thyroid C-cell carcinoma was 0/50 (control), 2/50 (100 ppm), 0/50 (300 ppm), and 1/50 (1000 ppm). In historical controls, the incidence of thyroid C-cell carcinoma for all routes of 2-year studies was 0/99 in females (incidence per study: 0/49, 0/50). The incidence of thyroid C-cell adenoma or carcinoma (combined) was significantly increased according to Poly-3 pairwise test at 100 ppm (P = 0.017) and 1000 ppm (P = 0.002), and Poly-3 trend test (P = 0.009).

In females, the incidence of benign pheochromocytoma in the adrenal medulla was significantly increased at 1000 ppm (P = 0.035, Poly-3 test). The incidence of adenocarcinoma in the uterus occurred with a significant positive trend (P = 0.017, Poly-3 trend test). The incidence of stromal polyp in the uterus was significantly increased at 300 ppm (P = 0.047, Poly-3 test).

Regarding non-neoplastic lesions, there was a significant increase in the incidence of chronic inflammation of the lung in all groups of exposed males and females, lung fibrosis in all groups of exposed males and in females at 300 and 1000 ppm, and adrenal medullary hyperplasia in females at 300 and 1000 ppm (NTP, 2018). [The Working Group noted this was a well-conducted GLP study conducted with multiple doses, a high number of animals per group, and use of males and females.]

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

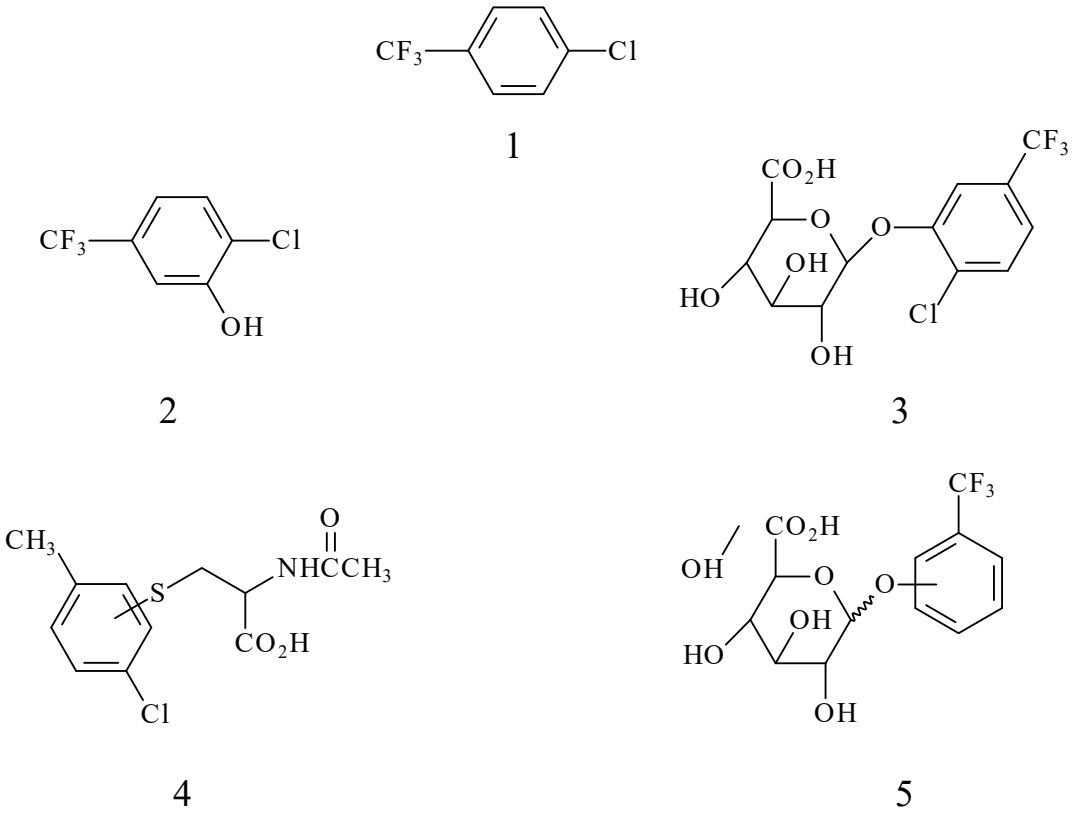
4.1.1 Humans

No direct data on absorption, distribution, metabolism, and excretion were available to the Working Group. Indirect evidence of absorption and distribution was provided by laboured breathing, dizziness, drowsiness, coughing, shortness of breath, chest pain, and oedema after exposure to 4-chlorobenzotrifluoride by inhalation (CAMEO-Chemicals, 2018).

4.1.2 Experimental systems

The absorption, distribution, metabolism, and excretion of 4-chlorobenzotrifluoride was assessed in rats in a study reported by Quistad & Mulholland (1983) and US EPA (1983a). In male and female Sprague-Dawley rats given a single oral dose of 4-chloro[trifluoromethyl-^{14}C]benzotrifluoride at 1 mg/kg body weight (bw), 62–82% of the administered dose was transported to the lungs and rapidly exhaled, while the remainder of the radiolabel was excreted in the urine (14–15%) and faeces (3–4%). 4-Chlorobenzotrifluoride was the major ^{14}C-labelled residue in the faeces. The major urinary metabolites were glucuronides of dihydroxybenzotrifluoride and 4-chloro-3-hydroxybenzotrifluoride (each representing 3–4% of the administered radiolabel); a minor amount of mercapturic acid *N*-acetyl-*S*-[chloro-(trifluoromethyl)phenyl]cysteine (0.1–0.2% of the administered radiolabel) was also observed (see Fig. 4.1). Less than 1% of the administered

Fig. 4.1 4-Chlorobenzotrifluoride and its observed metabolites in rats



(1) *p*-Chloro-α,α,α-trifluorotoluene [4-chlorobenzotrifluoride]; (2) 4-chloro-3-hydroxybenzotrifluoride; (3) glucuronide of chemical 2; (4) mercapturic acid conjugate of chemical 1; (5) glucuronide of dihydroxybenzotrifluoride.
Reprinted with permission from Quistad & Mulholland (1983). Metabolism of *p*-chlorobenzotrifluoride by rats. *J Agric Food Chem*. 31:585–589. Copyright (1983) American Chemical Society.

dose of 4-chlorobenzotrifluoride was found in tissues, mainly in fat, 4 days after dosing.

Bioavailability was shown to be complete in male F344 rats given a single oral dose of 4-chlorobenzotrifluoride (10, 50, or 400 mg/kg bw) (Yuan et al., 1991). The blood concentration increased proportionally with the administered dose.

The concentration of 4-chlorobenzotrifluoride in fat was ~10–33-fold that in the blood, liver, kidney, lung, or muscle in female Sprague-Dawley rats exposed via nose-only inhalation at a concentration of 50 ppm after exposure by whole-body inhalation for 13 weeks (Newton et al., 1998).

Levels of 4-chlorobenzotrifluoride were quantified in male and female F344/N rats and B6C3F$_1$ mice treated orally for 14 days at doses of 10, 50, 400, or 1000 mg/kg bw (NTP, 1992; Yuan et al., 1992). 4-Chlorobenzotrifluoride was detected in the blood, liver, and kidney of male and female rats, with kidney levels in males ~10-fold those in females; on the other hand, the substance could not be quantified in blood, kidney, and liver in the females and in most male mice (NTP, 1992; Yuan et al., 1992).

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether 4-chlorobenzotrifluoride is genotoxic; or alters cell proliferation, cell death, or nutrient supply. Insufficient data were available for the evaluation of other key characteristics of carcinogens.

4.2.1 Is genotoxic

Table 4.1, Table 4.2, Table 4.3, and Table 4.4 summarize the studies evaluated that report genetic and related effects of 4-chlorobenzotrifluoride.

(a) Humans

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

In human epithelial-like cells in vitro, 4-chlorobenzotrifluoride at 1–10 µL/mL induced unscheduled DNA synthesis in a dose-responsive manner (Carere & Morpurgo, 1981; Benigni et al., 1982; see Table 4.1).

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.2.

NTP (2018) investigated *Ctnnb1* and *Hras* mutations in hepatocellular carcinoma, either arising spontaneously or induced, in male and female B6C3F$_1$/N mice after chronic exposure to 4-chlorobenzotrifluoride by whole-body inhalation at 0, 100, 200, or 400 ppm. Genetic mutations in *Ctnnb1* and *Hras* are common in hepatocellular tumours in mice. No effect on *Ctnnb1* mutations was found on hepatocellular carcinoma in mice exposed to 4-chlorobenzotrifluoride compared with the control animals. On the other hand, a statistically significant difference in the frequency of *Hras* mutation (in the negative direction) was observed between hepatocellular carcinoma arising spontaneously and hepatocellular carcinoma resulting from chronic exposure to 4-chlorobenzotrifluoride at 400 ppm. [The Working Group noted that the lack of a dose–response relationship in the frequency of *Hras* mutations suggests that these were spontaneous lesions, rather than treatment-related.]

A single dose of 4-chlorobenzotrifluoride administered by gavage at 0.5, 1.7, or 5 mL/kg bw did not induce chromosomal aberrations in bone marrow cells of male or female Sprague-Dawley rats (US EPA, 1983b). After exposure to 4-chlorobenzotrifluoride at 2000 ppm by inhalation for 3 months, there was no effect on micronucleus formation in immature or mature peripheral blood erythrocytes in male or female Sprague-Dawley rats (NTP, 2018), while in B6C3F$_1$ mice a weak effect was seen in males, and no effect was detected in females (NTP, 2018).

Urine collected from male CD1 mice treated orally with 4-chlorobenzotrifluoride did not cause mutagenicity in *Salmonella typhimurium* strains TA1535, TA100, TA1537, and TA98 (US EPA, 1979b).

(ii) Non-human mammalian cells in vitro

See Table 4.3.

4-Chlorobenzotrifluoride did not induce gene mutations in L5178Y mouse lymphoma cells (US EPA, 1978a), or cause chromosomal aberration in Chinese hamster ovary (CHO) cells (US EPA, 1983c); however, sister-chromatid exchanges were found in L5178Y mouse lymphoma cells after treatment with 4-chlorobenzotrifluoride, with and without metabolic activation (US EPA, 1979a).

(iii) Non-mammalian experimental systems

See Table 4.4.

4-Chlorobenzotrifluoride did not induce mutagenicity in any of the tested strains of *Salmonella typhimurium* (TA1535, TA100, TA1537, TA1538, and TA98) with or without metabolic activation (US EPA, 1978b; Bignami &

Table 4.1 Genetic and related effects of 4-chlorobenzotrifluoride in human cells in vitro

End-point	Tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Unscheduled DNA synthesis	Epithelial-like human cells	+	NT	1 µL/mL	Purity, NR	Carere & Morpurgo (1981); Benigni et al. (1982)

HIC, highest ineffective concentration; LEC, lowest effective concentration, NR, not reported; NT, not tested.
[a] +, positive.

Table 4.2 Genetic and related effects of 4-chlorobenzotrifluoride in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Reference
Hras mutation (codon 61)	Mouse, B6C3F$_1$ (M, F)	Hepatocellular carcinoma (M, F)	– [b]	400 ppm	Whole-body inhalation, 2 years, 6 h/day, 5 days/wk, at 100, 200 or 400 ppm	NTP (2018)
Ctnnb1 mutation (codons 15–46)	Mouse, B6C3F$_1$ (M, F)	Hepatocellular carcinoma (M, F)	– [c]	400 ppm	Whole-body inhalation, 2 years, 6 h/day, 5 days/wk, at 100, 200, or 400 ppm	NTP (2018)
Chromosomal aberration	Rat, Sprague-Dawley (M, F)	Bone marrow cells	–	5 mL/kg	Gavage, 0.5, 1.7, 5.0 mL/kg bw, collection 6, 24, and 48 h after single treatment	US EPA (1983b)
Micronucleus formation	Rat, Sprague-Dawley (M, F); mouse, B6C3F$_1$/N (F)	Peripheral blood erythrocytes	–	2000 ppm	Inhalation, daily, for 3 mo	NTP (2018)
Micronucleus formation	Mouse, B6C3F$_1$/N (M)	Peripheral blood erythrocytes	± [d]	2000 ppm	Inhalation, daily, for 3 mo	NTP (2018)
Mutagenicity of urine	Mouse, CD1 (M)	Host-mediated assay; Ames test in *Salmonella typhimurium* strains TA1535, TA100, TA1537, and TA98	–	500 mg/kg bw per day	Gavage, 2 days	US EPA (1979b)

bw, body weight; F, female; h, hour; HID, highest ineffective dose; LED, lowest effective dose; M, male; mo, month; ppm, parts per million; wk, week.
[a] +, positive; –, negative; ±, equivocal.
[b] *Hras* mutation frequency in hepatocellular carcinomas of animals at 400 ppm (mutation observed in 29% of tissues assayed) was significantly lower ($P < 0.01$) than the mutation frequency in spontaneous hepatocellular carcinomas in the chamber-control animals (mutation in 73% of assayed tissues). No statistical difference at lower doses.
[c] *Ctnnb1* mutation frequency in hepatocellular carcinomas of animals at all doses up to 400 ppm was not significantly different from the mutation frequency in spontaneous hepatocellular carcinomas in the chamber-control animals.
[d] Effect considered equivocal by the Working Group.

Table 4.3 Genetic and related effects of 4-chlorobenzotrifluorotoluene in non-human mammals in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Gene mutation, *Tk* locus	L5178Y mouse, lymphoma	–	–	50 nL/mL		US EPA (1978a)
Sister-chromatid exchange	L5178Y mouse, lymphoma cells	+	+	2.5 nL/mL		US EPA (1979a)
Chromosomal aberration	Chinese hamster ovary cells	–	–	130 nL/mL	Purity, NR	US EPA (1983c)

HIC, highest ineffective concentration; LEC, lowest effective concentration, NR, not reported; Tk, thymidine kinase.
[a] +, positive; –, negative.

Table 4.4 Genetic and related effects of 4-chlorobenzotrifluoride 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 TA1535, TA100, TA1537, TA1538, and TA98	Reverse mutation	–	–	10 µL/plate	Purity, NR	US EPA (1978b)
Salmonella typhimurium TA1535 and TA100	Reverse mutation (induction of 8-azoguanine resistance)	–	NT	150 µg/plate		Bignami & Crebelli (1979)
Salmonella typhimurium TA1535, TA100, TA1537, and TA98,	Reverse mutation	–	–	0.4 µL/plate	Purity, NR	Carere & Morpurgo (1981); Benigni et al. (1982)
Salmonella typhimurium TA1535, TA100, TA1537, and TA98	Reverse mutation	–	–	1000 µg/plate	Purity, 96%	Haworth et al. (1983)
Salmonella typhimurium TA1535, TA100, TA1537, TA1538, and TA98	Reverse mutation	–	–	2500 µg/plate	Purity, NR	Mazza et al. (1986)
Salmonella typhimurium TA100 and TA98	Reverse mutation	–	–	5000 µg/plate (–S9) 6000 µg/plate (+S9)	Purity, NR	NTP (2018)
Escherichia coli WP2 *uvrA*/pKM101	Reverse mutation	–	–	5000 µg/plate (–S9) 6000 µg/plate (+S9)	Purity, NR	NTP (2018)
Escherichia coli W3110 polA$^+$ and P3478 polA$^-$	Reverse mutation	–	–	10 µL/plate	Purity, NR	US EPA (1978b)
Saccharomyces cerevisiae D4	DNA repair	–	–	10 µL/plate	Purity, NR	US EPA (1978b)
Saccharomyces cerevisiae 6117	Gene conversion and mitotic crossing over	–	–	2000 µg/mL	Purity, NR	Mazza et al. (1986)
Aspergillus nidulans	Mitotic recombination, spot test	–	NT	2.5 µL/plate, 2500 µg/plate	Purity, NR	Carere & Morpurgo (1981); Benigni et al. (1982)
Bacillus subtilis	DNA damage and repair	–	NT	10 000 µg/disc	Purity, NR	Mazza et al. (1986)

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

[a] –, negative.

Crebelli, 1979; Carere & Morpurgo, 1981; Benigni et al., 1982; Haworth et al., 1983; Mazza et al., 1986; NTP, 2018). It also gave consistently negative results in *Escherichia coli WP2 uvrA*/pKM101 and *E. coli W3110* polA+ and *P3478* polA– (NTP, 2018; US EPA, 1978b).

4-Chlorobenzotrifluoride did not induce genetic alterations in DNA repair in *Saccharomyces cerevisiae* strains D4 (US EPA, 1978b) and 6117 (Mazza et al., 1986), or mitotic recombination in *Aspergillus nidulans* (Carere & Morpurgo, 1981; Benigni et al., 1982), or DNA damage and repair in *Bacillus subtilis* (Mazza et al., 1986).

4.2.2 Alters cell proliferation, cell death, or nutrient supply

No data in humans were available to the Working Group.

Regarding repeated-dose treatment by the oral route, the results of a 14-day study in male and female F344/N rats and $B6C3F_1$ mice treated by gavage showed that 4-chlorobenzotrifluoride induced a consistent increase in liver and kidney weights, and adrenal cortex cytoplasmic vacuolization (NTP, 1992). These findings were confirmed in a 28-day study in Sprague-Dawley rats in which oral treatment with 4-chlorobenzotrifluoride caused increases in the relative weight of the liver and kidney (Macrì et al., 1987). In a 90-day study in Fischer 344 rats, 4-chlorobenzotrifluoride (0, 10, 40, 150, or 500 mg/kg bw per day, by gavage) increased the weight of the liver and kidney and caused centrilobular hypertrophy in the liver, effects that were generally dose-related (US EPA, 1983e).

Repeated-dose studies performed by the inhalation route confirmed some of the effects observed after oral administration. In a 13-week toxicity study in male and female $B6C3F_1$/N mice (NTP, 2018), exposure to 4-chlorobenzotrifluoride by whole-body inhalation resulted in increased absolute liver weight (≥ 250 ppm, in males and females), and significantly increased incidence of central lobular hepatocyte hypertrophy (≥ 250 ppm in males, ≥ 500 ppm in females), and hepatocyte necrosis and multinucleated hepatocytes (both lesions: ≥ 500 ppm in males, ≥ 1000 ppm in females). In $B6C3F_1$/N mice exposed to 4-chlorobenzotrifluoride by inhalation for up to 105 weeks (NTP, 2018), significant increases in the incidence of centrilobular hepatocyte hypertrophy, eosinophilic foci, multinucleated hepatocyte, and hepatocyte necrosis were reported in the liver of both males and females.

In a 28-day study in Sprague-Dawley rats treated by whole-body inhalation (6 hours per day, 5 days per week, at 100, 250, 500, or 1000 ppm), a significant increase in liver and kidney weights was observed, as well as in the frequency of hepatocellular hypertrophy (US EPA, 1993). Severity of effects was higher in males than in females. In a 90-day study in Sprague-Dawley rats treated by whole-body inhalation (6 hours per day, 5 days per week, at 10, 50, and 250 ppm (US EPA, 1994; Newton et al., 1998), an increase in relative liver weights, which correlated with hepatocellular hypertrophy, was found in males and females at the highest dose. Hypertrophy was not observed in the 90-day recovery animals. [The Working Group noted that liver enlargement is likely to be an indication of enzyme induction (see Section 4.2.3) rather than cell proliferation.] In a 13-week toxicity study in male and female Hsd:Sprague-Dawley rats (NTP, 2018), exposure to 4-chlorobenzotrifluoride by whole-body inhalation resulted in increased liver weight (≥ 125 ppm in males) and significantly increased incidence of centrilobular hepatocyte hypertrophy (≥ 250 ppm in males, ≥ 1000 ppm in females). In Hsd:Sprague-Dawley rats exposed to 4-chlorobenzotrifluoride by inhalation for up to 105 weeks (NTP, 2018), significant increases in the incidence of adrenal medullar hyperplasia and atypical hyperplasia of the endometrium were reported in females.

4.2.3 Evidence on other key characteristics of carcinogens

Modulation of metabolism enzymes provided evidence for receptor-mediated effects. A 13-week study in male and female Sprague-Dawley rats exposed to 4-chlorobenzotrifluoride by inhalation at 250 ppm, but not 10 or 50 ppm, resulted in increases of 5- and 2-fold in hepatic microsomal activity and levels of cytochrome P450 CYP2B in males and females, respectively, and an increase of 2-fold in levels of CYP1A1 and CYP1A2 in both males and females. CYP2E1 increased marginally in males exposed to 4-chlorobenzotrifluoride at 50 or 250 ppm, while CYP3A increased by 3-fold in females exposed at the highest dose only (Pelosi et al., 1998). When administered for 90 days by gavage to rat Fischer 344 at a dose of 10, 40, 150, or 500 mg/kg bw per day, 4-chlorobenzotrifluoride caused the induction of hepatic *para*-nitroanisole *O*-demethylase activity in males at the two higher doses and in females at the highest dose (US EPA, 1983d).

No changes in total or specific immunoglobulin IgM antibody activity to sheep erythrocytes were observed in a study that, in part, addressed immune suppression in female $B6C3F_1$ mice exposed dermally to 6–100% (v/v) 4-chlorobenzotrifluoride for 14 days (25 µL/ear) (Franko et al., 2011). In BALB/c mice, dermal exposure to 4-chlorobenzotrifluoride (75% and 100%, v/v) for three consecutive days significantly increased production of interferon-gamma (IFNγ) protein by stimulated draining lymphoid cells, but did not alter the immune response to a T-cell-dependent antigen (Franko et al., 2011). In female BALB/c mice, no treatment-related elevations in total or specific IgE were observed.

4-Chlorobenzotrifluoride failed to induce cell transformation in BALB/3T3 mouse cells (US EPA, 1980; US EPA, 1983e). In a study of chronic toxicity in Hsd:Sprague-Dawley rats exposed to 4-chlorobenzotrifluoride by inhalation for up to 105 weeks (NTP, 2018), significant increases in the incidence of chronic inflammation of the lung and of lung fibrosis were reported in males and females.

4.3 Other relevant evidence

Several studies reported effects related to α_{2u}-globulin in the kidney of male rats. In particular, kidney nephropathy, combined with a dose-dependent increase in kidney α_{2u}-globulin, was reported in a 14-day study in male F344/N rats treated by gavage (NTP, 1992). 4-Chlorobenzotrifluoride also increased the incidence of hyaline droplet-associated necrosis in the kidney in a 28-day study in Sprague-Dawley rats treated orally (Macrì et al., 1987). In a 90-day study in Fischer 344 rats treated by gavage, 4-chlorobenzotrifluoride increased the incidence of tubular degeneration in the kidneys (males only) in a dose-related manner (US EPA, 1983d). In a study in Sprague-Dawley rats treated by inhalation, intracytoplasmic eosinophilic granules were reported in the kidney (male only) after administration of 4-chlorobenzotrifluoride (6 hours per day, 5 days per week, for 4 weeks, at a dose of 100, 250, 500, or 1000 ppm) (US EPA, 1993). While such data can be informative interpreting the relevance to humans of kidney tumours observed in rodents (IARC, 1999), 4-chlorobenzotrifluoride did not induce kidney tumours in rodents (see Section 3).

4.4 Data relevant to comparisons across agents and end-points

The analysis of the bioactivity in vitro of the agents reviewed in *IARC Monographs* Volume 125 was informed by data from high-throughput screening assays generated by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the Government of the USA (Thomas et al., 2018). 4-Chlorobenzotrifluoride was one of thousands

of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes as of 1 September 2019 (US EPA, 2019). Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2019). [The Working Group noted that the metabolic capacity of the cell-based assays is variable, and generally limited, as acknowledged in Kavlock et al. (2012).]

Among the 428 assays in which 4-chlorobenzotrifluoride (at concentrations of up to 100 μM) was tested, it was found to be inactive in almost all assays. Active responses were observed in 10 assays (US EPA, 2019). For nuclear receptors, borderline activity (potency of < 50%, or half-maximal activity concentration, AC_{50}s, less than the lowest concentration tested) was found for estrogen receptor α (ERα) agonism and constitutive androstane receptor (CAR). For cell viability, 4-chlorobenzotrifluoride was shown to be cytotoxic in human HEPG2 and HEK293 cells at AC_{50}s of 0.001–0.01 μM.

5. Summary of Data Reported

5.1 Exposure data

4-Chlorobenzotrifluoride is a High Production Volume chemical widely used as a solvent and diluent for inks, paints, toners, and coatings. It is also extensively used in dispersive applications in the automotive industry. Additional applications may include its use as a major component in industrial and consumer formulations such as cleaners, degreasers, stain removers, rust inhibitors, and floor wax finishes and sealants. One study reported occupational exposures in paint- and vehicle-manufacturing facilities. The general population may be exposed via consumer products and contaminated water and fish; however, published studies documenting actual exposure levels were not available to the Working Group.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

In one well-conducted study that complied with good laboratory practice (GLP) in male and female mice treated by whole-body inhalation, 4-chlorobenzotrifluoride significantly increased the incidence, with a significant positive trend, of hepatocellular carcinoma and hepatoblastoma in males and females. The incidence of hepatocellular adenoma was significantly increased, with a significant positive trend, in females but not in males. The combined incidence of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma was significantly increased in males and females, with a significant positive trend. The incidence of Harderian gland adenoma and of Harderian gland adenoma or adenocarcinoma (combined) was significantly increased in females, with a significant positive trend.

In one well-conducted study that complied with GLP in male and female rats treated by whole-body inhalation, 4-chlorobenzotrifluoride significantly increased the incidence, with a significant positive trend, of thyroid C-cell adenoma in males and females, and of thyroid C-cell adenoma or carcinoma (combined) in females, and there was a significant positive trend in the incidence of bronchioloalveolar carcinoma in males. In females, there was a significant increase in the incidence, with a significant positive trend, of benign pheochromocytoma of the adrenal medulla. There was a significant positive trend in the incidence of uterine adenocarcinoma and of uterine stromal polyp in females.

5.4 Mechanistic evidence

No direct data on absorption, distribution, metabolism, or excretion in humans were available, but a study of other adverse effects in exposed humans indirectly confirmed absorption and distribution upon inhalation exposure. Several studies in rats exposed orally or by inhalation report the detection of 4-chlorobenzotrifluoride in multiple organs, mainly in fat.

There is suggestive evidence that 4-chlorobenzotrifluoride alters cell proliferation, cell death, or nutrient supply, based on a dose-related increase in the incidence of atypical hyperplasia in the endometrium in rats exposed chronically by inhalation. Regarding whether 4-chlorobenzotrifluoride is genotoxic, the findings were largely negative in rats and mice in vivo and in the Ames test, with the only positive results in single tests in vitro of unscheduled DNA synthesis in human cells and of sister-chromatid exchange in rodent cells. For other key characteristics of carcinogens, there is a paucity of available data. 4-Chlorobenzotrifluoride was largely inactive in the assay battery of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of 4-chlorobenzotrifluoride.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 4-chlorobenzotrifluoride.

6.3 Mechanistic evidence

There is *inadequate mechanistic evidence.*

6.4 Overall evaluation

4-Chlorobenzotrifluoride is *possibly carcinogenic to humans (Group 2B).*

6.5 Rationale

The evaluation of 4-chlorobenzotrifluoride as Group 2B is based on *sufficient evidence* of cancer in experimental animals. The evidence on cancer in humans is *inadequate* as no data were available. The *sufficient evidence* of carcinogenicity in experimental animals is based on the induction of malignant neoplasms in two species. The mechanistic evidence was *inadequate.*

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GLYCIDYL METHACRYLATE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

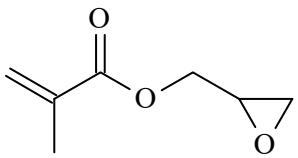
Chem. Abstr. Serv. Reg. No.: 106-91-2

Chem. Abstr. Serv. name: 2,3-epoxypropyl-methacrylate

IUPAC systematic name: (oxiran-2-yl)methyl 2-methylprop-2-enoate

Synonyms: glycidyl methacrylate; (*RS*)-2,3-epoxypropylmethacrylate; (±)-2,3-epoxypropylmethacrylate; 2-((methacryloxy)methyl) oxirane; 2-oxiranylmethyl ester; methacrylic acid-2,3-epoxypropylester; 2-propenoic acid, 2-methyl-; 1-propanol, 2,3-epoxy-,methacrylate.

1.1.2 Structural and molecular formula, and relative molecular mass



Molecular formula: $C_7H_{10}O_3$

Relative molecular mass: 142.15

1.1.3 Chemical and physical properties of the pure substance

Description: colourless, combustible liquid substance with a sweetish or fruity odour, which tends to polymerize spontaneously (HSDB, 2003)

Boiling point: 189 °C (HSDB, 2003)

Melting point: −41.5 °C (HSDB, 2003)

Density: 1.04–1.07 g/cm³ (20 °C) (ECHA, 2019; IFA, 2019)

Solubility: < 10–50 g/L (in water at 20–25 °C) (ECHA, 2019; IFA, 2019), very soluble in benzene, ethyl ether, and ethyl alcohol (HSDB, 2003)

Vapour pressure: 4.2 hPa (25 °C) (ECHA, 2019)

Flash point: 76–84 °C at 101.3 kPa (ECHA, 2019)

Auto-ignition temperature: 389 °C at 101.3 kPa (ECHA, 2019)

Vapour density: 4.91 (air = 1) (IFA, 2019)

Octanol/water partition coefficient (P): log K_{ow} = 0.96 (ILO, 2006)

Conversion factor: 1 ppm = 5.91 mg/m³ (at 20 °C and 101.3 kPa).

1.1.4 Technical grade and impurities

The purity of technical-grade glycidyl methacrylate is 92% (HSDB, 2003). Known potential impurities of the technical product can be epichlorohydrin (0.02%) and polymerization inhibitors such as monomethyl ether hydroquinone (≤ 0.01%) (HSDB, 2003; Dobrovolsky et al., 2016).

1.2 Production and use

1.2.1 Production process

Glycidyl methacrylate belongs to the group of substituted epoxides or substituted carboxylic acid esters and is produced by the esterification of methacrylic acid with either glycidol or epichlorohydrin (HSDB, 2003).

1.2.2 Production volume

Glycidyl methacrylate is listed as a High Production Volume chemical in the Screening Information Data Set (SIDS) of the Organisation for Economic Co-operation and Development (OECD, 2009). Currently, the majority of manufacturing sites are located in the USA and Europe, with fewer sites being situated in Asia (Chem Sources, 2019). The European Chemicals Agency (ECHA) reported that 1000–10 000 tonnes of glycidyl methacrylate per year are currently manufactured and/or imported in the European Economic Area (ECHA, 2019). The aggregate production volume in the USA in 2014 and 2015 has been reported to be between 10 000 000 and 50 000 000 lb [between approximately 4500 and 23 000 tonnes] (US EPA, 2016). The production volume in Japan for glycidyl methacrylate in 1995 was approximately 3000 tonnes (OECD-SIDS, 2000).

1.2.3 Uses

Glycidyl methacrylate is mainly used as co-monomer for the production of various composite materials and epoxy polymers, such as bisphenol A-glycidyl methacrylate (BisGMA) and triethylene glycol-dimethacrylate (TEGDMA). These are used as dental sealants (Pulgar et al., 2000; Gioka et al., 2005; Vervliet et al., 2018), or bone adhesives and tissue (Palussière et al., 2005; Middleton et al., 2008; Sanus et al., 2008; Monmaturapoj et al., 2017). Glycidyl methacrylate is also used as an adhesion promotion/crosslinking co-monomer in the manufacture of vinyl and acrylic resins (HSDB, 2003). These resins are used as industrial powder and metal coatings for household appliances, facades, and automotives (Pietschmann, 2010). Glycidyl methacrylate, as an acrylic copolymer, has also been classified as a food contact material substance by the United States Food and Drug Agency (US FDA, 2018a) for aqueous and fatty foods (US FDA, 2018b, and for components of paper and paperboard in contact with dry food (US FDA, 2018b). Glycidyl methacrylate is also used for the manufacture of epoxy polymers, which are increasingly proposed for new medical applications such as hydrogel contact lenses, medical imaging, 3D-printing biomaterials and targeted drug delivery (Hagit et al., 2010; Hardy et al., 2015; Li et al., 2015; Abbadessa et al., 2016; Musgrave & Fang, 2019; Pei et al., 2019).

In the European Economic Area, the ECHA reported that glycidyl methacrylate has active registrations under the regulations of Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and is used in articles, in formulation or re-packing, at industrial sites, and in manufacturing. Similarly, use as monomer in polymer synthesis has also been registered outside the European Union. The substance is used for the production of mixtures or articles by tabletting, compression, extrusion, or pelletization. Specifically, the industrial

use of monomers occurs in the manufacture of thermoplastics and as a process regulator for polymerization processes in the production of resins, rubbers, and polymers. Consequently, glycidyl methacrylate-based polymers can be found in products with plastic materials, such as food packaging and storage devices, toys, and mobile phones. In addition, there is an imported polymer product registered in the European Union that can contain the monomer in or on the article (ECHA, 2019).

1.3 Methods of measurement and analysis

For personal air sampling of glycidyl methacrylate and its analysis, the use of an XAD2 sorbent tube at a flow rate of 1 L/minute for sample collection, butyl acetate for desorption, and gas chromatography with flame ionization detection (GC-FID) has been used previously (OECD-SIDS, 2000). [The Working Group noted that no further methodological details were mentioned in this report; however, the United States Environmental Protection Agency (US EPA) Compendium Method TO-15 for the analysis of volatile organic compounds in air, including the analysis of ethyl acrylate and methyl methacrylate, or the United States National Institute for Occupational Safety and Health (NIOSH) method 1614 for the analysis of ethylene oxide could possibly be adapted to the determination of glycidyl methacrylate.]

No methods have been published for the measurement of glycidyl methacrylate in other environmental media such as water, soil or waste matrices.

No analytical methods for biological monitoring of glycidyl methacrylate in biological materials such as blood or urine samples from exposed individuals were available [The Working Group noted that previously published methods on the determination of epoxides such as ethylene oxide, i.e. measuring haemoglobin adducts in blood or mercapturic acids in urine, could possibly be adapted for glycidyl methacrylate.]

1.4 Exposure and occurrence

1.4.1 Environmental occurrence

Glycidyl methacrylate is not known to occur naturally in the environment. There are few data on the environmental occurrence of this chemical. On the basis of its low vapour pressure, glycidyl methacrylate is not expected to aerosolize readily (OECD-SIDS, 2000).

Glycidyl methacrylate can occur in the environment after release into waste water from chemical manufacturing; the amount released into air is negligible. It has been reported to be 100% biodegradable after 28 days using OECD 301C protocol and has a half-life of 3.66 days at pH 7 in water. On the basis of its low octanol/water partition coefficient, bioaccumulation of glycidyl methacrylate is expected to be low. It was reported that 99.1% will be distributed into the water phase when discharged into water; the remainder will be distributed between soil (0.4%) and air (0.4%). In Japan, approximately 3.3 tonnes per year were reported to be released into rivers by one manufacturer, and 1.62 tonnes per year by a second manufacturer, while release into air was negligible. The higher of the two releases resulted in a local predicted environmental concentration (PEC_{local}) of 8.9×10^{-3} mg/L as a worst-case scenario for water (OECD-SIDS, 2000).

1.4.2 Occupational exposure

Glycidyl methacrylate is manufactured in a closed system under well-controlled conditions, so air release is unlikely (OECD-SIDS, 2000). Some direct handling is required, such as during transfer at dedicated facilities and into small

containers, or laboratory work, when exposure can take place (ECHA, 2019).

The only sampling for occupational exposure available for glycidyl methacrylate was for Japan (OECD-SIDS, 2000). Glycidyl methacrylate was produced in a closed system. Sampling was conducted at two chemical-production sites for workers who were directly handling resin materials during sampling, maintaining, can filling, filtering, analysing, and removing sludge. The tasks that did not involve direct handling were transferring and treating waste. The highest personal air concentration was 2.3 mg/m^3 for filtration that was conducted three times per day and can filling that was conducted once every 7 days. For the other tasks, concentrations were below the limit of detection. Generally, dermal exposure, although short (5 minutes per day), was estimated to be 0.04 or 0.22 mg/kg body weight (bw) per day (OECD-SIDS, 2000).

Because glycidyl methacrylate is also used in the preparation of TEGDMA and BisGMA it can be assumed that workers preparing these dental and bone composite materials can also be potentially exposed (Olea et al., 1996). Specifically, some release of unreacted glycidyl methacrylate has been shown from a bone composite in an experimental setting, but the amount was not reported (Monmaturapoj et al., 2017). [The Working Group noted that short-term exposure to unreacted glycidyl methacrylate monomer might occur for workers during the preparation of dental and bone composite materials. Once the polymer is completely hardened, no exposure to glycidyl methacrylate is expected to occur. Hardening can take from a few minutes up to several days for some bone composites.]

Another study assessing dental-care personnel reported occupational exposure for respirable dust containing BisGMA and TEGDMA polymers, formed by reaction from bisphenol A and glycidyl methacrylate. The particles ranged in diameter from 6 nm to 5 μm and consisted of resin matrix. BisGMA and TEGDMA monomers were released from the polymer by the grinding process. Glycidyl methacrylate itself was not measured (Cokic et al., 2017). [The Working Group noted that the glycidyl methacrylate monomer is not likely to be released from the grinding process.]

Additionally, an occupation of potential concern is work in a chemical laboratory. Matura et al. (1995) reported a case study of a female laboratory worker with confirmed allergic contact dermatitis after exposure to glycidyl methacrylate via compounded emulsions.

1.4.3 Exposure of the general population

Exposure for the general population has not been well documented. Glycidyl methacrylate has a low vapour pressure but inhalation may still be possible. Estimates of consumption of glycidyl methacrylate via drinking-water and fish for locations near to chemical-manufacturing plants that produce or use this chemical are 2.97×10^{-4} mg/kg bw per day and 1.34×10^{-5} mg/kg bw per day, respectively, for an adult consuming 2 L per day of drinking-water or 90 g of fish, with a body weight of 60 kg (OECD-SIDS, 2000).

Patients, including young children, receive dental and bone composite materials containing TEGDMA and BisGMA (Olea et al., 1996; Nathanson et al., 1997; Pulgar et al., 2000; Gioka et al., 2005; Zimmerman-Downs et al., 2010; Vervliet at al., 2018). Bationo et al. (2016) reported use of monomers containing 3–5% glycidyl methacrylate to make an adhesive resin for orthodontic mineral fillers. The polymerization reaction for the dental resin occurs before the material is used in the patient, but often requires a blue visible light for a short time period to allow photo- or co-initiators to start the polymerization reaction. Curing time varies depending on the polymer, with some taking 20 seconds, while others, such as root canal sealer, taking 24 hours to set and 7 days to completely polymerize

(Vervliet et al., 2018), and bone composites taking as long as 10 days (Monmaturapoj et al., 2017). Release of bisphenol A, BisGMA, and TEDGMA was reported in many studies, but glycidyl methacrylate was not measured (Mair, 1994; Schmalz et al., 1999; Hagio et al., 2006; Lin et al., 2007).

[The Working Group noted that short-term exposure to unreacted glycidyl methacrylate monomer might occur for patients receiving these dental and bone composite materials while the polymerization process occurs. Once the polymer is completely hardened, no exposure to glycidyl methacrylate is expected to occur. Hardening can take few minutes up to several days for some bone composites.]

1.5 Regulations and guidelines

Glycidyl methacrylate has been listed by the ECHA as a carcinogen (Category 1B) and as a germ cell mutagen (Category 2) (ECHA, 2015a). An occupational exposure limit of 0.01 ppm [0.06 mg/m^3] has been recommended by the Japan Society for Occupational Health (JSOH, 2018), whereas a short-term limit value of 5 mg/m^3 is recommended in the People's Republic of China (IFA, 2019).

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

In a study that complied with good laboratory practice (GLP), groups of 50 male and 50 female B6D2F$_1$/Crlj [Crj:BDF$_1$] mice (age, 5 weeks) were exposed by whole-body inhalation to clean air (control) or 2,3-epoxypropyl methacrylate [glycidyl methacrylate] (purity, > 99.7%) vapours at a concentration of 0.6, 2.5, or 10 ppm (v/v) for 6 hours per day, 5 days per week, for 104 weeks (JBRC, 2015a, b). The mice were observed daily for clinical signs and mortality. Survival rates of males at 2.5 and 10 ppm and of females at 0.6, 2.5, and 10 ppm were significantly lower than those of their respective controls (males, 26/50 controls, 26/50, 15/50, 14/50; females, 27/50 controls, 15/50, 19/50, 9/50). There was no significant effect on body weight in exposed males and females. All mice underwent complete necropsy and histopathological examination.

In male and female mice, glycidyl methacrylate caused a significant increase in the incidence and/or a positive trend in the incidence of haemangiosarcoma of the nasal cavity in males (0/50 controls, 0/50, 1/50, 10/50; $P < 0.01$ at the highest dose, Fisher exact test; $P < 0.01$, Cochran–Armitage and Peto trend tests) and females (0/50 controls, 0/50, 1/50, 4/50; $P < 0.01$, Cochran–Armitage and Peto trend tests), and of haemangioma of the nasal cavity in males (0/50 controls, 0/50, 3/50, 8/50; $P < 0.01$ at the highest dose, Fisher exact test; $P < 0.01$, Cochran–Armitage and Peto trend tests) and females (0/50 controls, 0/50, 3/50, 7/50; $P < 0.01$ at high dose, Fisher exact test; $P < 0.01$, Cochran–Armitage and Peto trend tests). There was a significant increase in the incidence at the highest dose, and a positive trend in the incidence of haemangioma or haemangiosarcoma (combined) of the nasal cavity in male and female mice.

There was a significant positive trend in the incidence of adenoma of the Harderian gland

Table 3.1 Studies of carcinogenicity with glycidyl methacrylate in mice and rats exposed by inhalation (whole-body exposure)

Species, strain (sex) Age at start Duration Reference	Purity (vehicle) Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, B6D2F$_1$/Crlj (M) Age, 5 wk 104 wk JBRC (2015a, b)	Purity, > 99.7% (clean air) 0, 0.6, 2.5, 10 ppm (vapour) 6 h/day, 5 days/wk 50, 50, 50, 50 26, 26, 15, 14	*Nasal cavity* Haemangioma 0/50, 0/50, 3/50, 8/50*	$P < 0.01$ (Cochran–Armitage and Peto trend tests), *$P < 0.01$ (Fisher exact test)	Principal strengths: multiple-dose study; males and females used, GLP study, covered most of lifespan Other comments: survival rates of males exposed at 2.5 and 10 ppm significantly decreased; incidence in historical controls for laboratory: nasal cavity adenoma, 2/2545 (range, 0.1%, 0–2%); and forestomach squamous cell papilloma, 7/2545 (range, 0.3%, 0–2%)
		Haemangiosarcoma 0/50, 0/50, 1/50, 10/50*	$P < 0.01$ (Cochran–Armitage and Peto trend tests), *$P < 0.01$ (Fisher exact test)	
		Haemangioma or haemangiosarcoma (combined) 0/50, 0/50, 4/50, 16/50*	$P < 0.01$ (Cochran–Armitage and Peto trend tests), *$P < 0.01$ (Fisher exact test)	
		Adenoma 0/50, 0/50, 0/50, 3/50 (6%)	$P < 0.01$ (Cochran–Armitage and Peto trend tests)	
		Forestomach: squamous cell papilloma 0/50, 1/50, 0/50, 3/50 (6%)	$P < 0.05$ (Cochran–Armitage trend test), $P < 0.01$ (Peto trend test)	
		Harderian gland: adenoma 1/50, 1/50, 5/50, 5/50	$P < 0.05$ (Peto trend test)	
Mouse, B6D2F$_1$/Crlj (F) Age, 5 wk 104 wk JBRC (2015a, b)	Purity, > 99.7% (clean air) 0, 0.6, 2.5, 10 ppm (vapour) 6 h/day, 5 days/wk 50, 50, 50, 50 27, 15, 19, 9	*Nasal cavity* Haemangioma 0/50, 0/50, 3/50, 7/50*	$P < 0.01$ (Cochran–Armitage and Peto trend tests), *$P < 0.01$ (Fisher exact test)	Principal strengths: multiple-dose study, males and females used, GLP study, covered most of lifespan Other comments: survival rates of all three groups of treated females significantly decreased; incidence in historical controls for the laboratory, histiocytic sarcoma of the uterus, 534/2545 (21.0%, 10–34%)
		Haemangiosarcoma 0/50, 0/50, 1/50, 4/50	$P < 0.01$ (Cochran–Armitage and Peto trend tests)	
		Haemangioma or haemangiosarcoma (combined) 0/50, 0/50, 4/50, 11/50*	$P < 0.01$ (Cochran–Armitage and Peto trend tests), *$P < 0.01$ (Fisher exact test)	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity (vehicle) Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Mouse, B6D2F1/Crlj (F) Age, 5 wk 104 wk JBRC (2015a, b) (cont.)		*Lung*: bronchioloalveolar carcinoma 0/50, 2/50, 0/50, 5/50*	$P < 0.01$ (Cochran–Armitage and Peto trend tests), $^*P < 0.05$ (Fisher exact test)	
		Uterus: histiocytic sarcoma 11/50, 10/50, 12/50, 18/50	$P < 0.05$ (Cochran–Armitage trend test), $P < 0.01$ (Peto trend test)	
		Harderian gland: adenoma 1/50, 1/50, 2/50, 4/50	$P < 0.05$ (Peto trend test)	
Rat, Wistar (M+F, combined) NR (weight, 200 ± 20 g) Age, 6 months Ouyang et al. (1990)	Purity, NR (air) 0, 15.3, 206 mg/m^3 6 h/day, 6 days/wk 40, 40, 40 40, 40, 38	*All sites* No significant increase in the incidence of tumour		Principal strengths: males and females used Principal limitations: limited experimental details, short duration of the study Other comments: groups of 20 males and 20 females at start
Rat, F344/DuCrlCrlj (M) Age, 5 wk 104 wk JBRC (2015c, d)	Purity, > 99.7% (clean air) 0, 3.2, 8, 20 ppm (vapour) 6 h/day, 5 days/wk 50, 50, 50, 50 41, 44, 39, 9	*Nasal cavity* Squamous cell carcinoma 0/50, 0/50, 0/50, 29/50*	$P < 0.01$ (Cochran–Armitage and Peto trend tests), $^*P < 0.01$ (Fisher exact test)	Principal strengths: males and females used, multiple-dose study, GLP study, study covered most of lifespan Other comments: survival rates of males at the highest dose significantly decreased
		Esthesioneuroepithelioma [neuroepithelial carcinoma] 0/50, 0/50, 0/50, 7/50*	$P < 0.01$ (Cochran–Armitage and Peto trend tests), $^*P < 0.01$ (Fisher exact test)	
		Adenoma 0/50, 7/50*, 9/50*, 0/50	$^*P < 0.01$ (Fisher exact test)	
		Peritoneum: mesothelioma 1/50, 7/50*, 16/50**, 14/50**	$P < 0.01$ (Cochran–Armitage and Peto trend tests); $^*P < 0.05$, $^{**}P < 0.01$ (Fisher exact test)	

Table 3.1 (continued)

Species, strain (sex) Age at start Duration Reference	Purity (vehicle) Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Rat, F344/DuCrlCrlj (M) Age, 5 wk 104 wk JBRC (2015c, d) (cont.)		*Skin* Basal cell epithelioma 0/50, 1/50, 1/50, 4/50 Keratoacanthoma 0/50, 4/50, 3/50, 3/50 *Subcutis*: fibroma 5/50, 4/50, 4/50, 13/50*	 $P < 0.05$ (Cochran–Armitage trend test), $P < 0.01$ (Peto trend test) $P < 0.05$ (Peto trend test) $P < 0.01$ (Cochran–Armitage and Peto trend tests), *$P < 0.05$ (Fisher exact test)	
Rat, F344/DuCrlCrlj (F) Age, 5 wk 104 wk JBRC (2015c, d)	Purity, > 99.7% (clean air) 0, 3.2, 8, 20 ppm (vapour) 6 h/day, 5 days/wk 50, 50, 50, 50 39, 39, 35, 29	*Nasal cavity*: squamous cell carcinoma 0/50, 0/50, 0/50, 10/50* *Uterus*: endometrial stromal sarcoma 1/50, 1/50, 1/50, 5/50 (10%) *Mammary gland*: fibroadenoma 7/50, 14/50, 14/50, 23/50* *Subcutis*: fibroma 0/50, 2/50, 2/50, 3/50 *Thyroid*: C-cell adenoma 1/50, 1/50, 3/50, 4/50 *Clitoral gland*: adenoma 0/50, 0/50, 3/50, 4/50	$P < 0.01$ (Cochran–Armitage and Peto trend tests), *$P < 0.01$ (Fisher exact test) $P < 0.05$ (Cochran–Armitage trend test), $P < 0.01$ (Peto trend test) $P < 0.01$ (Cochran–Armitage and Peto trend tests), *$P < 0.01$ (Fisher exact test) $P < 0.05$ (Peto trend test) $P < 0.05$ (Peto trend test) $P < 0.05$ (Peto trend test)	Principal strengths: males and females used, multiple-dose study, GLP study, covered most of lifespan Other comments: survival rates of females at the highest dose significantly decreased; incidence in historical controls for the laboratory, endometrial stromal sarcoma, 54/2846 (1.9%, 0–8%)

F, female; GLP, good laboratory practice; h, hour; M, male; NR, not reported; ppm, parts per million; wk, week.

in male and female mice ($P < 0.05$, Peto trend test). In male mice, glycidyl methacrylate caused a significant positive trend in the incidence of adenoma of the nasal cavity (0/50 controls, 0/50, 0/50, 3/50; $P < 0.01$, Cochran–Armitage and Peto trend tests; with a historical control rate of 0.1% of 2545 male mice, range, 0–2%), and of squamous cell papilloma of the forestomach (0/50 controls, 1/50, 0/50, 3/50; $P < 0.05$, Cochran–Armitage trend test; $P < 0.01$, Peto trend test; with a historical control rate of 0.3% of 2545 male mice, range, 0–2%).

In female mice, glycidyl methacrylate caused a significant increase in the incidence at the highest dose ($P < 0.05$, Fisher exact test) and a positive trend in the incidence of bronchioloalveolar carcinoma ($P < 0.01$, Cochran–Armitage and Peto trend tests), and a positive trend in the incidence of histiocytic sarcoma in the uterus ($P < 0.05$, Cochran–Armitage trend test; $P < 0.01$, Peto trend test).

Regarding non-neoplastic lesions, transitional cell hyperplasia of the nasal cavity was observed in males and females exposed at 10 ppm and angiectasis of the nasal cavity was observed in females at 10 ppm (JBRC, 2015a, b). [The Working Group noted the strengths of this GLP study: the use of multiple doses and both males and females, while covering most of the lifespan.]

3.2 Rat

3.2.1 Inhalation

Three groups of 20 male and 20 female Wistar rats [age not reported; weight, 200±20 g] were exposed to glycidyl methacrylate [purity not reported] at a concentration of 0 (control), 15.3, or 206 mg/m^3 for 6 hours per day, 6 days per week, for 6 months. Two rats [sex unspecified] in the group at the highest dose died before the end of the study at 6 months. There was no significant increase in the incidence of any tumour type in exposed rats (Ouyang et al., 1990). [The Working Group noted the limited experimental details and short duration of the study.]

In a study that complied with GLP, groups of 50 male and 50 female F344/DuCrlCrlj (Fischer) rats (age, 5 weeks) were exposed by whole-body inhalation to clean air (control) or 2,3-epoxypropyl methacrylate [glycidyl methacrylate] (purity, > 99.7%) vapours at a dose of 3.2, 8, or 20 ppm (v/v) for 6 hours per day, 5 days per week, for 104 weeks (JBRC, 2015c, d). The rats were observed daily for clinical signs and mortality. Survival rates of males and females exposed at 20 ppm were significantly lower than their respective controls (males, 41/50 controls, 44/50, 39/50, 9/50; females, 39/50 controls, 39/50, 35/50, 29/50). Body weights were significantly decreased in males at 20 ppm throughout the 2-year exposure period, and in females at 20 ppm during the last half (from 54 weeks) of the 2-year exposure period and females at 8 ppm during the late period (from 82 weeks) of the exposure, compared with their respective controls. All rats underwent complete necropsy and histopathological examination.

In male and female rats, glycidyl methacrylate caused a significant increase in the incidence at the highest dose ($P < 0.01$, Fisher exact test), and a positive trend in the incidence of squamous cell carcinoma of the nasal cavity ($P < 0.01$, Cochran–Armitage and Peto trend tests).

In male rats, glycidyl methacrylate caused a significant increase in the incidence and a positive trend in the incidence of esthesioneuroepithelioma [neuroepithelial carcinoma] of the nasal cavity (0/50 controls, 0/50, 0/50, 7/50; $P < 0.01$ at the highest dose, Fisher exact test; $P < 0.01$, Cochran–Armitage and Peto trend tests), mesothelioma of the peritoneum (1/50 controls, 7/50, 16/50, 14/50; $P < 0.05$ at the lowest dose, $P < 0.01$ at the intermediate and highest doses, Fisher exact test; $P < 0.01$, Cochran–Armitage and Peto trend tests), subcutis fibroma (5/50 controls,

4/50, 4/50, 13/50; $P < 0.05$ at the highest dose, Fisher exact test; $P < 0.01$ Cochran–Armitage and Peto trend tests), and a significant positive trend in the incidence of basal cell epithelioma of the skin ($P < 0.05$, Cochran–Armitage trend test; $P < 0.01$, Peto trend test). There was also a significant increase ($P < 0.01$, Fisher exact test) in the incidence of adenoma of the nasal cavity in the groups at the lowest and intermediate doses, and a significant positive trend ($P < 0.05$, Peto trend test) in the incidence of skin keratoacanthoma.

In female rats, glycidyl methacrylate caused a significant positive trend in the incidence of endometrial stromal sarcoma of the uterus ($P < 0.05$, Cochran–Armitage trend test; $P < 0.01$, Peto trend test), and a significant increase in the incidence at the highest dose ($P < 0.01$, Fisher exact test) and a positive trend in the incidence of fibroadenoma of the mammary gland ($P < 0.01$, Cochran–Armitage and Peto trend tests). There was also a significant positive trend ($P < 0.05$, Peto trend test) in the incidence of subcutis fibroma, thyroid C-cell adenoma, and adenoma of the clitoral gland.

Regarding non-neoplastic lesions in the nasal cavity, squamous cell hyperplasia with atypia in males and females at 20 ppm, squamous cell metaplasia in the respiratory epithelium in females at 3.2 ppm and in males and females at 8 and 20 ppm, squamous cell metaplasia with atypia in males at 8 ppm and in males and females at 20 ppm, and transitional epithelium hyperplasia in males and females at 3.5 and 8 ppm and in females at 20 ppm, were observed in exposed groups (JBRC, 2015c, d). [The Working Group noted the strengths of this GLP study: the use of multiple doses and both males and females, while covering most of the lifespan.]

3.2.2 Oral administration (gavage)

In the study by Hadidian et al. (1968), five groups of three male and three female Fischer rats [age not reported, weanling] were given glycidyl methacrylate by gavage at a dose of 0.001, 0.003, 0.01, 0.03, or 0.3 mg (in 0.5 mL steroid) per rat, five times per week for a total of 260 individual doses, for 52 weeks. A sixth group of 15 male and 15 female Fischer rats underwent similar treatment with doses of 0.1 mg per rat. Two groups of 30 male and 30 female Fischer rats served as vehicle or untreated concurrent controls. The rats were observed for six additional months after treatment. At the end of the experiment (up to 600 days), full histopathology was performed. The pattern of tumour incidence observed with glycidyl methacrylate was similar to that observed in controls. [The Working Group noted the small number of animals per treated groups, the limited experimental details, the lack of statistics, and the limited reporting of results for controls. The Working Group judged the study inadequate for the evaluation.]

[The Working Group noted that the tumour site profile of glycidyl methacrylate in these studies is similar to that reported in carcinogenicity bioassays with glycidol. Specifically, in both male and female BDF_1 mice exposed by inhalation, glycidol induced significant increases in the incidence of malignant tumours (haemangiosarcoma, and adenoma/adenocarcinoma) of the nasal cavity (JBRC, 2003a, b). In these female mice, squamous cell carcinoma of the nasal cavity, and malignant tumours of the uterus (histiocytic sarcoma) and mammary gland (adenocarcinoma) were also reported. In F344 rats exposed by inhalation, glycidol induced malignant tumours of the nasal cavity (adenoma or adenocarcinoma in males and females, and squamous cell carcinoma in males), peritoneum (mesothelioma in males), and uterus (endometrial stromal sarcoma in females); other reported tumours in the rat were thyroid follicular cell carcinoma in males and splenic mononuclear cell leukaemia in females. In F344 rats treated with glycidol by gavage (IARC, 2000), there was an increased incidence of malignant tumours including of the peritoneum (males), mammary

gland (females), apocrine glands (males), brain (males and females), and gastrointestinal tract (males). In $B6C3F_1$ mice treated with glycidol by gavage, there was an increased incidence of malignant tumours including of the uterus (female), Harderian gland (males and females), and mammary gland (females).]

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

(a) Exposed humans

No data were available to the Working Group.

(b) Human tissues in vitro

The metabolism of ^{14}C-labelled glycidyl methacrylate (2 mM) was investigated in human liver homogenates over a 6-hour period (ECHA, 2015b). In the course of this study, the concentration of a single metabolite that was formed was mass balanced with the concentration of glycidyl methacrylate. This metabolite was identified as glycidol based on retention time match with [^{14}C] glycidol. [The Working Group considered such identification to be reasonable, taking also into account that maximum blood levels of glycidyl methacrylate were increased by 10-fold in rabbits in the presence of a carboxylesterase inhibitor (Shi et al., 1988); formation of both glycidol and methacrylic acid during ester cleavage remains to be definitely confirmed.]

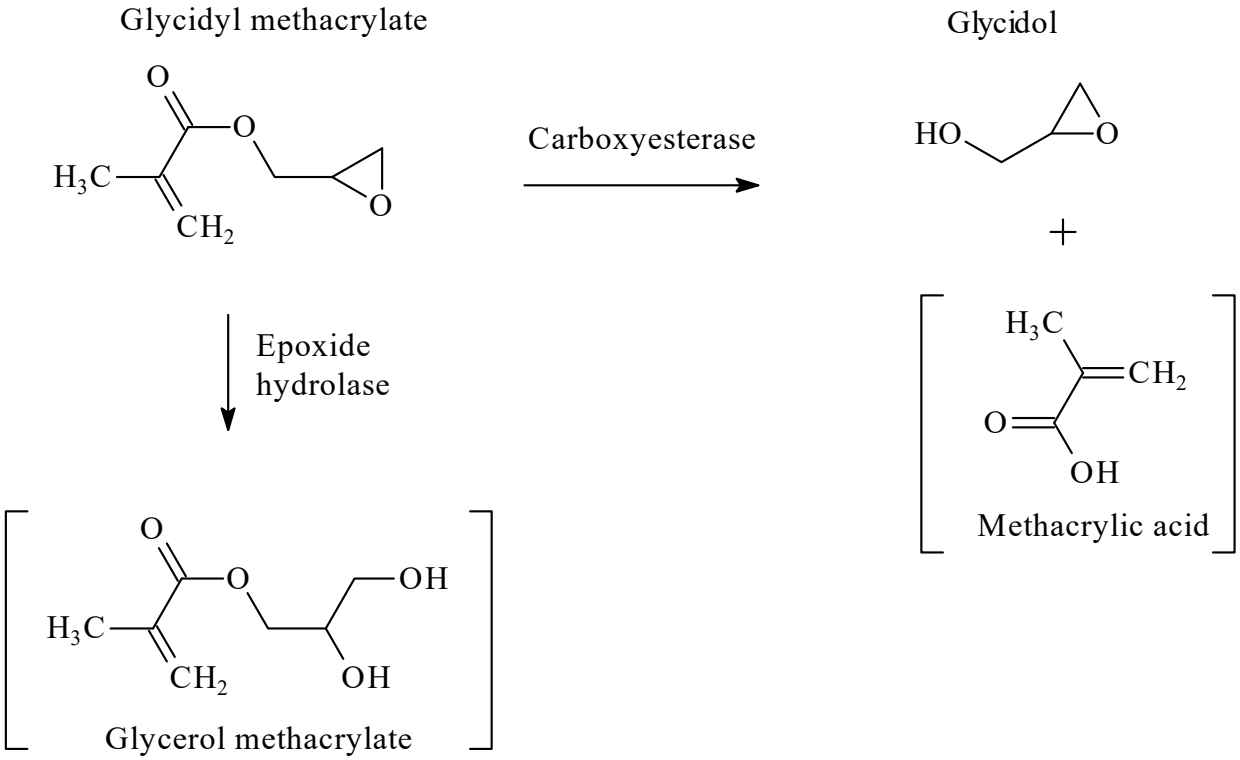
4.1.2 Experimental systems

The intravenous administration of glycidyl methacrylate (200 mg/kg bw) in male rabbits resulted in the elimination of more than 95% of the compound from the rabbit blood within 10 minutes [The Working Group noted the lack of experimental detail on sample collection]. In male rabbits treated with both glycidyl methacrylate (800 mg/kg bw, subcutaneously) and the carboxylesterase inhibitor tri-*ortho*-cresyl phosphate (100 µg/kg bw), maximum blood levels of glycidyl methacrylate were increased by 10-fold compared with administration of glycidyl methacrylate alone, indicating that glycidyl methacrylate was most probably metabolized by carboxylesterase (Shi et al., 1988). [The Working Group noted that this study reported on first-order kinetics of the parent compound; no metabolites were specifically measured.]

First-order elimination of glycidyl methacrylate occurred in rabbit blood, plasma, and tissue homogenates, with half-lives of 4.6–22.2 minutes. Liver homogenate eliminated glycidyl methacrylate most effectively. Co-incubation of blood and plasma with glycidyl methacrylate and tri-*ortho*-cresyl phosphate (0.1 mM) decreased the elimination rate of glycidyl methacrylate by several fold. The microsomal fraction of the rabbit liver homogenate eliminated glycidyl methacrylate faster than the mitochondrial and supernatant fractions. Co-incubation with tri-*ortho*-cresyl phosphate decreased the glycidyl methacrylate elimination rate in the supernatant, but not in the microsomes, indicating the role of microsomal oxidases in the biotransformation of glycidyl methacrylate (Shi et al., 1988).

The metabolism of [^{14}C]glycidyl methacrylate (2 mM) was investigated using liver homogenates and nasal epithelial tissues from Fischer 344 (F344) rats and New Zealand rabbits (ECHA, 2015b). The half-life of glycidyl methacrylate hydrolysis in liver homogenates was faster in tissues from rats and rabbits (30 minutes) than in those from humans (2 hours). In the course of this study, the concentration of a single metabolite that was formed was mass balanced with the concentration of glycidyl methacrylate. This metabolite was identified as glycidol based on retention time match with [^{14}C]glycidol

Fig. 4.1 Proposed metabolic pathways for glycidyl methacrylate in mammals



Two metabolic pathways have been proposed, one mediated by carboxyesterase and a second mediated by epoxide hydrolase. [The Working Group noted that metabolites other than glycidol (see Working Group comment in Section 4.1.1) are putative.]
Adapted with permission from ECHA (2015a). CLH report proposal for 2,3-epoxypropyl methacrylate (glycidyl methacrylate, GMA).

[see Working Group comment above, Section 4.1.1(b)].

Glycidyl methacrylate is likely to penetrate the skin in vivo given that the median lethal dose (LD_{50}) values for dermal exposure (480 mg/kg bw in rabbits) are in the same range as the LD_{50} values for oral and intraperitoneal administration (290–1050 mg/kg bw in various studies in mice, rats, and guinea-pigs) (ECHA, 2015a).

A proposed metabolic scheme for glycidyl methacrylate is presented in Fig. 4.1.

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether glycidyl methacrylate is electrophilic or can be metabolically activated to an electrophile; is genotoxic; alters cell proliferation, cell death, or nutrient supply; induces epigenetic alterations; induces oxidative stress; or causes immortalization. Insufficient data were available for the evaluation of other key characteristics of carcinogens.

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

No data on DNA adducts in humans or human systems were available.

Glycidyl methacrylate-specific DNA adducts were detected in the kidney, liver, testis and blood of rats treated with glycidyl methacrylate at a dose of 125 or 250 mg/kg bw using a nuclease P1-mediated ^{32}P-postlabelling method (Fang et al., 1999a; Tan et al., 1999).

Glycidyl methacrylate (62.2 µM) induced a shift in the calf thymus DNA absorbance spectrum, indicating binding of glycidyl methacrylate to DNA (Xie et al., 1990a, 1992). After reaction of glycidyl methacrylate with deoxyadenosine monophosphate (dAMP), deoxycytidine monophosphate (dCMP), deoxyguanosine monophosphate (dGMP), thymidine monophosphate (dTMP), and calf thymus DNA, covalent binding to all except dTMP at N^6 of adenine or *N*3 of cytosine was observed, and a main DNA adduct in the reaction of glycidyl methacrylate with calf thymus DNA was *N*3-methacrylate-2-hydroxypropyl–dCMP (Fang et al., 1999b).

Glycidol, a metabolite of glycidyl methacrylate that has been identified with reasonable certainty, is a reactive epoxide that has been demonstrated to alkylate DNA in several studies in vitro (Hemminki, 1979, Hemminki et al., 1980; Hemminki, 1983; Djurič & Sinsheimer, 1984a, b; Djurič et al., 1986; Segal et al., 1990).

4.2.2 Is genotoxic

Studies on glycidyl methacrylate have been carried out in human cells in vitro, in non-human mammalian cells in vivo, in non-human mammalian cells in vitro, and in non-mammalian systems, as summarized in Table 4.1, Table 4.2, Table 4.3, and Table 4.4, respectively.

(a) Glycidyl methacrylate

(i) Humans

See Table 4.1.

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

In several studies in primary human cells in vitro, induction of DNA strand breaks was reported after exposure to glycidyl methacrylate (Xie et al., 1990a; Poplawski et al., 2009; Styllou et al., 2015, 2017). Glycidyl methacrylate induced concentration-dependent increases in DNA double-strand breaks and single-strand breaks in human primary peripheral blood lymphocytes as assessed using neutral and alkaline comet assays (Poplawski et al., 2009). A concentration-dependent increase in the number of foci containing both gamma-H2A histone family member X and tumour protein p53 binding protein 1 (γ-H2AX/53BP1) was seen in primary human gingival fibroblasts (Styllou et al., 2015). This effect was reduced by the antioxidant *N*-acetylcysteine (Styllou et al., 2017). Glycidyl methacrylate induced unscheduled DNA synthesis in lymphocytes (Xie et al., 1990a).

In cultured human lung fibroblast 2BS cells, glycidyl methacrylate induced a significant, concentration-dependent increase in DNA single-strand breaks, as measured by the alkaline comet assay Yin et al. (2003). The highest tested concentration of glycidyl methacrylate (5 µg/mL [35 µM]) induced significant DNA damage after as little as 1 hour. Glycidyl methacrylate induced a significant and concentration-dependent increase in mutant frequencies in the hypoxanthine-guanine phosphoribosyltransferase (*HPRT)* gene in the absence of metabolic activation in cultured human lung fibroblasts (Yin et al., 2003). In transformed lung fibroblasts, mutations were observed in the *TP53* gene (Tan et al., 1996) and the migration of the *TP53* exon 8 amplicons was altered in the absence, but not presence, of metabolic activation (Tan et al., 1997). Glycidyl methacrylate induced phenotype

Table 4.1 Genetic and related effects of glycidyl methacrylate in human cells in vitro

End-point	Tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Unscheduled DNA synthesis	Human lymphocytes (primary)	+	NT	5.2 mM [739 µg/mL] GMA and hydroxyurea	Purity is described as refractive index, n_D^{30} = 1.4494	Xie et al. (1990a)
DNA strand breaks (alkaline or neutral comet assay)	Lymphocytes (primary)	+	NT	0.3 mM [42.5 µg/mL]	Purity, NR	Poplawski et al. (2009)
DNA strand breaks (pulse-field electrophoresis)	Lymphocytes (primary)	+	NT	1.2 mM [224 µg/mL]	Purity, NR	Poplawski et al. (2009)
DNA strand breaks, γ-H2AX/53BP1 foci)	Gingival fibroblasts (primary)	+	NT	0.012 mM [1.7 µg/mL]	Purity, NR	Styllou et al. (2015)
DNA strand breaks (alkaline comet assay)	Lung fibroblast	+	NT	0.5 µg/mL [0.0035 mM]		Yin et al. (2003)
Gene mutation, *HPRT* locus	Lung fibroblast	+	NT	1.0 µg /mL [0.007 mM]		Yin et al. (2003)
Mutation of DNA repair genes (*XRCC1, hMSH2, XPD, XRCC3*)	Human bronchial epithelial cells, 16HBE	+ (only for *hMSH2* gene)	NT	8 µg/mL [0.06 mM]	Single dose tested Cytotoxicity, NR	Dong et al. (2009)
Mutation of *TP53* gene	Human embryonic lung fibroblasts (cell line)	+	NT	8 µg/mL [0.06 mM]	Purity, NR Single dose tested Cytotoxicity, NR	Tan et al. (1996)
Chromosomal aberrations	Human embryonic lung fibroblasts (cell line)	+	NT	1 µg/mL [0.007 mM]	Purity, NR Cytotoxicity, NR	Tan et al. (1998)
Chromosomal aberrations	Human bronchial epithelial cell line, 16HBE	+	NT	Single exposure: 16 µg/mL [0.11 mM]		Wang et al. (2011)
		+	NT	Three rounds of exposure, 8 µg/mL		

53BP1, P53 binding protein 1; GMA, glycidyl methacrylate; γ-H2AX, gamma-histone 2AX; HIC, highest ineffective concentration; *HPRT*, hypoxanthine-guanine phosphoribosyltransferase; LEC, lowest effective concentration; NR, not reported; NT, not tested.

[a] +, positive.

Table 4.2 Genetic and related effects of glycidyl methacrylate in non-human mammals in vivo

End-point	Species, strain, (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Glycidyl methacrylate –DNA adducts	Rat, Wistar (M)	Kidney, liver, leukocytes, and testis	+	31.25 mg/kg bw per day	Gavage, 2 wk, 4× doses	Source and purity, NR	Tan et al. (1999); Fang et al. (1999a)
Unscheduled DNA synthesis	Mouse, Kunming hybrid strain (M)	Sperm cells	+	25 mg/kg bw per day	Intraperitoneal injection, 1×, killed 35 days after treatment	Purity is described as refractive index $n_D^{30} = 1.4494$ Magnitude of effect did not increase with increasing dose	Xie et al. (1990a)
DNA strand breaks, alkaline elution assay	Rat, Fischer 344 (M)	Liver, kidney, bone marrow	+	LED, 100 mg/kg bw per day (29 days, bone marrow and liver); HID, 150 mg/kg per day (29 days, kidney); LED, 250 mg/kg bw per day (3 days, bone marrow, liver, and kidney)	Gavage, 29 days or 3 days		Dobrovolsky et al. (2016)
Gene mutations by *Pig-a* assay	Rat, Fischer 344 (M)	Peripheral blood cells, reticulocytes	+	100 mg/kg bw per day (erythrocytes, 56 days), 150 mg/kg bw per day (reticulocytes, 15, 29, and 56 days)	Gavage, 15 or 29 days		Dobrovolsky et al. (2016)
Micronucleus formation	Rat, Fischer 344 (M)	Peripheral blood cells, reticulocytes	+	150 mg/kg bw per day	Gavage, 4 days		Dobrovolsky et al. (2016)
Micronucleus formation	Mouse, Kunming strain (M)	Polychromatic erythrocytes in bone marrow	+	25 mg/kg bw per day	Intraperitoneal injection; 2×, 24 h interval	Statistical test did not correct for multiple comparisons	Ouyang et al. (1988)

bw, body weight; h, hour; HID, highest ineffective dose; LED, lowest effective dose; M, male; NR, not reported; *Pig-a*, glycosylphosphatidyl inositol class A gene; wk, week.
[a] +, positive.

Table 4.3 Genetic and related effects of glycidyl methacrylate in non-human mammals 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 (strain and sex, NR), lymphocytes	+	NT	1300 µM [185 µg/mL]	Purity is described as refractive index n_D^{30} = 1.4494 Cytotoxicity, NR	Xie et al. (1990a)
Gene mutation, *Hprt* locus	Chinese hamster lung fibroblast cells, V79	+	–	–S9: 100 µM [14 µg/mL] (24 h), 200 µM [28 µg/mL] (4 h) +S9: 300 µM [42.5 µg/mL] (4 h)	Purity, NR	Schweikl et al. (1998)
Mutation frequency	Mouse, embryonic fibroblasts, BALB/c 3T3 cells	+	NT	64 µg/mL	Purity, NR Cytotoxicity, NR	Lei et al. (1998c)
Micronucleus formation	Chinese hamster lung fibroblast cells, V79	+	–	–S9: 200 µM [28 µg/mL] (4 h), 100 µM [14 µg/mL] (24 h) +S9: 500 µM [71 µg/mL] (4 h)		Schweikl et al. (2001)
Micronucleus formation	Chinese hamster lung fibroblast cells, V79	+	NT	100 µM [14 µg/mL]		Lee et al. (2006)
Sister-chromatid exchange	Chinese hamster lung fibroblast cells, V79	+	NT	78 µM [11 µg/mL]		von der Hude et al. (1991)

h, hour; HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; S9, 9000 × g supernatant.

[a] +, positive; –, negative.

Table 4.4 Genetic and related effects of glycidyl methacrylate in non-mammalian systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments on study quality	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA100	Reverse mutation	+	+	250 µg/plate	Purity, NR	Schweikl et al. (1998)
Salmonella typhimurium TA100	Reverse mutation	+	+	112 µg/plate	Source, NR Purity, 92% Cytotoxicity, NR	Ouyang et al. (1988)
Salmonella typhimurium TA98	Reverse mutation	–	–	5000 µg/plate	Purity, NR	Schweikl et al. (1998)
Salmonella typhimurium TA98	Reverse mutation	–	–	NR		Canter et al. (1986)
Salmonella typhimurium TA98	Reverse mutation	–	–	896 µg/plate	Source, NR Purity, 92% Cytotoxicity, NR	Ouyang et al. (1988)
Salmonella typhimurium TA97a, TA102	Reverse mutation	+/–	+/–	250 µg/plate (TA97a) 500 µg/plate (TA102)	Purity, NR	Schweikl et al. (1998)
Salmonella typhimurium TA97, 100, 1535	Reverse mutation	+	+	33 µg/plate for TA100 and TA1535 NR for TA97		Canter et al. (1986)
Klebsiella pneumoniae	Mutation	+	NT	1 mM [142 µg/plate]	Purity, 92% Cytotoxicity, NR	Voogd et al. (1981)
Escherichia coli PQ37 SOS chromotest	DNA damage	+	NT	0.3 mM [43 µg/plate]	Purity, 97%	von der Hude et al. (1990)

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

[a] +, positive; –, negative; +/–, equivocal.

transformation and chromosomal aberrations in cultured embryonic lung fibroblasts (Tan et al., 1998). Chromosomal aberrations were detected in cultured bronchial epithelial cells treated with glycidyl methacrylate (Wang et al., 2011).

(ii) Experimental systems

Non-human mammals in vivo

See Table 4.2.

In male F344 rats, oral exposure to glycidyl methacrylate induced a dose-dependent increase in the percentage of tail DNA in bone marrow, liver, and kidney cells, as measured by the alkaline comet assay, and in gene mutation in erythrocytes and reticulocytes as measured by *Pig-a* assay (Dobrovolsky et al., 2016). Glycidyl methacrylate increased micronucleus formation in erythrocytes from peripheral blood in male F344 rats treated orally with a dose of 150 mg/kg bw per day for 4 days, and in polychromatic erythrocytes in bone marrow of male Kunming hybrid mice exposed to glycidyl methacrylate as two doses of 25 mg/kg bw per day by intraperitoneal injection at an interval of 24 hours (Ouyang et al., 1988; Dobrovolsky et al., 2016).

Unscheduled DNA synthesis was increased in the sperm of male Kunming hybrid mice 35 days after being exposed to glycidyl methacrylate at a dose of 25 mg/kg bw per day or higher as a single intraperitoneal injection (Xie et al., 1990a). [The Working Group noted that the magnitude of the effect did not increase with increasing dose.]

Non-human mammalian cells in vitro

See Table 4.3.

Glycidyl methacrylate induced unscheduled DNA synthesis and semi-conservative DNA replication in rat lymphocytes (Xie et al., 1990a). Glycidyl methacrylate induced a concentration-dependent increase in hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) mutation in V79 Chinese hamster lung fibroblast cells, an effect completely abolished by the addition of rat liver S9 microsomal fraction (Schweikl et al., 1998). Glycidyl methacrylate induced a concentration-dependent increase in the frequencies of sister-chromatid exchange in Chinese hamster V79 cells (von der Hude et al., 1991). Schweikl et al. (2001) reported a dose-related increase in the frequencies of micronucleus formation in V79 Chinese hamster lung fibroblast cells exposed to glycidyl methacrylate (for 24 hours at 100, 150, or 200 μM or for 4 hours at 200, 300, 400, or 500 μM). The 24-hour exposure effects were reproduced by Lee et al. (2006). The effects disappeared in the presence of rat liver S9 microsomal fraction (only 4-hour exposure tested) (Schweikl et al., 2001).

Non-mammalian experimental systems

See Table 4.4.

Glycidyl methacrylate induced reverse mutations in *Salmonella typhimurium* strains TA97, TA100, TA1535, and TA102, but not TA98, both in the presence and in the absence of a rat liver S9 microsomal fraction (Canter et al., 1986; Ouyang et al., 1988; Schweikl et al., 1998). Glycidyl methacrylate was also mutagenic in *Klebsiella pneumoniae* (Voogd et al., 1981). Glycidyl methacrylate induced DNA damage in the SOS chromotest using *Escherichia coli* PQ37 (von der Hude et al., 1990).

Acellular systems

Glycidyl methacrylate did not introduce DNA breaks to isolated DNA, as assessed by the plasmid relaxation assay using pUC19 plasmids isolated from *E. coli* (Poplawski et al., 2009). *E. coli* HB101 transfected with glycidyl methacrylate-bound pBR322 was transformed to two stable and heritable mutants, from one of which deletion and insertion were detected in hot-spot regions (Xie et al., 1990b; Fang, 1991; Zuo et al., 1991; Zuo, 1991; Gao et al., 1994a, b).

(b) Glycidol

See Table 4.5, Table 4.6, Table 4.7, and Table 4.8.

Table 4.5 Genetic and related effects of glycidol in human cells in vitro

End-point	Tissue, cell line	Results[a]		Concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
Unscheduled DNA synthesis	WI-38 cells	–	+	0.33 µg/mL	Thompson et al. (1981)
Chromosomal aberrations	Lymphocytes (primary)	+	NT	29.6 µg/mL	Norppa et al. (1981)
Sister-chromatid exchange	Lymphocytes (primary)	+	NT	3.7 µg/mL	Norppa et al. (1981)

HID, highest ineffective dose; LED, lowest effective dose; NT, not tested.
[a] +, positive; –, negative.

Table 4.6 Genetic and related effects of glycidol in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Reference
Chromosomal aberrations	Rats, Sprague-Dawley (F)	Bone marrow	–	226 mg/kg bw per day	Oral × 5	Thompson & Hiles (1981)
Chromosomal aberrations	Rats, Sprague-Dawley (F)	Bone marrow	–	145 mg/kg bw per day	Intraperitoneal × 5	Thompson & Hiles (1981)
Micronucleus formation	Mouse, $B6C3F_1$ (M)	Polychromatic erythrocytes	+	37.5 mg/kg bw per day	Intraperitoneal × 2	NTP (1990)
Micronucleus frequency	Mouse, BalbC (M)	Polychromatic erythrocytes	+	120 mg/kg bw	Intraperitoneal × 1	Aasa et al. (2017)

bw, body weight; F, female; HID, highest ineffective dose; LED, lowest effective dose; M, male.
[a] +, positive; –, negative.

Table 4.7 Genetic and related effects of glycidol in non-human mammals in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA damage (alkaline comet assay)	Chinese hamster ovary cells	+	NT	20 µg/mL	Purity, NR	El Ramy et al. (2007)
Gene mutation, *Tk* locus	L5178Y mouse, lymphoma cells	+	+	8 µg/mL		Thompson et al. (1981)
Gene mutation, *Tk* locus	L5178Y mouse, lymphoma cells	+	NT	1.43 µg/mL		NTP (1990)
Gene mutation, *Hprt* locus	Chinese hamster ovary cells	+	NT	50 mM[b] [3.7 µg/mL]		Aasa et al. (2016)
Gene mutation, 6-thioguanine resistance	Chinese hamster lung V79 cells	+	NT	0.15 µg/mL		Smith et al. (1990)
Chromosomal aberrations	Chinese hamster cells	+	+	12.5 µg/mL		NTP (1990)
Sister-chromatid exchange	Chinese hamster cells	+	+	1.11 µg/mL		NTP (1990)
Sister-chromatid exchange	Chinese hamster V79 cells	+	NT	92.6 µg/mL		von der Hude et al. (1991)

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

[a] +, positive.

[b] Value of LEC was not provided by the authors, but was estimated from the relevant figure in the publication.

Table 4.8 Genetic and related effects of glycidol in non-mammalian systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
Drosophila melanogaster	Sex-linked recessive lethal mutations, heritable translocation test	+	NA	1230 ppm in feed	Foureman et al. (1994)
Neurospora crassa	Reverse mutation	+	NT	37 000[b] µg/mL (15 min)	Kølmark & Giles (1955)
Schizosaccharomyces pombe	Forward mutation	+	+	74 µg/mL	Migliore et al. (1982)
Klebsiella pneumoniae	Forward mutation	+	NT	14.8 µg/mL	Voogd et al. (1981)
Salmonella typhimurium TA1535	Reverse mutation	+	+	20.6 µg/plate	Thompson et al. (1981)
Salmonella typhimurium TA1535	Reverse mutation	+	+	500 µg/plate	Mamber et al. (1984)
Salmonella typhimurium TA1535	Reverse mutation	+	+	3 µg/plate	Canter et al. (1986)
Salmonella typhimurium TA1535	Reverse mutation	+	+	1 µg/plate	NTP (1990)
Salmonella typhimurium TA100	Reverse mutation	+	NT	100 µg/plate	Wade et al. (1979)
Salmonella typhimurium TA100	Reverse mutation	+	+	61.7 µg/plate	Thompson et al. (1981)
Salmonella typhimurium TA100	Reverse mutation	+	+	33 µg/plate	Canter et al. (1986)
Salmonella typhimurium TA100	Reverse mutation	+	NT	25 µg/plate	Claxton et al. (1991)
Salmonella typhimurium TA100	Reverse mutation	+	+	125 µg/plate	De Flora (1979)
Salmonella typhimurium TA100	Reverse mutation	+	+	100 µg/plate	NTP (1990)
Salmonella typhimurium TA98	Reverse mutation (spot test)	–	NT	10 000 µg/plate	Wade et al. (1979)
Salmonella typhimurium TA98	Reverse mutation	+	+	3333 µg/plate	NTP (1990)
Salmonella typhimurium TA1537	Reverse mutation	+[w]	+	1670 µg/plate	NTP (1990)
Salmonella typhimurium TA97	Reverse mutation	+	+	NR	Canter et al. (1986)

Table 4.8 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Reference
		Without metabolic activation	With metabolic activation		
Salmonella typhimurium TA97	Reverse mutation	+	+	1000 µg/plate	NTP (1990)
Escherichia coli (Sd-4)	Reverse mutation	+	NT	740 µg/mL	Hussain & Osterman-Golkar (1984)
Escherichia coli PQ37 SOS chromotest	DNA strand breaks, cross-links or related damage	+	NT	244.5 µg/mL	von der Hude et al. (1990)
Escherichia coli, rec assay	Differential toxicity	+	NT	NR	Mamber et al. (1984)
Prophage induction, SOS repair test	DNA strand breaks, cross-links or related damage	–	NT	500 µg/plate	Mamber et al. (1984)

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

[a] +, positive; –, negative; +[w], weakly positive.

[b] One dose tested; time-dependent response.

(i) Humans

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

Glycidol induced chromosomal aberrations and sister-chromatid exchange in primary human lymphocytes in the absence of metabolic activation (Norppa et al., 1981), while unscheduled DNA synthesis was induced in human fibroblast WI-38 cells only in the presence of metabolic activation (Thompson et al., 1981).

(ii) Experimental systems

Glycidol induced the formation of micronuclei in male mice B6C3F$_1$ and BalbC mice after intraperitoneal administration (NTP, 1990; Aasa et al., 2017). It was without effect on chromosomal aberrations in rats after oral or intraperitoneal treatment (Thompson & Hiles, 1981).

Tests performed in experimental systems in vitro gave consistently positive results. Glycidol induced DNA damage in the alkaline comet assay in Chinese hamster ovary cells (El Ramy et al., 2007). Glycidol induced *Tk* gene mutation in mouse lymphoma L5178Y cells (Thompson et al., 1981; NTP, 1990), *Hprt* gene mutation in Chinese hamster ovary cells (Aasa et al., 2016), and gave positive results in the 6-thioguanine resistance test in Chinese hamster lung V79 cells (Smith et al., 1990). Glycidol also induced chromosomal aberrations and sister-chromatid exchange in Chinese hamster cells (NTP, 1990; von der Hude et al., 1991).

Glycidol gave uniformly positive results in several assays for reverse mutation in *S. typhimurium* (De Flora, 1979; Wade et al., 1979; Thompson et al., 1981; Mamber et al., 1984; Canter et al., 1986; NTP, 1990; Claxton et al., 1991) and in two assays for mutation in fungi (Kølmark & Giles, 1955; Migliore et al., 1982). Glycidol was also mutagenic in *K. pneumoniae* (Voogd et al., 1981) and in *E. coli* (Hussain & Osterman-Golkar, 1984). Glycidol gave positive results in the assay for sex-linked recessive lethal mutation and in the heritable translocation test in *Drosophila melanogaster* (Foureman et al., 1994). Glycidol gave positive results in the *E. coli* PQ37 SOS chromotest, and negative results in the prophage-induction SOS repair test (Mamber et al., 1984; von der Hude et al., 1990).

4.2.3 Alters cell proliferation, cell death, or nutrient supply

In B6D2F$_1$/Crlj mice exposed to glycidyl methacrylate by inhalation for 104 weeks, increases in transitional cell hyperplasia of the nasal cavity were reported in males and females (JBRC, 2015a).

In F344/DuCrlCrlj rats exposed to glycidyl methacrylate by inhalation for 104 weeks, non-neoplastic lesions in the nasal cavity (squamous cell hyperplasia with atypia, squamous cell metaplasia in respiratory epithelium, and squamous cell metaplasia with atypia) and transitional cell hyperplasia were reported in males and females (JBRC, 2015b).

Gap-junctional intercellular communication, as measured by the scrape-loading/dye-transfer technique, was significantly inhibited in transformed human lung fibroblast cells treated with glycidyl methacrylate (2.5 or 5 µg/mL) (Yin et al., 2003).

4.2.4 Induces oxidative stress

Glycidyl methacrylate induced oxidative damage to DNA in primary human lymphocytes as assessed with the alkaline comet assay using the DNA repair enzymes endonuclease III (Endo III) and formamidopyrimidine-DNA glycosylase (Fpg) (Poplawski et al., 2009). The antioxidant *N*-acetylcysteine reduced the glycidyl methacrylate-induced double-strand breaks and nuclear chromatin condensation in primary human gingiva fibroblasts (Styllou et al., 2017). *N*-acetylcysteine reduced the frequency of micronucleus formation induced by glycidyl methacrylate in Chinese hamster lung fibroblast

V79 cells (Lee et al., 2006) (see Section 4.2.2(a)(ii)).

4.2.5 Evidence on other key characteristics of carcinogens

Glycidyl methacrylate (8.0 µg/mL [0.06 mM]) induced cell transformation of human lung fibroblasts (Yin et al., 2003). In addition, glycidyl methacrylate induced cell transformation of BALB/c mouse embryonic fibroblasts 3T3 cells or Golden Syrian hamster embryonic SHE cells in several studies (Xu et al., 1994; Yang et al., 1996; Zhang et al., 1996; Lei et al., 1998a, b).

In a series of studies, glycidyl methacrylate induced malignant transformation and methylation of the promoter regions or overexpression of several genes in human bronchial epithelial cells or embryonic lung fibroblast (Tan et al., 1998; Xu et al., 2001; Dong et al., 2009; Yang et al., 2009; Dong et al., 2010; Hu et al., 2012). Glycidyl methacrylate (8 µg/mL) induced changes in DNA methylation in stages of malignant transformation, as detected by a CpG (cytosine–phosphate–guanine) promoter methylation microarray (Wang et al., 2014), including of the opioid binding protein/cell adhesion molecule-like (*OPCML*) gene (Liu et al., 2015) in cultured human bronchial epithelial cells (16HBE).

Methylation of the *P16* [*CDKN2A*] gene promoter was detected at the early stage and protophase stage in the process of malignant transformation of human bronchial epithelial cells exposed to glycidyl methacrylate (Hu et al., 2012).

4.3 Data relevant to comparisons across agents and end-points

The analysis of the bioactivity in vitro of the agents reviewed in *IARC Monographs* Volume 125 was informed by data from high-throughput screening assays generated by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the Government of the USA (Thomas et al., 2018). Glycidyl methacrylate was one of thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes as of 1 September 2019 (US EPA, 2019a). Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2019a). [The Working Group noted that the metabolic capacity of the cell-based assays is variable, and generally limited, as acknowledged in Kavlock et al. (2012).]

Among the 402 assays in which glycidyl methacrylate (at concentrations up to 100 µM) was tested, it was found to be inactive in almost all assays. Active responses were observed in 8 assays (US EPA, 2019a). Effect on upregulation of nuclear factor erythroid 2-related factor 2 (NRF2) was reported at a half-maximal activity concentration (AC_{50}) of 38 µM. For nuclear receptors, borderline activity (potency of > 50% or activity observed only at the highest concentration tested) was found for the mouse embryonic cell-based assay (SSH_3T3_GLI3) at an AC_{50} of 82 µM. For cell viability, glycidyl methacrylate was shown to be cytotoxic only in human T47D, HEK293, and HEPG2 cells at AC_{50}s of 0.45–83 µM.

Glycidol was also tested (at concentrations up to 100 µM) in 673 assays and found to be inactive in almost all assays. Active responses were observed in 16 assays (US EPA, 2019b). Effects were observed in the form of TIMP metallopeptidase inhibitor 1 downregulation, retinoid X receptor α agonism and androgen receptor antagonism at concentrations between 10 and 38.9 µM. These were noted as borderline effects (< 50% efficacy, only highest concentration above baseline). For cell viability, glycidol was shown to be cytotoxic only in human HEK293 and HEPG2 cells at AC_{50}s of 33.6–65.5 µM.

5. Summary of Data Reported

5.1 Exposure characterization

Glycidyl methacrylate is a High Production Volume chemical that is mainly used as an intermediate in the production of epoxy polymers and vinyl and acrylic resins. These polymers are used in dental sealants, composites and adhesives; bone composite materials; powder coatings; and hydrogel lenses. There are emerging applications for the polymers in medical imaging and targeting drug delivery. Polymers formed of glycidyl methacrylate can also be used in food contact material. Occupational exposures by inhalation and dermal contact have been reported for chemical-production workers in a single study. Occupational exposure when preparing the dental and bone materials or exposure to patients receiving these materials may potentially occur, but has not been targeted for measurement. Exposure in the general population is not expected from use of the polymerized products.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

Glycidyl methacrylate was tested for carcinogenicity in one strain of male and female mice in one inhalation study, and in two different strains of male and female rats, respectively, in two inhalation studies, and one gavage study.

The inhalation study in mice was well-conducted under good laboratory practice, and resulted in a significant positive trend and increase in the incidence of haemangioma of the nasal cavity and of haemangioma or haemangiosarcoma (combined) of the nasal cavity in males and females, and of nasal cavity haemangiosarcoma in males and females (positive trend only for females). In female mice, there was a significant positive trend and increase in the incidence of bronchioalveolar carcinoma. There was also a significant positive trend in the incidence of uterine histiocytic sarcoma, and adenoma of the Harderian gland in female mice. There was a significant positive trend in the incidence of adenoma of the nasal cavity, squamous cell papilloma of the forestomach, and adenoma of the Harderian gland in male mice.

One inhalation study in male and female rats was well-conducted under good laboratory practice. In male and female rats, exposure to glycidyl methacrylate resulted in a significant positive trend and increase in the incidence of squamous cell carcinoma of the nasal cavity. In male rats, there was a significant positive trend and increase in the incidence of esthesioneuroepithelioma (neuroepithelial carcinoma) of the nasal cavity, and a significant increase in the incidence of adenoma of the nasal cavity. In male rats, there was also a significant positive trend and increase in the incidence of peritoneal mesothelioma and subcutis fibroma; significant positive trends in the incidence of skin basal cell epithelioma and skin keratoacanthoma were also observed. In female rats, exposure to glycidyl methacrylate resulted in a significant positive trend and increase in the incidence of fibroadenoma of the mammary gland; significant positive trends in the incidence of uterine endometrial stromal sarcoma, subcutis fibroma, thyroid C-cell adenoma, and adenoma of the clitoral gland were also observed.

The other inhalation study in rats gave negative results.

The study in male and female rats treated by gavage was inadequate for the evaluation of the carcinogenicity of glycidyl methacrylate in experimental animals.

5.4 Mechanistic evidence

No data on the absorption, distribution, metabolism or excretion of glycidyl methacrylate in exposed humans were available. No direct data on the absorption or excretion of glycidyl methacrylate were available from mammalian species. Indirect evidence of dermal and oral absorption is provided by tests for acute toxicity in various rodent species, which suggest that dermal absorption of glycidyl methacrylate is likely to be as efficient as oral absorption. In one study in rabbits, a carboxyesterase inhibitor markedly reduced the decline of glycidyl methacrylate in the blood. Studies in vitro in human, rabbit, and rat tissue homogenates showed the formation over time of a metabolite that was reasonably identified as glycidol.

There is consistent and coherent evidence that glycidyl methacrylate exhibits key characteristics of carcinogens (is genotoxic) in human primary cells. In all available studies in human primary cells, glycidyl methacrylate induced DNA damage, including double-strand breaks and unscheduled DNA synthesis. In different cultured human cell lines, consistent findings of various types of damage to DNA, including DNA strand breaks, gene mutations, and chromosomal aberrations were seen. In two studies in orally exposed rats, glycidyl methacrylate induced various types of damage to DNA, including strand breaks, gene mutations, and micronucleus formation. After intraperitoneal administration in mice, glycidyl methacrylate induced micronucleus formation and unscheduled DNA synthesis in two studies. In several studies in rodent cells in vitro, glycidyl methacrylate induced *Hprt* gene mutation, micronucleus formation, sister-chromatid exchanges, and unscheduled DNA synthesis; the effects were abolished by metabolic activation in the available tests. In addition, glycidyl methacrylate gave generally positive results in tests for base-pair substitution mutation in bacteria, both in the presence and in the absence of metabolic activation. Glycidyl methacrylate–DNA lesions in plasmid DNA yielded stable and heritable mutations.

Glycidol caused DNA damage, chromosomal aberrations, and sister-chromatid exchange in one study in human primary cells in vitro, and increased micronucleus formation in mice after intraperitoneal administration, but not chromosomal aberrations in rats after intraperitoneal or oral administration. Glycidol gave consistently positive results in a substantial number of genotoxicity assays in vitro. Glycidol gave positive results both in rodent cells, covering a range of end-points such as DNA damage, gene mutation and chromosomal aberrations, and in mutation tests in bacteria.

In experimental systems, there were consistent and coherent findings in male and female rats and mice exposed chronically by inhalation that glycidyl methacrylate alters cell proliferation, cell death, or nutrient supply. Glycidyl methacrylate induced a dose-related increase in the incidence of transitional cell hyperplasia of the nasal cavity in both sexes of mice and rats, and in squamous cell hyperplasia with atypia, squamous cell metaplasia in respiratory epithelium, and squamous cell metaplasia with atypia in male and female rats.

There is suggestive evidence that glycidyl methacrylate is electrophilic, based on the formation of DNA adducts in tissues of exposed rats and when glycidyl methacrylate was added to DNA or DNA bases, in the few available studies. In addition, glycidol is electrophilic. Glycidol is a reactive epoxide that alkylates DNA in vitro. There is also suggestive evidence from a narrow range of experiments in human and mammalian cells in vitro that glycidyl methacrylate induces oxidative stress or causes immortalization. Glycidyl methacrylate induced oxidative damage to DNA, and the antioxidant *N*-acetylcysteine reduced the effect of glycidyl methacrylate on several genotoxic end-points in human and

rodent cells. Glycidyl methacrylate also induced cell transformation in human and rodent cells in vitro.

Glycidyl methacrylate and glycidol were found to be mostly without effects in the assay battery of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes.

Overall, the mechanistic evidence is consistent and coherent that glycidyl methacrylate belongs, on the basis of mechanistic considerations, to a class of reactive glycidyl epoxides, for which one member has been classified as probably carcinogenic to humans. Glycidyl methacrylate bears structural similarity to other glycidyl epoxides, and there is close concordance with glycidol with respect to the genotoxicity profile as well as the tumour-site profile in chronic animal bioassays.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of glycidyl methacrylate.

6.2 Cancer in experimental animals

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

6.3 Mechanistic evidence

There is *strong evidence* that glycidyl methacrylate belongs, on the basis of mechanistic considerations, to a class of reactive glycidyl epoxides for which one member, glycidol, has been classified as *probably carcinogenic to humans (Group 2A)*. There is also *strong evidence* in human primary cells that glycidyl methacrylate exhibits key characteristics of carcinogens.

6.4 Overall evaluation

Glycidyl methacrylate is *probably carcinogenic to humans (Group 2A).*

6.5 Rationale

The Group 2A evaluation for glycidyl methacrylate is based on *sufficient evidence* of cancer in experimental animals and *strong* mechanistic evidence. The evidence regarding cancer in humans was *inadequate* as no data were available. The *sufficient evidence* of carcinogenicity in experimental animals is based on the induction of malignant neoplasms in two species.

There was *strong* mechanistic evidence, based on two distinct topics. There is *strong evidence* that glycidyl methacrylate belongs, based on mechanistic considerations, to a class of reactive glycidyl epoxides for which one member, glycidol, has been classified as *probably carcinogenic to humans*. Glycidyl methacrylate bears structural similarity to other members of this class, and there is close concordance with respect to the genotoxicity profile, and the target organs of carcinogenicity in chronic animal bioassays. There is also *strong evidence* in primary human cells that glycidyl methacrylate exhibits key characteristics of carcinogens; glycidyl methacrylate is genotoxic in all available tests in human primary cells, supported by consistent findings across several different test systems in various species. It also alters cell proliferation, cell death, or nutrient supply in experimental systems.

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LIST OF ABBREVIATIONS

AC_{50}	half-maximal activity concentration
ALMA	allylmercapturic acid
BisGMA	bisphenol A–glycidyl methacrylate
bw	body weight
CAR	constitutive androstane receptor
CYP	cytochrome P450
ECHA	European Chemicals Agency
ERα	estrogen receptor α
FDA	Food and Drug Agency
GC-ECD	gas chromatography with electron-capture detection
GC-FID	gas chromatography and flame ionization detection
GC-MS	gas chromatography-mass spectrometry
GLP	good laboratory practice
γ-H2AX	gamma-H2A histone family member X
HPMA	3-hydroxypropylmercapturic acid
HPV	high production volume
LD_{50}	median lethal dose
NBP	4-(4-nitrobenzyl)pyridine
NIOSH	National Institute for Occupational Safety and Health
OECD	Organisation for Economic Co-operation and Development
PEC_{local}	predicted environmental concentration, local
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
TEGDMA	triethylene glycol dimethacrylate
TPA	12-*O*-tetradecanoylphorbol-13-acetate
TWA	time-weighted average
UDS	unscheduled DNA synthesis
US EPA	United States Environmental Protection Agency
v/v	volume per volume

IARC MONOGRAPHS

This volume of the *IARC Monographs* provides evaluations of the carcinogenicity of five High Production Volume chemicals: glycidyl methacrylate, 1-butyl glycidyl ether, 1-bromo-3-chloropropane, 4-chlorobenzotrifluoride, and allyl chloride.

Glycidyl methacrylate is mainly used in the production of epoxy polymers and vinyl and acrylic resins. The polymers are subsequently used in dental sealants, composites and adhesives, bone composite materials, powder coatings, hydrogel lenses, and food contact material.

1-Butyl glycidyl ether is a reactive intermediate and viscosity-reducing solvent used in the manufacture of epoxy resins, and is also used as a surface modifier in the dyeing of cotton and wool.

1-Bromo-3-chloropropane is an intermediate in the manufacture of a wide range of pharmaceuticals, some pesticides, and other chemicals.

4-Chlorobenzotrifluoride is widely used as a solvent and diluent for inks, paints, toners, and coatings and in dispersive applications in the automotive industry. It is also a major component in industrial and consumer formulations such as cleaners, degreasers, stain removers, and sealants.

Allyl chloride is almost exclusively used in the production of epichlorohydrin, a basic building block for epoxy resins and the synthesis of glycerol, although it is also an intermediate in the synthesis of various pesticides, pharmaceuticals, adhesives, and personal-care products.

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

An *IARC Monographs* Working Group reviewed epidemiological evidence, animal bioassays, and mechanistic evidence to reach conclusions as to the carcinogenic hazard to humans of exposure to these agents.