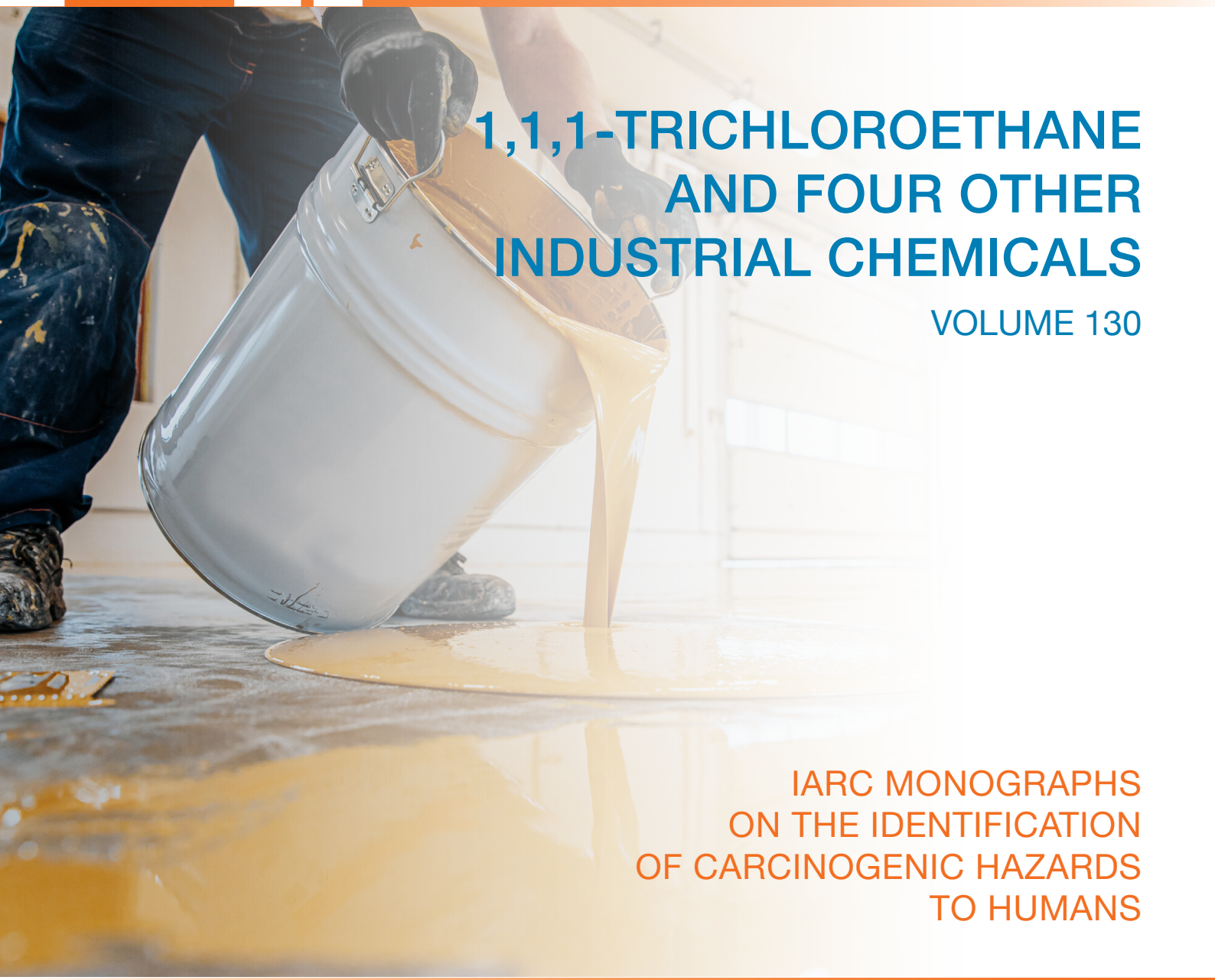
IARC MONOGRAPHS



1,1,1-TRICHLOROETHANE AND FOUR OTHER INDUSTRIAL CHEMICALS

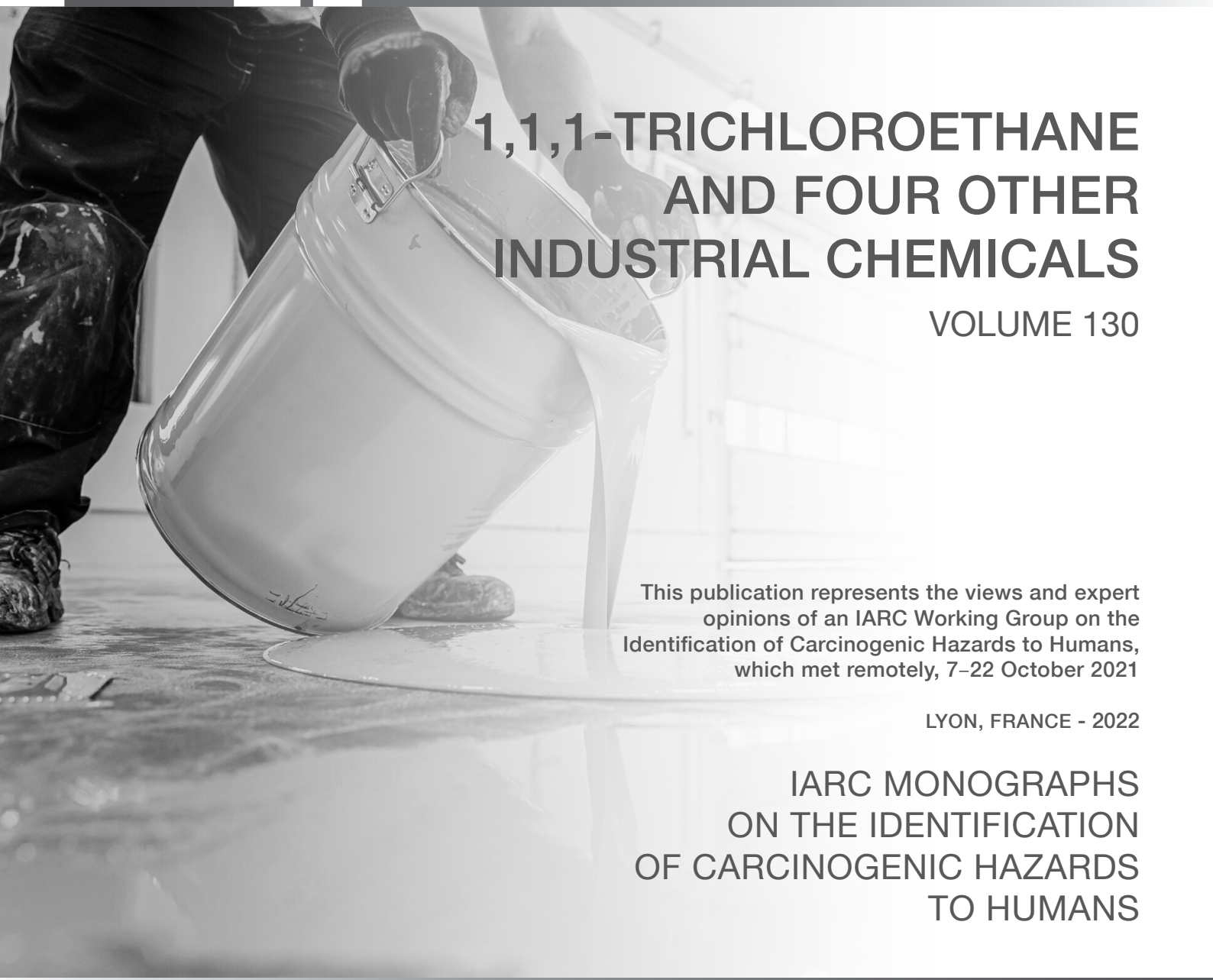
VOLUME 130

IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

World Health Organization

IARC MONOGRAPHS



1,1,1-TRICHLOROETHANE AND FOUR OTHER INDUSTRIAL CHEMICALS

VOLUME 130

This publication represents the views and expert opinions of an IARC Working Group on the Identification of Carcinogenic Hazards to Humans, which met remotely, 7–22 October 2021

LYON, FRANCE - 2022

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.who.int/.

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" (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.

Co-funded by the European Union

Published by the International Agency for Research on Cancer,
150 cours Albert Thomas, 69372 Lyon Cedex 08, France

Online publication, August 2022

IARC Monographs (and Corrigenda) are published online at https://publications.iarc.fr.
To report an error, please contact: imo@iarc.who.int.

Distributed by WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland
(tel.: +41 22 791 3264; fax: +41 22 791 4857; website: https://apps.who.int/bookorders; email: bookorders@who.int).

About the cover: Worker applying a yellow epoxy protective resin to a floor. 1,1,1-Trichloroethane, *N*-methylolacrylamide, and isophorone are used in commercial resins.

Source: © AdobeStock.com/Doralin

How to cite: IARC (2022). 1,1,1-Trichloroethane and four other industrial chemicals. *IARC Monogr Identif Carcinog Hazards Hum*. 130:1–368.

IARC Library Cataloguing-in-Publication Data

Names: IARC Working Group on the Identification of Carcinogenic Hazards to Humans.

Title: 1,1,1-Trichloroethane and four other industrial chemicals.

Description: Lyon: International Agency for Research on Cancer, 2022. | Series: IARC monographs on the identification of carcinogenic hazards to humans, ISSN 1017-1606; v. 130. | "This publication represents the views and expert opinions of an IARC Working Group on the Identification of Carcinogenic Hazards to Humans, which met remotely, 7–22 October 2021." | Includes bibliographical references.

Identifiers: ISBN 9789283201700 (pbk.) | ISBN 9789283201977 (ebook)

Subjects: MESH: Carcinogens--toxicity. | Neoplasms--chemically induced. | Trichloroethanes--adverse effects. | Phenylhydrazines--adverse effects. | Diphenylamine--adverse effects. | Acrylamides--adverse effects. | Cyclohexanones--adverse effects. | Risk Factors.

Classification: NLM W1

CONTENTS

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* programme, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France, or via email at imo@iarc.who.int, 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* programme. Corrigenda are published online on the relevant webpage for the volume concerned (IARC Publications: https://publications.iarc.fr/).

LIST OF PARTICIPANTS

Members [1]

Marc Audebert

Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE)
Toulouse
France

Fiorella Belpoggi (Meeting Chair)

Cesare Maltoni Cancer Research Centre
Ramazzini Institute
Bologna
Italy

Russell C. Cattley (Subgroup Chair, Cancer in Experimental Animals)

College of Veterinary Medicine
Auburn University
Auburn, AL
USA

Julie Cox

Health Canada
Ottawa, ON
Canada

Calvin Ge

Institute for Risk Assessment Sciences
Utrecht University
Utrecht
The Netherlands

Per Gustavsson

Karolinska Institutet
Stockholm
Sweden

[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 declare potentially relevant research, employment, and financial interests that are current or that have occurred during the past 4 years. Minimal interests are not disclosed here, 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 other non-publicly funded 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 disclosed as potentially significant conflicts of interests.

Heiko Käfferlein

Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IPA)
Ruhr University Bochum
Germany

Lawrence Lash

Department of Pharmacology
Wayne State University School of Medicine
Detroit, MI
USA

Annie Lumen [2]

National Center for Toxicological Research
United States Food and Drug Administration
Little Rock, AK
USA

Michiharu Matsumoto

Japan Bioassay Research Center
Japan Organization of Occupational Health and Safety (JOHAS)
Hadano
Japan

María Teresa Muñoz-Quezada

Universidad Católica del Maule
Talca
Chile

Cheryl Peters

Cumming School of Medicine, University of Calgary and Cancer Care Alberta, Alberta Health Services
Calgary, AB
Canada

Mark Purdue

Occupational and Environmental Epidemiology Branch
Division of Cancer Epidemiology and Genetics
National Cancer Institute
Rockville, MD
USA

Bradley Reisfeld (Subgroup Chair, Mechanistic Evidence)

Colorado State University
Wellington, CO
USA

Patricia Stewart [3] *(Subgroup Chair, Exposure Characterization)*

Stewart Exposure Assessments, LLC
Arlington, VA
USA

Camilla Svendsen

Norwegian Institute of Public Health
Oslo
Norway

[2] Dr Lumen became a salaried employee of Amgen, Inc., on 1 November 2021. This was determined not to present a conflict of interest restricting her participation at this meeting, as Amgen has no reported involvement with the agents under review.

[3] Dr Stewart is employed by Stewart Exposure Assessments, LLC, but the work conducted by this company does not involve competing interests for the topics covered by the present *IARC Monographs* Meeting.

Michelle Turner (Subgroup Chair, Cancer in Humans)

Barcelona Institute for Global Health (ISGlobal)
Barcelona
Spain

Mohammed Abbas Virji

Respiratory Health Division
National Institute for Occupational Safety and Health
Centers for Disease Control and Prevention
Morgantown, WV
USA

Marianna G. Yakubovskaya

Institute of Carcinogenesis
NN Blokhin National Medical Research Center of Oncology
Ministry of Health of the Russian Federation
Moscow
Russian Federation

James Yiin

National Institute for Occupational Safety and Health
Centers for Disease Control and Prevention
Cincinnati, OH
USA

Invited Specialists

None

Representatives

Amira Ben Amara

National Agency of Sanitary and Environmental Control of Products (ANCSEP)
Tunis
Tunisia

Sandrine Charles

French Agency for Food, Environmental and Occupational Health and Safety (ANSES)
Maisons-Alfort
France

Observer [4]

Audrey Batoon [5]

LANXESS Corporation
Shelton, CT
USA

IARC Secretariat

Lamia Benbrahim-Tallaa *(Rapporteur, Cancer in Experimental Animals)*
Nathan DeBono *(Rapporteur, Cancer in Humans)*
Aline de Conti *(Rapporteur, Mechanistic Evidence)*

[4] Each Observer agreed to respect the Guidelines for Observers at *IARC Monographs* meetings. Observers did not serve as Meeting Chair or Subgroup Chair, draft any part of a monograph, or participate in the evaluations. They also agreed not to contact participants before the meeting, not to lobby them at any time, not to send them written materials, and not to offer them meals or other favours. IARC asked and reminded Working Group Members to report any contact or attempt to influence that they may have encountered, either before or during the meeting.
[5] Dr Batoon attended as an Observer for LANXESS Corporation. She reports being a salaried employee of LANXESS Corporation (which formulates products using diphenylamine).

Fatiha El Ghissassi *(Rapporteur, Mechanistic Evidence)*
Samantha Goodman
Yann Grosse *(Responsible Officer; Rapporteur, Cancer in Experimental Animals)*
William Gwinn *(Rapporteur, Mechanistic Evidence)*
Simone Kühnle *(Rapporteur, Cancer in Experimental Animals)*
Jérôme Lavoué *(Rapporteur, Exposure Characterization)*
Heidi Mattock *(Scientific Editor)*
Daniel Middleton *(Rapporteur, Exposure Characterization)*
Adalberto Miranda Filho *(Rapporteur, Cancer in Humans)*
Felix Onyije
Mary Schubauer-Berigan *(Programme Head; Rapporteur, Cancer in Humans)*
Eero Suonio *(Rapporteur, Exposure Characterization)*
Susana Viegas *(Rapporteur, Exposure Characterization)*
Roland Wedekind

Administrative Assistance

Jennifer Nicholson
Sandrine Ruiz

Production Team

Niree Kraushaar
Solène Quennehen

Pre- and Post-Meeting Assistance

Kathryn Guyton
Misty Hein
Karen Müller *(Managing Editor)*

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.who.int/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 searching the literature and performing title and abstract screening, organizing conference calls to

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.

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.who.int/).

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).

The Working Group meets at IARC for approximately eight days to discuss and finalize

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 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 endpoint). 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 preneoplastic 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 effects", are based on empirical observations of the chemical and biological properties associated with the human carcinogens identified by

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).

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 evidenc*e; (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. *sufficient 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-thirtieth volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of five industrial chemicals: 1,1,1-trichloroethane, 1,2-diphenylhydrazine, diphenylamine, *N*-methylolacrylamide, and isophorone. Due to the coronavirus disease (COVID-19) pandemic, this meeting was held remotely.

1,1,1-Trichloroethane was considered previously by the *IARC Monographs* programme in 1978 (IARC, 1979), 1987 (IARC, 1987), and most recently in 1998, when it was evaluated as *not classifiable as to its carcinogenicity to humans (Group 3)* (IARC, 1999). *N*-Methylolacrylamide was considered previously by the *IARC Monographs* programme in 1994 and was also evaluated as *not classifiable as to its carcinogenicity to humans (Group 3)* (IARC, 1994). 1,2-Diphenylhydrazine, diphenylamine, and isophorone have not been evaluated previously by the *IARC Monographs* programme.

The Advisory Group to Recommend Priorities for the *IARC Monographs* that met in 2019 recommended that 1,1,1-trichloroethane be evaluated with high priority; 1,2-diphenylhydrazine, diphenylamine, and *N*-methylolacrylamide with medium priority; and isophorone with low priority (IARC, 2019a; Marques et al., 2019). A summary of the findings of this volume appears in *The Lancet Oncology* (Belpoggi et al., 2021).

Occupational exposure in the past

National surveys and epidemiological studies indicate that 2–6% of North American and European populations (e.g. Silvestri et al., 1983; Talibov et al., 2014) were occupationally exposed to 1,1,1-trichloroethane in the 1980s and 1990s, although extreme reductions in use have occurred since the adoption of the Montreal Protocol on Substances that Deplete the Ozone Layer, in 1987 (UNEP, 2021). The substantial use of 1,1,1-trichloroethane in the past may have an enduring impact on cancer incidence rates for cancer types with long latency. Historical groundwater contamination with 1,1,1-trichloroethane could also remain a source of exposure in the future (Palau et al., 2016).

Availability of epidemiological data

There was a paucity of epidemiological data for agents other than 1,1,1-trichloroethane. Information on use of this agent is more limited in low- and middle-income countries than in high-income countries. Given the large number and variety of chlorinated organic solvents in commercial use, many human cancer studies only described the group or class of compounds under assessment (e.g. chlorinated aliphatic hydrocarbons), and did not name 1,1,1-trichloroethane explicitly in the abstract of their

publication. This made the identification of relevant studies in the literature search challenging. As a result, multiple literature search terms were used in title and abstract searches to capture various chemical class names in addition to the agent name. Full-text literature searches were also conducted to identify studies that did not state the agent name in the title or abstract, but that reported analyses specific to 1,1,1-trichloroethane in the body of the article. This feature of the agent is likely to be a challenge for future monographs investigating individual chemicals that belong to a broader class of compounds.

The human cancer studies investigating epidemiological associations between 1,1,1-trichloroethane exposure and cancer risk were primarily limited by challenges in the assessment of exposure. Studies with assessment of biomarkers of exposure to 1,1,1-trichloroethane were a notable research gap, as only one such study was identified. There was one study on transgenerational effects of exposure, which was another notable research gap. More generally, the number of studies per cancer site was small, and there were only two cohort studies of workers occupationally exposed to the agent.

For diphenylamine, information was available about occupations known to have a high probability of exposure; however, there was very little epidemiological research about cancer risk among exposed workers.

Data from high-throughput screening assays

The analysis of the in vitro bioactivity of all evaluated agents, except *N*-methylolacrylamide, 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). Three compounds (i.e. 1,2-diphenylhydrazine, diphenylamine, and isophorone) were considered active in low numbers of assay end-points mapped to the following key characteristics of carcinogens (Smith et al., 2016): "is genotoxic", "induces oxidative stress", "modulates receptor-mediated effects", and "alters cell proliferation, cell death, or nutrient supply". Specifically, 1,2-diphenylhydrazine was considered active in four assay end-points for "is genotoxic", four assay end-points for "induces oxidative stress", seven assay end-points for "modulates receptor-mediated effects", and nine assay end-points for "alters cell proliferation, cell death, or nutrient supply"; diphenylamine was considered active in six assay end-points for "modulates receptor-mediated effects", and eight assay end-points for "alters cell proliferation, cell death, or nutrient supply"; and isophorone was considered active in five assay end-points for "modulates receptor-mediated effects", and two assay end-points for "alters cell proliferation, cell death, or nutrient supply". The results were generated with the software "kc-hits" (key characteristics of carcinogens – high-throughput screening discovery tool) (available from: https://gitlab.com/i1650/kc-hits). The mapping of assay end-points to each key characteristic follows that described in *IARC Monographs* Volume 123 (IARC, 2019b). All ToxCast/Tox21 data were obtained from the United States Environmental Protection Agency CompTox Chemicals Dashboard 10th Release (US EPA, 2021) at the time of the evaluations performed for the *IARC Monographs* Volume 130 in October 2021. These programmes are constantly being improved and new assays are added over time. However, at present, the general lack of metabolic activation and the small number of genotoxicity assays in these high-throughput screening programmes restrict their value in determining whether a chemical is genotoxic as part of an assessment of carcinogenicity.

Scope of systematic review

Standardized searches of the PubMed database (NCBI, 2021) were conducted for the 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 the agent, including the full set of search terms for the agent name and each outcome type, are available online.[1]

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[1] The literature trees for the present volume are available at: https://hawcproject.iarc.who.int/assessment/661/ (1,1,1-trichloroethane), https://hawcproject.iarc.who.int/assessment/658/ (1,2-diphenylhydrazine), https://hawcproject.iarc.who.int/assessment/662/ (diphenylamine), https://hawcproject.iarc.who.int/assessment/659/ (*N*-methylolacrylamide), and https://hawcproject.iarc.who.int/assessment/663/ (isophorone).

1,1,1-TRICHLOROETHANE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 71-55-6

EC/List No.: 200-756-3

Chem. Abstr. Serv. name: 1,1,1-trichloroethane

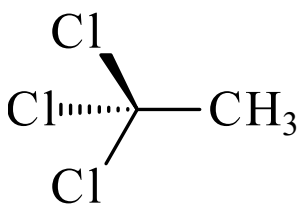
IUPAC systematic name: 1,1,1-trichloroethane

Synonyms: methylchloroform; trichloroethane; methyltrichloromethane; trichloromethylmethane; ethane, 1,1,1-trichloro-; α-trichloroethane; chlorothene; Solvent 111, Inhibisol, and other depositor-supplied synonyms and acronyms (NCBI, 2021).

1.1.2 Structural and molecular information

Relative molecular mass: 133.40 (IFA, 2021a)

Chemical structure:



Molecular formula: $C_2H_3Cl_3$

1.1.3 Chemical and physical properties

Description: colourless liquid with a mildly sweet, ethereal, and chloroform-like odour (IFA, 2021a, NCBI, 2021)

Odour threshold: odour may be noticeable at concentrations near 100 ppm [555 mg/m³] and has been described as strong and unpleasant at 1500–2000 ppm [8.32–11.1 g/m³] (NCBI, 2021)

Boiling point: 74 °C (IFA, 2021a)

Melting point: –30 °C (NCBI, 2021)

Density: 1.34 g/cm³ at 20 °C (IFA, 2021a)

Relative vapour density: 4.61 (air = 1) (IFA, 2021a)

Vapour pressure: 133.3 hPa at 20 °C (IFA, 2021a)

Auto-ignition temperature: 490 °C (IFA, 2021a)

Lower explosion limit: 9.5 vol.% (529 g/m³) (IFA, 2021a)

Upper explosion limit: 15.5 vol.% (860 g/m³) (IFA, 2021a)

Solubility: 1 g/L at 25 °C (IFA, 2021a), < 1 g/L at 20 °C, soluble in all common organic solvents including acetone, benzene, methanol, carbon tetrachloride, and ether; very good solvent for fats, paraffins, and other organic compounds (NCBI, 2021)

Viscosity: 0.86 mPa.s at 20 °C (NCBI, 2021)

Octanol/water partition coefficient (P): log K_{ow} = 2.49 (IFA, 2021a)

Reactivity: decomposes on exposure to light and high temperatures with carbon monoxide, carbon dioxide, hydrogen chloride, chlorine, and trace amounts of phosgene, polychlorinated dioxins, and related chlorine compounds as decomposition products. Risk of explosion on contact with alkali metals, nitrogen oxides, and oxygen, and at increased pressures and heat. Readily corrodes aluminium and aluminium alloys, and moderately corrodes iron and zinc (IFA, 2021a; NCBI, 2021)

Conversion factor: 1 ppm is equivalent to 5.55 mg/m^3 at 101 kPa and 20 °C (IFA, 2021a).

1.1.4 Impurities

Commercial-grade 1,1,1-trichloroethane has a purity of 90–95% and contains stabilizers at 3–8% (WHO, 1992; Doherty, 2000). Known impurities of 1,1,1-trichloroethane include trace amounts of 1,2-dichloroethane, chloroform, 1,1-dichloroethane, carbon tetrachloride, trichloroethylene, 1,1,2-trichloroethane, and vinylidene chloride (Stewart et al., 1969; NCBI, 2021).

1.2 Production and use

1.2.1 Production process

1,1,1-Trichloroethane is mainly manufactured from the catalytic hydrochlorination of ethylene to 1,2-dichloroethane, followed by thermal dehydrochlorination to vinyl chloride, conversion to 1,1-dichloroethane via catalytic hydrochlorination, and finally to 1,1,1-trichloroethane through a chlorination process (Doherty, 2000; Marshall & Pottenger, 2016). 1,1,1-Trichloroethane is also produced by the catalytic hydrochlorination of 1,1-dichloroethylene, which is derived from 1,1,2-trichloroethane, which in turn is derived from vinyl chloride or 1,2-dichloroethane via chlorination (Marshall & Pottenger, 2016). Alternatively, 1,1,1-trichloroethane and various other chlorinated ethanes and ethenes can also be produced via non-catalytic chlorination of ethane, as was the case until 1979 in the USA (US EPA, 1994a; Doherty, 2000).

1.2.2 Production volume

1,1,1-Trichloroethane is classified as a High Production Volume chemical, indicating that it is manufactured or imported in amounts greater than 1 million pounds [454 tonnes] per year (US EPA 2021b). However, production volumes of 1,1,1-trichloroethane were historically much higher and since the adoption of the Montreal Protocol on Substances that Deplete the Ozone Layer, in 1987, and the Clean Air Act, USA, in 1990, the production of 1,1,1-trichloroethane has been phased out for most non-essential uses, both in the USA and globally.

Total world production of 1,1,1-trichloroethane was 155 000 tonnes in 1970, which gradually increased and peaked at 725 000 tonnes in 1990, after which it rapidly declined to 301 000 tonnes in 1993 (Midgley & McCulloch, 1995), 184 000 tonnes in 2009, and 160 000 tonnes in 2014 (Marshall & Pottenger, 2016). Production in the USA was 245 000 tonnes in 1973 and peaked at 394 000 tonnes in 1985 (WHO, 1992), after which it declined to 450 million pounds [204 000 tonnes] in 1993 (ATSDR, 2006). In the USA, during the period 2012–2015, 163 million pounds [74 000 tonnes] to 192 million pounds [87 000 tonnes] of 1,1,1-trichloroethane were produced in the industrial sectors "industrial gas manufacturing" and "plastic material and resin manufacturing" (US EPA, 2016).

The use of and demand for 1,1,1-trichloroethane in the USA was estimated at 273 000 tonnes in 1987, with a peak at

312 000 tonnes in 1989, followed by a gradual decline in subsequent years to 282 000 tonnes in 1992 (US EPA, 1994a); total world demand for 1,1,1-trichloroethane in 1987 was 578 000 tonnes (IARC, 1999). Production and use of 1,1,1-trichloroethane was last reported in 2000–2001 in the lubricants category at 0.9–1.6 tonnes in Norway, and in 2009 at 0.6 tonnes for cleaning/washing agents in Denmark (SPIN, 2021).

In 1989, the biggest producers of 1,1,1-trichloroethane were the USA, followed by Japan, UK, Germany, France, Canada, and Brazil. All these countries, except Canada, reported continued production in 2004, albeit at much lower volumes. A similar decline was reported for the global consumption of 1,1,1-trichloroethane. The highest users of 1,1,1-trichloroethane in 1989 were the USA, Japan, European Community, Canada, Brazil, and Australia; most countries globally had zero or significantly reduced consumption levels by 2004 (UNEP, 2005).

Atmospheric measurements of 1,1,1-trichloroethane are a stable long-term indicator of its emissions, although localized small-scale emissions may be missed (Prinn et al., 2001). Various studies conducted in the USA (Millet & Goldstein, 2004), Europe (Krol et al., 2003), and globally (Prinn et al., 2001) reported an exponential decline in atmospheric levels of 1,1,1-trichloroethane from a peak in 1992 to 2000, when levels were below those of 1978, when measurements began (Prinn et al., 2001; Reimann et al., 2005). Fig. 1.1 illustrates the decline in global atmospheric levels of 1,1,1-trichloroethane (Prinn et al., 2018).

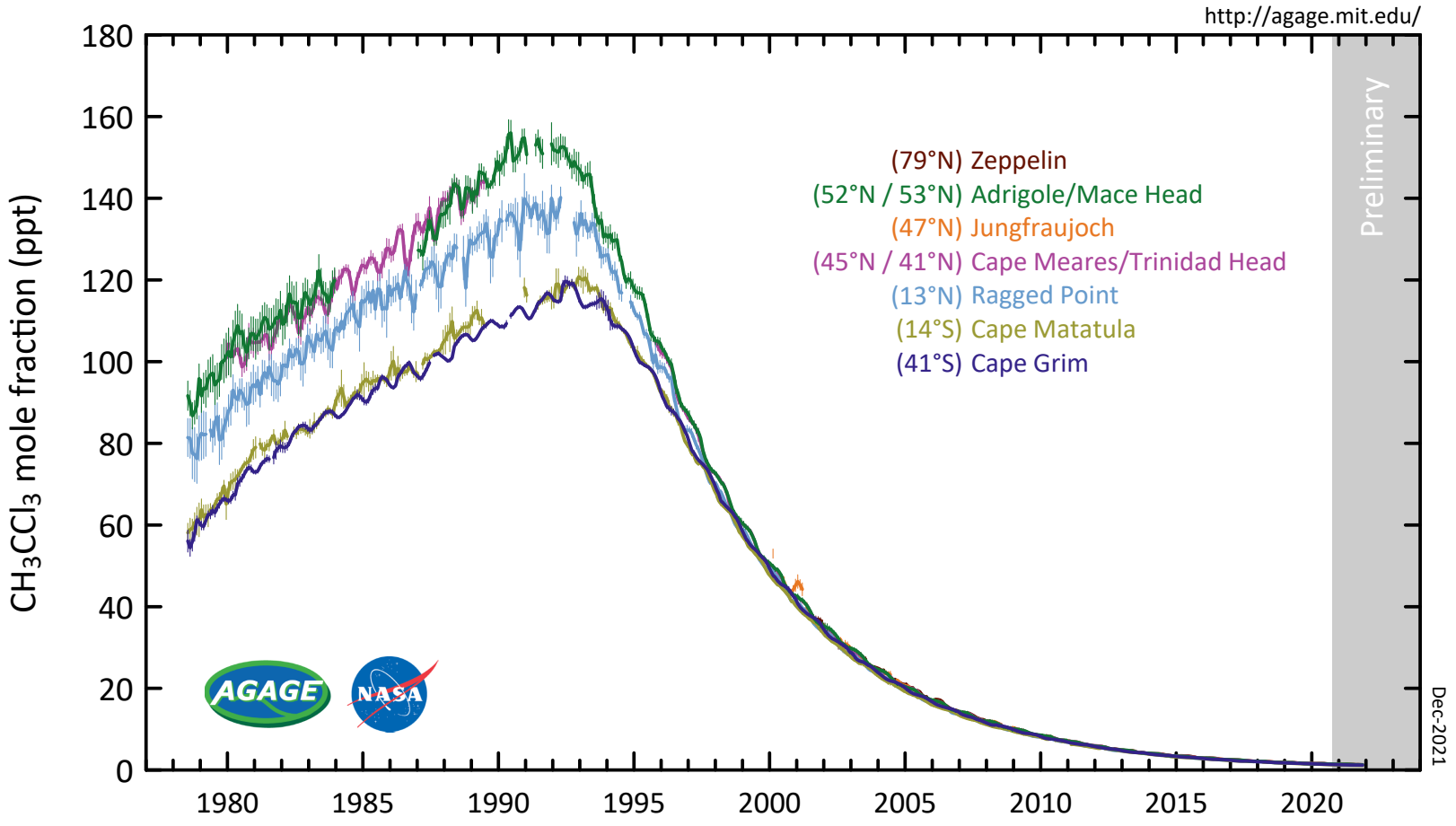
[The Working Group noted that, while continued localized and smaller-scale use of 1,1,1-trichloroethane may be occurring, the data in Fig. 1.1 serves as a useful indicator of the large reduction in production and use of this chemical worldwide.]

The Montreal Protocol resulted in a significant decline in production of 1,1,1-trichloroethane in developed countries in the 1990s. However, production of 1,1,1-trichloroethane for export to low- and middle-income countries (LMICs) that were signatories to the Montreal Protocol may have continued until 2012 (ATSDR, 2006). [The Working Group noted that, while the downward trend in the production of 1,1,1-trichloroethane is clear, reliable data on current production volumes, particularly in LMICs, were hard to identify.]

1.2.3 Uses

1,1,1-Trichloroethane was among the most widely used degreasing solvents in the USA in the 1970s and 1980s. Before the 1950s, 1,1,1-trichloroethane was reported to be a contaminant in the production of chlorinated hydrocarbons, as a rubber solvent, and in a list of dyes. 1,1,1-Trichloroethane was commercially applied in the 1950s–1960s as a cold-cleaning solvent for some metals and as an aerosol propellant for products, e.g. hair spray. In the 1970s, 1,1,1-trichloroethane was primarily used for cold cleaning, vapour degreasing, and ultrasonic cleaning of metal parts. Between 1975 and 1985, cold cleaning and vapour degreasing accounted for 63% of 1,1,1-trichloroethane produced in the USA, with the remainder spread over the manufacture of copolymers (20.5%), exports (11.8%), and miscellaneous purposes (5.1%) (Doherty, 2000). In 1995, the major use of 1,1,1-trichloroethane was as an intermediate in the production of hydrochlorofluorocarbons (~60%), followed by vapour degreasing and cold cleaning (25%), as a solvent for adhesives (5%), in coatings and inks (3%), textiles (2%), and in miscellaneous applications including electronics (5%) (ATSDR, 2006). In 1995, the Montreal Protocol banned all non-essential uses of 1,1,1-trichloroethane by 2002 (Marshall & Pottenger, 2016; UNEP, 2021) [the Working Group noted that, other than the use as an intermediate, most of the uses cited above (ATSDR, 2006) are probably nonessential uses]. Essential uses – defined by the Montreal Protocol

Fig. 1.1 Concentrations of 1,1,1-trichloroethane (CH_3CCl_3) in the lower atmosphere, as measured by the Advanced Global Atmospheric Gases Experiment at stations around the world, 1978–2020



Abundances are given as pollution-free monthly mean mole fractions in parts per trillion. The Advanced Global Atmospheric Gases Experiment (AGAGE) has been measuring the composition of the global atmosphere continuously since 1978.

as those "necessary for the health and safety or for the critical functioning of society" – such as for medical devices and aviation safety testing, may have continued (ATSDR, 2006). By the early 2000s, 1,1,1-trichloroethane was almost entirely used as a precursor for hydrofluorocarbons (ATSDR, 2006). Toxic release inventory (TRI) data from 2009 to 2020 show that 46.8% of toxic releases were reported by the hazardous-waste industry sectors and 49.1% by the chemical industry (US EPA, 2021c). Other nonessential uses of 1,1,1-trichloroethane may also have occurred after 2000 to consume stockpiles of the chemical accumulated earlier. In LMICs, some current use of 1,1,1-trichloroethane that would not be considered essential under the Montreal Protocol is still evident; for example, one online chemical supplier in India lists this chemical for use as a "fumigant herbicide" (Ottokemi, 2021). [The Working Group noted that, aside from the obvious reduction in the widespread use of 1,1,1-trichloroethane since its prohibition, reliable data on current use patterns, particularly in LMICs, were not available.]

1.3 Detection and quantification

1,1,1-Trichloroethane is quantified in air, water, soil, consumer products, and various biological samples (including breath, blood, and urine) by a variety of analytical methods that use chromatography for separation of the constituents plus various detectors (ATSDR, 2006). Representative methods in different matrices are summarized in Table 1.1.

1.3.1 Air

Several standard methods for workplace evaluations of 1,1,1-trichloroethane in air samples include sample collection on coconut shell/activated charcoal tube, or in an adsorption tube filled with Chromosorb 106, and analysis by gas chromatography with flame ionization detection (GC-FID) following National Institute for Occupational Safety and Health (NIOSH) Method 1003 or the German Deutsche Gesetzliche Unfallversicherung (DGUV) information 213-565 Method 02 or 03 (NIOSH, 2003; DGUV, 2017a, b). Another standard method includes the collection of 1,1,1-trichloroethane in evacuated stainless-steel canisters, followed by preconcentration, and separation and analysis by gas chromatography-mass spectrometry (GC-MS) according to United States Environmental Protection Agency (US EPA) Method TO-15A (US EPA, 2019). A variation of this method includes the collection and preconcentration of samples in a sorbent tube filled with activated charcoal and analysis by GC-MS (Russell & Shadoff, 1977).

1.3.2 Water

Water and wastewater samples are analysed by bubbling an inert gas through a sample to transfer the volatile sample components to a vapour phase, followed by trapping the purged vapour onto sorbent material, and finally desorbing and transferring the purgeables onto a gas chromatography (GC) column for separation. The sample can be quantified for 1,1,1-trichloroethane using a variety of detectors including electrolytic conductivity or microcoulometric detector using US EPA Method 601 (US EPA, 1994b) or mass spectrometry using US EPA Method 624 for wastewater samples (US EPA, 1984). Similarly, 1,1,1-trichloroethane in drinking-water samples is measured by purging and trapping the volatile sample components, then separating with GC-MS using US EPA Method 524.2 (US EPA, 1995; Zoccolillo et al., 2005). Groves et al. (2006) describe the development of a field-portable instrument for the quantification of 1,1,1-trichloroethane in drinking-water samples, based on measuring changes in the mass and viscoelastic properties of an array of polymer-coated surface-acoustic-wave microsensors,

Table 1.1 Representative methods for the detection and quantification of 1,1,1-trichloroethane and its metabolites (trichloroethanol and trichloroacetic acid) in various matrices

Sample matrix (method number)	Sample preparation	Analytical technique	LOD (unless otherwise stated)	Reference
Workplace air				
Air (NIOSH Method 1003)	Coconut shell charcoal tube and extraction in carbon disulfide; sample target volume 3 L	GC-FID	1.0 µg/sample	NIOSH (2003)
Air (German DGUV Information 213-565 Method 02)	Activated charcoal tube and extraction in carbon disulfide	GC-FID	0.6 ng/sample (LOQ) [0.18 ng/sample (LOD)]	DGUV (2017a)
Air (German DGUV Information 213-565 Method 03)	Sorbent tube with Chromosorb 106 followed by thermal desorption	GC-FID/MSD	0.85 µg/sample (LOQ)	DGUV (2017b)
Air (US EPA Method TO-15A)	Collection in evacuated stainless-steel canisters with flow controllers and preconcentration before injection	GC-MS	1 pptv	US EPA (2019)
Ambient air				
Air	Stainless steel tubes packed with Porapak N porous polymer and thermal desorption	GC-ECD/MS	NR	Russell & Shadoff (1977)
Water and wastewater				
Water (US EPA Method 601 for municipal and industrial discharges)	Purge and trap onto adsorbent followed by rapid heating	GC-ECD*	0.03 µg/L	US EPA (1994b)
Water (US EPA Method 624 for wastewater)	Purge and trap onto adsorbent followed by thermal desorption	GC-MS	3.8 µg/L	US EPA (1984)
Water (US EPA Method 524.2 for surface water, ground water, and drinking-water)	Purge and trap onto adsorbent followed by thermal desorption	GC-MS	0.08 µg/L	US EPA (1995)
Soil, sediment, consumer products				
Liquid or solid beverages, and grains	Extraction in isooctane	GC-ECD GC-HECD	3 ppb [µg/L] (LOQ) 7 ppb [µg/L] (LOQ)	Daft (1987)
Table-ready foods and various grains	Purged in 100 °C bath with nitrogen gas, collected on Tenax TA and XAD-4 resin trap, and eluted with hexane	GC-ECD/ HECD	0.3 ppb [µg/kg] (LOQ)	Heikes & Hopper (1986); Heikes (1987)
PVC containers used for food packaging, foodstuffs	PVC sample dissolved in *N*,*N*-dimethyl-formamide followed by headspace analysis	GC-MS (PVC) GC-ECD (foods)	1 ppm [mg/kg] (LOQ) 0.002–0.01 ppm [mg/kg]	Gilbert et al. (1978)
Pharmaceutical products	*N*,*N*-Dimethylformamide as dispersive and 1,2-dibromoethane as extraction solvents	GC-FID	0.05 µg/g in solid sample; 5 µg/L in solution	Farajzadeh et al. (2012)
	Dispersive liquid–liquid microextraction	GC-MS	1.5 µg/mL in solution	Heydari & Azizi (2015)

Table 1.1 (continued)

Sample matrix (method number)	Sample preparation	Analytical technique	LOD (unless otherwise stated)	Reference
Raw landfill leachates	HS-SPME	HS-SPME-GC-MS	0.05 ng/mL	Flórez Menéndez et al. (2004)
		HS-GC-MS	0.1 ng/mL	
Biological samples				
Exhaled air	Stainless-steel devise with charcoal cloth and desorbed in carbon disulfide	GC-FID	3.8 µg/mL (LOQ) [1.15 µg/mL (LOD)]	Glaser & Arnold (1989)
Blood and exhaled air, TCOH and TCA	Headspace analysis for blood Collection to Tedlar bag during 1 minute and direct injection of exhaled air sample	GC-ECD	0.06 mg/L TCOH in blood ; 0.03 mg/L TCA in blood; 0.01 µg/L TCOH in exhaled air	Monster & Boersma (1975)
Exhaled air	Collection of alveolar breath i.e. end-expired air into silanized glass tubes; direct injection	GC-ECD	0.08 ng/L	Stein et al. (1996)
Blood	Headspace analysis	GC-FID and ECD	0.1 mg/L	Ramsey & Flanagan (1982)
		GC-MS	0.1 mg/L (LOQ)	Dills et al. (1991)
Urine	Headspace analysis	GC-MSD	NR	Ghittori et al. (1987)
Urine and blood	Headspace analysis	GC-MSD	1 µg/L	Imbriani et al. (1988)
Blood and urine, TCA, TCOH, and trichloro-compounds	Multiple extraction steps	GC-ECD	< 1 mg/L (LOQ)	Ogata & Saeki (1974); Humbert & Fernández (1976)
Blood (1,1,1-trichloroethane) and urine (TCA)	Modified purge and trap with dynamic headspace analysis	GC-MS	0.8 µg/L (LOQ), blood 0.009 µg/mL (LOQ), TCA in urine	Johns et al. (2005)

DGUV, Deutsche Gesetzliche Unfallversicherung (German Social Accident Insurance); ECD, electron capture detection; ECD*, electrolytic conductivity detection; FID, flame ionization detector; GC, gas chromatography; HECD, Hall electroconductivity detector; HS, static headspace; LOD, limit of detection; LOQ, limit of quantification; MS, mass spectrometry; MSD, mass selective detector; NIOSH, National Institute for Occupational Safety and Health; NR, not reported; ppm, parts per million; ppt, parts per trillion; pptv, parts per trillion by volume; PVC, polyvinyl chloride; SPME, solid-phase microextraction; TCA, trichloroacetic acid; TCOH, trichloroethanol; US EPA, United States Environmental Protection Agency.

that occur when a substance of interest is absorbed.

1.3.3 Soil, sediment, consumer products, and food

Various methods for the detection and quantification of 1,1,1-trichloroethane in food products, soil, and various other media have been reported. Trace levels of 1,1,1-trichloroethane in beverages and grains treated with fumigants were measured by gas chromatography with electron capture detection and Hall electroconductivity detector (GC-ECD/HECD) in isooctane extract (Daft, 1987). GC-ECD/HECD was also used to quantify 1,1,1-trichloroethane in table-ready foods and various grains after purging samples in a 100 °C bath with nitrogen gas, collecting on a Tenax TA and XAD-4 resin trap, and eluting with hexane (Heikes & Hopper, 1986; Heikes, 1987). 1,1,1-Trichloroethane in polyvinyl chloride containers used in food packaging as well as in the food itself has been quantified using GC-MS (Gilbert et al., 1978). Dispersive liquid–liquid microextraction (DLLME) combined with GC-FID or GC-MS detection was used to quantify 1,1,1-trichloroethane and other residual solvents in pharmaceutical products (Farajzadeh et al., 2012; Heydari & Azizi, 2015). Two extraction and preconcentration procedures – static headspace and solid-phase microextraction – combined with GC-MS were used to quantify 1,1,1-trichloroethane in raw landfill leachates (Flórez Menéndez et al., 2004).

1.3.4 Biological specimens

1,1,1-Trichloroethane and its metabolites, trichloroethanol and trichloroacetic acid, have been quantified in blood, end-exhaled air (not trichloroacetic acid), and urine samples from exposed humans (Monster, 1986; ATSDR, 2006); this is described in detail in Section 4.1.

After inhalation, 1,1,1-trichloroethane is poorly metabolized, and a large fraction (up to 90%) of the absorbed dose is rapidly excreted unaltered in exhaled air (ATSDR, 2006) where it can be measured by methods based on GC-FID (Glaser & Arnold, 1989) or electron capture detection (ECD) (Monster & Boersma, 1975; Stein et al., 1996). Various combinations of sample collection and detection are used for the quantification of 1,1,1-trichloroethane in exhaled air samples (ATSDR, 2006). A direct reading method based on colorimetry has also been described (Droz et al., 1988). In general, unchanged 1,1,1-trichloroethane is measurable in blood and exhaled air within 5–15 minutes of exposure, whereas metabolites such as trichloroacetic acid are detected in the urine 64 hours after exposure (Monster, 1986; ATSDR, 2006). Real-time direct measurement of 1,1,1-trichloroethane in exhaled air was achieved in a laboratory study where the exhaled air was directly channelled from the participants's face mask through a glow discharge ionization source to an ion trap mass spectrometer for quantification (Giardino et al., 1999). The parent compound can also be analysed in blood via headspace analysis and detection using GC with both FID and ECD (Ramsey & Flanagan, 1982) or mass spectrometry (Dills et al., 1991). Urine samples have also been analysed using GC with mass selective detector (Ghittori et al., 1987; Imbriani et al., 1988). Additionally, the sum of the free and conjugated trichloroethanol (i.e. total trichloroethanol) in blood and urine have also been described as human biomarkers of exposure. A method that includes acidic hydrolysis for sample preparation and that is based on GC and ECD has been reported (Ogata & Saeki, 1974; Humbert & Fernández, 1976). A similar method has been used for trichloroacetic acid in urine, an additional human biomarker of 1,1,1-trichloroethane. Trichloroacetic acid in urine and 1,1,1-trichloroethane in blood have also been quantified using a headspace GC-MS method (Johns et al., 2005). [In contrast to total

trichloroethanol in blood and urine for which sampling time is critical for exposure assessment (end of shift at end of work week), timing (end of work week) is less critical for trichloroacetic acid in urine.]

Airborne exposure levels of 1,1,1-trichloroethane are shown to be well correlated with levels in exhaled air, blood, and urine (ACGIH, 2001; ATSDR, 2006). Therefore, 1,1,1-trichloroethane level in exhaled air, blood, and urine is the primary biomarker of exposure, with established biological limit values reported in Section 1.5.2 (ACGIH, 2001; DFG, 2020).

1.4 Occurrence and exposure

There are no natural sources of 1,1,1-trichloroethane. The main sources of emission into the environment are anthropogenic, from air emissions, release to surface water and soil, and leachates from landfills and wastewater during the production and use of industrial and consumer products. [The Working Group noted that most of the studies reviewed in this section evaluated exposures during the period of peak use and production of 1,1,1-trichloroethane (i.e. from the 1970s to the early 1990s). Few studies were identified that evaluated occurrence and exposure after the year 2000, when a decline in production and use occurred. Therefore, the Working Group does not expect the levels described to reflect current exposures (e.g. see Fig. 1.1 in Section 1.2 in relation to atmospheric emissions).]

1.4.1 Environmental occurrence

Once in the atmosphere, 1,1,1-trichloroethane is slowly eliminated through reaction with hydroxyl radicals, while an estimated 15% migrates to the stratosphere where it depletes ozone (ATSDR, 2006). Owing to its long half-life, 1,1,1-trichloroethane can migrate far from its original source, while its moderate solubility in water means that it evaporates from surface water and soil into the atmosphere, and easily leaches out of landfills and soil. Depending on the sample-collection location, 1,1,1-trichloroethane has been detected at varying levels in urban, rural, indoor and personal air; surface, ground, drinking-water and rainwater; soil and sediment; and waste (ATSDR, 2006).

(a) Air

The worldwide average atmospheric concentration of 1,1,1-trichloroethane increased from about 0.06 ppb [0.33 μg/m^3] in 1974 to about 0.15 ppb [0.83 μg/m^3] in 1991 and then declined rapidly thereafter as production and use declined (Midgley & McCulloch, 1995). In remote areas, 1,1,1-trichloroethane concentrations in the air increased during 1975–1980 from 87 to 156 ppt [0.48 to 0.87 μg/m^3] in the Pacific north-western region of the USA and from 45 to 102 ppt [0.25 to 0.57 μg/m^3] in Antarctica (Rasmussen et al., 1981).

1,1,1-Trichloroethane has been measured in air samples from all over the USA. 1,1,1-Trichloroethane concentrations were typically 0.1–1 ppb [0.55–5.55 μg/m^3] in urban areas and < 0.2 ppb [< 1.11 μg/m^3] in rural areas but could reach 1000 ppb [5.55 mg/m^3] in large urban areas and near waste sites (ATSDR, 2006). Urban 24-hour average air concentrations ranged from 0.13 to 28.4 ppb [0.72 to 158 μg/m^3] in 1987–1990 in various cities in California, USA (Hisham & Grosjean, 1991). Measurements collected at 20 landfill sites for non-hazardous municipal trash indicated 24-hour air concentrations of 1,1,1-trichloroethane as high as 3.6 ppm [20 mg/m^3] (Wood & Porter, 1987). Overnight indoor and outdoor concentrations of 1,1,1-trichloroethane measured between 1980 and 1984 during various seasons in residential areas at five geographical locations in the USA were variable, being influenced by numerous factors; estimated median and maximum indoor concentrations were 1.5–24 μg/m^3 and 14–880 μg/m^3, while estimated

median and maximum outdoor concentrations were 0.6–29 μg/m³ and 7.6–190 μg/m³, respectively (Pellizzari et al., 1986). A National Human Exposure Assessment Survey (NHEXAS) conducted in six midwestern states in the USA in 1995–1997 measured an average indoor concentration of 1,1,1-trichloroethane of 6.29 μg/m³, with a maximum of 186.4 μg/m³ (Bonanno et al., 2001).

Outside the USA, 1,1,1-trichloroethane was measured in the atmosphere in Italy in 1987–1989, with a median concentration of 3.72 μg/m³ in Turin (a city) and 1.48 μg/m³ in Cuorgnè (a rural site) (Gilli et al., 1992). Additionally, median concentrations of 1,1,1-trichloroethane in Turin were 9 and 2.67 μg/m³ indoors, 8.55 and 2.44 μg/m³ outdoors, and 12.1 and 3.03 μg/m³ in personal samples collected during winter and summer, respectively. A study conducted by an organochlorine-manufacturing company in the United Kingdom (UK) reported the highest concentration of 1,1,1-trichloroethane (16 ppb [89 μg/m³]) in the air near the manufacturing facility in Runcorn (an industrial town located between the cities of Liverpool and Manchester). Concentrations decreased as distance from the facility increased, from 6.2–11 ppb [34–61 μg/m³] in Runcorn Heath, < 0.1–6 ppb [< 0.56–33 μg/m³] in a Liverpool/Manchester suburban area, and to even lower levels further away (Pearson & McConnell, 1975). Atmospheric air samples collected from multiple urban and rural locations in western Europe in 1972–1976 indicated 1,1,1-trichloroethane concentrations ranging from 0.03 to 1.01 ppb [0.17–5.6 μg/m³] at rural locations in the UK, < 0.02–0.13 ppb [0.11–0.72 μg/m³] at urban locations in the Netherlands, not detected (ND) to 6.55 ppb [ND to 36.4 μg/m³] at urban locations in Germany, ND to 0.39 ppb [ND to 2.2 μg/m³] in Brussels, Belgium, and < 0.84–2.01 ppb [< 4.7–11.2 μg/m³] in Lyon, France (Correia et al., 1977). Average concentrations of 1,1,1-trichloroethane in the air in rural Hokkaido, Japan, in 1979–1980 ranged from 0.54 to 0.65 μg/m³ (WHO, 1992).

(b) Water

1,1,1-Trichloroethane has been measured in a variety of water sources, from surface water and groundwater to rain runoff at sites near sources of emission. Concentrations were < 1 ppb [< 1 μg/L] in surface water at a distance from emission sources such as industrial or waste sites, 0–18 ppb [0–18 μg/L] in groundwater samples, 0.01–3.5 ppb [0.01–3.5 μg/L] in drinking-water from surface water or groundwater, and up to 11 000 ppb [11 mg/L] in groundwater near or at sources of emission, as reported in numerous studies in cities throughout the USA (ATSDR, 2006). Between 1981 and 1983, 5000 samples of drinking-water were collected from 400 respondents in New Jersey, North Carolina, and North Dakota, USA. The mean and maximum concentrations of 1,1,1-trichloroethane ranged from 0.03 to 0.6 μg/m³ and from 0.05 to 5.3 μg/m³, respectively (Wallace et al., 1987b). 1,1,1-Trichloroethane concentrations in 945 samples collected from water supplies using groundwater sources across USA states in 1981–1982 ranged from non-quantifiable (< 0.2 μg/L) to 21 μg/L (Westrick et al., 1984). Drinking-water samples collected from 100 cities in Germany contained 1,1,1-trichloroethane at concentrations ranging from < 0.1 to 1.7 μg/L (Bauer, 1981; WHO, 1992). The combined concentration of 1,1,1-trichloroethane plus carbon tetrachloride was found to be 3 ppb [3 μg/L] in municipal surface-water supplies of the cities of Liverpool, Manchester, and Chester in the UK (Pearson & McConnell, 1975).

(c) Soil and sediment

Limited data have been reported on the contamination of soil with 1,1,1-trichloroethane, partly because 1,1,1-trichloroethane rapidly evaporates or leaches out. In the USA, grab samples taken from sludge at a solvent-recovery plant measured 1,1,1-trichloroethane concentrations

in the range of 23 000 to 120 000 ppb [µg/L]. 1,1,1-Trichloroethane concentrations averaged 0.4 ppb [µg/kg] in samples taken from river sediments passing through an industrial area in Japan and were non-detectable in samples taken from a river going through non-industrial areas. In the USA, 1,1,1-trichloroethane was found in nearly half of the hazardous waste sites that are on the National Priorities List, which is a list of sites with hazardous waste of serious concern and are targeted by the US EPA for clean-up (ATSDR, 2006).

1.4.2 Dietary exposure

1,1,1-Trichloroethane was measured in a variety of food products ranging from meats and dairy to cereals, baked products, nuts, fruit, and vegetables. Some of the highest concentrations were reported in seafood products such as clams and oysters, and some dairy products such as butter, ice cream, and cheese in samples obtained in the USA (ATSDR, 2006). 1,1,1-Trichloroethane was detected in 138 out of 231 food items tested from the market basket collection by the United States Food and Drug Administration (US FDA), and the levels in these food products were highly variable, with 3–35 ng/g [ppb] in cereals, 1–9 ng/g in raw, canned or cooked vegetables, 2–40 ng/g in baked goods, 10–228 ng/g in nuts/nut products, 1–520 ng/g in dairy products, 15 ng/g in a chocolate candy, 2–76 ng/g in meat dishes, 6 ng/g in one infant/toddler blend, 2–32 ng/g in raw, canned, or dried fruits, and 2–3 ng/g in clear beverages (Daft, 1988). A Canadian study reported 1,1,1-trichloroethane concentrations ranging from ND (limit of detection, approximately 0.01 µg/g for both detectors) to 0.39 µg/g (electron capture detector) or 0.47 µg/g (Coulson electrolytic conductivity detector) in several samples of breakfast cereal (Page & Charbonneau, 1977).

1.4.3 Consumer products

1,1,1-Trichloroethane was extensively used as a functional ingredient in many household products, including adhesives and adhesive cleaners, lubricants, general-purpose liquid cleaners and spray degreasers, various automotive products, oven cleaners, spot removers, shoe polish, glues, typewriter correction fluid, fabric finishes, and some fumigation products for grains (ATSDR, 2006). A survey of 1159 household products purchased in shops in six cities in the USA in the late 1980s measured 1,1,1-trichloroethane in 18.6% of the products, with average concentrations (w/w%) varying from 36.4% in automotive products, 30.2% in household cleaners and polishes, 12.7% in paint-related products, 66.5% in fabric- and leather-treatment products, 21.2% in cleaners for electronic equipment, 43.3% in oils, greases, and lubricants, 38.3% in adhesive-related products, and 57.1% in miscellaneous products (Sack et al., 1992). In the USA, average concentrations of 1,1,1-trichloroethane in emissions from household products and building material were 696 µg/m^3 for cleaning agents and pesticides, 4.9 µg/m^3 for painted sheetrock, 13 µg/m^3 from glued wallpaper, and 22 µg/m^3 from glued carpet; 1,1,1-trichloroethane was present in 8 of the 15 products tested (Wallace et al., 1987a). 1,1,1-Trichloroethane was also used as a solvent in some cosmetic products, such as aerosol hair-colour spray, a manicuring product, and a personal hygiene product (Hooker, 2008). A study on concentrations of volatile organic compounds in 666 sanitary products obtained from retail stores in the Republic of Korea reported that all measurements of 1,1,1-trichloroethane were below the lower limit of quantification [limit unspecified] (Kim et al., 2019). Some pharmaceutical products, such as aerosol drug products intended for inhalation, contained 1,1,1-trichloroethane, but these products were withdrawn from the market by the US FDA in 1973 at which time a new drug application was required for all

such products (US FDA, 1973). [The Working Group noted that, due to the adoption of the Montreal Protocol and subsequent drop in production and use of 1,1,1-trichloroethane, many of the abovementioned occurrences may no longer be applicable.]

1.4.4 Occupational exposure

[The Working Group noted that most of the studies reviewed in this section evaluated occupational exposures during the pre-Montreal Protocol era.]

The NIOSH National Occupational Exposure Survey (NOES) of 1981–1983 estimated that approximately 2 528 300 workers were potentially exposed to 1,1,1-trichloroethane in 42 broad industry activities in the USA (NIOSH, 1983). In 1982, 101 510 000 workers were employed in the USA (Silvestri et al., 1983); thus 2.5% of the working population of the USA in 1982 was potentially occupationally exposed to 1,1,1-trichloroethane. Qualitative information on jobs with the potential for occupational exposure to 1,1,1-trichloroethane was available from several epidemiological studies investigating health outcomes. Degreasing was the primary operation identified by several epidemiological studies (Anttila et al., 1995; Gold et al., 2011; Purdue et al., 2017; Callahan et al., 2018). Exposure in metal plating and coating work was also prevalent (Hadkhale et al., 2017; Talibov et al., 2017). Printing was identified in two case reports in Japan (Kubo et al., 2014a; Kumagai, 2014). 1,1,1-Trichloroethane was also noted as a component in cleaning fluids (Anttila et al., 1995; Zarchy, 1996) and glues (Anttila et al., 1995; Gold et al., 2011). Occupations reported in epidemiological studies as having exposure to 1,1,1-trichloroethane were airplane maintenance workers (Stewart et al., 1991; Gold et al., 2011); automobile workers (Gold et al., 2011); upholsterers; smelters; shoe lasters and sole fitters; machine and engine mechanics (Talibov et al., 2017); mechanics and repairmen; metal-machining occupations; occupations related to fabricating, assembling, installing and repair of electrical, electronic and related equipment; metal shaping and forming occupations, except machining; and occupations in the physical sciences (Christensen et al., 2013). [The Working Group noted that some of these occupations overlap.]

The potential for high exposure existed in 1,1,1-trichloroethane manufacture, industrial organic chemistry, and five broad industry-activity groups that used the largest amount of 1,1,1-trichloroethane for cleaning, including furniture and fixtures, fabricated metal products, electric and electronic equipment, transportation equipment, and miscellaneous manufacturing industries. Smaller amounts were used for cleaning in food and kindred products, primary metals, nonelectric machinery, instruments and clocks, and in non-manufacturing industries such as maintenance facilities (railroad, bus, aircraft and truck), automotive and electric-tool repair shops, automobile dealers, and service stations (US EPA, 1994a).

Exposure to 1,1,1-trichloroethane also occurred in industries where it was used as a raw material to manufacture paints and inks, aerosol products (e.g. hair sprays), adhesive products (e.g. holding adhesives), other chemical products (e.g. chlorofluorocarbons used as refrigerants), and textile products (e.g. spotting fluid) (US EPA, 1994a). The report noted that downstream application and use of these products could have caused exposures during, for example, the application of surface-coating products in the paper and paperboard industries, in wood and flatwood product plants, in printing and publishing facilities, and in the production of adhesives and sealants. Other end uses included use as a coolant and lubricant in cutting oils, a component in plastic film cleaners, and a carrier solvent for silicone paper coatings and protective coatings (US EPA, 1994a).

In these diverse workplaces, 1,1,1-trichloroethane is absorbed via all routes, but inhalation is the major route of exposure, while exposure via the skin contributes < 0.1% to the absorbed dose (Riihimäki & Pfäffli, 1978; ACGIH, 2001).

NIOSH conducted numerous workplace assessments for 1,1,1-trichloroethane through the Health Hazard Evaluation Program (HHE) and Industrywide Studies (IWS) in the USA (Hein et al., 2010). The 1441 measurements of exposure for 1,1,1-trichloroethane were compiled from 89 HHE reports, 9 IWS reports, and 2 studies published between 1970 and 1996. The assessments were conducted across a wide range of industries, and 1,1,1-trichloroethane exposures ranged from 0.0004 to 1500 ppm, [0.002 to 8300 mg/m^3] with a median concentration of 0.95 ppm [5.3 mg/m^3], and 2.1% of the measurements exceeded the threshold limit value (TLV) established by the American Conference of Governmental Industrial Hygienists (ACGIH) in 2001 (ACGIH, 2001). 1,1,1-Trichloroethane exposure summaries by industry activity group, obtained only from HHE reports (NIOSH, 2016) for which five or more personal samples were available for the industry activity group are reported in Table S1.2 (Annex 1, Supplementary material for 1,1,1-trichloroethane, Section 1, Exposure Characterization, available from: https://publications.iarc.fr/611). Exposures above 100 ppm [555 mg/m^3] occurred in many industries, including electrical parts, rubber products, glass products, iron and steel, plastic products, fabricated metals, books and binders, electronics, aircraft, printed material, and ship repair. Low exposures were measured for bituminous coal, textile, some plastics and paper and miscellaneous chemicals. [The Working Group noted that 1,1,1-trichloroethane exposure may not have been of interest in some of these investigations and was measured as part of a panel of analytes. Furthermore, the NIOSH HHEs can often identify emerging issues or trends in exposures, and the review by Hein et al. (2010) did not identify an HHE for 1,1,1-trichloroethane after 2000, probably because its use was restricted by the Montreal Protocol.]

Similar results were reported by ATSDR (2006) in a table summarizing 1,1,1-trichloroethane exposures, which identified high exposures in cleaning and degreasing of fabricated metals, manufacture of electronics components, mixing commercial resins, and spray painting and gluing. Published literature in 1973–1996 reported personal exposures to 1,1,1-trichloroethane in the range of 83 to 367 ppm [460 to 2950 mg/m^3] in a brake repair shop during simulation (Gitelman & Dement, 1996), 14 to 2490 mg/m^3 among degreasing workers (Tay et al., 1995), as high as 214 mg/m^3 in foam manufacturing (Boeniger, 1991), means of ND to 838 ppm [ND to 4650 mg/m^3] for various jobs in textile manufacturing (Kramer et al., 1978), and lower levels during a visit to dry cleaners (median, 675 µg/m^3), in the paper industry (range, ND to 4.5 µg/m^3), and working in a laboratory (median, 24–86 µg/m^3), albeit based on very few measurements (Wallace et al., 1989; Rosenberg et al., 1991). A more recent study in university students using solvents during printmaking quantified average personal exposure to 1,1,1-trichloroethane as 40.5 µg/m^3 (Ryan et al., 2002). In two national databases on occupational exposure in France and the USA, more than 95% of the available measurements for 1,1,1-trichloroethane were made before 2000. The few measurements made after 2000 were mostly non-detectable (USA) or corresponded to uses possibly deemed essential (i.e. manufacture of medical and dental instruments and supplies, France). [The Working Group noted that few workplace exposure data after the mid-1990s were available, and few other data were available from outside the USA. This is probably due to the restricted use of 1,1,1-trichloroethane since the adoption of the Montreal Protocol (see Section 1.2.2).]

An assessment of intensity of exposure to chlorinated solvents, including 1,1,1-trichloroethane, was conducted for several epidemiological studies (Neta et al., 2012; Ruder et al., 2013; Purdue et al., 2017; Callahan et al., 2018). Published measurement data (n = 947) were linked to a set of exposure determinants and applied in a regression model to identify significant exposure determinants (Hein et al., 2010). Significant determinants of 1,1,1-trichloroethane were: active application of energy to a solvent (e.g. stirring, mixing, and agitation) and aerosolization as the primary or secondary mechanisms of release; location (outdoors and outdoors/indoors versus only indoors); local exhaust ventilation (present and effective versus absent or present but ineffective); proximity (near and near/far versus far (≥ 0.9 m) only); and the presence of industrial mechanical dilution (versus not present).

Few studies have conducted biomonitoring of 1,1,1-trichloroethane or its urinary metabolites in the workplace. In a study on aircraft-maintenance workers at an Air Force base in the USA, 1,1,1-trichloroethane concentrations in air ranged from ND to 4.7 ppm [ND to 26.1 mg/m^3, the Working Groups noted that the definition of ND was not provided], 0.1 to 51.0 ppb [0.56 to 283 µg/m^3] in breath, and ND in blood, while levels of trichloroacetic acid in the urine were ND to 0.0024 mg/mL (Lemasters et al., 1999a). A study in workers in printing companies in Japan reported average air concentrations of 1,1,1-trichloroethane of 4.3, 24.6, and 53.4 ppm [23.9, 136.5, and 296.4 mg/m^3] at three plants, which corresponded to urinary concentrations of trichloroethanol of 1.2, 5.5, and 9.9 mg/L, trichloroacetic acid of 0.6, 2.4, and 3.6 mg/L, and total trichloro-compounds of 2.0, 8.2, and 13.9 mg/L, respectively (Seki et al., 1975). In Germany, the average blood concentration of 1,1,1-trichloroethane was 633 µg/L in 3 priming workers, whereas levels were undetectable in 4 other priming workers and 28 varnishing workers (Angerer & Wulf, 1985). [The Working Group noted that several additional occupational biomonitoring studies have been conducted with the objective of evaluating the relations between exposure to 1,1,1-trichloroethane and its biological markers in various media (e.g. Monster, 1986; Ghittori et al., 1987; Mizunuma et al., 1995). As such, these studies reported correlation or regression coefficients between various metrics and not summary values for 1,1,1-trichloroethane or its biological markers.]

1.4.5 Exposure of the general population

The general population was probably exposed to low levels of 1,1,1-trichloroethane between the 1970s and the 1990s because of the widespread use of 1,1,1-trichloroethane in a variety of consumer and household products, background concentrations in air, water, and food, and the potential for occupational exposure. Blood concentrations of 1,1,1-trichloroethane in a sample of the general public drawn from participants in the Third National Health and Nutrition Examination Survey (NHANES III) in 1988–1994 ranged from below the limit of detection (0.086 µg/L) to 14 mg/L, with a geometric mean of 0.16 µg/L (Wu et al., 2006); a different sample of non-occupationally exposed participants drawn from NHANES III had a mean of 0.34 µg/L and a median of 0.13 µg/L (Ashley et al., 1994). [The Working Group noted that the studies by Wu et al. (2006) and Ashley et al. (1994) drew different samples from the 1988–1994 NHANES III data and reported different summary metrics. Wu et al. (2006) reported a geometric mean, whereas Ashley et al. (1994) reported the mean and the median. In a lognormal distribution, the geometric mean is closer to the median, and both are lower than the mean.] As a result of prohibition of the production and use of 1,1,1-trichloroethane after the 1990s, exposure of the general public has diminished, as indicated by the NHANES survey results from 2003–2010 and 2011–2016, none of which detected 1,1,1-trichloroethane in blood

samples from participating adults (CDC, 2021a, b). Blood concentrations of 1,1,1-trichloroethane in children from two poor, minority neighbourhoods in Minneapolis, USA, in 2000–2001, were mostly below the limit of detection, with 0–2% being above the limit of detection for the four sampling campaigns, and a mean of 0.03 ng/mL (Sexton et al., 2005). [The Working Group did not identify data on exposure of the general population outside the USA.]

1.5 Regulations and guidelines

1.5.1 Exposure limits and guidelines

(a) Occupational exposure limits

Australia, Switzerland, and Turkey have established the same airborne exposure limits as the European Union and its Member States, that is, 555 mg/m^3 (100 ppm) for the 8-hour time-weighted average (TWA), and 1110 mg/m^3 (200 ppm) for 15-minute short-term measurements. Singapore and the provinces of Ontario and Quebec in Canada all use the same limits as the ACGIH TLV of 1910 mg/m^3 (350 ppm) for the 8-hour TWA, and 2460 mg/m^3 (450 ppm) for 15-minute short-term measurements. The Republic of Korea has an 8-hour TWA of 1900 mg/m^3 (350 ppm) and a short-term limit of 2450 mg/m^3 (450 ppm). In Denmark, Sweden, and Norway, 8-hour TWAs are 275, 300, and 270 mg/m^3, respectively (50 ppm), and short-term limits are 550 mg/m^3 (100 ppm) in Denmark and 1100 mg/m^3 (200 ppm) in Sweden, while none has been established in Norway (IFA, 2021b). In the USA, NIOSH has established an "immediately dangerous to life and health" limit of 700 ppm [3800 mg/m^3], and a 15-minute ceiling recommended exposure limit of 1900 mg/m^3 (350 ppm) based on data on acute toxicity by inhalation in humans (NIOSH, 2021). Table 1.3 summarizes the occupational exposure limits for 1,1,1-trichloroethane in selected countries (IFA, 2021b).

(b) Environmental exposure limits

WHO has calculated a health-based value of 2 mg/L drinking-water for 1,1,1-trichloroethane but did not consider it necessary to derive a formal guideline value for 1,1,1-trichloroethane in drinking-water (WHO, 2017). In the USA, a maximum concentration level of 0.2 mg/L was established for 1,1,1-trichloroethane in the public water supply by the US EPA in 1989 under the Safe Drinking-water Act, and the same limit was set by the US FDA for bottled drinking-water (Doherty, 2000; Hooker, 2008; US EPA, 2021a). The Agency for Toxic Substances and Disease Registry (ATSDR) has derived minimal risk levels (MRLs), which are the daily human exposures to 1,1,1-trichloroethane that are likely to be without an appreciable risk of adverse effects over specified time periods. The derived inhalation MRLs are 2 ppm [11.1 mg/m^3] for an acute exposure duration of less than 14 days and 0.7 ppm [3.9 mg/m^3] for an intermediate exposure duration of 15–364 days. The derived oral MRL is 20 mg/kg per day for an intermediate exposure duration of 15–364 days (ATSDR, 2006).

According to the harmonized classification and labelling system implemented in the European Union (Classification, Labelling and Packaging of Substances and Mixtures, Regulation (EC) No. 1272/2008), 1,1,1-trichloroethane has the following classification: acute toxicity, category 4; ozone, category 1 (ECHA, 2021). Employers are obliged under this regulation to minimize worker exposure to 1,1,1-trichloroethane and must arrange for medical surveillance of exposed workers (European Council, 1998).

1.5.2 Reference values for biological monitoring of exposure

The ACGIH has established various biological exposure indices (BEI) for 1,1,1-trichloroethane in different biological media (ACGIH, 2001). A BEI of 20 ppm [109 mg/m^3] was established

Table 1.3 Occupational exposure limits for 1,1,1-trichloroethane in various countries

Country	8-hour TWA (mg/m³)	Short-term (15 minutes) (mg/m³)	Reference
China	900	–	IFA (2021b)
Denmark	275	550	IFA (2021b)
European Union[a]	555	1110	IFA (2021b)
Germany	550	550	IFA (2021b)
Israel	1100	1910	IFA (2021b)
Japan	1100	–	IFA (2021b)
New Zealand	680	680	IFA (2021b)
Norway	270	–	IFA (2021b)
Poland	300	–	IFA (2021b)
Republic of Korea	1900	2450	IFA (2021b)
Sweden	300	1100	IFA (2021b)
United Kingdom	1110	2220	IFA (2021b)
USA – ACGIH[b]	1910	2460	ACGIH (2001)
USA – NIOSH	–	1910 (ceiling)	NIOSH (2021)
USA – OSHA	1900	–	NIOSH (2021)

ACGIH, American Conference of Governmental Industrial Hygienists; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; TWA, time-weighted average.

[a] The same occupational exposure limits are also required in Australia, Austria, Belgium, Finland, France, Hungary, Ireland, Italy, Latvia, the Netherlands, Romania, Spain, Switzerland, and Turkey.

[b] The same occupational exposure limits are also required in the provinces of Ontario and Quebec in Canada, and in Singapore.

for 1,1,1-trichloroethane in samples of exhaled air taken before the last shift of the work week (ACGIH, 2020). A BEI of 700 µg/L was established for 1,1,1-trichloroethane in urine samples taken at the end of the work shift after 2–3 days of exposure (ACGIH, 2020). The ACGIH does not have a BEI for 1,1,1-trichloroethane in blood. Previously, the ACGIH had established a BEI of 40 ppm [220 mg/ m³] for 1,1,1-trichloroethane in end-exhaled air collected before the last shift of the work week, 10 mg/L for trichloroacetic acid in urine collected at the end of the work week, 30 mg/L for total trichloroethanol in urine collected at end of shift at the end of the work week (ACGIH, 2001), and 1 mg/L for total trichloroethanol in blood collected at end of shift at the end of the work week (ACGIH, 2012). The German Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area of the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) has established a biological tolerance value (BAT) of 275 µg/L for 1,1,1-trichloroethane in blood taken at the beginning of shift after multiple work shifts of exposure (Bolt et al., 2018; DFG, 2020). [The Working Group noted that information on biological reference values outside of the USA and Germany was not available.]

1.6 Quality of exposure assessment in key epidemiological studies of cancer and mechanistic studies in humans

Two cohort studies, five nested case–control studies, 16 case–control studies, three case reports, and two mechanistic studies relevant to human cancer were available to the Working Group. Details on the selected domains of the exposure assessment review for these studies are

summarized in Table S1.4 and Table S1.5 (Annex 1, Supplementary material for 1,1,1-trichloroethane, Section 1, Exposure Characterization, available from: https://publications.iarc.fr/611).

1.6.1 *Exposure assessment methods in epidemiological studies of cancer and mechanistic studies in humans*

The exposure assessment methods employed by these studies are organized below by study design.

(a) Cohort studies

Anttila et al. (1995) compiled measurements of trichloroethylene in urine, and of perchloroethylene [tetrachloroethylene] and 1,1,1-trichloroethane in blood collected in 1975–1983 by the Finnish Institute of Occupational Health. The authors reported that sampling methods may have changed over time. The timing of sample collection was not specified. A single measurement was available for 61% of the cohort exposed to 1,1,1-trichloroethane, and the only exposure metric developed was "exposed".

The study by Radican et al. (2008) presented a cohort of 14 455 aircraft-maintenance workers in the USA who were exposed to a variety of solvents (including 1,1,1-trichloroethane) and other chemicals. Work histories and employer records, job descriptions, walk-through surveys, air monitoring results, and interviews of employees were compiled to create a job-exposure matrix (JEM) comprising job titles that linked to study participants in a "yes/no" exposure evaluation for 14 solvents, including 1,1,1-trichloroethane (Stewart et al., 1991). Relative exposure levels were estimated semiquantitatively for "mixed solvents" (including 1,1,1-trichloroethane).

(b) Case–control studies

Three primary groups provided most of the human cancer studies available for critical review by the Working Group: the Montreal studies, the United States National Cancer Institute (NCI), and groups using the Nordic Occupational Cancer Study (NOCCA) JEM/FINJEM (job-exposure matrix/Finnish job-exposure matrix). In these and the other case–control studies reviewed, work histories had generally been collected (by interview, and for all jobs or jobs held for ≥ 6 or ≥ 12 months) and included job title, type of employer, tasks, materials and chemicals used, and frequency (referred to below as "standard work histories"). Typically, experts (chemists or industrial hygienists) reviewed the published literature (but not participant-specific air measurements) to estimate categorical levels of exposure probability, duration, and intensity (referred to below as "standard exposure assessment methods"). Other solvent exposures (trichloroethylene, perchloroethylene [tetrachloroethylene], methylene chloride [dichloromethane], and less often, carbon tetrachloride and chloroform) were typically evaluated. Unless otherwise identified, the case–control studies reviewed here used these methods.

The Montreal studies (Infante-Rivard et al., 2005; Christensen et al., 2013; Vizcaya et al., 2013) collected standard work histories with specialized questionnaires for technical information (Gérin et al., 1985). All information provided by the study participant, accrued from other studies by these experts, and personal or consultants' knowledge was considered when assessing participant-specific categories of the experts' degree of confidence [the Working Group noted that confidence was the assessors' confidence that exposure had actually occurred (possible, probable, definite), which is similar to probability or prevalence in other studies described here] that exposure to 1,1,1-trichloroethane had occurred and the frequency of exposure. Concentration of the agent (low, medium, high) was referenced to benchmark occupations. The studies assessed exposures to multiple solvents.

Three case–control studies used data from the Surveillance, Epidemiology, and End Results

(SEER) programme of the NCI (NCI-SEER; Gold et al., 2011; Purdue et al., 2017; Callahan et al., 2018). The studies collected standard work histories but also administered 20–39 job-specific modules. Standard assessment methods were followed. Assessments were participant-specific, but all three studies developed task- and job-exposure matrices for imputing estimates when participant-specific information was not available. Categorical estimates of probability, frequency, and confidence were assessed for 1,1,1-trichloroethane. Experts also estimated determinants of exposure by combining 947 measurements of 1,1,1-trichloroethane from the literature (Hein et al., 2010) to develop intensity estimates for task- and job-exposure matrices comprising probability, intensity, frequency, and confidence to impute exposure metrics when participant-specific information was unavailable. Probability was defined in these three studies as the theoretical probability of exposure to the solvent. Dermal exposure was considered for all. Other chlorinated solvents were evaluated.

Five other case–control studies were available from the NCI, in addition to a study by NIOSH in which the same general methodologies (although less sophisticated) for the assessment of exposure to 1,1,1-trichloroethane were employed as in the NCI-SEER studies, i.e. standard work histories for Neta et al. (2012), Ruder et al. (2013), and Heineman et al. (1994). In Neta et al. (2012), additional information was collected from 64 job-specific interview modules. The study by Ruder et al. (2013), a case–control study carried out by NIOSH and the NCI, included exposure modules for "solvents, thinners, glues, inks, varnishes, stains or paint strippers", rather than job modules. The interview questionnaire used by Dosemeci et al. (1999) only collected information on tasks, task duration, and full-time/part-time status for the most recent and usual occupation and industry, although duration of employment was collected for 20 jobs of interest [the Working Group noted that jobs or exposures were not identified]. Standard assessment procedures were used by Neta et al. (2012) and Ruder et al. (2013), except that intensity was estimated using the methodology from Hein et al. (2010). Dosemeci et al. (1999) and Heineman et al. (1994) used JEMs that relied on standard information sources and exposure studies to develop an NCI-JEM for 1,1,1-trichloroethane by assigning categorical values for probability and intensity separately for jobs and for industry codes, which were then combined into a single estimate (Gomez et al., 1994) for probability and intensity. In the studies by Neta et al. (2012) and Ruder et al. (2013), exposure categories were assigned for probability, frequency, and confidence, and for continuous estimates of intensity. Other exposures evaluated by these four NCI studies included at least four other chlorinated solvents. Kernan et al. (1999) investigated exposure to 1,1,1-trichloroethane by coding jobs identified on death certificates from 24 states of the USA into broad categories of the 1980 USA census job codes. A JEM, for which the methods were not described, used categorical estimates of probability and intensity for several 11 specific chlorinated hydrocarbons, all chlorinated hydrocarbons, and all organic solvents combined.

Of the seven studies based in Nordic populations, five (Talibov et al., 2014, 2017, 2019; Hadkhale et al., 2017; Le Cornet et al., 2017) used self-reports to each participating country's 10-year census to obtain job titles resulting in multiple jobs over the censuses. [The Working Group noted that Talibov et al. (2014, 2017, 2019), and Hadkhale at al. (2017) are nested case–control studies from a larger cohort.] Pedersen et al. (2020) used a Danish register for the source of occupational histories. The jobs were coded to each country's standard occupational coding system. In the nested case–control study by Videnros et al. (2020), questionnaires were administered to the participants for the three latest occupations, collecting dates and tasks. Exposures for all seven studies were assigned to study participants

via NOCCA-JEM, which is based on FINJEM (Kauppinen et al., 2009, 2014). FINJEM used prevalence of jobs and measurement data from various Finnish databases to develop continuous estimates of prevalence and, for the exposed, the mean intensity of exposure to 1,1,1-trichloroethane (Kauppinen et al., 2014). Prevalence in these studies was defined as the percentage of people exposed in the job among those employed in the job. FINJEM information was reviewed by NOCCA-JEM experts in each of the countries and modified if the difference between the JEM and the study participant was likely to result in a substantial difference, on the basis of local expertise and country-specific data sets (Kauppinen et al., 2009). [The Working Group noted that no information was provided as to how this was done.] Five other chlorinated solvents were evaluated in each of the NOCCA-FINJEM studies, apart from Pedersen et al. (2020), in which the only other chlorinated solvent was trichloroethylene. Several studies examined other solvents (e.g. benzene) and non-solvent exposures.

The studies by Sciannameo et al. (2019) and Miligi et al. (2006) took place in Italy. No description was provided as to the "detailed" work histories collected in the former, whereas the latter used job- or industry-specific questionnaires. Sciannameo et al. (2019) used FINJEM; for Miligi et al. (2006), experts developed a JEM that served as a baseline to reduce differences between raters, but participant-specific estimates were assigned that incorporated categorical levels of probability and intensity of exposure to five chlorinated hydrocarbons, including 1,1,1-trichloroethane. Sciannameo et al. (2019) evaluated exposure to 29 agents previously classified by the *IARC Monographs* programme as *carcinogenic to humans* (Group 1) or *probably carcinogenic to humans* (Group 2A).

The exposure assessment in the Occupational Exposure and Brain Cancer (INTEROCC) study by McLean et al. (2014) collected standard work histories in seven countries (Australia, Canada, France, Germany, Israel, New Zealand, and the UK). An expert from each country coded the jobs to international occupation and industry coding systems using a guideline to increase consistency across the study sites (van Tongeren et al., 2013). FINJEM was modified by the exposure estimates used in the Montreal case–control studies to create an INTEROCC-JEM. The INTEROCC-JEM includes continuous estimates of probability of exposure to 1,1,1-trichloroethane. In this study, McLean et al. (2014) did not assign intensity values for participants with probability estimates of < 25%. Trichloroethylene, perchloroethylene [tetrachloroethylene], and methylene chloride [dichloromethane] were also evaluated in these studies, as were a limited number of other solvents.

(c) *Case studies*

Three case studies reported on individuals exposed to 1,1,1-trichloroethane. Zarchy (1996) described two cases in the USA in people with exposure from cleaning metal for 2–4 years for a frequency of 5–15 days per month. Kubo et al. (2014a, b) reported on 3 cases in Japan in people who worked in printing shops removing ink residues. Kumagai (2014) reported on a single case in Japan in a person who worked in a printing company from 1984 to 1995 and was exposed to 1,1,1-trichloroethane at an estimated concentration of 240 ppm [1330 mg/m^3].

(d) *Mechanistic studies*

Two studies were available on mechanistic evidence of end-points related to the key characteristics of carcinogens in humans.

The study by Muttray et al. (1999) used an exposure chamber and a crossover design. Controlled exposures were to 1,1,1-trichloroethane (purity, > 99%) at 200.4 ppm [1112 mg/m^3] and 22 ppm [122 mg/m^3] (as measured by a MIRAN infrared analyser) for 4 hours at two separate time-points, 1 week apart. No other exposures occurred at the time of the experiment.

The study by Lemasters et al. (1999b) on aircraft-maintenance workers in the USA comprised two substudies. The first substudy (an exposure assessment pilot) assessed 1,1,1-trichloroethane exposures in air, breath, blood, and urine samples, and investigated correlations between these exposure measurements. The second substudy used a prospective, repeated-measures design to investigate the genotoxic effects of exposure to selected chlorinated and aromatic solvents, including 1,1,1-trichloroethane. On the basis of the results of the pilot study, only breath samples and industrial hygiene samples were used in the genotoxicity study. Three air samples from 8-hour shifts were taken on 5 consecutive days for "total solvents", which included 1,1,1-trichloroethane, with participants' breath sampled at the end of the 3 days. The mean concentration of total solvents was < 6 ppm (ranging up to 106 ppm, n = 286) [the Working Group noted that no information was provided on 1,1,1-trichloroethane]. Other solvents present included under total solvents were methyl ethyl ketone, xylenes, toluene, and methylene chloride [dichloromethane].

1.6.2 Critical review of exposure assessment

(a) Studies of cancer in humans

(i) Cohort studies

Anttila et al. (1995) provided limited information on exposure with which to interpret the epidemiological results. Blood concentration of 1,1,1-trichloroethane reflects short-term exposure [the Working Group noted that 90–95% of 1,1,1-trichloroethane is eliminated from the blood within 50 hours (NCBI, 2021)]. It is not known how representative of actual exposures the blood levels were, either within or outside the 9 years of reported measurements, especially since major changes in exposure levels in industry were believed to have taken place during the measurement period (1970s to 1980s). The assessment only stated "exposed", with no indication of the exposure levels these workers had experienced, since only the annual means for the entire 1,1,1-trichloroethane-exposed cohort were reported. It was not known whether decreases in mean measurements reflected changes in air concentrations of 1,1,1-trichloroethane in the work environment in the same job, in different jobs, or in different people exposed at different times, or whether the variability observed was attributable to day-to-day variability. [The Working Group noted that making inferences about the relation between air concentrations and expected corresponding blood concentrations is challenging (see Section 1.3.4). Other carcinogens, particularly chlorinated solvents, may have been confounders, because at this time in Finland, the same primary industries used both 1,1,1-trichloroethane and trichloroethylene. A strength of this study was that those participants identified as "exposed" were truly exposed. The limitations were likely to result in attenuation of the disease risk estimate to the null, since only "exposed" participants, many of whom may have low exposures to 1,1,1-trichloroethane, were identified.]

In the cohort study by Radican et al. (2008), a variety of sources of detailed data (both qualitative and quantitative) were used to assess exposure to solvents and other hazards, but the linkage between study participant and exposure was weak. Exposure to 1,1,1-trichloroethane was limited to "exposed" and "unexposed", and to "all solvents" (including 1,1,1-trichloroethane), so the disease risk estimates may be attenuated to the null, since both the "exposed" and "all solvents" category may contain participants with very low exposures to 1,1,1-trichloroethane, or for the "all solvents", with no exposure. Finally, exposure was only assessed up to 1982.

(ii) Case–control studies

All the case–control studies on 1,1,1-trichloroethane generally had the same limitations. First, there may have been differential recall bias (cases reporting differently than controls),

although Vizcaya et al. (2013) found no difference in the number of jobs reported per participant or in the interviewers' subjective ratings of interview quality. Jobs and industries were typically coded according to standard coding systems, which may result in the grouping of heterogeneously exposed study participants. Estimates of intensity were affected by substantial measurement limitations: few measurements were available, particularly before the 1970s when they were often non-existent; it is likely that no measurements were made on the study participants; and most measurements available probably represent companies with higher and lower exposure, so it is not known how representative the measurement results are of the participants' actual exposures. Details were rarely provided on how exposures were assessed when measurements were not available, making it difficult to interpret results. Estimates of probability were affected by the limited availability of historical use patterns for 1,1,1-trichloroethane. There was often limited or a lack of information available on the frequency of exposed tasks, so that participants exposed at a lower frequency may have been included in a higher-than-appropriate exposure category. JEMs were often used. The major weakness of JEMs is that they assign the same value to all participants with a particular set of exposure determinants (such as job/industry), although there is often high variability within jobs. JEMs are generally weighted to male workers' exposures, which may over- or underestimate women's exposures, depending on the work setting. Also, most estimates relied heavily on the experts' experience and knowledge, with little factual data to support the assessment. Exposures were generally semiquantitative. Dermal exposure was often not considered. Exposures were often low, increasing the chance that a potential association could be missed. Also, chlorinated solvents have been used interchangeably over the years for many purposes (particularly degreasers and glues) in the workplace, which could have resulted in confounding; however, most of the studies did not adjust for exposure to other solvents. Correlation between exposures could thus have occurred, particularly between exposures to chlorinated solvents, either because the exposure assessor had coded a job as having some probability of exposure to several of these solvents or because of actual exposures experienced by the study participants. Table S1.6 identifies the correlations observed by the studies under review (see Annex 1, Supplementary material for 1,1,1-trichloroethane, Section 1, Exposure Characterization, available from: https://publications.iarc.fr/611).

Several of the studies included prevalence or confidence in the calculation of cumulative exposure. In cases where prevalence is multiplied by intensity to calculate a cumulative metric, bias has been found to be negligible when the prevalence of exposure in the studied population is either very low or very high (Burstyn et al., 2012). Moreover, although some of the limitations described above may be differential, measurement error generally results in non-differential misclassification (Armstrong, 1998). In general, then, the exposure assessment is likely to result in non-differential misclassification, which probably results in a decrease in calculated disease risk, although the exposure unit per outcome unit may be affected. Unless otherwise specified, generally the exposure assessment conducted in the case–control studies identified below is likely to attenuate disease risks to the null, with studies of lower quality probably having greater attenuation than those of higher quality.

Of the case–control studies on 1,1,1-trichloroethane, the Montreal studies (Infante-Rivard et al., 2005; Christensen et al., 2013; Vizcaya et al., 2013) were considered to have the highest quality of exposure assessment. The strength of these studies lies in the greater breadth of detailed information available from the study participants and from other sources compared with that in most of the other case–control

studies. The assessments were participant-specific estimates reached by consensus. Confidence (i.e. probability) was assessed in addition to frequency and intensity. Dermal exposure (yes/no) was considered. Although approximately 300 substances were evaluated, adjustment for possible confounding exposures was not performed. The exposure assessment was evaluated for reliability (Goldberg et al., 1986; Fritschi et al., 2003). A specific weakness of the study by Infante-Rivard et al. (2005), which assessed exposures in mothers of patients with childhood cancer, was that exposures were not evaluated in fathers, which could have confounded results.

The three case–control studies that used data from either the full or partial NCI-SEER data (i.e. Gold et al., 2011; Purdue et al., 2017; and Callahan et al., 2018) were also high-quality studies that developed participant-specific estimates, supported by job-specific modules and an extensive literature review. Experts were blinded to case status. The job- and task-exposure matrices were developed to impute missing data, which probably increased consistency in the evaluations. Exposure metrics were probability, frequency, duration, and confidence, which allowed exploration of multiple toxicity mechanisms.

A limitation of the studies based on FINJEM and NOCCA (NOCCA for Hadkhale et al., 2017; Talibov et al., 2014, 2017, 2019; NORD-TEST for Le Cornet et al., 2017) is that a brief job title with little detail was collected via the census once every 10 years, and there was no information on the date or duration of each job. Additionally, it was not possible to account for job changes after 1990 since this was the last year of job-code data linkage for the NOCCA cohort. Pedersen et al. (2020) contained slightly more information, noting registry-based jobs, but also industries, and dates. In contrast, Sciannameo et al. (2019) and Videnros et al. (2020) used a questionnaire to collect work-history information but used FINJEM for the exposure assessment. Videnros et al. (2020) additionally updated each prevalence value on the basis of questionnaire data. The emphasis in the development of FINJEM was on highly prevalent occupations with substantial exposure, resulting in lower confidence for jobs that are less prevalent or have a lower exposure (Kauppinen et al., 2014). Later studies (after 2009) and those using NOCCA-JEM (which is based on FINJEM) may have had different distributions of jobs, and these exposure estimates may have had greater misclassification than the earlier FINJEM studies. It is not clear whether exposure situations in Finland are comparable to those in the other Nordic countries of NOCCA-JEM. While FINJEM has been compared to other JEMs, it is difficult to properly interpret its agreement or disagreement with exposure estimates in other Nordic countries.

A strength of FINJEM is that a substantial body of information was used in its development. [The Working Group noted that FINJEM and NOCCA-JEM are well-developed and strong JEMs, but that the NOCCA and NORD-TEST jobs are a source of much uncertainty due to their being job titles with little detail that were collected only once per 10 years.] FINJEM requires a certain minimum level of exposure and excludes all exposures whose prevalence in an occupation is < 5%, increasing specificity. [The Working Group noted that although 5% appears to be low, only two other studies (McLean et al., 2014 and Pedersen et al., 2020) used higher values. Most studies did not indicate any exclusion in the exposure assessment, and it is difficult to imagine the exposure assessors doing so without actual prevalence figures.] The JEM considered the intermittency of exposure in an annual mean exposure [although the source of this information was not identified]. Arithmetic means of the measurement results were calculated as long-term (1-year average during working hours) concentrations. [The Working Group noted that this strength was diminished by the categorization of intensity.] For NOCCA-JEM, FINJEM was reviewed and modified by a team of Nordic

experts to be country-specific, but it typically relied on FINJEM unless available information supported a substantial change. FINJEM is likely to be one of the better JEMs because the exposure prevalence in an occupation was based on actual worker populations and workplace measurements. A particular strength of Pedersen et al. (2020) is the use of the minimum criteria for defining exposure of > 10% probability and at least 1 year of employment, thus increasing specificity.

Gold et al. (2011), Purdue et al. (2017), and Callahan et al. (2018) all made use of detailed and well-described expert assessment processes supported by full occupational histories, the use of job-specific modules, and JEMs to derive a series of participant-specific detailed exposure metrics of probability, intensity, frequency, confidence, and duration of exposure. The experts assessing the exposures were blinded to case status, which reduces the risk of differential bias across cases and controls. No direct exposure-monitoring data were available, but the exposure assessment process was carefully considered and semiquantitative in nature, which is a key strength in the absence of monitoring data.

The strengths and weaknesses of four other studies (Heineman et al., 1994; Dosemeci et al., 1999; Neta et al., 2012; Ruder et al., 2013) varied. The strengths and weaknesses of Neta et al. (2012) were similar to those of the SEER-based studies. Exposures may have been missed by Ruder et al. (2013) owing to the use of exposure modules, and certainly were missed by Dosemeci et al. (1999) owing to the incomplete nature of the occupational history and the inclusion of only the most recent and longest-held jobs. In addition, it was unclear whether the method used by Heineman et al. (1994) and Dosemeci et al. (1999) involving an algorithm based on separate semiquantitative estimates for job and for industry developed valid exposure estimates.

Kernan et al. (1999) was the weakest of the case–control studies in terms of quality of exposure assessment. Only one job was collected per participant, and no information was available on dates or duration of the job, making the validity of the assessments questionable since exposures levels changed over time and across industries even in the same job.

The study by Miligi et al. (2006) benefited from job-specific questionnaires. One weakness was that it was unclear whether jobs with a duration of ≥ 5 years were included in this assessment, as was the case for a previous analysis of the same data set by the same authors. The authors did not indicate a minimum job duration for inclusion in the exposure assessment, but if they repeated their earlier cut point of 5 years, this would have led to exposed people being erroneously included in the unexposed category if critical jobs with solvent exposure occurred for shorter durations. The experts categorized intensity of exposure on the basis of the presence of exposure controls presumed in place, which was probably highly variable across jobs, years, and industries, and which might limit the validity of the exposure assessment.

The INTEROCC case–control analysis across seven countries by McLean et al. (2014) used a specialized JEM based on FINJEM and data from the Montreal exposure-assessment team, which is expected to have improved the quality of the exposure assessment. It was unclear how differences across countries were considered and how workplace exposures compared between Finland (the baseline) and the other countries in the study. Exposure intensity was assigned to participants with ≥ 25% probability of exposure, increasing specificity.

(b) Mechanistic studies in humans

The study by Muttray et al. (1999) was appropriately designed. Exposure occurred only 20 minutes before the biological measures were taken, which may have been insufficient

for some markers. The analytical methods used were appropriate. It would have been informative to have included a time-point at which the participants were not exposed (0 ppm) to aid in interpretation of the effect of differences between exposure at 20 ppm [111 mg/m^3] and 200 ppm [1110 mg/m^3].

In the study by Lemasters et al. (1999b), genotoxicity end-points were assessed before beginning work and then at intervals of 15 and 30 weeks. The comparison group (controls) was unlikely to have had any significant exposure. The results are presented as aggregate "solvent" values by breath and industrial hygiene measurements, which hinders interpretation, particularly for 1,1,1-trichloroethane. The number of measurements of 1,1,1-trichloroethane was not stated, and it was not clear which analytical method was used to measure "total solvents". [The Working Group noted that the air and breath measurements of 1,1,1-trichloroethane made in the pilot study are of unknown significance to the genotoxicity study, since the exposure assessments were conducted separately over different time periods for different purposes. Owing to typical within-worker and between-worker variability in occupational exposure levels and the small number of workers included in the pilot study, no further inferences were made on the relevance of the pilot study to the genotoxicity study.]

2. Cancer in Humans

In this section, a review of the evidence from studies of cancer in humans exposed to 1,1,1-trichloroethane is presented. 1,1,1-Trichloroethane was previously considered in *IARC Monographs* Volumes 20 and 71 (IARC, 1979, 1999). For *IARC Monographs* Volume 20, no case reports or epidemiological studies were available to the Working Group. For *IARC Monographs* Volume 71, there was one cohort study in biologically monitored workers (Anttila et al., 1995), one population-based case–control study on astrocytic brain cancer (Heineman et al., 1994), and one hospital- and population-based case–control study on multiple cancer types (Siemiatycki, 1991). Results from several new studies have subsequently been published, including an updated analysis of Siemiatycki (1991) with a more refined exposure assessment, additional control for covariates, and more complete reporting (Christensen et al., 2013; Vizcaya et al., 2013).

The epidemiological database for this evaluation consisted of two cohort studies on biologically monitored workers in Finland (Anttila et al., 1995) and aircraft-maintenance workers in the USA (Radican et al., 2008), four large-scale case–control studies nested in the NOCCA study (Talibov et al., 2014, 2017, 2019; Hadkhale et al., 2017), one nested case–control study in a population-based cohort of Swedish women (Videnros et al., 2020), and sixteen largely population-based case–control studies conducted mainly in North America and Europe (Heineman et al., 1994; Dosemeci et al., 1999; Kernan et al., 1999; Infante-Rivard et al., 2005; Miligi et al., 2006; Gold et al., 2011; Neta et al., 2012; Christensen et al., 2013; Ruder et al., 2013; Vizcaya et al., 2013; McLean et al., 2014; Le Cornet et al., 2017; Purdue et al., 2017; Callahan et al., 2018; Sciannameo et al., 2019; Pedersen et al., 2020). There were also two case series described in multiple reports of biliary–pancreatic cancers in workers exposed to 1,1,1-trichloroethane in Japan and the USA. One of these concerned a cluster of cholangiocarcinoma cases, and the other reported on two cases of cholangiocarcinoma and ampullary carcinoma; they were included in the present review owing to the rarity of the outcomes (Zarchy, 1996; Kumagai et al., 2013, 2016; Kubo et al., 2014a, 2014b).

For this evaluation, the Working Group considered only studies that presented findings specifically for measured or estimated exposure to

1,1,1-trichloroethane. The quality of the exposure assessment was a critical consideration for the evaluation of the included studies and detailed critiques of each study are provided in Section 1.6. Although there were also other studies, including in specific occupational groups exposed to 1,1,1-trichloroethane, such as aircraft or electronics workers (Sung et al., 2007; Lipworth et al., 2011; DeBono et al., 2019a, b), or studies examining associations for grouped solvent exposures, including 1,1,1-trichloroethane combined with other solvents (Lee et al., 2002; Chang et al., 2003a, b, 2005; Dryver et al., 2004; Ojajärvi et al., 2007; Miligi et al., 2013; Silver et al., 2014; Olsson et al., 2018), such studies were considered by the Working Group to be uninformative and were excluded here since the independent contribution of 1,1,1-trichloroethane to any observed association with cancer was unclear. Also excluded here was an ecological study on drinking-water contamination (Cohn et al., 1994).

Where there were multiple publications derived from the same study, only the most relevant (i.e. longest follow-up, most detailed exposure assessment) was considered here (as such, Siemiatycki, 1991; Spirtas et al., 1991; Blair et al., 1998; and Videnros et al., 2019 were excluded). In one study, cumulative occupational exposures to 1,1,1-trichloroethane were estimated among study participants; however, owing to the very small number of exposed participants, associations with risk of glioma were not examined, and the study was not further considered here (Benke et al., 2017).

Identification of studies assessing cancer risk in humans exposed to 1,1,1-trichloroethane was initially performed through a comprehensive search of biomedical databases, using standard keyword searches of titles and abstracts as well as of MeSH terms (described in Section 6 of the *IARC Monographs* Preamble; IARC, 2019). After this, an expanded database search was conducted to identify studies for which the agent was not explicitly mentioned in the title or abstract, since in some studies 1,1,1-trichloroethane was examined along with multiple other occupational agents and not specifically mentioned in these search fields. The expanded search was performed both by including a broader range of search terms related to the chemical class, i.e. including expanded search terms for solvents, chlorinated solvents, chlorinated hydrocarbons, or aliphatic solvents or hydrocarbons, as well as additional synonyms for the agent not included in the initial search (e.g. methyl chloroform). Additionally, keyword searches were also performed (uniquely for the expanded search) in the full text of the manuscript in available databases, by searching beyond the title and abstract to further identify potentially relevant studies in which the agent name was mentioned only in the body of the manuscript. This expanded search resulted in an approximate doubling of the number of studies included in the evidence evaluation, but for most of these studies, 1,1,1-trichloroethane was not the main focus of the study.

Studies included in the evaluation assessed a range of cancer types, with the largest number of studies being on cancers of the haematopoietic and lymphoid tissues, followed by cancers of the genitourinary system, brain and nervous system, breast, and digestive tract; there were fewer studies on other cancer sites. Owing to the largely population-based nature of the available studies, the participants reported a wide range of occupations, although the prevalence of 1,1,1-trichloroethane exposure in most studies was generally low, and the intensity of exposure was probably also low. No cohort or case–control studies were found on environmental exposure to 1,1,1-trichloroethane and cancer.

2.1 Cancers of the haematopoietic and lymphoid tissues

2.1.1 Cohort studies

See Table 2.1.

The Working Group identified two cohort studies and two case–control studies nested within population-based cohorts in which the relation between occupational exposure to 1,1,1-trichloroethane and risk of cancers of the haematopoietic and lymphoid tissues had been investigated (Anttila et al., 1995; Radican et al., 2008; Talibov et al., 2014, 2017).

Anttila et al. (1995) conducted a retrospective cohort study in Finland that was constructed from a database of workers undergoing biological monitoring for occupational exposures to three chlorinated solvents. The cohort included 2050 male and 1924 female workers monitored by the Finnish Institute of Occupational Health via blood measurements of 1,1,1-trichloroethane (recorded between 1975 and 1983) and tetrachloroethylene (1974–1983) or urinary measurements of trichloroacetic acid, a metabolite of trichloroethylene (1965–1982). Approximately 94% of the workers were monitored for one solvent only; only for a small subset of the cohort (n = 271) were measurements available for 1,1,1-trichloroethane exposure. Mean age at the time of first measurement of 1,1,1-trichloroethane was 38.2 years and 39.9 years for men and women, respectively. Among those participants monitored for 1,1,1-trichloroethane, only one measurement was available for 61% and fewer than three measurements were available (average, 2.0 measurements per individual) for 79%. The workers were followed up for cancer incidence between 1967 and 1992 through linkage to the Finnish cancer registry; the mean duration of follow-up was 18 years. The observed incidence rates for exposed workers were compared with rates in the Finnish population categorized by sex, 5-year age group, and three calendar periods, using standardized incidence ratios (SIRs). The standardized incidence ratio for any lymphohaematopoietic malignancy among workers with a 1,1,1-trichloroethane measurement was 4.23 (95% CI, 0.87–12.3; 3 cases). An excess of multiple myeloma was also observed, although the confidence limits were wide (SIR, 15.98; 95% CI, 1.93–57.7; 2 cases). [The strengths of the study included documentation of workers' exposure to 1,1,1-trichloroethane through blood measurements and long-term follow-up for cancer incidence through linkage to a national registry. An important limitation was the small sample size of workers exposed to 1,1,1-trichloroethane, which limited power and precluded more detailed analyses across exposure levels. The quantitative exposure level for these cases in the biological samples was not considered in analyses.]

Radican et al. (2008) conducted the most recent update to a cohort study on cancer mortality in 10 730 male and 3725 female civilian aircraft-maintenance workers employed at a United States Air Force base for at least 1 year between 1952 and 1956. In this cohort update, mortality was followed-up between 1953 and 2000. By the end of follow-up, approximately 60% of cohort members had died, and the average age of survivors was 75 years (standard deviation, 7). A comprehensive assessment was undertaken to characterize various exposures and was informed by walk-through surveys of the base by an industrial hygienist, interviews with employees, review of historical facility records, position descriptions, and monitoring data providing exposure measurements. A JEM was developed primarily on job title and, where known, department, creating 43 000 job-department code combinations. The most detailed exposure assessment was conducted for trichloroethylene; only qualitative (ever/never) assessments were performed for exposure to 1,1,1-trichloroethane and 12 other solvents, including other chlorinated solvents (methylene chloride [dichloromethane], carbon tetrachloride, *O*-dichlorobenzene,

Table 2.1 Cohort studies on exposure to 1,1,1-trichloroethane and cancers of the haematopoietic and lymphoid tissues

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Anttila et al. (1995) Finland Enrolment, 1965–1983 (1,1,1-TCE: 1975–1983)/ follow-up, 1967–1992	3974 workers (2050 men and 1924 women), 271 of whom were monitored for exposure to 1,1,1-TCE; workers biologically monitored for occupational exposure to three halogenated hydrocarbon solvents in Finland Exposure assessment method: quantitative measurements; a database of measurements in urine from trichloroethylene-exposed participants, and blood from tetrachloroethylene- and 1,1,1-trichloroethane-exposed participants was used to identify ever exposed to the chemicals	Lymphatic and haematopoietic (ICD-7, codes 200–204), incidence	Compared with the general population (SIR): Any 1,1,1-TCE exposure	3	4.23 (0.87–12.3)	Age, sex, calendar period	*Exposure assessment critique*: Exposed were truly exposed. Blood levels only reflect short-term (days) exposures for 9 yr. No information was provided on the interpretation of the measurements or the participants' exposures, including possible exposures to 1,1,1-TCE outside the 1975–1983 window or to other agents. *Strengths*: documented exposure to 1,1,1-TCE via blood measurements; long-term follow-up for cancer incidence ascertained through linkage to national cancer registry. *Limitations*: small sample size; no assessment of exposure–response relations.
		NHL (ICD-7, codes 200 and 202), incidence	Compared with the general population (SIR): Any 1,1,1-TCE exposure	1	3.87 (0.10–21.5)		
		Multiple myeloma (ICD-7, code 203), incidence	Compared with the general population (SIR): Any 1,1,1-TCE exposure	2	15.98 (1.93–57.7)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Radican et al. (2008) Utah, USA Enrolment, 1952–1956/ follow-up, 1953–2000	14 455 (10 730 men and 3725 women); civilian workers employed at Hill Air Force Base, an aircraft-maintenance facility, for ≥ 1 yr between 1952 and 1956 who were followed up for cancer mortality through linkage to the national death index. Exposure assessment method: review of facility records, jobs, walk-through surveys, interviews, measurements used to assign yes/no exposed by job group	NHL, mortality	Exposure to 1,1,1-TCE, men (HR):			Age, race	*Exposure assessment critique*: Extensive data collection, including measurements. Linkage of jobs to exposures was limited due to the limited information in the available records. Given 1,1,1-TCE was often interchanged with other chlorinated solvents, the difficulty in making these links was a non-trivial limitation. Job information used to assign yes/no. *Strengths*: exposure assessment conducted by industrial hygienists with access to base facilities and records; long follow-up period; internal comparison group. *Limitations*: small number of deaths among exposed workers; qualitative exposure assessment; potential co-exposures to other organic solvents.
			No exposure to solvents or chemicals	NR	1		
			Ever	12	1.51 (0.61–3.73)		
		NHL, mortality	Exposure to 1,1,1-TCE, women (HR):				
			No exposure to solvents or chemicals	NR	1		
			Ever	0	–		
		Multiple myeloma, mortality	Exposure to 1,1,1-TCE, men (HR):				
			No exposure to solvents or chemicals	NR	1		
			Ever	4	0.64 (0.18–2.30)		
		Multiple myeloma, mortality	Exposure to 1,1,1-TCE, women (HR):				
			No exposure to solvents or chemicals	NR	1		
			Ever	3	14.46 (3.24–64.63)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Talibov et al. (2014) Sweden, Finland, Norway, Iceland 1961–2005	Cases: 14 982 incident cases of AML diagnosed between 1961–2005 and identified within NOCCA, a registry-based cohort study of Nordic country residents who participated in censuses in 1960, 1970, 1980/81, or 1990 and were followed up through linkage to national cancer registries Controls: 74 505; 5 controls per case randomly selected from NOCCA cohort members alive and free of AML on the case's date of diagnosis and further matched on year of birth, sex, and country. Exposure assessment method: records; used self-reported jobs to the census and NOCCA-JEM that includes semiquantitative estimates of prevalence exposed, mean level of exposure, and duration	AML, incidence	Cumulative exposure to 1,1,1-TCE (HR):			Age, year of birth, sex, country, aliphatic and alicyclic hydrocarbon solvents, benzene, toluene, trichloroethylene, methylene chloride [dichloromethane], perchloroethylene [tetrachloroethylene], other organic solvents, formaldehyde, ionizing radiation	*Exposure assessment critique*: NOCCA-JEM is a robust and well-developed JEM. NOCCA-JEM was normalized to the country. Intensity and prevalence estimates based on actual data. Could be missing exposed jobs due to 10 yr census collection. Prevalence was included in cumulative exposure but is not a component of toxicity. Other comments: conducted sensitivity analyses with 3, 5, 7, 10, and 20 yr exposure lags. Note: some of the reported exposure categories overlap. *Strengths*: very large study size; a detailed, time-specific, and quantitative JEM was applied; cancer diagnoses were ascertained through linkage to national cancer registries. *Limitations*: exposure estimates were based on census data on jobs, giving limited information on jobs held during the lifetime; no data on smoking habits were available
			No solvent exposures	NR	1		
			≤ 5.6 ppm-years	566	0.89 (0.76–1.04)		
			5.6–12.7 ppm-years	244	0.86 (0.71–1.05)		
			> 12.7 ppm-years	86	0.81 (0.61–1.08)		
			Trend-test *P* value, 0.58				

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Talibov et al. (2017) Sweden, Finland, Norway, Iceland 1961–2005	Cases: 20 615; incident cases of CLL diagnosed between 1961 and 2005 with no previous history of cancer, identified within NOCCA, a registry-based cohort study of Nordic country residents who participated in censuses in 1960, 1970, 1980/81 or 1990 and were followed up through linkage to national cancer registries Controls: 103 075; 5 controls per case, randomly selected from NOCCA cohort members alive and with no previous history of cancer as of the case's date of diagnosis and further matched on year of birth, sex, and country. Exposure assessment method: records; used self-reported jobs to the census and NOCCA-JEM that includes semiquantitative estimates of prevalence exposed, mean level of exposure, and duration	NHL (CLL), incidence	Cumulative exposure to 1,1,1-TCE, men (OR):			Age, year of birth, country, benzene, methylene chloride [dichloromethane], perchloroethylene [tetrachloroethylene], trichloroethylene, other organic solvents, formaldehyde, ionizing radiation	*Exposure assessment critique*: NOCCA-JEM is a robust and well-developed JEM. NOCCA-JEM was normalized to the country. Intensity and prevalence estimates based on actual data. Could be missing exposed jobs due to 10 yr census collection. Prevalence was included in cumulative exposure but is not a component of toxicity. Metrics were all categorical. Other comments: Conducted sensitivity analyses with 5, 10, and 20 yr exposure lags. Some of the reported exposure categories overlap. *Strengths*: very large study size; a detailed, time-specific, and quantitative JEM was applied; cancer diagnoses were ascertained through linkage to national cancer registries. *Limitations*: exposure estimates were based on census data on jobs, giving limited information on jobs held during the lifetime.
			No solvent exposures	NR	1		
			≤ 5.6 ppm-years	884	0.99 (0.86–1.13)		
			5.6–12.9 ppm-years	352	0.95 (0.81–1.12)		
			> 12.9 ppm-years	180	1.18 (0.95–1.45)		
			Trend-test *P* value, 0.39				
		NHL (CLL), incidence	Cumulative exposure to 1,1,1-TCE, women (OR):				
			No solvent exposures	NR	1		
			≤ 5.6 ppm-years	96	1.11 (0.76–1.62)		
			5.6–12.9 ppm-years	41	1.19 (0.73–1.96)		
			> 12.9 ppm-years	6	0.70 (0.28–1.75)		
			Trend-test *P* value, 0.19				

AML, acute myeloid leukaemia; CI, confidence interval; CLL, chronic lymphocytic leukaemia; HR, hazard ratio; ICD, International Classification of Diseases; JEM, job-exposure matrix; NHL, non-Hodgkin lymphoma; NOCCA, Nordic Occupational Cancer Study; NOCCA-JEM, Nordic Occupational Cancer Study job-exposure matrix; NR, not reported; OR, odds ratio; ppm, parts per million; SIR, standardized incidence ratio; 1,1,1-TCE, 1,1,1-trichloroethane; yr, year.

tetrachloroethylene, and chloroform). Correlations between solvent exposures were not reported. Using Cox regression models, hazard ratios were calculated to estimate cancer risks for exposed versus unexposed workers using attained age as the time scale and adjusting for race. The authors observed a statistically non-significant association between exposure to 1,1,1-trichloroethane and mortality from non-Hodgkin lymphoma (NHL) among male workers (hazard ratio, HR, 1.51; 95% CI, 0.61–3.73; 12 exposed cases). No deaths attributable to NHL among exposed women were observed. For multiple myeloma, an association with 1,1,1-trichloroethane with wide confidence limits was observed among women (HR, 14.46; 95% CI, 3.24–64.63; 3 exposed cases). No association was apparent for men (HR, 0.64; 95% CI, 0.18–2.30; 4 exposed cases). [The study had several strengths, including a long period of follow-up and the use of an internal comparison group of unexposed workers for the analysis, avoiding potential "healthy worker effect" bias from comparisons with the general population. The exposure assessment was performed by industrial hygienists with access to the workplace facilities and records. Limitations included the small number of mortality end-points among the exposed, the qualitative nature of the exposure assessment, the difficulty in linking participants to estimates often associated with no more detail than job title, the lack of continued exposure assessment after 1982, and the potential for confounding from co-exposure to other organic solvents.]

A case–control study nested within a registry-based study on cancer caused by occupational exposures in the Nordic countries, known as the Nordic Occupational Cancer Study (NOCCA), investigated the risk of acute myeloid leukaemia (AML) in relation to occupational exposure to 1,1,1-trichloroethane and other solvents (Talibov et al., 2014). NOCCA is a cohort study including 14.9 million persons from Denmark, Finland, Iceland, Norway, and Sweden who participated in one or more population censuses in 1960, 1970, 1980/1981, and/or 1990. For Sweden and Norway, data used were from censuses in 1960 and later, and for Finland from 1970 or later. For Iceland, data from the census in 1981 were used. Participants from Denmark were not included in the case–control study since individual-level records were not accessible. Cases of AML diagnosed between 1961 and 2005 in the cohort were identified from the cancer registries in the respective countries. Five controls per case were randomly sampled from cohort members who were alive and free of AML on the date of diagnosis of the case and were further matched on year of birth, sex, and country. A JEM (the NOCCA-JEM) assigned exposure estimates for six individual solvents and four solvent groups to more than 300 occupations across four time periods: 1945–1959, 1960–1974, 1975–1984, and 1985–1994. Exposure to 1,1,1-trichloroethane for the study participants was estimated by application of the JEM to the job titles in the available censuses, and lifetime cumulative exposure was calculated as the product of exposure prevalence, exposure intensity, and exposure duration, summed over job titles in the censuses from ages 20 to 65 years. Conditional logistic regression was applied, adjusting for exposure to solvents other than 1,1,1-trichloroethane, formaldehyde, and ionizing radiation.

There were 7751 cases of AML among men and 7231 among women. The risk of AML was not related to cumulative exposure to 1,1,1-trichloroethane, with hazard ratios below 1 observed for each category of cumulative exposure. [The Working Group noted that the study had a strength in the very large sample size but that the use of census data for occupational titles gave limited information on jobs held over the lifetime. For early periods of follow-up only one census may have been available, and there was a long period (from 1990 until end of follow-up in 2005) for which exposure was not known. The JEM was well developed, but JEMs have limited

ability to identify persons with low and high exposure within an occupation and this issue, with the limited information from the census jobs and lack of information on industry, contributed to misclassification of exposure.]

Talibov et al. (2017) reported a study on chronic lymphocytic leukaemia (CLL) and exposure to solvents conducted within the NOCCA cohort using a nested case–control design similar to that of Talibov et al. (2014). The same cohort was used and analysed with similar methods, here focusing on 20 615 cases of CLL diagnosed in 1961–2005, and 103 075 controls, selected as in Talibov et al. (2014). A small difference in the exposure assessment was that occupational titles from the census in 1990 were not used for Norway. Exposure to six specific solvents and two groups of solvents were assessed by the NOCCA-JEM. In addition to cumulative exposure, peak exposure and average exposure levels were also assessed. Conditional logistic regression was applied, adjusting for exposure to the other included solvents, formaldehyde, and ionizing radiation.

The odds ratio for CLL in relation to occupational exposure to 1,1,1-trichloroethane was close to unity in all three categories of cumulative exposure and there was no evidence of an exposure–response relation for men (*P* for trend, 0.39) or women (*P* for trend, 0.19). Sensitivity analyses incorporating lag time, peak exposure, or average exposure level in the model gave no further evidence of an association with exposure to 1,1,1-trichloroethane. [The Working Group noted that the study had a strength in its very large sample size, but that the use of census data for occupational titles gave limited information on jobs held over the lifetime. For early periods of follow-up, only one census may have been available, and there was a long period (from 1990 until the end of follow-up in 2005) for which exposure was not known. The JEM was well developed, but JEMs have limited ability to identify persons with low or high exposure within an occupation, and this issue, with the limited information from the census jobs and lack of information on industry, contributed to misclassification of exposure.]

2.1.2 Case–control studies

See Table 2.2.

The Working Group identified five case–control studies on the association between exposure to 1,1,1-trichloroethane and cancers of the haematopoietic and lymphoid tissues. All studies were population-based.

A case–control study investigating the association between childhood leukaemia and maternal exposure to organic solvents before and during pregnancy was performed in the province of Quebec, Canada (Infante-Rivard et al., 2005). Cases of acute lymphoblastic leukaemia (n = 790) were identified from hospitals with regional coverage. Children aged 0–9 years at diagnosis were included for the period 1980–1993, and children aged up to 14 years at diagnosis were included for 1994–2000. Controls (n = 790) individually matched on age and sex were identified from registers representing the population of the area. The response rate was high for cases (93.1%) and for controls (86.2%). The parents were contacted by telephone and a structured questionnaire was used to obtain occupational histories of the mothers from age 18 years up to birth of the child. For the 2 years before pregnancy and up to birth, a semi-structured questionnaire was used to investigate details of each occupation held, including job titles, industry type with address and location, and information about materials handled and produced, and the specific work environment. Job-specific questionnaires were used for certain occupations. Exposures to 21 specific solvents and 6 mixtures were assigned by a team of chemists and industrial hygienists using expert assessment methods. There were also questions about exposures to solvents during a hobby. Conditional logistic

Table 2.2 Case–control studies on exposure to 1,1,1-trichloroethane and cancers of the haematopoietic and lymphoid tissues

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Infante-Rivard et al. (2005) Province of Quebec, Canada 1980–2000	Cases: 790 cases of incident childhood ALL were identified from hospitals with regional coverage; mothers responded to the questionnaire Controls: 790 controls; mothers of children (matched on age and sex of the case and identified from registers representing the population of the area) responded to the questionnaire Exposure assessment method: expert judgement; full work histories, specialized questionnaires [presumed measurement data], and extensive review used to assign participant-specific semiquantitative estimates of confidence, frequency, and intensity for each job held	Childhood cancer (ALL), incidence	Maternal exposure to 1,1,1-TCE from 2 yr before pregnancy up to birth (OR):			Age and sex of the child, maternal age, and education	*Exposure assessment critique*: Substantial data available for assessment including [presumably] published measurement data. Evaluation was participant-specific. Careful consideration of each job held by each participant (i.e. confidence, frequency, and intensity) is a key strength. Cumulative exposure did not include intensity. Metrics were all categorical. *Strengths*: large study size and a very detailed and thorough process for exposure classification; high participation rate among cases and controls; cancer cases were ascertained through clinical diagnoses at hospitals. *Limitations*: very wide confidence intervals gave imprecise risk estimates.
			Never	NR	1		
			Ever	NR	7.55 (0.92–61.97)		
		Childhood cancer (ALL), incidence	Maternal exposure to 1,1,1-TCE during pregnancy (OR):				
			Never	NR	1		
			Ever	NR	4.07 (0.45–36.7)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Miligi et al. (2006) Italy, 8 areas 1991–1993	Cases: 1428 cases of NLL, 304 cases of HD; incident cases in people aged 20–74 yr identified from hospitals and pathology departments; cases of CLL were included among the NHL cases Controls: 1530 controls were selected randomly from population register, frequency-matched on age and sex Exposure assessment method: expert judgement; full work histories and job- or industry-specific questionnaires used to assign participant-specific semiquantitative estimates of probability and intensity of exposure for each job; cited Costantini et al. (2001), which indicated that only jobs held for ≥ 5 yr more than 5 yr before diagnosis were considered	NHL	1,1,1-TCE exposure intensity, 5 yr lag (OR): No exposure to any solvent Very low or low Medium or high Trend-test *P* value, 0.24	 820 15 5	 1 0.7 (0.3–1.3) 0.7 (0.2–2.2)	Age, sex, study area, education	*Exposure assessment critique*: Slightly more information was available for assessment because of the job- and industry-specific modules. Evaluation was participant-specific. Careful consideration of each job held by each participant (i.e. probability and intensity) is a key strength. [Assumed from Costantini et al., 2001)] that only jobs with ≥ 5 yr of employment more than 5 yr before diagnosis were considered which could have resulted in exposed participants being assigned to the unexposed group. Cumulative exposure was not evaluated. Metrics were all categorical. *Strengths*: high participation rate; detailed exposure assessment method harmonized between centres; cancer diagnoses ascertained through hospital and pathology departments with additional review of doubtful cases. *Limitations*: low numbers precluded analysis according to exposure duration and subtypes of NHL.

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Gold et al. (2011) USA, Seattle-Puget Sound region and Metropolitan Detroit 1 January 2000 to 31 March 2002	Cases: 180 incident cases of multiple myeloma identified from regional cancer registries Controls: 481 controls obtained from a parallel study on NHL from the population in same regions, obtained by random-digit dialling and from medical service files. Exposure assessment method: expert judgement; full work histories, job-specific modules, literature review, measurement data (for deterministic modelling of intensity) and [presumed] study-specific task- and job-exposure matrices (for imputation when participant-specific information was missing) used to assign participant-specific semiquantitative estimates of probability, frequency, and intensity for each job held for ≥ 1 yr	Multiple myeloma	Exposure to 1,1,1-TCE (OR):			Age, sex, race, education, study area	*Exposure assessment critique*: Substantial data available for assessment including published measurement data. Evaluation was participant-specific. Job- and task-specific matrices (when participant-specific information was missing) probably increased consistency. Careful consideration of each job held by each participant (i.e. probability, frequency, intensity, and confidence of exposure) is a key strength. Metrics were all categorical. *Strengths*: study size and detailed exposure assessments; cancer diagnoses ascertained through regional cancer registries and medical record review. *Limitations*: low participation rate among controls; potential for survival bias as 18% of eligible cases died before they could be contacted.
			Unexposed	144	1		
			Ever	36	1.8 (1.1–2.9)		
		Multiple myeloma	Duration of exposure to 1,1,1-TCE (OR):				
			Unexposed	144	1		
			1–3 yr	7	1.6 (0.6–4.3)		
			4–8 yr	11	2.3 (1.0–5.3)		
			9–21 yr	11	1.9 (0.8–4.5)		
			22–45 yr	7	1.3 (0.5–3.3)		
			Trend-test *P* value, 0.17				
		Multiple myeloma	Cumulative 1,1,1-TCE exposure index (OR):				
			Unexposed	144	1		
			1–53	7	1.7 (0.7–4.4)		
			54–605	10	2.2 (0.9–5.3)		
			606–3750	8	1.4 (0.5–3.4)		
			3751–57 000	11	1.9 (0.8–4.4)		
			Trend-test *P* value, 0.19				
		Multiple myeloma	Cumulative 1,1,1-TCE exposure index, 10 yr lag (OR):				
			Unexposed	147	1		
			1–49	7	1.8 (0.7–4.6)		
			50–342	7	1.5 (0.6–3.3)		
			343–2781	8	1.3 (0.5–3.3)		
			2782–49 500	11	1.8 (0.8–4.1)		
			Trend-test *P* value, 0.21				
		Multiple myeloma	Reanalysis with jobs assessed with low confidence considered unexposed: any exposure to 1,1,1-TCE (OR):				
			Unexposed	163	1		
			Ever	17	2.2 (1.1–4.4)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Gold et al. (2011) USA, Seattle-Puget Sound region and Metropolitan Detroit 1 January 2000 to 31 March 2002 (cont.)		Multiple myeloma	Reanalysis with jobs assessed with low confidence considered unexposed: duration of exposure to 1,1,1-TCE (OR):			Age, sex, race, education, study area	
			Unexposed	163	1		
			1–5 yr	5	1.8 (0.6–5.7)		
			6–16 yr	6	6.7 (1.5–29)		
			17–25 yr	4	1.6 (0.4–6.0)		
			26–45 yr	2	1.3 (0.2–7.4)		
			Trend-test *P* value, 0.27				
		Multiple myeloma	Reanalysis with jobs assessed with low confidence considered unexposed: cumulative 1,1,1-TCE exposure index (OR):				
			Unexposed	163	1		
			1–378	5	3.7 (1.0–13)		
			379–1938	2	1.1 (0.2–5.8)		
			1939–10 012	6	3.0 (0.9–10)		
			10 013–57 000	4	1.5 (0.4–5.8)		
			Trend-test *P* value, 0.33				
		Multiple myeloma	Reanalysis with jobs assessed with low confidence considered unexposed: cumulative 1,1,1-TCE exposure index, 10 yr lag (OR):				
			Unexposed	164	1		
			1–303	5	3.1 (0.9–11)		
			304–1690	0	–		
			1691–4500	5	2.3 (0.6–8.0)		
			4501–49 500	6	2.8 (0.8–9.9)		
			Trend-test *P* value, 0.07				

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Christensen et al. (2013) Montreal, Canada 1979–1985	Cases: 3730 cancer cases at 11 organ sites, including 215 NHL cases; male incident cases of histologically confirmed NHL from 18 large hospitals in Montreal metropolitan area, Canadian citizens aged 35–70 yr (median, 57 yr) Controls: 533 population controls, 2341 other cancer controls; population controls obtained randomly from population-based electoral lists, stratified by sex and age; other cancer controls from other participating cases Exposure assessment method: expert judgement; full work histories and specialized questionnaires, [presumed measurement data], and extensive review to assign participant-specific semiquantitative estimates of confidence, frequency and intensity for each job held	NHL (ICD-9, codes 200 and 202)	Any exposure to 1,1,1-TCE, 5 yr lag, men (OR):			Age, census tract median income, education, ethnicity, self/proxy, smoking (cigarette-years)	*Exposure assessment critique*: Substantial data available for assessment including [presumably] published measurement data. Evaluation was participant-specific. Careful consideration of each job held by each participant (i.e. confidence, frequency, and intensity) is a key strength. Cumulative exposure included confidence, which is not a component of toxicity. Metrics were all categorical. *Strengths*: very detailed process for exposure classification; ascertained histologically confirmed cancer diagnoses through hospitals. *Limitations*: very low number of exposed cases (n = 5).
			No chlorinated solvent exposure	155	1		
			Any	5	1.2 (0.4–4.0)		
		NHL (ICD-9, codes 200 and 202)	Substantial exposure to 1,1,1-TCE, 5 yr lag, men (OR)				
			No chlorinated solvent exposure	155	1		
			Substantial	2	0.8 (0.1–4.0)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size, description, exposure assessment method	Cancer type (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Callahan et al. (2018) USA (Iowa, Los Angeles, Seattle, Detroit) July 1998 to June 2000	Cases: 1189 incident cases of NHL identified from the NCI-SEER registry; response rate, 76%; cases of CLL were included among the NHL cases Controls: 982 controls, frequency-matched on age, sex, race, and area, were recruited via random-digit dialling for ages < 65 yr and from Medicare files for ages 65–74 yr; response rate, 52% Exposure assessment method: expert judgement; full work histories, job-specific modules, literature review, measurement data (for deterministic modelling of intensity), [presumed] study-specific task- and job- and task-specific matrices (for imputation when participant-specific information was missing) used to assign participant-specific semiquantitative estimates of probability, frequency, and intensity for each job held	NHL (ICD-O-3, codes 967–972) NHL (ICD-O-3, codes 967–972)	1,1,1-TCE exposure probability (OR): Unexposed < 50% ≥ 50% Duration of 1,1,1-TCE exposure at probability ≥ 50% (OR): Unexposed ≤ 312 h > 312 h Trend-test *P* value, 0.47	 619 551 14 619 2 11	 1 1.1 (0.9–1.3) 1.0 (0.4–2.1) 1 0.3 (0.1–1.6) 1.5 (0.6–4.3)	Age, sex, study area, race, education	*Exposure assessment critique*: Substantial data available for assessment including published measurement data. Evaluation was participant-specific. Deterministic modelling of intensity and job- and task-specific matrices (when participant-specific information was missing) probably increased consistency. Careful consideration of each job held by each participant (i.e. probability, frequency, intensity, and confidence of exposure) is a key strength. Published measurement data modelled to estimate intensity but was not used. Intensity not used in analyses. Metrics were all categorical. Other comments: conducted sensitivity analyses with 5 and 15 yr exposure lags *Strengths*: large study size; very detailed assessment of individual exposure; cancer diagnoses ascertained through regional cancer registries and medical record review. *Limitations*: a somewhat low response rate among controls.

ALL, acute lymphoblastic leukaemia; CI, confidence interval; CLL, chronic lymphocytic leukaemia/small lymphocytic lymphoma; HD, Hodgkin disease; ICD-9, International Classification of Diseases, 9th Revision; ICD-O-3, International Classification of Diseases for Oncology, 3rd Revision; NCI-SEER, United States National Cancer Institute-Surveillance, Epidemiology, and End Results Program; NHL, non-Hodgkin lymphoma; NR, not reported; OR, odds ratio; 1,1,1-TCE, 1,1,1-trichloroethane; yr, year.

regression was applied, adjusting for maternal age and education.

The exposure prevalence for specific solvents was not reported, with only the number of discordant exposure pairs provided. There were only eight discordant pairs for exposure to 1,1,1-trichloroethane from 2 years before pregnancy up to birth, and five discordant pairs for exposure during pregnancy. For the 2-year period before pregnancy and up to birth, the odds ratio associated with exposure to 1,1,1-trichloroethane was 7.55 (95% CI, 0.92–61.97). For exposures during pregnancy, the odds ratio was 4.07 (95% CI, 0.45–36.7). [The Working Group noted that there was a detailed exposure assessment process but very imprecise risk estimates, and it was not possible to examine exposure–response associations, which limited the informativeness of this study.]

The association between occupational exposure to organic solvents and risk of NHL and Hodgkin disease was investigated in a population-based case–control study in Italy (Miligi et al., 2006). Incident cases in people aged 20–74 years were identified between 1991 and 1993 from hospitals and pathology departments in eight study areas where manufacturing industries using solvents were prevalent. Controls were selected randomly from population registers in the same areas, frequency-matched on age and sex. Cases of CLL were included among the NHL cases since NHL and CLL were considered to represent the same disease entity. Occupational histories were obtained by interviews primarily carried out at the home of the study participants. A small proportion of interviews were performed via proxies. The response rate was 83% among NHL cases, 88% among cases of Hodgkin disease, and 73% among controls. Job-specific questionnaires were used, and exposure to specific solvents and groups of solvents were coded blindly by expert judgement. A JEM was developed to aid in harmonizing assessments between centres. Probability (low/medium/high) and intensity (very low/low/medium/high) of exposure were coded for eight specific solvents and five groups of solvents. Logistic regression models were applied adjusting for sex, age, area, and education, and using participants not exposed to any solvent as referents. For each agent, analyses were based on participants with a medium or high probability of exposure, while those assigned a low probability were excluded.

There were 1428 cases of NHL (including CLL), 304 cases of Hodgkin disease, and 1530 controls included in the final data set. There was a relatively low prevalence of exposure to 1,1,1-trichloroethane (20 cases of NHL and 32 controls were exposed), and odds ratios for NHL were below 1 regardless of exposure intensity. Analyses across categories of exposure duration and for individual NHL subtypes gave very low numbers and odds ratios were not estimated. The risk of Hodgkin disease in relation to exposure to 1,1,1-trichloroethane was not reported because numbers of cases were small. [The Working Group noted that there was a high participation rate and a detailed exposure assessment procedure. The classification of exposure as having had at least 5 years of employment more than 5 years before diagnosis may have reduced study informativeness, as those with a shorter exposure duration were included in the unexposed group.]

Gold et al. (2011) conducted a case–control study on the association between six chlorinated solvents and the risk of multiple myeloma. The study was based on cases and controls from two urban areas in the USA: the Seattle-Puget Sound region of Washington State and the Detroit metropolitan area of Michigan. The study included 180 incident cases (55% men), aged 35–74 years, diagnosed between 1 January 2000 and 31 March 2002, identified from regional cancer registries. Controls from a parallel study on NHL in the same areas (Chatterjee et al., 2004) were used as controls in the present study. Controls under age 65 years were recruited by random-digit dialling, and controls aged 65–74 years were identified

from medical service files. In total, 481 controls were included. The response rate among cases that were alive, could be located, and confirmed to be eligible was 71%. The response rate among controls used for this study was 52%. An occupational history (from 1941 for cases and 1946 for controls) was obtained by personal interviews, including description and main duties for each job held for at least 1 year. Job-specific questionnaires were used for 20 occupations involving potential exposure to solvents. Exposures were assessed by an occupational epidemiologist and reviewed by an industrial hygienist. Exposure probability, frequency, intensity, and confidence were assessed for each of six chlorinated solvents. Cumulative exposure was calculated as the product of intensity, frequency, and duration summed over jobs with a probability category of 2 or higher (i.e. participants with an exposure probability of 10% or more in the occupation) in the work history. Individuals with a probability of exposure of ≥ 10% to 1,1,1-trichloroethane included 36 cases (20%) and 65 controls (14%). Unconditional logistic regression was applied, adjusting for age, sex, race, education, and study area, and using those unexposed to the respective solvents as referents.

Ever versus never exposure to 1,1,1-trichloroethane was associated with an increased risk of multiple myeloma (OR, 1.8; 95% CI, 1.1–2.9), and this association remained in a sensitivity analysis reassigning jobs with low confidence in the assessment to the unexposed category. In analyses across categories of exposure duration, cumulative exposure, and 10-year lagged cumulative exposure, odds ratios were above unity but with no indication of an exposure–response trend. The risk was systematically higher in all categories of exposure versus the unexposed, but with an absence of trend with increasing exposure to any of the exposure metrics. Trend tests results gave $P = 0.17$ for duration, $P = 0.19$ for cumulative exposure, and $P = 0.21$ for cumulative exposure lagged 10 years. Similar findings were obtained in a sensitivity analysis reassigning jobs with low confidence to the unexposed category. [The Working Group noted that there was a detailed exposure assessment procedure and that ever exposure to 1,1,1-trichloroethane was associated with a significantly increased risk of multiple myeloma. However, there was no exposure–response trend in terms of exposure duration, cumulative exposure, or 10-year lagged cumulative exposure. A lower participation rate among controls than among cases may have introduced bias. It was noted but not considered to be an important limitation that work histories for controls did not cover the period 1941–1946 (as it did for cases), since exposure to 1,1,1-trichloroethane was not common at that time.]

A case–control study on a large set of cancers was carried out in Montreal, Canada. Detailed data on methods and basic results were published earlier by Siemiatycki (1991). Findings regarding 11 selected cancers in relation to exposure to chlorinated solvents were investigated by Christensen et al. (2013). The study was based on incident cases of cancer among male Canadian citizens aged 35–70 years identified from the 18 largest hospitals in the Montreal area from 1979 to 1985. Population controls were selected randomly among men from electoral lists, frequency matched on age. The present report concerned 11 specific cancers sites, among them 215 cases of NHL (ICD-9, codes 200 and 202). The response rate among all cancer cases was 82%, but the response rate among NHL cases was not reported. There were 533 population controls (response rate, 72%). For certain analyses, cases of cancer at other organ sites than the one under study were used as controls (cancer controls) and were combined (weighted equally) with the population controls. Study participants were interviewed regarding demographic and lifestyle factors according to a structured questionnaire. For occupational history, a semi-structured questionnaire was used that included detailed

questions on job tasks, company, and workplace characteristics. Job-specific questionnaires were used for certain jobs. Exposures were assessed from the questionnaires by a team of chemists and industrial hygienists. For each job, the team coded confidence, frequency, and relative level of concentration of the exposure. Exposure was coded for two groups of chlorinated solvents and six specific chlorinated solvents, including 1,1,1-trichloroethane. Exposures occurring in the past 5 years were excluded owing to latency considerations. Unconditional logistic regression was applied, and adjusted for age, median income in neighbourhood of residence, education, ethnicity, self versus proxy respondent, and tobacco smoking (cigarette-years). Persons never exposed to chlorinated solvents were used as the referent category.

Exposure to 1,1,1-trichloroethane was relatively rare, with 1.9% of the population controls having been exposed. There were 5 cases of NHL in people who had been exposed to 1,1,1-trichloroethane. Using general population controls, no statistically significant elevated odds ratios were observed, either in those with any exposure to 1,1,1-trichloroethane (OR, 1.2; 95% CI, 0.4–4.0; 5 cases) or in those with substantial exposure to 1,1,1-trichloroethane (OR, 0.8; 95% CI, 0.1–4.0; 2 cases). Findings were similar using the general population and cancer controls combined. [The Working Group noted that there was a detailed process for exposure assessment but that the very low number of exposed cases of NHL limited the precision in risk estimates. In addition, intensity and/or cumulative exposure metrics were not specifically evaluated.]

The relation between cancer and occupational exposure to chlorinated organic solvents was investigated in the NCI-SEER study, a population-based case–control study performed in the USA (Callahan et al., 2018). This study investigated the risk of NHL in relation to exposure to 1,1,1-trichloroethane and four other specific chlorinated organic solvents. The study was based on data from four regions: the state of Iowa, Los Angeles county, and the metropolitan areas of Seattle and Detroit. Incident cases of NHL (ICD-O-3, codes 967–972) in people aged 20–74 years were identified between July 1998 and June 2000. Controls, frequency matched on age, sex, race, and area, were recruited via random-digit dialling for ages under 65 years and from Medicare files for ages 65–74 years. Among participants who could be traced, the response rate was 76% for cases and 52% for controls. Participants were interviewed in their homes using computer-aided questionnaires. Background data, occupational history, and various details about the work environment were recorded for every occupation held for 6 months or longer. Thirty-two job- or industry-specific modules were used to identify details regarding exposure to organic solvents, including type of solvent used, frequency and time spent on solvent-related tasks, work practices, and use of personal protective equipment. An industrial hygienist classified exposure to five specific chlorinated organic solvents by first developing JEMs specific for jobs and tasks for each of the five substances. The hygienist then used these matrices in addition to participant-specific work task information to assess the probability, frequency, and intensity of exposure. Levels of confidence were assessed for all estimates. Assessments were combined into metrics of duration, cumulative hours, and weekly average of exposure levels for each of the substances. Unconditional logistic regression was applied adjusting for age, sex, study area, race, and education.

The study showed no evidence of an association between exposure to 1,1,1-trichloroethane and NHL when investigating risk in relation to exposure probability (< 50% or ≥ 50%) or cumulative hours of exposure (≤ 312 hours and > 312 hours). There was evidence for an association between NHL and exposure to carbon tetrachloride. [The Working Group noted that there was a detailed exposure classification process but a low response rate, especially among controls.

The number of cases with a high probability of exposure to 1,1,1-trichloroethane was low.]

2.2 Cancers of the brain and nervous system

See Table 2.3.

The Working Group identified four case–control studies and one cohort study investigating the risk of cancer of the brain and nervous system associated with exposure to 1,1,1-trichloroethane. Two of the case–control studies were population-based, one was hospital-based, and one was a multicentre study.

A population-based case–control study on mortality from astrocytic brain cancer in White men was performed in three areas of the USA where exposure to organic solvents was prevalent in petroleum-refining and chemical-manufacturing industries (Heineman et al., 1994). The study included deaths from astrocytic brain cancer in southern Louisiana from 1 January 1978 to 30 June 1980, and in northern New Jersey and Philadelphia from 1 January 1979 to 31 December 1981. Controls were selected randomly among White male residents deceased from causes other than brain cancer, cerebrovascular disease, epilepsy, suicide, and homicide, and frequency-matched on age, year of death, and study area. The next of kin of cases and controls were interviewed regarding occupational history, including data on job titles, tasks, company, industry type, and products. Of the 741 cases and 741 controls, next of kin could be traced for 88% of the cases and 83% of the controls. Of these next of kin, 74% provided complete interviews for cases and 63% for controls. After exclusion of non-astrocytic tumours, the final data set comprised 300 cases. Of the 386 controls with completed interviews, 320 remained after exclusion of deaths from lung cancer, liver cancer, leukaemia, Hodgkin disease, NHL, and cirrhosis of the liver. Exposure to six specific chlorinated organic solvents, including methyl chloroform [1,1,1-trichloroethane], was assessed by a set of JEMs, specific to a level of intensity and probability of exposure for time periods where exposure had been deemed to occur for each job title and industry (Gomez et al., 1994). The matrices were applied to the job histories by an algorithm that considered whether the job or the industry was the primary generator of exposure to incorporate the estimates into a single cumulative exposure estimate. Three semiquantitative exposure metrics were derived: exposure duration, cumulative exposure score, and average intensity of exposure. Adjusted logistic regression was applied in a stratified analysis using maximum likelihood estimates, with those unexposed to the specific substance as referents. Trends in the odds ratio over strata of exposure were evaluated by the Mantel method.

The risk of death from astrocytoma was evaluated through analysis of risk in relation to a large number of combinations of exposure probability (low/medium/high), intensity (low–medium or high), duration (2–20 or ≥ 21 years) and cumulative exposure score (low/medium/high). Probability of exposure to 1,1,1-trichloroethane was assessed as low for most of the cases and controls. Little indication of an association with exposure to 1,1,1-trichloroethane was found. There was no consistent evidence of increasing risk with exposure probability or cumulative exposure; however, risk increased with exposure duration (all probabilities combined) when compared with the unexposed (OR for 2–20 years, 1.1; 95% CI, 0.7–1.7; OR for ≥ 21 years, 1.8; 95% CI, 1.0–3.3; *P* for trend, < 0.05). There were some indications of a trend with exposure intensity in those exposed for ≥ 21 years: OR for low and medium intensity, 1.6 (95% CI, 0.9–3.1); OR for high intensity, 3.7 (95% CI, 0.7–27.9); *P* for trend, < 0.05. The risk associated with the individual chlorinated solvents with simultaneous adjustment for the other solvents in the study was investigated, but 1,1,1-trichloroethane was not

Table 2.3 Cohort and case–control studies on exposure to 1,1,1-trichloroethane and cancers of the brain and nervous system

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Heineman et al. (1994) USA, 3 areas 1978–1980 (area I) or 1979–1981 (area II+III) Case–control	Cases: 300 men; deaths from astrocytic brain cancer initially identified from death certificates and confirmed by hospital diagnoses Controls: 320 men; deaths other than brain cancer and excluding deaths from cerebrovascular disease, epilepsy, suicide, homicide, selected cancers (lung, liver, leukaemia, HD, NHL) and cirrhosis of the liver and frequency-matched to cases on age, year of death, and study area. Exposure assessment method: questionnaire; full work histories taken by proxy and expert JEM used; estimated (semiquantitative estimates) intensity and probability by assigning probability and intensity separately to each job and to each industry and then combining them using an algorithm	Brain (astrocytoma), mortality	Duration of exposure to methyl chloroform [1,1,1-TCE], all probabilities (OR):			Age, year of death, study area	*Exposure assessment critique*: Evaluated several metrics. Unclear whether exposure assessment method produced valid results. Jobs limited by proxy reporting of full job history, which may miss key exposures. Metrics were all categorical. Other comments: conducted sensitivity analyses with 10 and 20 yr exposure lags. *Strengths*: detailed work histories and detailed assessments of individual exposure using a set of JEMs developed for this study. *Limitations*: job histories from next of kin; exposure metrics were semiquantitative.
			Unexposed	188	1		
			2–20 yr	63	1.1 (0.7–1.7)		
			≥ 21 yr	38	1.8 (1.0–3.3)		
			Trend-test *P* value, < 0.05				
		Brain (astrocytoma), mortality	Cumulative methyl chloroform [1,1,1-TCE] exposure score, all probabilities (OR):				
			Unexposed	188	1		
			Low score	34	1.0 (0.6–1.8)		
			Medium score	47	1.6 (1.0–2.7)		
			High score	20	1.3 (0.6–2.6)		
			Trend-test *P* value, > 0.05				
		Brain (astrocytoma), mortality	Average intensity of methyl chloroform [1,1,1-TCE] exposure, exposure duration 2–20 yr (OR):				
			Unexposed	188	1		
			Low and medium intensity	54	1.1 (0.7–1.8)		
			High intensity	9	0.9 (0.3–2.6)		
			Trend-test *P* value, > 0.05				
		Brain (astrocytoma), mortality	Average intensity of methyl chloroform [1,1,1-TCE] exposure, exposure duration ≥ 21 yr (OR):				
			Unexposed	188	1		
			Low and medium intensity	32	1.6 (0.9–3.1)		
			High intensity	6	3.7 (0.7–27.9)		
			Trend-test *P* value, < 0.05				

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Heineman et al. (1994) USA, 3 areas 1978–1980 (area I) or 1979–1981 (area II+III) Case–control (cont.)		Brain (astrocytoma), mortality	Methyl chloroform [1,1,1-TCE] exposure probability (OR): Unexposed Low probability Medium probability High probability Trend-test *P* value, > 0.05	 188 97 11 4	 1 1.2 (0.8–1.7) 2.2 (0.7–7.6) 1.2 (0.2–7.3)	Age, year of death, study area	

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Anttila et al. (1995) Finland Enrolment, 1965–1983 (1,1,1-TCE: 1975–1983)/ follow-up, 1967–1992 Cohort	3974 workers (2050 men and 1924 women), 271 of whom were monitored for exposure to 1,1,1-TCE; workers biologically monitored for occupational exposure to three halogenated hydrocarbon solvents in Finland Exposure assessment method: quantitative measurements; a database of measurements in urine from trichloroethylene-, and blood from tetrachloroethylene- and 1,1,1-TCE-exposed participants was used to identify ever-exposed to the chemicals	[Brain and] nervous system (ICD-7, code 193), incidence	Compared with the general population (SIR):			Age, sex, calendar period	*Exposure assessment critique*: Exposed were truly exposed. Blood levels only reflect short-term (days) exposures for 9 yr. No information was provided on the interpretation of the measurements or the participants' exposures, including possible exposure to 1,1,1-TCE outside the 1975–1983 window or to other agents. *Strengths*: exposure assessment was based on biological monitoring of exposure; long-term follow-up for cancer incidence ascertained through linkage to national cancer registry. *Limitations*: findings for brain cancer were based on only 3 cases; the quantitative exposure for these cases was not reported.
			Any 1,1,1-TCE exposure	3	6.05 (1.25–17.7)		

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Neta et al. (2012) USA, three hospitals 1994–1998 Case–control	Cases: 484 gliomas, 197 meningiomas; identified from referrals, diagnoses verified by microscopy Controls: 797; controls were selected among patients referred for non-malignant conditions: injuries, cardiovascular diseases, musculoskeletal conditions, digestive disorders, and other diagnoses, and frequency-matched to cases on sex, age class, race, hospital, and proximity to the hospital Exposure assessment method: questionnaire; full work histories, job-specific modules, literature review, measurement data, and [presumed] study-specific task- and JEM (for imputation when participant-specific information was missing) used to assign participant-specific semiquantitative estimates of probability, frequency, intensity and confidence for each job	Brain (glioma; ICD-O-2, codes 9380–9473), incidence	Probability of exposure to 1,1,1-TCE (OR):			Sex, age, race, hospital, proximity to hospital	*Exposure assessment critique*: Substantial data available for assessment, including published measurement data. Evaluation was participant-specific. Deterministic modelling of intensity and job- and task-specific matrices (when participant-specific information was missing) probably increased consistency. Careful consideration of each job held by each participant (i.e. probability, frequency, intensity, and confidence of exposure) is a key strength. Metrics were all categorical. Other comments: conducted sensitivity analyses with 10 yr exposure lag. *Strengths*: detailed exposure assessment procedure; cancer diagnoses ascertained through hospitals. *Limitations*: use of hospital-based controls may attenuate observed risks.
			Unexposed	334	1		
			Possible	140	0.8 (0.6–1.0)		
			Probable	10	1.0 (0.4–2.4)		
		Brain (glioma), incidence	Years of probable exposure to 1,1,1-TCE (OR):				
			Unexposed	334	1		
			Low	5	1.0 (0.3–3.4)		
			High	5	0.8 (0.2–2.7)		
			Trend-test *P* value, 0.76				
		Brain (glioma), incidence	Cumulative probable exposure to 1,1,1-TCE (OR):				
			Unexposed	334	1		
			Low	6	1.1 (0.3–3.5)		
			High	4	0.7 (0.2–2.6)		
			Trend-test *P* value, 0.70				
		Brain (glioma), incidence	Average weekly probable 1,1,1-TCE exposure (OR):				
			Unexposed	334	1		
			Low	6	1.0 (0.3–3.3)		
			High	4	0.8 (0.2–2.8)		
			Trend-test *P* value, 0.76				
		Brain (glioma), incidence	Highest probable 1,1,1-TCE exposure (OR):				
			Unexposed	334	1		
			Low	5	0.9 (0.3–3.1)		
			High	5	0.9 (0.3–3.0)		
			Trend-test *P* value, 0.8				
		Brain (meningioma, ICD-O-2, codes 9530–9538), incidence	Probability of exposure to 1,1,1-TCE (OR):				
			Unexposed	146	1		
			Possible	46	0.8 (0.5–1.2)		
			Probable	5	2.3 (0.7–7.2)		

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Ruder et al. (2013) Non-metropolitan areas of Iowa, Michigan, Minnesota and Wisconsin, USA 1995–1997 Case–control	Cases: 457 men and 341 women; cases of histologically verified glioma were identified from participating medical facilities and neurosurgeon offices Controls: 648 men and 527 women; 2 controls per case, frequency-matched on sex and age, were selected from driving license registers (for ages < 65 yr) and from Medicare data tapes (ages 65–80 yr) Exposure assessment method: questionnaire; full work histories, exposure modules, literature review and measurement data for modelling of intensity used to assign participant-specific semiquantitative estimates of probability, frequency, and confidence for each job held	Brain (glioma), incidence Brain (glioma), incidence Brain (glioma), incidence Brain (glioma), incidence	Any exposure to 1,1,1-TCE (OR): Never Ever Any exposure to 1,1,1-TCE, men (OR): Never Ever Any exposure to 1,1,1-TCE, women (OR): Never Ever Natural logarithm of cumulative 1,1,1-TCE exposure (ppm): Per 1-unit increase	 494 304 243 214 251 90 304	 1 0.75 (0.61–0.90) 1 0.83 (0.64–1.06) 1 0.64 (0.47–0.88) 0.97 (0.96–0.99)	Age, sex, education	*Exposure assessment critique*: Substantial data available for assessment available, including published measurement data. Evaluation was participant-specific. Deterministic modelling of intensity and job- and task-specific matrices (when participant-specific information was missing) probably increased consistency. Careful consideration of each job held by each participant (i.e. probability, frequency, intensity, and confidence of exposure) is a key strength. Metrics were all categorical. *Strengths*: detailed exposure assessment; histologically confirmed cancer diagnoses were ascertained through medical facilities. *Limitations*: uniformly, and largely statistically significantly, low risks in association with all six studied solvents raises the question of bias.

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McLean et al. (2014) Australia, Canada, France, Germany, Israel, New Zealand, UK 2000–2004 Case–control	Cases: 1906 incident cases of meningioma in ages 30–59 yr (age range varied between centres) Controls: 5565 controls were randomly selected from the population in each centre, individually or frequency-matched to the cases on year of birth, sex, and study region Exposure assessment method: questionnaire; full work histories were used and coded using ISCO and ISIC and a coding guideline to help with consistency across study site along with a study-specific JEM (INTEROCC-JEM) that was based on FINJEM plus modification from Montreal data to assign prevalence and intensity for all jobs held for ≥ 6 months	Brain (meningioma), incidence	Ever exposed (probability ≥ 25%) to 1,1,1-TCE, 5 yr lag (OR): Never Ever	 1811 1	 1 1.35 (0.10–17.55)	Age, sex, region, education	*Exposure assessment critique*: Stronger study than many of the other FINJEM/NOCCA-JEM studies because work histories were self-reports from interviews that gathered more information than job only. FINJEM is a robust and well-developed JEM. FINJEM was normalized to the country. Intensity and prevalence estimates based on actual data. Definition of cumulative was unclear but may include prevalence, which is not a component of toxicity. The JEM was modified with Montreal data but unclear how. Differences across countries were taken into account during the exposure assessment, but details on this were not provided. Metrics were all categorical. Other comments: conducted sensitivity analyses with 1 and 10 yr exposure lags.

Table 2.3 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McLean et al. (2014) Australia, Canada, France, Germany, Israel, New Zealand, UK 2000–2004 Case–control (cont.)							*Strengths*: large multicentre study; ascertained histologically confirmed or diagnostically unequivocal cancer diagnoses. *Limitations*: the method for exposure classification was not sensitive enough to identify persons with exposure to 1,1,1-TCE

CI, confidence interval; FINJEM, Finnish Job Exposure Matrix; HD, Hodgkin disease; ICD, International Classification of Diseases; ICD-O, International Classification of Diseases for Oncology; INTEROCC, Occupational Exposures and Brain Cancer study; ISCO, International Standard Classification of Occupations; ISIC, International Standard Industrial Classification; JEM, job-exposure matrix; NHL, non-Hodgkin lymphoma; NOCCA, Nordic Occupational Cancer Study; NOCCA-JEM, Nordic Occupational Cancer Study job-exposure matrix; OR, odds ratio; SIR, standardized incidence ratio; 1,1,1-TCE, 1,1,1-trichloroethane; UK, United Kingdom; yr, year.

included in this analysis since the evidence for a primary effect of this substance was assessed as weak. [The Working Group noted that there were some weak indications of an increased risk of mortality from brain astrocytoma associated with exposure to 1,1,1-trichloroethane, but the study did not evaluate whether this could be caused by concurrent exposure to other chlorinated organic solvents. Work histories from next of kin and the use of an imprecise exposure assessment algorithm reflected limited consideration of temporal trends in use when estimating probability and intensity of exposure. Additionally, the use of semiquantitative exposure metrics and low specificity in the exposure assessment may have contributed to misclassification of exposure, leading to attenuated risks.]

The incidence of cancer was investigated in a cohort of Finnish workers undergoing mandatory biological monitoring for occupational exposure to trichloroethylene, tetrachloroethylene, or 1,1,1-trichloroethane (Anttila et al., 1995). Details of the study have been reviewed in Section 2.1.1. The cohort comprised 3974 male and female workers. Of these, 140 men and 131 women had been exposed to 1,1,1-trichloroethane, monitored from 1975 to 1983, with on average two blood measurements of 1,1,1-trichloroethane per person. Cancer incidence was ascertained from date of first exposure to 1,1,1-trichloroethane up to 1992. Expected numbers of cancer cases specific to sex, age, and calendar period were derived from the Finnish general population, and SIRs were calculated by the person-year method.

The risk of cancer of the [brain and] nervous system (ICD-7, code 193) was significantly elevated among those exposed to 1,1,1-trichloroethane (SIR, 6.05; 95% CI, 1.25–17.7), although the estimate was based on only 3 exposed cases. There was no significantly elevated risk of brain cancer in those exposed to trichloroethylene or tetrachloroethylene. [The Working Group noted as a strength that exposure was defined from biological monitoring, but that the positive finding for brain cancer was based on few exposed cases.]

The association between glioma and meningioma and exposure to six specific chlorinated organic solvents was investigated in a hospital-based case–control study in the USA (Neta et al., 2012). Study participants were recruited from three hospitals (all of which were regional referral centres for brain tumours) in Boston, Pittsburgh, and Phoenix. Cases of glioma and meningioma were identified from 1994 to 1998. Controls were selected among patients referred for non-malignant conditions: injuries, cardiovascular diseases, musculoskeletal conditions, digestive disorders, and other diagnoses, and frequency-matched on the cases by sex, age, race, hospital, and proximity to the hospital. The participation rate among cases was 92% for glioma, 94% for meningioma, and 86% for controls. There were 484 gliomas, 197 meningiomas, and 797 controls in the final data set. Study participants or, in some cases, next of kin, were interviewed regarding demographic factors and lifetime history of occupations held for at least six months, including information on job title, employer, full-time/part-time job, type of business or service, tasks, and materials. In all, 64 job-specific modules were developed to assess exposure to a variety of agents, including chlorinated organic solvents. Additional interviews were performed for clarification after initial assessment by an industrial hygienist. An industrial hygienist assessed the exposure to six solvents (1,1,1-trichloroethane, dichloromethane, trichloromethane, carbon tetrachloride, trichloroethylene, and tetrachloroethylene) during each job. Task-exposure matrices were developed for this assessment. For each participant's job, the hygienist estimated the exposure probability and frequency. In addition, eight known or inferred exposure determinants (mechanism of release, process condition, temperature, usage rate, type of ventilation, location, confined space,

proximity to the source), along with confidence in the estimations were assigned to each job. Each participant's job exposure intensity (continuous, in ppm) was modelled on the basis of a database of measurements extracted from the literature and the same exposure determinants. For participants with an exposure probability of ≥ 50%, the duration of exposure, cumulative exposure (ppm hours), average exposure, and highest exposure were assessed. Unconditional logistic regression was applied, adjusting for the variables used for frequency matching of the controls.

There was no consistent evidence of increased risk of glioma or meningioma associated with exposure to any of the six chlorinated organic solvents investigated. For glioma and exposure to 1,1,1-trichloroethane, there was no association with exposure probability, or indicators (low/high) for years exposed, cumulative exposure, average weekly exposure, or highest exposure. The risk of meningioma for those with probable exposure to 1,1,1-trichloroethane was non-significantly elevated (OR, 2.3; 95% CI, 0.7–7.2; 5 cases). In sensitivity analyses, participants categorized as probably exposed but with low confidence and participants with information from proxy respondents were not included, certain diagnoses in the control series were excluded, and a 10-year latency was applied. None of these analyses changed the risk estimates appreciably. [The Working Group noted that a strength of the study was the detailed exposure assessment; however, there were very few cases with probable exposure to 1,1,1-trichloroethane, and the use of hospital controls may have tended to attenuate the observed risks.]

A population-based case–control study on brain glioma was performed in non-metropolitan areas of Iowa, Michigan, Minnesota, and Wisconsin in the USA. The study was initiated by NIOSH with the primary purpose of investigating health risks related to farming and is known as the Upper Midwest Health Study. This study was used to investigate the risk of glioma associated with exposure to six chlorinated organic solvents in non-farming jobs, since exposure to chlorinated solvents was considered to be low in farming jobs (Ruder et al., 2013). Cases of histologically verified glioma were identified from participating medical facilities and neurosurgeon offices from 1995 to 1997. Two controls per case (872 cases), frequency-matched on sex and age, were selected from driving licence registers (for ages < 65 years) and from Medicare data tapes (for ages 65–80 years). The participation rate was 91.5%, among cases (or their next of kin) and 70.4% among controls. Of the cases, 438 were interviewed in person and 360 via proxy respondents. All respondents were interviewed about their lifetime history of occupations held for at least 1 year, including data on employer name, industry, job titles, tasks, materials used, and employment frequency. Specific questions were asked regarding exposure to organic solvents. An industrial hygienist coded occupational exposure to 1,1,1-trichloroethane, carbon tetrachloride, chloroform, methylene chloride [dichloromethane], tetrachloroethylene, and trichloroethylene, based on job histories and databases of exposure levels. For each job, the industrial hygienist assessed the exposure probability, frequency of exposure, and confidence of probability and of frequency. In addition, the industrial hygienist used exposure determinants for jobs assigned a non-zero probability of exposure to estimate exposure intensity (ppm) using methods described above for the hospital-based case–control study (Neta et al., 2012). Duration, frequency, and intensity associated with each job, across all jobs, were used to calculate cumulative exposures in ppm-years. Unconditional logistic regression was applied to estimate associations for the six solvents, adjusting for the variables used for frequency matching, in addition to age (as a continuous variable) and education.

The study showed low risks of glioma associated with exposure to the studied solvents. The risk associated with any exposure to

1,1,1-trichloroethane was low (OR, 0.75; 95% CI, 0.61–0.90) for men and women combined. Exclusion of next-of-kin respondents did not change the results. A significantly negative exposure–response relation was found, with the odds ratio for a one-unit increase in natural-log transformed cumulative exposure to 1,1,1-trichloroethane (ppm-years) being 0.97 (95% CI, 0.96–0.99). Findings for the other five solvents were similar. Exclusion of unexposed cases and controls from the analysis still gave a significantly negative exposure–response relation. Exclusion of proxy respondents gave similar results. The potential reasons for the uniformly low risks for all studied solvents were discussed in terms of a possible selection of healthy individuals into exposed occupations or selection of less healthy individuals out of exposed occupations. Controls also were slightly older than cases, giving more opportunities to have worked in exposed occupations during earlier periods. [The Working Group noted that a strength of this study was the detailed exposure assessment; however, the uniformly negative, and partly statistically significantly negative association with exposure to any of the studied substances may have been attributable to bias caused by unidentified methodological problems.]

The relation between incidence of meningioma and exposure to seven specific and four groups of organic solvents was investigated in the INTEROCC study, a multicentre case–control study (McLean et al., 2014). The INTEROCC study was initially set up as and used data from the INTERPHONE study, the aim of which was to investigate the risk of brain cancer associated with mobile phone use. The study included ten centres in seven countries: Australia, Canada, France, Germany, Israel, New Zealand, and the UK. Cases and controls were identified from 2000 to 2004. Details in recruitment of cases and controls varied between countries. In most centres the study included residents aged 30–59 years in each region associated with the study centre. Cases were either verified histologically or by unequivocal diagnostic imaging. Controls were randomly selected from the population in each centre, individually or frequency-matched on the cases by year of birth, sex, and study region. The final data set comprised 1906 cases and 5565 controls. Individuals were interviewed face-to-face, with a small number of proxy interviews. The interview covered background factors and a full occupational history, including job title, tasks, company name, and company activities. Occupational hygienists from each country coded job title and industry branch for all jobs held for at least 6 months. A JEM, the INTEROCC-JEM, was developed specifically for this study, and was based on adaptions of the FINJEM (Kauppinen et al., 1998) to reflect local conditions. The matrix linked quantitative estimates of exposure probability and intensity for seven specific organic solvents (including 1,1,1-trichloroethane) and four groups of organic solvents to each job in the job histories of the study participants. For each substance, participants with an exposure probability of ≥ 25% were classified as exposed, and participants with an exposure probability of ≥ 5% but < 25% were excluded from the analysis. [The Working Group noted that participants with an exposure probability of < 5% had already been classified as unexposed by the JEM.] Conditional logistic regression was applied, adjusting for the variables in matching of controls, and education.

No associations with any of the studied organic solvents were found. One case and three controls were classified as exposed to 1,1,1-trichloroethane, giving an odds ratio of 1.35 with a very wide confidence interval (95% CI, 0.10–17.55). [The Working Group noted that the JEM was well developed and based on more information than most of the other studies reviewed, although it was limited in identifying individuals with low and high exposure in a job title. The prevalence of exposure to 1,1,1-trichloroethane was very low, < 0.1% among cases and

controls, and the risk estimates were imprecise owing to low numbers.]

2.3 Cancer of the breast

See Table 2.4.

The Working Group identified one cohort study, two nested case–control studies, and one population-based case–control study in Nordic countries and the USA that investigated associations between risk of breast cancer and exposure to 1,1,1-trichloroethane.

As detailed in Section 2.1.1, Radican et al. (2008) extended the follow-up of a cohort of 14 455 civilian aircraft-maintenance workers employed for at least 1 year between 1952 and 1956 at a United States Air Force base to evaluate cancer mortality risks in relation to potential exposure to trichloroethylene and other chemicals according to job titles from personnel records. The follow-up was extended to 2000 using exclusively the national death index and included non-White workers. The cohort was mostly male (74%) and non-White workers accounted for only 2.7% of the cohort. The most detailed exposure assessment was for trichloroethylene, which was replaced by 1,1,1-trichloroethane in the degreasers after 1978. Exposure to 1,1,1-trichloroethane was only evaluated qualitatively as ever versus never in the analysis. Cox proportional hazard regression models were applied to estimate the risk for exposed versus unexposed workers. In this follow-up, there was an elevated risk of mortality attributable to breast cancer (HR, 2.35; 95% CI, 0.83–6.64) among women exposed to 1,1,1-trichloroethane, although this was based on only 4 exposed deaths. [The Working Group noted that this was a relatively large cohort with a long follow-up period. Limitations of the exposure assessment included its qualitative nature, the difficulty in linking participants to estimates often associated with no more detail than job title, and the lack of continued exposure assessment after 1982. There were very few breast cancer deaths among the exposed.]

Talibov et al. (2019) conducted a nested case–control study within the NOCCA cohort to evaluate occupational exposures in relation to breast cancer in men in Sweden, Finland, and Iceland. Occupational titles were available only for census years. The study included 1469 incident cases of breast cancer in men identified from national registries, and five controls per case matched on country, sex, and year of birth who were randomly selected from the NOCCA cohort. Information on occupation during the follow-up was obtained from computerized census records from 1960 in Sweden, 1970 in Finland and 1981 in Iceland. Occupational exposures were estimated by linking job titles of study participants to the NOCCA-JEM. A cumulative exposure index was derived as a product of exposure prevalence and annual average exposure each year over the employment period of the study participants, as assessed from the census data. Conditional logistic regression was applied, with adjustment for socioeconomic status using single (each exposure agent one at a time) and multiple (all 24 exposure agents, except those that were highly correlated, were added simultaneously) exposure models. Analyses were conducted with dichotomous (ever/never) or polytomous exposure (categorized by using 50th and 90th percentiles of exposure distribution among exposed controls with the unexposed group as the reference category). None of the odds ratios for exposure to 1,1,1-trichloroethane were statistically significant in these models. [The Working Group noted that a strength was the large sample size for cases and controls in the study and fairly accurate and complete cancer incidence data. A limitation was that the information on work histories was based on census data only. The JEM was well developed, but it was limited in its ability to identify persons with low and high exposure in a population-based study.]

Table 2.4 Cohort and case–control studies on exposure to 1,1,1-trichloroethane and cancer of the breast

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Radican et al. (2008) Utah, USA Enrolment, 1952–1956/ follow-up, 1953–2000 Cohort	14 455 (10 730 men and 3725 women); civilian workers employed at Hill Air Force Base, an aircraft-maintenance facility, for ≥ 1 yr between 1952 and 1956, who were followed up for cancer mortality through linkage to the national death index Exposure assessment method: expert judgement; review of facility records, jobs, walk-through surveys, interviews, measurements used to assign yes/no exposed by job group	Breast, mortality	Exposed to 1,1,1-TCE, women (HR): No chemical exposures Ever	 NR 4	 1 2.35 (0.83–6.64)	Age, race	*Exposure assessment critique*: Extensive data collection, including measurements. Linkage of jobs to exposures was limited owing to the limited information in the available records. Given 1,1,1-TCE was often interchanged with other chlorinated solvents, the difficulty in making these links is a non-trivial limitation. Job information used to assign yes/no. *Strengths*: relatively large cohort with a long follow-up period; exposure assessment was based on information regarding exposure and work processes provided by the United States Air Force. *Limitations*: very few cases for breast cancer deaths from exposure to 1,1,1-TCE; exposure not mutually exclusive; cancer incidence was not updated; data on lifestyle and other non-occupational risk factors, which might be confounders or effect modifiers, were not available for the cohort.

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Talibov et al. (2019) Sweden, Finland, Iceland Sweden (77%, 1960–2005), Finland (21%, 1970–2005), Iceland (2%, 1981–2004) Nested case–control	Cases: 1469 cases in men with breast cancer diagnosed 1961–2005 in Sweden, Finland, and Iceland within the NOCCA cohort; participants from the NOCCA cohort had to be aged ≥ 20 yr at the date of diagnosis of the case (index date) and had to have at least one census record before index date Controls: 7345; 5 controls for each case, randomly selected from the NOCCA cohort, matched on country, sex, and year of birth Exposure assessment method: records; used self-reported jobs to the census and NOCCA-JEM that includes semiquantitative estimates of prevalence exposed, mean level of exposure, and duration	Breast (men), incidence	Exposed to 1,1,1-TCE (OR): Never Ever	 1288 181	 1 1.01 (0.84–1.20)	Country, year of birth, socioeconomic status	*Exposure assessment critique*: NOCCA-JEM is a robust and well-developed JEM. NOCCA-JEM was normalized to the country. Intensity and prevalence estimates based on actual data. Could be missing exposed jobs owing to 10 yr census collection. Prevalence is included in cumulative exposure but is not a component of toxicity. Other comments: conducted sensitivity analyses with 5 and 10 yr exposure lags. *Strengths*: accuracy and completeness of cancer incidence data from this well-established large cohort in Nordic countries; ran models with one agent at a time as well as all agents simultaneously. *Limitations*: information was not available on potential confounders such smoking, alcohol, leisure time, physical activity, and obesity.
		Breast (men), incidence	Exposed to 1,1,1-TCE (OR): Never Ever	 1288 181	 1 1.02 (0.67–1.57)	Country, year of birth, socioeconomic status, up to 23 additional exposures (solvents, metals, gases, and others)	
		Breast (men), incidence	Cumulative 1,1,1-TCE exposure index (OR): Not exposed ≤ 5.6 ppm-years 5.7–13 ppm-years > 13 ppm-years Trend-test *P* value, 0.83	 1288 122 41 18	 1 0.98 (0.80–1.20) 1.10 (0.77–1.55) 1.01 (0.60–1.69)	Country, year of birth, socioeconomic status	
		Breast (men), incidence	Cumulative 1,1,1-TCE exposure index (OR): Not exposed ≤ 5.6 ppm-years 5.7–13 ppm-years > 13 ppm-years Trend-test *P* value, 0.73	 1288 122 41 18	 1 1.18 (0.70–1.98) 1.36 (0.74–2.50) 1.10 (0.50–2.41)	Country, year of birth, socioeconomic status, up to 23 additional exposures (solvents, metals, gases, and others)	

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Videnros et al. (2020) Malmö city, Sweden 1991–1996 with follow-up to 31 December 2013 Nested case–control	Cases: 731 women with first-time diagnosis of invasive breast cancer in 1991–2013, identified through the Swedish cancer registry, excluding premenopausal cases, those with no self-reported work history, and breast cancer diagnosis before baseline; women were born in 1923–1950, living in Malmö city, Sweden, 1991–1996, and enrolled for a population-based prospective cohort study (MDCS) Controls: 1669; 2 controls per case, matched on age using density-based selection from the cohort	Breast (postmenopausal), incidence	Exposed to 1,1,1-TCE (OR): Never Ever	 721 10	 1 1.06 (0.50–2.24)	Age	*Exposure assessment critique*: Stronger study than many of the other FINJEM/NOCCA-JEM studies because work histories were self-reports from interviews that gathered more information than job only. Although only 3 jobs collected, they generally covered most of work history. FINJEM is a robust and well-developed JEM. Normalized FINJEM to countries. Modified FINJEM/NOCCA-JEM to reflect study participants. Intensity and prevalence estimates based on actual data. Prevalence included in the mean intensity metric, although prevalence is not a component of toxicity. Metrics were all categorical. Leisure time physical activity covariate was confirmed with the authors.
		Breast (postmenopausal), incidence	Exposed to 1,1,1-TCE (OR): Never Ever	 721 10	 1 1.17 (0.53–2.56)	Age, parity, age at first [full-] term pregnancy, months of breastfeeding per child, hormone replacement therapy, alcohol consumption, height, BMI, leisure time physical activity	
		Breast (postmenopausal), incidence	Duration of 1,1,1-TCE exposure (OR): Unexposed 1–10 yr > 10 yr Trend-test *P* value, 0.51	 721 2 8	 1 0.60 (0.13–2.89) 1.55 (0.61–3.94)		
		Breast (postmenopausal), incidence	Mean 1,1,1-TCE exposure intensity (OR): Unexposed > 0–0.41 ppm (mean, 0.32 ppm) 0.47–1.34 ppm (mean, 0.83 ppm)	 721 5 5	 1 1.20 (0.42–3.49) 0.94 (0.33–2.69)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Videnros et al. (2020) Malmö city, Sweden 1991–1996 with follow-up to 31 December 2013 Nested case–control (cont.)	Exposure assessment method: expert judgement; used questionnaires administered to participants for three jobs; reviewed FINJEM, NOCCA-JEM and participant-specific data to assign participant-specific semiquantitative estimates of prevalence, intensity, and duration for each job held	Breast (postmenopausal), incidence	Mean 1,1,1-TCE exposure intensity (OR): Unexposed > 0–0.41 ppm (mean, 0.32 ppm) 0.47–1.34 ppm (mean, 0.83 ppm) Trend-test *P* value, 0.76	 721 5 5	 1 1.23 (0.42–3.63) 1.10 (0.36–3.39)	Age, parity, age at first [full-] term pregnancy, months of breastfeeding per child, hormone replacement therapy, alcohol consumption, height, BMI, leisure time, physical activity	*Strengths*: this nested case–control study updated the authors' previous cohort study with improved exposure estimates on individual levels from an occupational hygienist; cancer diagnoses ascertained through linkage with national registry. *Limitations*: only 2 controls per case; low study power as exposures to 1,1,1-TCE and other chemicals were quite rare.

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pedersen et al. (2020) Denmark Women born in Denmark in or after 1946; breast cancer cases identified by 2016 Case–control	Cases: 38 375 first primary breast cancer cases identified via the Danish cancer registry (established 1942) through 2016 and born in Denmark in or after 1946 with registration in the Danish Supplementary Pension Fund Register (ATP) for employment history (since 1964) Controls: 191 875; 5 random controls per case from the Danish Civil Registration System (established in 1968), matched on year of birth. Born in Denmark ≥ 1946 with employment history; alive and free of breast cancer at the date of diagnosis of the corresponding case (index date)	Breast, incidence	Any exposure to 1,1,1-TCE, women aged < 50 yr (OR):			Parity, age at first live birth, heavy physical activity at work	*Exposure assessment critique*: NOCCA-JEM is a robust and well-developed JEM. NOCCA-JEM was normalized to the country. Prevalence and intensity were based on actual data. Prevalence, which is not a component of toxicity, was included in cumulative exposure. Metrics were all categorical. Other comments: covariate adjustment for parity may have been unnecessary in parity stratified estimates. Considered exposure windows of 1–9, 10–20, and > 20 yr to evaluate latency *Strengths*: population-based case–control study with established exposure assessment methods; potential confounders related to breast cancer were included in analysis; analysis by breast cancer subtypes (ER + and ER–) reported; cancer diagnoses ascertained through linkage with national registry.
			Never	17 234	1		
			Ever	98	1.06 (0.85–1.32)		
		Breast, incidence	Any exposure to 1,1,1-TCE, women aged ≥ 50 yr (OR):				
			Never	20 885	1		
			Ever	158	0.95 (0.80–1.13)		
		Breast, incidence	Duration of 1,1,1-TCE exposure, women aged < 50 yr (OR):				
			Unexposed	17 234	1		
			1–9 yr	90	1.06 (0.85–1.34)		
			≥ 10 yr	8	1.05 (0.49–2.26)		
			Trend-test *P* value, 0.69				
		Breast, incidence	Duration of 1,1,1-TCE exposure, women aged ≥ 50 yr (OR):				
			Unexposed	20 885	1		
			1–9 yr	138	0.97 (0.81–1.17)		
			≥ 10 yr	20	0.85 (0.53–1.39)		
			Trend-test *P* value, 0.48				
		Breast, incidence	Cumulative 1,1,1-TCE exposure quartile, women aged < 50 yr (OR):				
			Unexposed	17 234	1		
			> 0–25%	32	1.43 (0.96–2.11)		
			> 25–50%	25	1.06 (0.69–1.64)		
			> 50–75%	23	1.03 (0.66–1.62)		
			> 75%	18	0.75 (0.45–1.23)		
			Trend-test *P* value, 0.66				

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pedersen et al. (2020) Denmark Women born in Denmark in or after 1946; breast cancer cases identified by 2016 Case–control (cont.)	Exposure assessment method: records; using job information from a Danish register and NOCCA-JEM that includes semiquantitative estimates of prevalence exposed, mean level of exposure, and duration	Breast, incidence	Cumulative 1,1,1-TCE exposure quartile, women aged ≥ 50 yr (OR):			Parity, age at first live birth, heavy physical activity at work	*Limitations*: low prevalence of exposure to 1,1,1-trichloroethane among women (0.7%) may reduce power and result in limited positive findings; crosswalk between Nordic Classification of Occupations (NYK) based NOCCA-JEM and Danish industry code (DSE) may lead to exposure misclassification; JEM did not entail measurements of exposure > 1995 so metrics in the latest era (1985–95) were assumed.
			Unexposed	20 885	1		
			> 0–25%	6	0.37 (0.16–0.85)		
			> 25–50%	68	1.04 (0.80–1.35)		
			> 50–75%	47	1.13 (0.82–1.55)		
			> 75%	37	0.88 (0.62–1.25)		
			Trend-test *P* value, 0.65				
		Breast, incidence	Latency of 1,1,1-TCE exposure, women aged < 50 yr (OR):				
			Unexposed	17 234	1		
			1–9 yr	9	0.82 (0.40–1.67)		
			10–20 yr	38	1.36 (0.95–1.96)		
			> 20 yr	51	0.96 (0.71–1.29)		
		Breast, incidence	Latency of 1,1,1-TCE exposure, women aged ≥ 50 yr (OR):				
			Unexposed	20 885	1		
			1–9 yr	5	1.06 (0.40–2.78)		
			10–20 yr	13	1.59 (0.85–2.97)		
			> 20 yr	140	0.92 (0.76–1.10)		
		Breast, incidence	Timing of first job exposed to 1,1,1-TCE, parous women aged < 50 yr (OR):				
			Unexposed	17 234	1		
			Before first live birth	62	1.14 (0.86–1.51)		
			After first live birth	24	0.81 (0.52–1.26)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pedersen et al. (2020) Denmark Women born in Denmark in or after 1946; breast cancer cases identified by 2016 Case–control (cont.)		Breast, incidence	Timing of first job exposed to 1,1,1-TCE, parous women aged ≥ 50 yr (OR):			Parity, age at first live birth, heavy physical activity at work	
			Unexposed	20 885	1		
			Before first live birth	87	0.96 (0.74–1.21)		
			After first live birth	53	0.85 (0.63–1.15)		
		Breast (ER+), incidence	Any exposure to 1,1,1-TCE, women aged < 50 yr (OR):				
			Never	NR	1		
			Ever	51	0.99 (0.73–1.34)		
		Breast (ER+), incidence	Any exposure to 1,1,1-TCE, women aged ≥ 50 yr (OR):				
			Never	NR	1		
			Ever	127	1.08 (0.89–1.31)		
		Breast (ER–), incidence	Any exposure to 1,1,1-TCE, women aged < 50 yr (OR):				
			Never	NR	1		
			Ever	49	1.32 (0.88–1.97)		
		Breast (ER–), incidence	Any exposure to 1,1,1-TCE, women aged ≥ 50 yr (OR):				
			Never	NR	1		
			Ever	19	0.65 (0.40–1.06)		
		Breast (ER+), incidence	Duration of 1,1,1-TCE exposure, women aged ≥ 50 yr (OR):				
			Unexposed	NR	1		
			1–9 yr	111	1.12 (0.91–1.37)		
			≥ 10 yr	16	0.89 (0.52–1.51)		

Table 2.4 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pedersen et al. (2020) Denmark Women born in Denmark in or after 1946; breast cancer cases identified by 2016 Case–control (cont.)		Breast (ER+), incidence	Cumulative 1,1,1-TCE exposure quartile, women aged ≥ 50 yr (OR):			Parity, age at first live birth, heavy physical activity at work	
			Unexposed	NR	1		
			> 0–25%	24	0.83 (0.54–1.28)		
			> 25–50%	37	1.29 (0.90–1.85)		
			> 50–75%	34	1.15 (0.79–1.67)		
			> 75%	32	1.06 (0.72–1.55)		
		Breast (ER+), incidence	Latency of 1,1,1-TCE exposure, women aged ≥ 50 yr (OR):				
			Unexposed	NR	1		
			1–9 yr	5	1.48 (0.54–4.01)		
			10–20 yr	11	1.81 (0.91–3.63)		
			> 20 yr	111	1.03 (0.84–1.26)		
		Breast, incidence	Any exposure to 1,1,1-TCE, parous women aged < 50 yr (OR):				
			Never	NR	1		
			Ever	86	1.03 (0.81–1.30)		
		Breast, incidence	Any exposure to 1,1,1-TCE, parous women aged ≥ 50 yr (OR):				
			Never	NR	1		
			Ever	140	0.92 (0.76–1.10)		
		Breast, incidence	Any exposure to 1,1,1-TCE, nulliparous women aged < 50 yr (OR):				
			Never	NR	1		
			Ever	12	0.41 (0.13–1.28)		
		Breast, incidence	Any exposure to 1,1,1-TCE, nulliparous women aged ≥ 50 yr (OR):				
			Never	NR	1		
			Ever	18	2.33 (0.66–8.14)		

BMI, body mass index; CI, confidence interval; ER, estrogen receptor; FINJEM, Finnish job-exposure matrix; JEM, job-exposure matrix; MDCS, Malmö Diet and Cancer Study; NOCCA, Nordic Occupational Cancer Study; NOCCA-JEM, Nordic Occupational Cancer Study job-exposure matrix; NR, not reported; OR, odds ratio; ppm, parts per million; 1,1,1-TCE, 1,1,1-trichloroethane; yr, year.

Videnros et al. (2020) conducted a follow-up nested case–control study, using exposure estimates that had been improved compared with those in the original study, to examine the association between workplace chemical exposures and postmenopausal breast cancer. The original study (Videnros et al., 2019) included 16 084 women born in 1923–1950, living in Malmö city, Sweden, in 1991–1996, and participating in the Malmö Diet and Cancer Study, a population-based prospective cohort study. Each participant at baseline filled out an extensive questionnaire on lifestyle, reproductive factors, and working history with specific tasks. Exposure to 1,1,1-trichloroethane and other chemicals was assessed through the NOCCA-JEM and FINJEM, adapted for Swedish working conditions. In this follow-up, two controls per case matched on age were included in analyses after excluding 239 cases with a missing questionnaire, for a total of 731 cases and 1669 controls. Also excluded were women with no self-reported work history (n = 42), a diagnosis of breast cancer before baseline (n = 50), and premenopausal status until the end of follow-up (n = 55). An occupational hygienist reviewed and reclassified the prevalence estimates in the NOCCA-JEM and FINJEM to reflect participant-specific data on work tasks. Both conditional and unconditional logistic regression was applied with adjustment for potential confounders (not including any other chemicals of interest in the study), however only results from unconditional logistic regression were reported. Women exposed to 1,1,1-trichloroethane had a slightly increased risk of breast cancer compared with unexposed women (OR, 1.17; 95% CI, 0.53–2.56). Exposure duration of > 10 years was associated with an odds ratio of 1.55 (95% CI, 0.61–3.94). This was not statistically significant and there was no significant trend. When investigating the risk according to mean intensity (ppm), there was no clear evidence of a trend in increasing risk of breast cancer with increasing mean intensity, with odds ratios changing from 1.23 (95% CI, 0.42–3.63) in the lower class (range, > 0–0.41 ppm; mean, 0.32 ppm) to 1.10 (95% CI, 0.36–3.39) in the higher class (range, 0.47–1.34 ppm; mean, 0.83 ppm) compared with women with no exposure to 1,1,1-trichloroethane. [The Working Group noted that a major strength was the exposure assessment by an occupational hygienist to estimate each woman's probability of exposure according to the specific work task specified in the baseline questionnaire. There was also extensive individual information on hormonal and reproductive factors as a control for confounding. The questionnaires for about 22% of the cases were lost before detailed work information could be extracted so they had to be excluded from this study. Only two controls per case were selected owing to feasibility concerns to allow exposure assessment by an occupational hygienist. Few participants in this population-based cohort had been exposed to 1,1,1-trichloroethane and, for those who had been exposed, exposure intensity was low. The highest average exposure intensity for an individual was 1.34 ppm (the current Swedish occupational exposure limit is 50 ppm).]

A population-based case–control study by Pedersen et al. (2020) was conducted to investigate the risk of breast cancer, including hormonal subtypes, among Danish women. It included 38 375 first primary breast cancer cases identified via the nationwide Danish Cancer Registry (established in 1942) through 2016, under age 70 years at the time of diagnosis, born in Denmark in or after 1946, and registered in the Danish Supplementary Pension Fund Register (ATP) (to ensure access to complete employment history). Five controls per case matched on year of birth were randomly selected using the Danish Civil Registration System (established in 1968) for a total of 191 875 controls with employment history who were alive and free of breast cancer at the date of diagnosis of the corresponding case (index date). Data retrieved from the ATP, which has obtained employment history

on all wage earners since 1964, included start and end of employment dates, company name, and a Danish five-digit branch/industry code (Danmarks Statistisk Erhvervsgrupperingskode, DSE) based on an extended version of the International Standard Industrial Classification of all Economic Activities (ISIC). Four of the historically most commonly used organic solvents in Denmark, including 1,1,1-trichloroethane, were selected for the study. Exposure to each of the four solvents was classified, based on each woman's employment history, using the Danish version of the NOCCA-JEM. A crosswalk between the Nordic Classification of Occupations (used in the NOCCA-JEM) and DSE codes was developed for exposed jobs in the Danish version. Conditional logistic regression was applied among women ever versus never exposed to each organic solvent and by different metrics for exposure, with adjustment for potential confounders (including reproductive variables and heavy physical activity at work), stratified by age at the index date (ages < 50 years and ≥ 50 years, approximating menopausal status) and further by estrogen hormone receptor status. The results showed no positive associations between exposure to 1,1,1-trichloroethane and breast cancer. Evaluations of the risk of breast cancer with exposure to 1,1,1-trichloroethane by various metrics including duration of exposure, quartiles of cumulative exposure, latency, and timing of first job with exposure (before or after first live birth) did not show any positive patterns of association. [The Working Group noted that this study included a large number of cases and controls. Participants who had a probability of exposure of < 10% and a job duration of < 1 year were classified as unexposed, probably increasing specificity. The ability of a JEM to identify participants with high or low exposure in a population-based study is limited. Further misclassification could be present since the JEM was not sex-specific.]

2.4 Cancers of the kidney and urinary bladder

See Table 2.5.

A total of six studies evaluated the association between exposure to 1,1,1-trichloroethane and cancers of the kidney or urinary bladder, including one retrospective cohort study on multiple cancer types (Anttila et al., 1995), one nested case–control study (Hadkhale et al., 2017), and four case–control studies (Dosemeci et al., 1999; Christensen et al., 2013; Purdue et al., 2017; Sciannameo et al., 2019).

In Finland, a cohort of 2050 men and 1924 women who were monitored biologically for regular occupational exposure to halogenated hydrocarbons at the Finnish Institute of Occupational Health were followed for cancer incidence through 1992; the cohort included 140 men and 131 women exposed to 1,1,1-trichloroethane between 1975 and 1983 (Anttila et al., 1995). There were no cases of kidney cancer observed among workers exposed to 1,1,1-trichloroethane, similar to the number of 0.40 expected. [The Working Group noted that despite the documented exposure of workers and complete follow-up, the small number of workers exposed to 1,1,1-trichloroethane (and lack of observed kidney cancer cases) limited the informativeness of the study.]

A population-based case–control study in Minnesota, USA, recruited 438 White newly diagnosed histologically confirmed cases of renal cell carcinoma (273 men and 165 women) from a state-wide cancer registry, and 687 White age- and sex-matched population controls (462 men and 225 women) in 1988–1990 (Dosemeci et al., 1999). Response rates were 87% for cases and 86% for controls for the overall interview. Trained interviewers captured information on a range of personal factors, including the most recent and usual occupation and industry, job activities, year of start and end, part-time or full-time status, and duration of employment in specific

Table 2.5 Cohort and case–control studies on exposure to 1,1,1-trichloroethane and cancers of the kidney and urinary bladder

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Anttila et al. (1995) Finland Enrolment, 1965–1983 (1,1,1-TCE: 1975–1983)/follow-up, 1967–1992 Cohort	3974 workers (2050 men and 1924 women), 271 of whom were monitored for exposure to 1,1,1-TCE; workers biologically monitored for occupational exposure to three halogenated hydrocarbon solvents in Finland Exposure assessment method: quantitative measurements; a database of measurements in urine from trichloroethylene-exposed participants, and blood from tetrachloroethylene- and 1,1,1-TCE-exposed participants was used to identify ever-exposed to the chemicals	Kidney, incidence	Compared with the general population (SIR): Any 1,1,1-TCE exposure Expected cases	 0 0.4	 0 (0–9.16) –	Age, sex, calendar period	*Exposure assessment critique*: Exposed were truly exposed. Blood levels only reflect short-term (days) exposures for 9 yr. No information was provided on the interpretation of the measurements or the participants' exposures, including possible exposures to 1,1,1-TCE outside the 1975–1983 window or to other agents. *Strengths*: documented exposure; complete follow-up for cancer incidence through linkage with national registry. *Limitations*: small number of workers exposed to 1,1,1-TCE; limited exposure information (timing of measurements, exposure duration,

Table 2.5 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Anttila et al. (1995) Finland Enrolment, 1965–1983 (1,1,1-TCE: 1975–1983)/follow-up, 1967–1992 Cohort (cont.)							multiple solvent exposures (94.4% of worke rmonitored for one solvent, multiple exposures probably underestimated), limited information on potential confounders, worker selection unclear (estimated 4000 workers in Finland occupationally exposed at end of follow-up period).

Table 2.5 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Dosemeci et al. (1999) Minnesota, USA 1988–1990 Case–control	Cases: 438 newly diagnosed cases of histologically confirmed RCC (273 White men and 165 White women) from Minnesota Cancer Surveillance System aged 20–85 yr with in-person interviews Controls: 687 (462 White men and 225 White women); random-digit dialling (20–64 yr) and Health Care Financing Administration (65–85 yr), age and sex-stratified controls with in-person interview Exposure assessment method: partial work histories and expert-developed JEM used; estimated (semiquantitative estimates) intensity and probability by assigning probability and intensity separately to each job and each industry and then combining using an algorithm	Kidney (RCC), incidence	Exposure to methyl chloroform [1,1,1-TCE] (OR):			Age, sex, smoking, hypertension and/or use of diuretics and/or hypertension drugs, BMI	*Exposure assessment critique*: Proxy respondents (next of kin) were required for 35% of cases, so these were excluded from the analysis. Unclear whether exposure assessment method produced valid results. Work histories limited to longest and most recent jobs, which may miss key exposures. Metrics were all categorical. *Strengths*: objective exposure assessment; histologically confirmed cancer diagnoses ascertained through state-wide registry
			Never	NR	1		
			Ever	66	0.94 (0.7–1.3)		
		Kidney (RCC), incidence	Exposure to methyl chloroform [1,1,1-TCE], men (OR):				
			Never	NR	1		
			Ever	53	0.88 (0.6–1.3)		
		Kidney (RCC), incidence	Exposure to methyl chloroform [1,1,1-TCE], women (OR):				
			Never	NR	1		
			Ever	13	1.26 (0.6–2.8)		

Table 2.5 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Dosemeci et al. (1999) Minnesota, USA 1988–1990 Case–control (cont.)							*Limitations*: small number of exposed participants; lack of. lifetime occupational history information; lack of exposure specificity; limited consideration of multiple CAHC exposures; potential survival bias (35% of cases who had died excluded).

Table 2.5 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Christensen et al. (2013) Montreal, Canada 1979–1985 Case–control	Cases: 3730 cancer cases at 11 sites, including 177 kidney and 484 bladder cancer cases; male incident histologically confirmed kidney and bladder cancer cases from 18 large hospitals in Montreal metropolitan area, Canadian citizens aged 35–70 yr (median, 59 and 60 yr, respectively) Controls: 533 population controls, 1999 and 2299 other cancer controls respectively; population controls obtained randomly from population-based electoral lists, stratified by sex and age, other cancer controls from other participating cases	Kidney (ICD-9, code 189), incidence	Any exposure to 1,1,1-TCE, 5 yr lag, men (OR): No chlorinated solvent exposure Ever-analysis limited to population controls Ever-analysis including both population and other cancer controls	 134 4 4	 1 1.1 (0.3–3.7) 1.3 (0.4–4.0)	Age, census tract median income, education, ethnicity, self/proxy, smoking, coffee, beer, wine, and spirit intake	*Exposure assessment critique*: Substantial data available for assessment including [presumably] published measurement data. Evaluation was participant-specific. Careful consideration of each job held by each participant (i.e. confidence, frequency, and intensity) is a key strength. Cumulative exposure included confidence, which is not a component of toxicity. Metrics were all categorical.

Table 2.5 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Christensen et al. (2013) Montreal, Canada 1979–1985 Case–control (cont.)	Exposure assessment method: expert judgement; full work histories and specialized questionnaires, [presumed measurement data], and extensive review to assign participant-specific semiquantitative estimates of confidence, frequency and intensity for each job held	Kidney (ICD-9, code 189), incidence	Substantial exposure to 1,1,1-TCE, 5 yr lag, men (OR):			Age, census tract median income, education, ethnicity, self/proxy, smoking, coffee, beer, wine, and spirit intake	*Strengths*: detailed lifetime occupational histories and expert exposure assessment, some semiquantitative exposure estimates, multiple control groups; histologically confirmed cancer diagnoses ascertained through hospitals. *Limitations*: small numbers of workers exposed to 1,1,1-TCE; retrospective exposure assessment.
			No chlorinated solvent exposure	134	1		
			Ever-analysis limited to population controls	3	1.2 (0.3–5.0)		
			Ever-analysis including both population and other cancer controls	3	1.5 (0.4–5.3)		
		Urinary bladder (ICD-9, code 188), incidence	Any exposure to 1,1,1-TCE, 5 yr lag, men (OR):			Age, census tract median income, education, ethnicity, self/proxy, smoking, coffee intake, aromatic amines exposure	
			No chlorinated solvent exposure	372	1		
			Ever-analysis limited to population controls	5	0.6 (0.2–1.8)		
			Ever-analysis including both population and other cancer controls	5	0.7 (0.2–1.9)		
		Urinary bladder (ICD-9, code 188), incidence	Substantial exposure to 1,1,1-TCE, 5 yr lag, men (OR):			Age, census tract median income, education, ethnicity, self/proxy, smoking, coffee intake, aromatic amines exposure	
			No chlorinated solvent exposure	372	1		
			Ever-analysis limited to population controls	3	0.5 (0.1–2.2)		
			Ever-analysis including both population and other cancer controls	3	0.6 (0.2–2.4)		

Table 2.5 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Purdue et al. (2017) USA; Detroit and Chicago, USKC study 2002–2007 Case–control	Cases: 1217 histologically confirmed incident cases of kidney cancer identified in Metropolitan Cancer Surveillance System (Detroit), and review of pathology reports from 56 hospitals (Chicago), patients aged 20–79 yr Controls: 1235; Department of Motor Vehicle records (ages 20–64 yr) and Medicare files (ages 65–79 yr) frequency-matched on sex, age, and race Exposure assessment method: expert judgement; full work histories, job-specific modules, literature review, measurement data, and [presumed] study-specific job- and task-specific matrices (for imputation when participant-specific information was missing) used to assign participant-specific semiquantitative estimates of probability, frequency, intensity and confidence for each job held	Kidney, incidence	Probability of exposure to 1,1,1-TCE (OR):			Age, sex, race, study centre, education level, smoking status, BMI, history of hypertension	*Exposure assessment critique*: Substantial data available for assessment including published measurement data modelled to estimate intensity but was not used. Evaluation was participant-specific. Use of job- and task-specific matrices (when participant-specific information was missing) probably increased consistency. Careful consideration of each job held by each participant (i.e. probability, frequency, and confidence of exposure) is a key strength. Cumulative exposure did not include intensity. Metrics were all categorical. Other comments: conducted sensitivity analyses with 5 and 15 yr exposure lags.
			Unexposed	579	1		
			< 50%	562	1.2 (1.0–1.4)		
			50–89%	41	1.0 (0.6–1.6)		
			≥ 90%	7	1.2 (0.4–4.1)		
		Kidney, incidence	Cumulative hours exposed to 1,1,1-TCE at a high intensity (OR):				
			Unexposed	579	1		
			Low: ≤ 520 h	9	0.6 (0.2–1.6)		
			Medium: 521–1456 h	14	0.8 (0.3–2.0)		
			High: > 1456 h	21	1.6 (0.8–3.2)		
			Trend-test *P* value, 0.3				

Table 2.5 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Purdue et al. (2017) USA; Detroit and Chicago, USKC study 2002–2007 Case–control (cont.)							*Strengths*: detailed lifetime occupational histories and expert exposure assessment, some quantitative exposure estimates; histologically confirmed cancer diagnoses ascertained through regional cancer registries. *Limitations*: small numbers of workers exposed to 1,1,1-TCE; retrospective exposure assessment; low rate of participation in controls.

Table 2.5 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hadkhale et al. (2017) Finland, Iceland, Norway, Sweden, NOCCA database 1961–2005 Nested case–control	Cases: 113 343 incident bladder cancer cases from NOCCA cohort (14.9 million persons), aged ≥ 20 yr, occupational information from at least one census from 1960–1990 before index date, cases identified through linkage with cancer registries Controls: 566 715 controls from NOCCA cohort matched on country, sex, birth year at index date Exposure assessment method: used self-reported jobs to the census and NOCCA-JEM that includes semiquantitative estimates of prevalence mean level of exposure, and duration	Urinary bladder, incidence	Cumulative exposure to 1,1,1-TCE, 10 yr lag (HR):			Age, year of birth, sex, country, trichloroethylene, perchloroethylene [tetrachloroethylene], aromatic hydrocarbon solvents, benzene, toluene, chlorinated hydrocarbon solvents, other organic solvents, ionizing radiation, asbestos, benzo[*a*]-pyrene, diesel engine exhaust, sulfur dioxide	*Exposure assessment critique*: NOCCA-JEM is a robust and well-developed JEM. NOCCA-JEM was normalized to the country. Prevalence and intensity were based on actual data. Could be missing exposed jobs due to 10 yr census collection. Prevalence was included in cumulative exposure, but it is not a component of toxicity. Metrics were all categorical. Other comments: Conducted sensitivity analyses with 0, 10 and 20 yr exposure lags. *Strengths*: large-scale population-based study, quantitative cumulative exposure estimates, consideration of other occupational exposures; cancer diagnoses ascertained through linkage to national cancer registries.
			Unexposed	105 469	1		
			< 5.60 ppm-years	6011	0.98 (0.93–1.02)		
			5.60–10.15 ppm-years	1160	1.00 (0.92–1.07)		
			> 10.15 ppm-years	703	1.00 (0.89–1.07)		
			Trend-test *P* value, 0.67				
		Urinary bladder, incidence	Cumulative exposure to 1,1,1-TCE, 10 yr lag, age < 50 yr (HR):				
			Unexposed	54 167	1		
			< 5.60 ppm-years	2897	1.00 (0.91–1.05)		
			5.60–10.15 ppm-years	283	0.85 (0.73–1.00)		
			> 10.15 ppm-years	101	0.90 (0.70–1.11)		
			Trend-test *P* value, 0.12				
		Urinary bladder, incidence	Cumulative exposure to 1,1,1-TCE, 10 yr lag, age ≥ 50 yr (HR):				
			Unexposed	51 302	1		
			< 5.60 ppm-years	3114	1.00 (0.90–1.03)		
			5.60–10.15 ppm-years	877	1.08 (1.00–1.20)		
			> 10.15 ppm-years	602	1.03 (0.92–1.14)		
			Trend-test *P* value, 0.06				

Table 2.5 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hadkhale et al. (2017) Finland, Iceland, Norway, Sweden, NOCCA database 1961–2005 Nested case–control (cont.)		Urinary bladder, incidence	Cumulative exposure to 1,1,1-TCE, 10 yr lag, men (HR):			Age, year of birth, country, trichloroethylene, perchloroethylene [tetrachloroethylene], aromatic hydrocarbon solvents, benzene, toluene, chlorinated hydrocarbon solvents, other organic solvents, ionizing radiation, asbestos, benzo[*a*]-pyrene, diesel engine exhaust, sulfur dioxide	*Limitations*: no information on other potential personal confounding variables such as cigarette smoking; limited occupational information; occupational titles updated infrequently (every 10 yr).
			Unexposed	77 107	1		
			< 5.60 ppm-years	5711	1.00 (0.92–1.01)		
			5.60–10.15 ppm-years	1120	1.00 (0.91–1.07)		
			> 10.15 ppm-years	691	1.00 (0.90–1.07)		
			Trend-test *P* value, 0.6				
		Urinary bladder, incidence	Cumulative exposure to 1,1,1-TCE, 10 yr lag, women (HR):				
			Unexposed	28 362	1		
			< 5.60 ppm-years	300	1.04 (0.85–1.30)		
			5.60–10.15 ppm-years	40	1.15 (0.80–1.70)		
			> 10.15 ppm-years	12	1.11 (0.58–2.20)		
			Trend-test *P* value, 0.98				
Sciannameo et al. (2019) Turin and Brescia, Italy 1992–2012 Case–control	Cases: 893 incident cases of histologically confirmed bladder cancer diagnosed at a local hospital in Turin in men aged 40–74 yr; or at the urology department of two local hospitals in Brescia in men aged 20–80 yr	Urinary bladder (ICD-9, code 188), incidence	Exposed to [1,1,1-]TCE, 10 yr lag, men (OR):			Age, smoking status, intensity of smoking, study	*Exposure assessment critique*: Stronger study than many of the other FINJEM/NOCCA-JEM studies because work histories were self-reports from interviews that gathered more information than job only. NOCCA-JEM is a robust and well-developed JEM. FINJEM was normalized to the country.
			Never	531	1		
			Ever (≥ 2 yr)	362	1.18 (0.96–1.46)		
		Urinary bladder (ICD-9, code 188), incidence	Cumulative exposure to [1,1,1-]TCE, 10 yr lag, men (OR):				
			Never	531	1		
			Low	181	1.33 (1.02–1.73)		
			High	181	1.08 (0.83–1.41)		

Table 2.5 (continued)

Reference, location, enrolment/follow-up period, study design	Population size, description, exposure assessment method	Organ site (histology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Sciannameo et al. (2019) Turin and Brescia, Italy 1992–2012 Case–control (cont.)	Controls: 978; controls in Turin were males aged 40–74 yr, hospitalized in same hospital as cases in general medicine, otolaryngology, orthopaedic, and cardiology departments without neoplastic, metabolic, urological, or smoking-related disease; controls in Brescia were hospitalized males at the same hospital as cases for urological non-neoplastic diseases, frequency-matched on age, period, and hospital. Exposure assessment method: used self-reported work histories and assessed by FINJEM that included semiquantitative estimates of prevalence exposed, mean level of exposure, and duration	Urinary bladder (ICD-9, code 188), low grade, incidence	Exposed to [1,1,1-]TCE, 10 yr lag, men (OR):			Age, smoking status, intensity of smoking, study	Intensity and prevalence estimates were based on actual data. Definition of cumulative is unclear but may include prevalence, which, is not a component of toxicity. *Strengths:* examination of tumour grade, semiquantitative exposure estimates; histologically confirmed cancer diagnoses ascertained through medical facilities. *Limitations:* hospital-based design; representativeness of study participants unclear; large proportion of exposed workers; limited consideration of multiple occupational exposures.
			Never	327	1		
			Ever (≥ 2 yr)	229	1.23 (0.97–1.55)		
		Urinary bladder (ICD-9, code 188), low grade, incidence	Cumulative exposure to [1,1,1-]TCE, 10 yr lag, men (OR):				
			Never	327	1		
			Low	111	1.31 (0.97–1.76)		
			High	118	1.15 (0.86–1.55)		
		Urinary bladder (ICD-9, code 188), high grade, incidence	Exposed to [1,1,1-]TCE, 10 yr lag, men (OR):				
			Never	209	1		
			Ever (≥ 2 yr)	136	1.16 (0.87–1.54)		
		Urinary bladder (ICD-9, code 188), high grade, incidence	Cumulative exposure to [1,1,1-]TCE, 10 yr lag, men (OR):				
			Never	209	1		
			Low	72	1.35 (0.95–1.93)		
			High	64	0.98 (0.68–1.42)		

BMI, body mass index; CAHC, chlorinated aliphatic hydrocarbons; CI, confidence interval; FINJEM, Finnish job-exposure matrix; HR, hazard ratio; ICD, International Classification of Diseases; JEM, job-exposure matrix; NOCCA, Nordic Occupational Cancer Study; NR, not reported; OR, odds ratio; ppm, parts per million; RCC, renal cell carcinoma; SIR, standardized incidence ratio; USKC, United States Kidney Cancer study; 1,1,1-TCE, 1,1,1-trichloroethane; yr, year.

occupations/industries of interest. Participants with complete and personal (excluding next of kin) interviews comprised 63% of cases and 97% of controls. Exposure to six specific chlorinated organic solvents, including methyl chloroform [1,1,1-trichloroethane], was assessed by a set of JEMs specific to a level of intensity and to the probability of exposure for time periods where exposure had been deemed to occur for each job title and each industry (Gomez et al., 1994). The matrices were applied to the job histories by an algorithm that considered whether the job or the industry was the primary generator of exposure and incorporated the estimates into a single cumulative exposure estimate. A total of 15% of cases and 17% of controls were exposed to methyl chloroform [1,1,1-trichloroethane] (19% and 21%, respectively, of men, and 8% and 7%, respectively, of women). [The Working Group noted the relatively high exposure prevalence in this population-based study and the low specificity in the exposure assessment.] Logistic regression was applied adjusting for age, sex, smoking, hypertension and/or use of diuretics and/or anti-hypertension drugs, and body mass index in overall analyses. For methyl chloroform [1,1,1-trichloroethane], there was no clear association with ever exposure observed overall (OR, 0.94; 95% CI, 0.7–1.3). In findings by sex, there was a weak positive although imprecise and non-significant association observed in women (OR, 1.26; 95% CI, 0.6–2.8), but not men (OR, 0.88; 95% CI, 0.6–1.3). [The Working Group noted the limited occupational history and lack of lifetime work information, the small size of the study and inability to examine level of exposure, and the lack of consideration of multiple exposures to chlorinated aliphatic hydrocarbons in the analysis. These factors limited the informativeness of the study. The use of an exposure assessment algorithm with limited consideration of temporal trends in use when estimating probability and intensity of exposure, as well as the use of semiquantitative exposure metrics and low specificity in the exposure assessment (Gomez et al., 1994), may have resulted in misclassification. The analysis also included only surviving cases, excluding the 35% who had died.]

A population-based case–control study in Montreal, Quebec, Canada, recruited 3730 incident cases of histologically confirmed cancer at 11 different cancer sites in men in 1979–1985, and included 177 cases of kidney cancer and 484 cases of bladder cancer, and 533 population controls (Christensen et al., 2013). For certain analyses, cases of cancer at sites other than the one under study were used as controls (cancer controls) and were combined with equal weight with the population controls. Detailed interviews captured a range of information on each job held during working life. Expert chemists and industrial hygienists assigned categories of confidence of exposure, frequency of exposure, and relative exposure level for a total of 294 agents, including six chlorinated solvents (two chlorinated alkenes, and four chlorinated alkanes, including 1,1,1-trichloroethane). Exposures occurring in the past 5 years were excluded due to latency considerations. A total of 2.3% of kidney cancer cases, 1.9% of population controls, and 1.3% of other cancer controls had any exposure to 1,1,1-trichloroethane. For kidney cancer, unconditional logistic regression was applied adjusting for age, census tract median income, education, ethnicity, self/proxy, smoking, coffee, beer, wine, and spirit intake. There was no clear association between any or substantial exposure to 1,1,1-trichloroethane and kidney cancer risk (odds ratios were elevated, ranging from 1.1 to 1.5, but were imprecise). For bladder cancer, 1.0% of cases had any exposure to 1,1,1-trichloroethane. There was also no clear association between any or substantial exposure to 1,1,1-trichloroethane (odds ratios ranged from 0.5 to 0.7 and were imprecise) and bladder cancer risk in analysis adjusting for age, census tract median income, education, ethnicity, self/proxy, smoking, coffee, and exposure to aromatic amines. Similar results

[not reported] in the analysis of self-respondents (excluding proxies) were also observed. [The Working Group noted that the detailed exposure assessment was a strength of the study, while the small number of workers exposed to 1,1,1-trichloroethane limited its informativeness. Also, intensity and/or cumulative exposure metrics were not specifically evaluated.]

A population-based case–control study in Detroit and Chicago, USA, recruited 1217 incident cases of histologically confirmed kidney cancer and 1235 controls in 2002–2007 (Purdue et al., 2017). The sampling strategy was designed to oversample Black participants. Response rates were 77% among cases and 54% among controls. Participants completed a mailed work history calendar and responded to additional occupational and job-specific modules in interviews focusing on solvent exposures. An expert industrial hygienist assigned levels of exposure probability, frequency, and intensity for six chlorinated solvents, including 1,1,1-trichloroethane, to each job. A total of 4.0% of cases and 4.4% of controls had a 50% or greater probability of exposure to 1,1,1-trichloroethane. Unconditional logistic regression was applied adjusting for age, sex, race, study centre, education level, smoking status, body mass index, and history of hypertension. There was no clear association between categories of probability of exposure to 1,1,1-trichloroethane and kidney cancer risk. The odds ratio among those with a < 50% probability of exposure relative to those who were unexposed to 1,1,1-trichloroethane was 1.2 (95% CI, 1.0–1.4); the odds ratio for a ≥ 90% probability of exposure was 1.2 (95% CI, 0.4–4.1) based on 7 exposed cases. In the analysis of categories of cumulative hours of exposure among high-intensity jobs, there was a positive although imprecise estimate in the highest tertile (> 1456 hours) (OR, 1.6; 95% CI, 0.8–3.2; *P* for trend, 0.30; 21 exposed cases). [The Working Group noted that the detailed exposure assessment was a strength of the study, while the small number of highly exposed workers, correlations of varying strength with other occupational exposures to solvents, and low response rate among controls limited its informativeness.]

A population-based case–control study nested in the NOCCA database included 113 343 incident cases of bladder cancer (84 629 men and 28 714 women) and 566 715 matched controls from four countries (Finland, Iceland, Norway, and Sweden) from 1961 to 2005 (Hadkhale et al., 2017). The NOCCA-JEM was used to estimate the proportion and level of exposure to selected solvents, including 1,1,1-trichloroethane, based on occupational titles in census records. A total of 6.9% of cases and 6.4% of controls were occupationally exposed to 1,1,1-trichloroethane with a 10-year lag (8.9% of cases and 8.2% of controls among men, and 1.2% of cases and 1.0% of controls among women). Conditional logistic regression was applied in the overall analysis adjusting for age, sex, country, and exposure to trichloroethylene, perchloroethylene [tetrachloroethylene], aromatic hydrocarbon solvents, benzene, toluene, chlorinated hydrocarbon solvents, other organic solvent, ionizing radiation, asbestos, benzo[*a*]pyrene, diesel engine exhaust, and sulfur dioxide. Although positive associations were observed with occupational exposure to some solvents, no association was observed between categories of cumulative exposure to 1,1,1-trichloroethane and risk of bladder cancer risk, with the estimate in the highest category (> 10.15 ppm-years) being 1.00 (95% CI, 0.89–1.07; *P* for trend, 0.67) relative to those unexposed to 1,1,1-trichloroethane. There were also no clear associations observed in results stratified by age at diagnosis (< 50 years and ≥ 50 years). Although there were some weakly elevated hazard ratios in some categories of cumulative exposure among women, findings were imprecise and there was no evidence for a trend (*P* for trend, 0.98). [The Working Group noted that the large-scale population-based design was a strength of the study, as was the consideration of occupational exposures

to other solvents through adjustment of study findings for other such agents. The study also used a well-developed JEM. The lack of data on other personal potentially confounding factors (i.e. cigarette smoking), the limited information on occupational history (based on census records updated only every 10 years), and the inability of a JEM to identify workers with high and low exposure within a population, limited the informativeness of the study.]

Two hospital-based case–control studies in Brescia and Turin, Italy, were pooled to include a total of 893 incident cases of histologically confirmed bladder cancer in men diagnosed in local hospitals and clinics and 978 hospitalized controls (Sciannameo et al., 2019). Response rates were > 90% for both cases and controls at both study sites. Information on lifetime occupational history was obtained and linked to FINJEM, assigning probability and intensity of exposure for 29 selected agents, including trichloroethane for the years 1960–1984. [The Working Group noted that the published manuscript did not explicitly specify 1,1,1-trichloroethane, but rather "trichloroethane", as the agent examined here. The manuscript also apparently incorrectly noted the current IARC classification of [1,1,1-] trichloroethane as Group 2A or 2B, rather than Group 3.] After the application of a 10-year lag, a total of 40.5% of cases and 36.6% of controls were ever exposed (2 years or longer) to [1,1,1-]trichloroethane. [The Working Group noted the large proportion of exposed participants in this study in contrast to that in most other studies reviewed here.] Logistic regression was applied adjusting for age, smoking status, intensity of smoking, and study. A positive, non-significant estimate of 1.18 (95% CI, 0.96–1.46) for ever exposure to [1,1,1-]trichloroethane was observed relative to never exposure; among the highly exposed, the odds ratio was 1.08 (95% CI, 0.83–1.41). Results were generally similar when stratified by high- or low-grade disease. [The Working Group noted the hospital-based design and questions regarding the representativeness of study participants as limitations of the study, as well as a lack of consideration of multiple occupational exposures in analysis. The JEM was well developed, but the exposure assignment and large proportion of exposed participants was of concern, possibly reflecting low specificity in the JEM-based approach.]

2.5 Cancers of the digestive, respiratory, or genital tract, and other solid cancers

See Table 2.6.

Several studies (two cohort, four case–control, two case series) reported on occupational exposure to 1,1,1-trichloroethane in relation to cancers not covered in Sections 2.1–2.4 of the present monograph (Anttila et al., 1995; Zarchy, 1996; Kernan et al., 1999; Radican et al., 2008; Christensen et al., 2013; Kumagai et al., 2013; Vizcaya et al., 2013; Kubo et al., 2014a, b; Kumagai et al., 2016; Le Cornet et al., 2017). The malignancies included melanoma and cancers of the bone, lung, oesophagus, stomach, colon, rectum, liver, pancreas, bile duct, cervix, prostate and testis.

Anttila et al. (1995) conducted a 26-year cancer incidence follow-up of Finnish workers undergoing biological monitoring for exposure to 1,1,1-trichloroethane, trichloroethylene and tetrachloroethylene. In the analysis of 271 workers exposed to 1,1,1-trichloroethane, standardized incidence ratios were reported for all cancers (SIR, 1.58; 95% CI, 0.92–2.52; 17 exposed cases) and cancers of the lung (SIR, 1.31; 95% CI, 0.16–4.71; 2 exposed cases) and cervix (SIR, 8.28; 95% CI, 0.21–46.1; 1 exposed case). [Strengths of the study included its documentation of workers' exposure to 1,1,1-trichloroethane through blood measurements and long-term follow-up for cancer incidence through linkage to a national registry. An important limitation was the small sample size of workers exposed to 1,1,1-trichloroethane,

Table 2.6 Cohort and case–control studies on exposure to 1,1,1-trichloroethane and cancers of the digestive, respiratory, and genital tract, and other solid cancers

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Anttila et al. (1995) Finland Enrolment, 1965–1983 (1,1,1-TCE: 1975–1983)/follow-up, 1967–1992 Cohort	3974 workers (2050 men and 1924 women), 271 of whom were monitored for exposure to 1,1,1-TCE; workers biologically monitored for occupational exposure to three halogenated hydrocarbon solvents in Finland Exposure assessment method: quantitative measurements; a database of measurements in urine from trichloroethylene-exposed participants, and blood from tetrachloroethylene- and 1,1,1-TCE-exposed participants was used to identify ever exposed to the chemicals	All cancers combined, incidence	Compared with the general population (SIR): Any 1,1,1-TCE exposure	17	1.58 (0.92–2.52)	Age, sex, calendar period	*Exposure assessment critique*: Exposed were truly exposed. Blood levels only reflect short-term (days) exposures for 9 yr. No information was provided on the interpretation of the measurements or the participants' exposures, including possible exposures to 1,1,1-TCE outside the 1975–1983 window or to other agents. *Strengths*: documented exposure to 1,1,1-TCE via blood measurements; long-term follow-up for cancer incidence through linkage to national cancer registry. *Limitations*: small sample size; no assessment of exposure–response relationships.
		Lung, incidence	Compared with the general population (SIR): Any 1,1,1-TCE exposure	2	1.31 (0.16–4.71)		
		Uterine cervix, incidence	Compared with the general population (SIR): Any 1,1,1-TCE exposure	1	8.28 (0.21–46.1)	Age, calendar period	

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Radican et al. (2008) Utah, USA Enrolment, 1952–1956/ follow-up, 1953–2000 Cohort	14 455 (10 730 men and 3725 women); civilian workers employed at Hill Air Force Base, an aircraft-maintenance facility, for ≥ 1 yr between 1952 and 1956, who were followed up for cancer mortality through linkage to the national death index Exposure assessment method: review of facility records, jobs, walk-through surveys, interviews, measurements used to assign yes/no exposed by job group	Bone, mortality	Exposure to 1,1,1-TCE, women (HR):			Age, race	*Exposure assessment critique*: Extensive data collection, including measurements. Linkage of jobs to exposures was limited due to the limited information in the available records. Given 1,1,1-TCE was often interchanged with other chlorinated solvents, the difficulty in making these links is a non-trivial limitation. Job information used to assign yes/no. *Strengths*: large cohort size and long follow-up period; internal comparison group. *Limitations*: small number of deaths among exposed workers; qualitative exposure assessment; potential co-exposures with other organic solvents.
			No chemical exposures	NR	–		
			Ever	1	17.87 (1.12–286)		
		Bone, mortality	Exposure to 1,1,1-TCE, men (HR):				
			No chemical exposures	NR	–		
			Ever	0	–		

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kernan et al. (1999) USA 1984–1993 Case–control	Cases: 63 097; death certificates from 24 states, with pancreatic cancer listed as the underlying cause of death Controls: 252 386; death certificates from 24 states, with other underlying cause of death (excluding cancer, pancreatitis, and other pancreatic diseases), frequency-matched on state, race, sex, and 5 yr age group Exposure assessment method: source of job information was death certificates, and assessed for probability and intensity using a JEM	Pancreas, mortality	Intensity of methyl chloroform [1,1,1-TCE] exposure, Black women (OR):			Age, metropolitan status, region of residence, marital status	*Exposure assessment critique*: Weakest of the case–control studies reviewed. Death certificates provide only a single job, so other exposed jobs were likely to have been missed. No important other information available (industry, dates, tasks, etc.). No information was provided as to the development of the JEM. Estimates of cumulative exposure were not developed. Metrics were all categorical. *Strengths*: large sample size. *Limitations*: death certificate information may not accurately capture usual job; as only one job is listed, exposures from other jobs were probably missed; no information was available regarding duration of usual employment or potential confounders.
			Unexposed	NR	1		
			Low	312	1.0 (0.8–1.1)		
			Medium	22	1.1 (0.7–1.7)		
			High	42	0.8 (0.5–1.1)		
		Pancreas, mortality	Intensity of methyl chloroform [1,1,1-TCE] exposure, Black men (OR):				
			Unexposed	NR	1		
			Low	926	0.9 (0.9–1.0)		
			Medium	101	1.1 (0.9–1.5)		
			High	83	1.2 (0.9–1.5)		
		Pancreas, mortality	Intensity of methyl chloroform [1,1,1-TCE] exposure, White women (OR):				
			Unexposed	NR	1		
			Low	1003	1.1 (1.0–1.2)		
			Medium	236	1.0 (0.8–1.1)		
			High	382	1.1 (1.0–1.2)		
		Pancreas, mortality	Intensity of methyl chloroform [1,1,1-TCE] exposure, White men (OR):				
			Unexposed	NR	1		
			Low	5359	1.0 (0.9–1.0)		
			Medium	1027	1.0 (0.9–1.1)		
			High	507	0.9 (0.8–0.9)		
		Pancreas, mortality	Probability of methyl chloroform [1,1,1-TCE] exposure, Black women (OR):				
			Unexposed	NR	1		
			Low	274	0.9 (0.8–1.1)		
			Medium	25	0.7 (0.5–1.1)		
			High	4	1.2 (0.4–3.7)		

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kernan et al. (1999) USA 1984–1993 Case–control (cont.)		Pancreas, mortality	Probability of methyl chloroform [1,1,1-TCE] exposure, Black men (OR):			Age, metropolitan status, region of residence, marital status	
			Unexposed	NR	1		
			Low	673	0.9 (0.9–1.1)		
			Medium	5	0.5 (0.2–1.3)		
			High	8	2.9 (1.2–7.5)		
		Pancreas, mortality	Probability of methyl chloroform [1,1,1-TCE] exposure, White women (OR):				
			Unexposed	NR	1		
			Low	762	1.1 (1.1–1.2)		
			Medium	36	0.7 (0.4–0.9)		
			High	41	1.0 (0.7–1.4)		
		Pancreas, mortality	Probability of methyl chloroform [1,1,1-TCE] exposure, White men (OR):				
			Unexposed	NR	1		
			Low	3943	0.9 (0.9–1.0)		
			Medium	47	1.0 (0.7–1.3)		
			High	48	0.9 (0.7–1.3)		

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Christensen et al. (2013) Montreal, Canada 1979–1985 Case–control	Cases: 3730 cancer cases at 11 organ sites, including 103 melanoma and 99 oesophagus, 251 stomach, 496 colon, 248 rectum, 48 liver, 116 pancreas, and 449 prostate cancer cases; male incident histologically confirmed cancers from 18 large hospitals in the Montreal metropolitan area, Canadian citizens, aged 35–70 yr Controls: 533 population controls, 1295–2525 other cancer controls; population controls obtained randomly from population-based electoral lists, stratified by sex and age, other cancer controls from other participating cases	Prostate, incidence	Any exposure to 1,1,1-TCE, 5 yr lag (OR):			Age, census tract median income, educational attainment, ethnicity, questionnaire respondent (self vs proxy), smoking, beer, wine, and spirit intake	*Exposure assessment critique*: Substantial data available for assessment including [presumably] published measurement data. Evaluation was participant-specific. Careful consideration of each job held by each participant (i.e. confidence, frequency, and intensity) is a key strength. Metrics were all categorical. *Strengths:* detailed expert-based exposure assessment; histologically confirmed cancer diagnoses ascertained through hospitals. *Limitations:* small case sample sizes; no quantitative exposure metrics.
			No chlorinated solvent or hydrocarbon exposure	335	1		
			Ever-analysis limited to population controls	5	0.7 (0.2–2.1)		
			Ever-analysis including both population and other cancer controls	5	0.8 (0.3–2.4)		
		Prostate, incidence	Substantial exposure to 1,1,1-TCE, 5 yr lag (OR):				
			No chlorinated solvent or hydrocarbon exposure	335	1		
			Ever-analysis limited to population controls	5	1.3 (0.4–4.6)		
			Ever-analysis including both population and other cancer controls	5	1.6 (0.5–5.1)		

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Christensen et al. (2013) Montreal, Canada 1979–1985 Case–control (cont.)	Exposure assessment method: expert judgement; full work histories and specialized questionnaires, [presumed measurement data], and extensive review to assign participant-specific semiquantitative estimates of confidence, frequency and intensity for each job held	Colon, incidence	Any exposure to 1,1,1-TCE, 5 yr lag (OR):			Age, census tract median income, educational attainment, ethnicity, questionnaire respondent (self vs proxy), smoking, beer, wine, and spirit intake	
			No chlorinated solvent or hydrocarbon exposure	365	1		
			Ever-analysis limited to population controls	5	0.6 (0.2–1.7)		
			Ever-analysis including both population and other cancer controls	5	0.6 (0.2–1.7)		
		Colon, incidence	Substantial exposure to 1,1,1-TCE, 5 yr lag (OR):				
			No chlorinated solvent or hydrocarbon exposure	365	1		
			Ever-analysis limited to population controls	4	0.7 (0.2–2.7)		
			Ever-analysis including both population and other cancer controls	4	0.8 (0.3–2.6)		

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Christensen et al. (2013) Montreal, Canada 1979–1985 Case–control (cont.)		Stomach, incidence	Any exposure to 1,1,1-TCE, 5 yr lag (OR):			Age, census tract median income, educational attainment, ethnicity, questionnaire respondent (self vs proxy), smoking, beer, wine, and spirit intake	
			No chlorinated solvent or hydrocarbon exposure	195	1		
			Ever-analysis limited to population controls	4	1.1 (0.3–3.8)		
			Ever-analysis including both population and other cancer controls	4	1.2 (0.4–3.8)		
		Stomach, incidence	Substantial exposure to 1,1,1-TCE, 5 yr lag (OR):				
			No chlorinated solvent or hydrocarbon exposure	195	1		
			Ever-analysis limited to population controls	2	0.8 (0.2–4.3)		
			Ever-analysis including both population and other cancer controls	2	0.9 (0.2–4.4)		

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Christensen et al. (2013) Montreal, Canada 1979–1985 Case–control (cont.)		Rectum, incidence	Any exposure to 1,1,1-TCE, 5 yr lag (OR):			Age, census tract median income, educational attainment, ethnicity, questionnaire respondent (self vs proxy), smoking, beer intake	
			No chlorinated solvent or hydrocarbon exposure	192	1		
			Ever-analysis limited to population controls	2	0.4 (0.1–2.0)		
			Ever-analysis including both population and other cancer controls	2	0.4 (0.1–1.8)		
		Rectum, incidence	Substantial exposure to 1,1,1-TCE, 5 yr lag (OR):				
			No chlorinated solvent or hydrocarbon exposure	192	1		
			Ever-analysis limited to population controls	2	0.6 (0.1–3.3)		
			Ever-analysis including both population and other cancer controls	2	0.6 (0.1–3.0)		

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Christensen et al. (2013) Montreal, Canada 1979–1985 Case–control (cont.)		Melanoma, incidence	Any exposure to 1,1,1-TCE, 5 yr lag (OR):			Age, census tract median income, educational attainment, ethnicity, questionnaire respondent (self vs proxy), smoking	
			No chlorinated solvent or hydrocarbon exposure	69	1		
			Ever-analysis limited to population controls	2	0.9 (0.2–4.5)		
			Ever-analysis including both population and other cancer controls	2	0.9 (0.2–4.3)		
		Melanoma, incidence	Substantial exposure to 1,1,1-TCE, 5 yr lag (OR):				
			No chlorinated solvent or hydrocarbon exposure	69	1		
			Ever-analysis limited to population controls	1	0.5 (0.1–4.8)		
			Ever-analysis including both population and other cancer controls	1	0.6 (0.1–5.3)		

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Christensen et al. (2013) Montreal, Canada 1979–1985 Case–control (cont.)		Pancreas, incidence	Any exposure to 1,1,1-TCE, 5 yr lag (OR):			Age, census tract median income, educational attainment, ethnicity, questionnaire respondent (self vs proxy), smoking, coffee, beer, wine, and spirit intake	
			No chlorinated solvent or hydrocarbon exposure	95	1		
			Ever-analysis limited to population controls	1	0.6 (0.1–5.7)		
			Ever-analysis including both population and other cancer controls	1	0.8 (0.1–6.0)		
		Pancreas, incidence	Substantial exposure to 1,1,1-TCE, 5 yr lag (OR):				
			No chlorinated solvent or hydrocarbon exposure	95	1		
			Ever-analysis limited to population controls	1	0.8 (0.1–7.5)		
			Ever-analysis including both population and other cancer controls	1	1.1 (0.1–8.8)		

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Christensen et al. (2013) Montreal, Canada 1979–1985 Case–control (cont.)		Oesophagus, incidence	Any exposure to 1,1,1-TCE, 5 yr lag (OR):			Age, census tract median income, educational attainment, ethnicity, questionnaire respondent (self vs proxy), smoking, coffee, tea, beer, wine, and spirit intake	
			No chlorinated solvent or hydrocarbon exposure	75	1		
			Ever-analysis limited to population controls	2	1.4 (0.3–7.5)		
			Ever-analysis including both population and other cancer controls	2	1.9 (0.4–8.7)		
		Oesophagus, incidence	Substantial exposure to 1,1,1-TCE, 5 yr lag (OR):				
			No chlorinated solvent or hydrocarbon exposure	75	1		
			Ever-analysis limited to population controls	1	1.1 (0.1–10)		
			Ever-analysis including both population and other cancer controls	1	1.4 (0.2–12)		

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Christensen et al. (2013) Montreal, Canada 1979–1985 Case–control (cont.)		Liver (hepatocellular carcinoma), incidence	Any exposure to 1,1,1-TCE, 5 yr lag (OR):			Age, census tract median income, educational attainment, ethnicity, questionnaire respondent (self vs proxy), smoking, beer, wine, and spirit intake	
			No chlorinated solvent or hydrocarbon exposure	33	1		
			Ever-analysis limited to population controls	1	1.8 (0.2–17)		
			Ever-analysis including both population and other cancer controls	1	2.3 (0.3–19)		
		Liver (hepatocellular carcinoma), incidence	Substantial exposure to 1,1,1-TCE, 5 yr lag (OR):				
			No chlorinated solvent or hydrocarbon exposure	33	1		
			Ever-analysis limited to population controls	1	2.2 (0.2–22)		
			Ever-analysis including both population and other cancer controls	1	3.2 (0.4–28)		

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Vizcaya et al. (2013) Montreal, Canada 1980–1986 and 1995–2001 Case–control	Cases: 1586 (study I, *n* = 851; study II, *n* = 735); male Montreal residents (study I, aged 35–70 yr; study II, aged 35–75 yr) diagnosed with lung cancer at one of 18 local hospitals Controls: 1431 (study I, *n* = 533; study II, *n* = 898); male Montreal residents on electoral list, frequency-matched to sex and age distributions of cases Exposure assessment method: expert judgement; full work histories and specialized questionnaires, [presumed measurement data], and extensive review to assign participant-specific semiquantitative estimates of confidence, frequency and intensity for each job held	Lung, incidence	Any exposure to 1,1,1-TCE (OR):			Age, educational attainment, socioeconomic status, ethnicity, questionnaire respondent (self vs proxy), exposure to eight known carcinogens, smoking habit, study	*Exposure assessment critique*: Substantial data available for assessment including [presumably] published measurement data. Evaluation was participant-specific. Careful consideration of each job held by each participant (i.e. confidence, frequency, and intensity) is a key strength. Lifetime exposure included confidence, which is not a component of toxicity. Metrics were all categorical. *Strengths*: detailed expert-based exposure assessment; large sample size. *Limitations*: absence of quantitative exposure metrics.
			No chlorinated solvent or vinyl chloride exposure	1313	1		
			Ever	22	1.1 (0.5–2.3)		
		Lung, incidence	Substantial exposure to 1,1,1-TCE (OR):				
			No chlorinated solvent or vinyl chloride exposure	1313	1		
			Ever	13	1.1 (0.4–2.7)		

Table 2.6 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Le Cornet et al. (2017) Finland, Norway, Sweden Finland, 1988–2012; Norway, 1978–2010; Sweden, 1979–2011 Case–control	Cases: 8112; first-primary testicular germ cell tumour cases, aged 14–49 yr, captured in national cancer registries of Finland, Norway and Sweden Controls: 26 264; cancer-free men sampled from central population registries individually matched to cases (4:1 ratio) by year and country of birth Exposure assessment method: used self-reported jobs to the census and NOCCA-JEM that includes semiquantitative estimates of prevalence exposed and mean level of exposure	Testis, incidence	Maternal exposure to 1,1,1-TCE (OR):			Year, country of child's birth	*Exposure assessment critique*: NOCCA-JEM is a robust and well-developed JEM. NOCCA-JEM was normalized to the country. Prevalence and intensity were based on actual data. Could be missing exposed jobs due to 10 yr census collection. Exposure metric unclear. Authors indicate exposure is binary but then discuss none, low and high [could be based on prevalence × intensity]. *Strengths*: large sample size; opportunity to link to census records to estimate parental exposure before participants' birth; use of country-specific JEM; cancer diagnoses ascertained through linkage with national registries. *Limitations*: the use of census data for occupational titles and absence of data on industry of employment provided little information for use in exposure assessment.
			Unexposed	6937	1		
			Low	45	0.95 (0.68–1.33)		
			High	36	1.14 (0.77–1.67)		
		Testis, incidence	Paternal exposure to 1,1,1-TCE (OR):			Year, country of child's birth	
			Unexposed	7017	1		
			Low	380	1.10 (0.98–1.25)		
			High	458	1.07 (0.95–1.19)		

CI, confidence interval; HR, hazard ratio; JEM, job-exposure matrix; NOCCA, Nordic Occupational Cancer Study; NR, not reported; OR, odds ratio; ppm, parts per million; SIR, standardized incidence ratio; 1,1,1-TCE, 1,1,1-trichloroethane; yr, year; vs, versus.

which limited power and precluded more detailed analyses across exposure levels.]

In an updated mortality follow-up of 10 730 male and 3725 female civilian aircraft-maintenance workers at a United States Air Force base in a study conducted by Radican et al. (2008), a hazard ratio for bone cancer of 17.87 (95% CI, 1.12–286) among exposed versus unexposed women was observed based on a single death. However, as no bone cancer deaths were observed among male workers, it is possible that this finding is attributable to chance. [Study strengths included a long period of follow-up and the use of internal comparisons with unexposed workers to estimate relative risk. Also, the exposure assessment was performed by industrial hygienists with access to the base facilities and records. Limitations included the small number of exposed mortality end-points for 1,1,1-trichloroethane, the qualitative nature of the exposure assessment, the difficulty in linking participants to estimates often associated with no more detail than job title, and the lack of continued exposure assessment after 1982.]

Kernan et al. (1999) conducted a case–control study on occupational risk factors for pancreatic cancer using death certificate records from 24 states in the USA, with controls selected from death records unrelated to cancer or non-malignant pancreatic disease, frequency-matched on state, race, sex, and 5-year age group. Decedents' usual occupation and industry were coded from death certificates and a JEM was applied to the coded occupational data to assess potential exposure to formaldehyde and 11 chlorinated hydrocarbons, including 1,1,1-trichloroethane [referred to as methyl chloroform]. Odds ratios were estimated separately for Black women, Black men, White women, and White men using logistic regression models adjusted for age, marital status, and metropolitan and residential status. A statistically significant elevated odds ratio for pancreatic cancer mortality in relation to high probability of exposure to 1,1,1-trichloroethane (versus those never exposed to the solvent) was observed among Black men (OR, 2.9, 95% CI, 1.2–7.5; 8 exposed deaths). However, as null findings were observed for all other sex and race strata, as well as in analyses of 1,1,1-trichloroethane exposure intensity, it is possible that this finding is attributable to chance. [A strength of this study was its large sample size, although the one statistically significant finding was based on a small number of exposed deaths. Death certificates provided only a single job; other exposed jobs were likely to have been missed. No information regarding industry, duration of usual employment, or potential confounders was available.]

Christensen et al. (2013) investigated occupational exposure to 1,1,1-trichloroethane and other chlorinated solvents in relation to several cancer sites in a case–control study in male residents of Montreal, Canada. Cancers of interest in this analysis included melanoma (n = 103) and cancers of the oesophagus (n = 99), stomach (n = 251), colon (n = 496), rectum (n = 248), liver (n = 48), pancreas (n = 116), and prostate (n = 449). Participants completed a detailed in-person interview that included a semi-structured occupational history questionnaire collecting information regarding employer details, tasks performed, use of protective equipment and other workplace characteristics for each job held for at least 6 months. Interviews were conducted with proxy respondents if a participant had died or could not otherwise be interviewed. A team of industrial chemists and hygienists reviewed participants' occupational histories and translated each job into potential exposures from a list of 293 substances. Odds ratios were estimated using unconditional logistic regression in relation to two different control groups: population controls only (n = 533) and population controls combined with cases of other cancers (n = 1295 to n = 2525). All models adjusted for age, ethnicity, and socioeconomic status. Additional covariates were adjusted for depending on the cancer

type (oesophagus: smoking, coffee, tea, and alcohol intake; stomach, colon and liver: smoking, coffee, tea, and alcohol intake; rectum: smoking and beer intake; pancreas: smoking, coffee, and alcohol intake; prostate: smoking and alcohol intake). Exposures occurring in the previous 5 years were excluded due to latency considerations. For 1,1,1-trichloroethane exposure, the odds ratios for these cancers were close to the null or based on very small numbers of exposed participants. [A strength of this analysis was the detailed expert-based retrospective exposure assessment methodology. Study limitations included the absence of quantitative exposure metrics and the small case sample sizes.]

Vizcaya et al. (2013) conducted an analysis of exposure to chlorinated solvents and lung cancer risk among men, using data from two studies: the Montreal case–control study on different cancer sites analysed by Christensen et al. (2013) and a subsequent case–control study on lung cancer conducted in Montreal using a nearly identical study design and exposure assessment approach. Unconditional logistic regression was applied with adjustment for age, census median income, ethnicity, educational attainment, respondent type (self versus proxy), smoking, and exposure to occupational lung carcinogens (asbestos, crystalline silica, chromium(VI), arsenic compounds, diesel exhaust emissions, soot, wood dust, and benzo[*a*]pyrene). In the pooled analysis, exposure to 1,1,1-trichloroethane was not associated with lung cancer risk, with odds ratios of 1.1 observed for any exposure and for "substantial" exposure. [Strengths of this analysis included the detailed expert-based retrospective exposure assessment methodology and the large pooled sample size. A limitation was the absence of quantitative exposure metrics.]

Le Cornet et al. (2017) performed a registry-based case–control study on testicular germ cell tumours within three Nordic countries to investigate associations with parental occupational exposures to several organic solvents, including 1,1,1-trichloroethane, during the prenatal period. Unique personal identification codes assigned to residents of each country provided the opportunity to create linkages between cancer and other population registries, including parents' census records. Testicular cancer cases in men diagnosed between ages 14 and 49 years from 1988 to 2012 in Finland, 1978 to 2010 in Norway, and 1979 to 2011 in Sweden were selected for the study (n = 8112). Four controls randomly selected from the national population registers were individually matched on each case by year and country of birth. Job codes for the parents of each participants were retrieved from the last census conducted before the participant's birth and the first census conducted afterward. Parental occupational exposures to 1,1,1-trichloroethane and five other individual solvents were estimated using the NOCCA-JEM. Odds ratios for high exposure to 1,1,1-trichloroethane, estimated using conditional logistic regression, were close to unity for both maternal and paternal occupations (OR, 1.14; 95% CI, 0.77–1.67; and OR, 1.07; 95% CI, 0.95–1.19, respectively) versus no exposure to 1,1,1-trichloroethane). Findings were similar in sensitivity analyses restricting to solvent exposure within the year before childbirth and excluding participants exposed to other solvents. [A strength of this study was the unique opportunity within Nordic countries to create linkages across different administrative data records, which enabled the capture of census-defined parental occupations in the prenatal period of cases, and controls for exposure assessment. Other strengths included the large sample size and the availability of a well-developed country-specific JEM to enable semiquantitative assessments of exposure to 1,1,1-trichloroethane and other solvents. The use of census data for occupational titles and absence of data on industry of employment provided little information for use in exposure assessment, and the JEMs had limited ability to identify individuals with low and high exposure.]

In addition to the previously mentioned cohort and case–control studies, two case studies on biliary–pancreatic cancers diagnosed among workers exposed to 1,1,1-trichloroethane and other chemicals have also been reported. A cluster of 17 cases of cholangiocarcinoma diagnosed at a relatively young age among former and current employees of an offset proof-printing plant in Osaka, Japan, was described in a series of reports by Kumagai et al. (2013) and Kubo et al. (2014a, b). While some of the cases had been exposed to 1,1,1-trichloroethane, all shared a history of high-level, long-term exposure to 1,2-dichloropropane. A subsequent retrospective cohort study among workers employed at the same company demonstrated a strong exposure–response relation between exposure to 1,2-dichloropropane and cholangiocarcinoma (Kumagai et al., 2016). [These findings were influential in the classification of 1,2-dichloropropane as *carcinogenic to humans*, IARC Group 1, in *IARC Monographs* Volume 110 (IARC, 2016).] A small case study in the USA by Zarchy (1996), reporting on two cases of cholangiocarcinoma and ampullary carcinoma diagnosed in workers exposed to 1,1,1-trichloroethane, tetrachloroethylene, and other unspecified chemicals, provided no evidence of value towards clarifying the carcinogenicity of this agent.

2.6 Evidence synthesis for cancer in humans

The epidemiological database for this evaluation comprised two cohort studies, five nested case–control studies, and sixteen population-based case–control studies, with most of these having been published since the previous evaluations of 1,1,1-trichloroethane in *IARC Monographs* Volumes 20 and 71 (IARC, 1979, 1999). The largest number of studies examined cancers of the haematopoietic and lymphoid tissues, followed by cancers of the kidney and urinary bladder, the brain and nervous system, and the breast. There were a smaller number of studies on other cancers at other sites, including digestive tract, skin (melanoma), and cancers of the bone, lung, cervix, prostate, and testis. There were also two case studies on cholangiocarcinoma and ampullary carcinoma.

2.6.1 Studies evaluated

In the assessment of the carcinogenicity of 1,1,1-trichloroethane in humans, some studies were considered to be somewhat more informative on the basis of study quality, since they included aspects of study power, exposure assessment, potential co-exposure to other occupational agents, and confounding and selection bias (further discussed below). In some studies on 1,1,1-trichloroethane, there was a low prevalence of exposure and/or small study size, leading to very few exposed cases, and the resulting effect estimates were imprecise (Anttila et al., 1995; Infante-Rivard et al., 2005; Radican et al., 2008; Christensen et al., 2013; McLean et al., 2014). Low prevalence of exposure was a limitation observed in most studies, and lead to small numbers of exposed cases.

The Working Group determined that reports from two case studies on cholangiocarcinoma and ampullary carcinoma were uninformative for assessing the association between exposure to 1,1,1-trichloroethane and cancer and are not further discussed here (Zarchy, 1996; Kumagai et al., 2013, 2016; Kubo et al., 2014a, b).

2.6.2 Exposure assessment and misclassification of exposure

The Working Group considered that the quality of the exposure assessment was a major factor in the evaluation of epidemiological studies on the carcinogenicity of occupational exposure to 1,1,1-trichloroethane. A summary and detailed evaluation of the strengths and limitations of the

exposure assessment in previous epidemiological studies is provided in Sections 1.6.1 and 1.6.2, respectively.

Exposure assessment in cohort studies was performed using either data on biological monitoring of workers (Anttila et al., 1995) or a detailed exposure assessment approach, including review of facility records, jobs, walk-through surveys, interviews, and measurements to assign exposure status by job group (Radican et al., 2008). Although exposure was documented among monitored workers, there are concerns regarding the representativeness of measurements as well as their small number (Anttila et al., 1995). In Radican et al. (2008), there was limited information on participant job history with which to assign exposure estimates. In both studies, there were no quantitative exposure–response analyses and limitations in exposure assessment are likely to result in attenuation of disease risk towards the null.

In several large-scale nested case–control studies based in the NOCCA cohort, estimates of cumulative exposure to 1,1,1-trichloroethane were assigned on the basis of census job data using the well-developed NOCCA-JEM. However, census job data was limited to job titles captured every 10 years and ending in 1990; this may have led to non-differential misclassification of exposure history (Talibov et al., 2014, 2017, 2019; Hadkhale et al., 2017). In another nested case–control study in a population-based cohort, an expert hygienist review was conducted of exposure prevalence in the NOCCA-JEM/FINJEM based on more detailed questionnaire data captured on work tasks in recent jobs, although exposure prevalence and intensity were low in this study (Videnros et al., 2020).

Case–control studies were largely population-based, and exposure assessment ranged from studies assessing detailed participant-specific quantitative or semiquantitative estimates of exposure based on combinations of work histories, job- or task-specific modules, literature/measurement data, and expert review (Infante-Rivard et al., 2005; Miligi et al., 2006; Gold et al., 2011; Neta et al., 2012; Christensen et al., 2013; Ruder et al., 2013; Vizcaya et al., 2013; Purdue et al., 2017; Callahan et al., 2018), to studies relying on study-specific JEMs or the NOCCA-JEM/FINJEM assigned to participant lifetime job history or to the longest or most recent job(s) (Heineman et al., 1994; Dosemeci et al., 1999; Kernan et al., 1999; McLean et al., 2014; Le Cornet et al., 2017; Sciannameo et al., 2019; Pedersen et al., 2020). A weakness of JEM-based studies is the fact that the JEM does not take into account variability between workers in the same occupation, leading to limited ability to identify participants with high exposure, as well as low specificity (Dosemeci et al., 1999; Sciannameo et al., 2019). In some JEM-based studies, higher probabilities of exposure (for example of > 10% or > 25%) were applied (instead of the 5% typically used) in an attempt to improve specificity (McLean et al., 2014; Pedersen et al., 2020). JEMs were also not sex-specific.

In general, non-differential misclassification is expected to result in attenuation of risk estimates towards the null in case–control studies, with attenuation probably greater in lower-quality studies than in higher-quality studies. There may also be some degree of Berkson-type error from JEM or other group-based exposure estimation, probably resulting in a reduction in precision of the effect estimate (but not bias). Recall bias may also be present in retrospective studies based on occupational information reported when disease status is known. In some studies, interviews relied fully or partially on proxy or next-of-kin respondents, possibly leading to misclassification in occupational histories, although findings in sensitivity analysis (where performed) excluding such respondents did not materially change study findings (Heineman et al., 1994; Miligi et al., 2006; Neta et al., 2012; Christensen et al., 2013; Ruder et al., 2013). One study comparing findings using either general-population

controls or other cancer cases combined (and weighted equally) with general-population controls reported similar findings by control group (Christensen et al., 2013). In one study, jobs held for at least 2 years were captured in an attempt to minimize recall bias, although there may be misclassification of exposures in jobs held for shorter periods of time (Sciannameo et al., 2019).

There is also co-exposure to other occupational agents that may pose a carcinogenic hazard (see below). With few exceptions (Anttila et al., 1995; Dosemeci et al., 1999; Radican et al., 2008), most studies examined several quantitative or semiquantitative exposure categories such as exposure duration, intensity, probability, or cumulative exposure. Some studies assigned exposure using a lag period, ranging from approximately 3 to 20 years, in either the overall or the sensitivity analysis (Heineman et al., 1994; Gold et al., 2011; Neta et al., 2012; Christensen et al., 2013; McLean et al., 2014; Talibov et al., 2014, 2017, 2019; Hadkhale et al., 2017; Purdue et al., 2017; Sciannameo et al., 2019; Pedersen et al., 2020). The appropriate lag period for 1,1,1-trichloroethane may differ substantially according to the cancer site evaluated (e.g. in adults, latency for acute leukaemia may be much shorter than for CLL or other types of NHL). In other studies, there was little information on the timing of jobs or exposure for individual study participants (Kernan et al., 1999; Talibov et al., 2014, 2017, 2019; Hadkhale et al., 2017).

Owing to the correlated nature of exposures to several chlorinated solvents, and their interchangeable use over time, there may also be some degree of misclassification and uncertainty in the assignment of exposure to a specific solvent over time (see also below). In one study, published information was used to assign a probability of exposure that the solvent was used in a particular time period, although uncertainties remain (Gold et al., 2011).

2.6.3 Co-exposures to other occupational agents of relevance for cancer hazard identification

Although all studies assessed exposure not only to 1,1,1-trichloroethane, but also to multiple other solvents or agents with occupational exposures, few explicitly provided information on the correlation structure with exposure to such agents (Dosemeci et al., 1999; Gold et al., 2011; McLean et al., 2014; Le Cornet et al., 2017; Purdue et al., 2017; Callahan et al., 2018; Talibov et al., 2019; Pedersen et al., 2020; Videnros et al., 2020). Other solvents assessed typically included trichloroethylene (IARC Group 1, with *sufficient* evidence for kidney cancer and *limited* evidence for cancers of the liver and bile duct and for NHL other than multiple myeloma and CLL), tetrachloroethylene (Group 2A, with *limited* evidence for bladder cancer), dichloromethane (Group 2A, with *limited* evidence for cancer of the biliary tract and for NHL other than multiple myeloma and CLL) and, less often, carbon tetrachloride (Group 2B) and chloroform (Group 2B). In some studies, moderate to strong correlations between 1,1,1-trichloroethane and other occupational exposures to solvents were observed (see Table S1.6; Annex 1, Supplementary material for 1,1,1-trichloroethane, Section 1, Exposure Characterization, available from: https://publications.iarc.fr/611). There were also moderate to strong correlations with exposure to other occupational agents (i.e. metals such as chromium, nickel, and lead; and welding fumes, a Group 1 carcinogen with *sufficient* evidence for lung cancer and *limited* evidence for kidney cancer). It may therefore be difficult to distinguish the agent responsible for any positive association observed, depending on the cancer site, and there may be confounding by other occupational exposures (see also below). In Anttila et al. (1995), workers were typically monitored for exposure to one solvent only. As noted above, due to interchanges in the occupational use of solvents over time, there

may also be some degree of misclassification and uncertainty in the assignment of exposures to specific solvents over the study period (Radican et al., 2008; Gold et al., 2011). Owing to the multiple solvents or agents assessed for occupational exposure in each study, multiple testing is also of concern. There was no information on chemicals added to 1,1,1-trichloroethane as stabilizers or solvents, or other impurities, in epidemiological studies described here.

2.6.4 Confounding, selection bias, and outcome measurement error

Studies generally adjusted in their design and/or analysis for personal data, such as age, sex, race, or education, which were usually captured from personal interviews; registry or death certificate-based studies adjusted for fewer personal data. Some studies adjusted for other known cancer site-specific risk factors, such as reproductive factors for breast cancer (Pedersen et al., 2020; Videnros et al., 2020) and hypertension for kidney cancer (Dosemeci et al., 1999; Purdue et al., 2017). For several other studies, there was no information available on other personal or lifestyle factors to control for their potentially confounding effects. For example, although Hadkhale et al. (2017) adjusted for a range of occupational agents in their analysis of 1,1,1-trichloroethane and cancer of the urinary bladder, no data were available on cigarette smoking or other personal factors within the linked population registers used in that study. For census-based studies in particular, limited data were available on other potential risk factors, including exposure to other occupational carcinogens. For studies on some other cancer sites for which there are fewer known risk factors, there is less concern regarding potential residual confounding (e.g. brain and nervous system tumours, bone cancer, multiple myeloma).

The potential for selection bias is expected to be minimal in the large-scale NOCCA-based studies, given their composition of records from comprehensive national registries of all residents in the Nordic countries participating in decennial population censuses, and diagnoses from nation-wide registries of cancer incidence. Some case–control studies had low participation rates, particularly among proxy or next-of-kin controls, possibly leading to some degree of selection bias and underrepresentation of exposed controls (Heineman et al., 1994; Gold et al., 2011; Purdue et al., 2017; Callahan et al., 2018). There are also concerns regarding potential selection or other methodological sources of bias in some hospital-based studies (Neta et al., 2012; Sciannameo et al., 2019), as well as in studies in which consistently inverse associations were observed (Miligi et al., 2006; Neta et al., 2012; Ruder et al., 2013). The selection of workers for monitoring in the study by Anttila et al. (1995) was not clear. There may be some degree of survival bias in studies excluding large proportions of deceased cases (Dosemeci et al., 1999; Gold et al., 2011).

In most studies, case identification was comprehensive and of high quality. In several studies, cancer cases were identified from comprehensive, population-wide cancer registry or surveillance systems (Anttila et al., 1995; Dosemeci et al., 1999; Gold et al., 2011; Talibov et al., 2014, 2017, 2019; Hadkhale et al., 2017; Le Cornet et al., 2017; Purdue et al., 2017; Callahan et al., 2018; Pedersen et al., 2020; Videnros et al., 2020). Other studies used extensive hospital- or treatment centre-based recruitment (Infante-Rivard et al., 2005; Miligi et al., 2006; Neta et al., 2012; Christensen et al., 2013; Ruder et al., 2013; Vizcaya et al., 2013; McLean et al., 2014). In one study, the representativeness of included cases was unclear (Sciannameo et al., 2019). In other studies, case identification was based on death certificates or death registries (Heineman et al., 1994; Kernan et al., 1999; Radican et al., 2008). Heineman et al. (1994) confirmed cause of death with hospital diagnostic records.

2.6.5 Cancers of the haematopoietic and lymphoid tissues

Two cohort studies (Anttila et al., 1995; Radican et al., 2008), two nested case–control studies (Talibov et al., 2014, 2017), and five case–control studies (Infante-Rivard et al., 2005; Miligi et al., 2006; Gold et al., 2011; Christensen et al., 2013; Callahan et al., 2018) investigated the association between haematopoietic and lymphatic malignancies and exposure to 1,1,1-trichloroethane.

Findings from studies on NHL showed no clear association with exposure to 1,1,1-trichloroethane. Anttila et al. (1995) reported a positive although imprecise standardized incidence ratio based on a single exposed case. Radican et al. (2008) reported a weak positive but non-statistically significant association between ever exposure to 1,1,1-trichloroethane and mortality attributable to NHL in men (HR, 1.51; 95% CI, 0.61–3.73; 12 exposed cases). There were no deaths from NHL among exposed women. Co-exposure with other organic solvents having suggested associations with NHL (i.e. trichloroethylene and dichloromethane) in this study remains of concern. Talibov et al. (2017) reported odds ratios close to unity for incident cases of NHL (CLL) in both men and women (1416 and 143 exposed cases, respectively) among categories of cumulative exposure based on the NOCCA-JEM in a large-scale nested case–control study, with no evidence for a trend. Among the case–control studies, Miligi et al. (2006) reported odds ratios of < 1.0 for incident cases of NHL in association with expert-derived categories of low-very low (15 exposed cases), or medium-high intensity of exposure (5 exposed cases). Only jobs held for at least 5 years and for more than 5 years before diagnosis were considered. Christensen et al. (2013) reported no association between NHL and any or substantial exposure to 1,1,1-trichloroethane; there were few exposed cases. Callahan et al. (2018) reported no association between NHL and categories of exposure probability or cumulative hours of exposure to 1,1,1-trichloroethane in a case–control study with detailed individual exposure assessments (565 exposed cases).

Among studies on multiple myeloma, some positive although sometimes imprecise associations were observed in the available studies. Anttila et al. (1995) reported a significant positive standardized incidence ratio for multiple myeloma, in men and women combined, of 15.98 (95% CI, 1.93–57.7; 2 exposed cases). Radican et al. (2008) reported a significant positive association between ever exposure to 1,1,1-trichloroethane and mortality attributable to multiple myeloma in women (HR, 14.46; 95% CI, 3.24–64.63; 3 exposed cases), but not in men (HR, 0.64; 95% CI, 0.18–2.30; 4 exposed cases); an overall HR for the cohort was not estimated. Gold et al. (2011), in a case–control study including 180 incident cases and 481 controls, reported a significant positive association between ever exposure to 1,1,1-trichloroethane and multiple myeloma (OR, 1.8; 95% CI, 1.1–2.9; 36 exposed cases). The association remained in a sensitivity analysis that assigned jobs with low confidence in the assessment to the referent (unexposed) category (OR, 2.2; 95% CI, 1.1–4.4; 17 exposed cases). Odds ratios were elevated across most categories of exposure duration, unlagged cumulative exposure, and cumulative exposure with a 10-year lag, although no evidence of a positive trend with increasing exposure category was observed. The limitations of this study included the small numbers of exposed participants, misclassification and uncertainties in the assignment of correlated chlorinated solvent exposures, potential survival bias, and selection bias, possibly resulting in some bias in the findings observed.

There was no clear association between maternal prenatal exposure to 1,1,1-trichloroethane and incident cases of childhood ALL; odds ratios were elevated although imprecise (Infante-Rivard et al., 2005). There was also no association between exposure to 1,1,1-trichloroethane

and incident cases of adult AML in a large-scale study, with odds ratios of < 1.0 observed in all categories of cumulative exposure, based on 896 exposed cases (Talibov et al., 2014).

Overall, the Working Group considered that in the body of available evidence, a positive association between exposure to 1,1,1-trichloroethane and multiple myeloma was credible; however, associations were imprecise in two cohort studies and, to a lesser extent, in one case–control study. The small numbers of exposed participants, potential misclassification in exposure assessment, and potential selection bias were further limitations of these studies. The available studies in humans were not sufficiently informative to permit a conclusion to be drawn about the presence of a causal association between exposure to 1,1,1-trichloroethane and NHL, AML, or childhood ALL.

2.6.6 Cancers of the brain and nervous system

One retrospective cohort study (Anttila et al., 1995) and four case–control studies (Heineman et al., 1994; Neta et al., 2012; Ruder et al., 2013; McLean et al., 2014) evaluated the association between 1,1,1-trichloroethane exposure and cancers of the brain and nervous system.

Overall, there was no clear association between cancers of the brain and nervous system and exposure to 1,1,1-trichloroethane. Although Heineman et al. (1994) observed an elevated odds ratio for astrocytoma in the highest category of exposure duration (all probabilities combined) (OR, 1.8; 95% CI, 1.0–3.3; 38 exposed cases) and a significant trend compared with the unexposed (P < 0.05) in a death certificate-based study, there were no clear associations with categories of cumulative exposure or exposure probability. Limitations in exposure assessment, next-of-kin interviews, and small numbers of exposed cases reduced the informativeness of the study. Anttila et al. (1995) observed a significantly elevated standardized incidence ratio for cancer of the nervous system (SIR, 6.05; 95% CI, 1.25–17.7) based on 3 exposed cases. Neta et al. (2012) reported no evidence of an increased risk of incident glioma with categories of exposure probability, duration, cumulative exposure, average weekly exposure, or highest exposure to 1,1,1-trichloroethane using a detailed individual exposure assessment approach. For meningioma, there was a non-significantly elevated odds ratio for probable exposure to 1,1,1-trichloroethane (OR, 2.3; 95% CI, 0.7–7.2) based on 5 exposed cases. McLean et al. (2014) observed no clear association between ever exposure to 1,1,1-trichloroethane and meningioma risk (based on 1 exposed case). There also was no evidence of an increased risk of incident glioma with exposure to 1,1,1-trichloroethane (overall or across categories of cumulative exposure) in Ruder et al. (2013), with odds ratios significantly lower than 1.0 observed, possibly due to selection or other methodological sources of bias.

Overall, the Working Group considered that the available studies in humans were not sufficiently informative to permit a conclusion to be drawn about the presence of a causal association between exposure to 1,1,1-trichloroethane and cancers of the brain and nervous system.

2.6.7 Cancer of the breast

One cohort study (Radican et al., 2008), one nested case–control study (Videnros et al., 2020), and one population-based case–control study (Pedersen et al., 2020) evaluated the association between exposure to 1,1,1-trichloroethane and breast cancer in women. There was one nested case–control study on breast cancer in men (Talibov et al., 2019).

Findings from studies on breast cancer in women showed no association with exposure to 1,1,1-trichloroethane. A positive although imprecise association (HR, 2.35; 95% CI, 0.83–6.64) based on only 4 exposed cases was observed

in the cohort study by Radican et al. (2008). Videnros et al. (2020) observed no association between ever exposure to 1,1,1-trichloroethane and incidence of post-menopausal breast cancer on the basis of expert hygienist review of exposure prevalence in the NOCCA-JEM/FINJEM; there were 10 exposed cases. There was also no trend with categories of exposure duration or mean exposure intensity, although levels of exposure intensity were low. Pedersen et al. (2020) observed no association between ever exposure to 1,1,1-trichloroethane based on the NOCCA-JEM and incident breast cancer risk by age group (< 50 and ≥ 50 years, including 98 and 158 exposed cases, respectively) overall or by categories of duration of exposure, cumulative exposure, or latency among women, or timing of first exposed job among parous women. There were also no clear associations according to tumour estrogen receptor or parity status.

There was also no evidence of an association between breast cancer in men and exposure to 1,1,1-trichloroethane. Talibov et al. (2019) reported no association with NOCCA-JEM-based categories of ever exposure or cumulative exposure in a large-scale nested case–control study, based on 181 exposed cases. Data on occupational history were limited, and there were few highly exposed participants.

Overall, the Working Group considered that the available studies in humans were not sufficiently informative to permit a conclusion to be drawn about the presence of a causal association between exposure to 1,1,1-trichloroethane and risk of breast cancer in either women or men.

2.6.8 Cancers of the kidney and urinary bladder

One retrospective cohort study (Anttila et al., 1995) and three case–control studies (Dosemeci et al., 1999; Christensen et al., 2013; Purdue et al., 2017) evaluated the association between exposure to 1,1,1-trichloroethane and kidney cancer. Findings from studies on kidney cancer showed no association with 1,1,1-trichloroethane. Anttila et al. (1995) observed no cases of kidney cancer (compared with 0.4 expected). Dosemeci et al. (1999) observed no association between ever exposure to 1,1,1-trichloroethane and incidence of renal cell carcinoma, either overall (66 exposed cases) or by sex. There was the potential for survival bias, given the exclusion of 35% of deceased cases from the analysis. There were no clear associations with kidney cancer incidence in Christensen et al. (2013); odds ratios were weakly elevated although imprecise, based on 4 exposed cases. Purdue et al. (2017), using a detailed expert-based exposure assessment approach, observed no association between incidence of kidney cancer and categories of probability or cumulative hours of exposure (610 exposed cases). Although there was a positive non-significant odds ratio (1.6; 95% CI, 0.8–3.2) in the highest tertile of cumulative hours of exposure among high-intensity jobs, there were few exposed cases (n = 21) and no evidence for a trend. Potential selection bias from low participation rates among controls, and occupational co-exposure to other solvents that cause kidney cancer are also of concern.

One nested case–control study (Hadkhale et al., 2017) and two case–control studies (Christensen et al., 2013; Sciannameo et al., 2019) evaluated the association between exposure to 1,1,1-trichloroethane and cancer of the urinary bladder. Findings from studies on bladder cancer showed no clear association with 1,1,1-trichloroethane. In a large-scale study, Hadkhale et al. (2017) reported no association with NOCCA-JEM-based categories of cumulative exposure, both overall and by sex or age group, after adjustment for a range of other occupational solvents and agents. The study was large (7874 exposed cases) but limited census-based data on occupational history were available. Christensen et al. (2013) reported imprecise inverse associations, based on 5 exposed cases. Although Sciannameo

et al. (2019) observed a weakly positive non-significant association between ever exposure to 1,1,1-trichloroethane based on FINJEM estimates and incidence of urinary bladder cancer (OR, 1.18; 95% CI, 0.96–1.46, 362 exposed cases), potential selection bias and limitations in exposure assessment remain of concern.

Overall, the Working Group considered that the available studies in humans were not sufficiently informative to permit a conclusion to be drawn about the presence of a causal association between exposure to 1,1,1-trichloroethane and cancers of the kidney or urinary bladder.

2.6.9 Cancers of the digestive, respiratory, or genital tract, and other solid cancers

Two cohort studies (Anttila et al., 1995; Radican et al., 2008) and four case–control studies (Kernan et al., 1999; Christensen et al., 2013; Vizcaya et al., 2013; Le Cornet et al., 2017) evaluated the association between exposure to 1,1,1-trichloroethane and cancers of the digestive, respiratory, and genital tract, or other solid cancers.

Anttila et al. (1995) reported a positive but imprecise association between biologically monitored 1,1,1-trichloroethane and total cancer incidence (SIR, 1.58; 95% CI, 0.92–2.52; 17 exposed cases). There were few exposed cases for cancer at other sites (Anttila et al., 1995; Radican et al., 2008). Study weaknesses including the small number of exposed cases, limited monitoring data, and potential co-exposure to other occupational solvents are of concern. Kernan et al. (1999) in a death certificate-based study reported a significantly elevated odds ratio (2.9; 95% CI, 1.2–7.5; 8 exposed cases) for mortality attributable to pancreatic cancer among Black males with a high probability of exposure; however, there were no positive associations in other sex/race strata or according to intensity of exposure. There are also limitations in exposure assessment in the death certificate-based study. Christensen et al. (2013) and Vizcaya et al. (2013) reported no clear association between ever exposure (any or substantial) to 1,1,1-trichloroethane exposure and several cancer types, including melanoma and cancers of the prostate, colon, stomach, rectum, pancreas, oesophagus, liver, and lung; there were few exposed cases. Le Cornet et al. (2017) reported odds ratios close to unity in a large-scale registry-based study; they included semi-quantitative categories of prenatal maternal and paternal occupational exposure to 1,1,1-trichloroethane in the year of or before birth, and testicular germ cell tumours in the child.

Overall, the Working Group considered that the few available studies in humans were not sufficiently informative to permit a conclusion to be drawn about the presence of a causal association between exposure to 1,1,1-trichloroethane and cancers of the digestive, respiratory, or genital tract, or other solid cancers.

3. Cancer in Experimental Animals

See Table 3.1.

3.1 Mouse

3.1.1 Inhalation

In a well-conducted chronic toxicity and carcinogenicity study that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female Crj:BDF_1 mice (age, 6 weeks) were exposed by inhalation (whole-body exposure) to 1,1,1-trichloroethane (purity, >95%; one of the impurities was identified as *para*-dioxane [1,4-dioxane], present at 3.34–3.50%) at a concentration of 0, 200, 800, or 3200 ppm for the control group, and the groups at the lowest, intermediate, and highest doses, respectively, for 6 hours per day, 5 days per week, for 104 weeks (Ohnishi et al., 2013). In the group of male mice at the highest dose, the survival rate was slightly

Table 3.1 Studies of carcinogenicity in experimental animals exposed to 1,1,1-trichloroethane

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, Crj:BDF_1 (M) 6 wk 104 wk Ohnishi et al. (2013)	Inhalation (whole-body exposure) 1,1,1-Trichloroethane, > 95% (impurity, 1,4-dioxane ranging from 3.34% to 3.50%) Air 0, 200, 800, 3200 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 40, 34, 34, 31	*Lung* Bronchioloalveolar adenoma 4/50, 8/50, 4/50, 1/50 Bronchioloalveolar carcinoma 3/50, 5/50, 6/50, 10/50 Bronchioloalveolar adenoma or carcinoma (combined) 7/50, 13/50, 10/50, 11/50 *Liver* Hepatocellular adenoma 10/50, 8/50, 12/50, 15/50 Hepatocellular carcinoma 14/50, 12/50, 10/50, 15/50 Hepatocellular adenoma or carcinoma (combined) 23/50, 19/50, 21/50, 26/50 *Spleen*: malignant lymphoma 3/50 (6%), 4/50 (8%), 3/50 (6%), 9/50 (18%) *Harderian gland*: adenoma 1/50 (2%), 4/50 (8%), 4/50 (8%), 8/50 (16%)*	 NS $P < 0.01$, Peto trend test $P < 0.05$, Peto trend test $P < 0.05$, Peto trend test NS NS $P < 0.01$, Peto trend test $P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test	Principal strengths: males and females used; multiple doses used; adequate duration of exposure and observation; well-conducted GLP study; adequate number of mice per group Historical controls: spleen lymphoma, 24/597 (4%); range, 2–8%; Harderian gland adenoma, 30/598 (5%); range, 2–10%

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, Crj:BDF$_1$ (F) 6 wk 104 wk Ohnishi et al. (2013)	Inhalation (whole-body exposure) 1,1,1-Trichloroethane, > 95% (impurity, 1,4-dioxane ranging from 3.34% to 3.50%) Air 0, 200, 800, 3200 ppm 6 h/day, 5 days/wk 50, 48, 50, 49 29, 28, 29, 29	*Lung* Bronchioloalveolar adenoma 0/50, 0/48, 0/50, 5/49 (10.2%) Bronchioloalveolar carcinoma 1/50, 3/48, 1/50, 2/49 Bronchioloalveolar adenoma or carcinoma (combined) 1/50 (2%), 3/48 (6%), 1/50 (2%), 7/49 (14%)* *Liver* Hepatocellular adenoma 2/50 (4%), 9/48 (19%)*, 14/50 (28%)**, 19/49 (39%)** Hepatocellular carcinoma 2/50 (4%), 1/48 (2%), 2/50 (4%), 1/49 (2%) Hepatocellular adenoma or carcinoma (combined) 4/50 (8%), 10/48 (20%), 16/50 (32%)*, 20/49 (40%)**	 $P < 0.01$, Peto trend test NS $P < 0.01$, Peto trend test; *$P < 0.05$, Fisher exact test $P < 0.01$, Peto trend test; *$P < 0.05$ and **$P < 0.01$, Fisher exact test NS $P < 0.01$, Peto trend test; *$P < 0.05$ and **$P < 0.01$, Fisher exact test.	Principal strengths: males and females used; multiple doses used; adequate duration of exposure and observation; well-conducted GLP study; adequate number of mice per group Historical controls: bronchioloalveolar adenoma, 23/599 (3.8%); range, 0.0–10.0%; bronchioloalveolar adenoma or carcinoma (combined), 40/599 (6.7%); range, 2.0–12.0%; hepatocellular adenoma, 29/599 (4.8%); range, 2.0–10.0%; hepatocellular adenoma or carcinoma (combined), 40/599 (6.7%); range, 2.0–12.0%; hepatocellular carcinoma, 12/599 (0.2%); range, 0.0–4.0%
Full carcinogenicity Mouse, B6C3F$_1$ (M) 5–6 wk 24 mo Quast et al. (1988)	Inhalation (whole-body exposure) 1,1,1-Trichloroethane, ~94% (5% stabilizers (butylene oxide, *tert*-amyl alcohol, methyl butynol, nitroethane, and nitromethane), and < 1% minor impurities) Air 0, 150, 500, 1500 ppm (equivalent to 0, 0.82, 2.73, or 8.19 mg/L in air); 6 h/day, 5 days/wk (except holidays) 50, 50, 50, 50 NR	*Lacrimal/Harderian gland*: adenoma or cystadenoma (combined) 8/50, 8/49, 5/50, 4/50	 NS	Principal strengths: males and females used; adequate duration of exposure and observation; multiple doses used; well-conducted study; adequate number of mice per group Principal limitations: number of mice at study termination was not reported Other comments: no effect of treatment on survival [range, 40–70% across groups; read from Figure]

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 5–6 wk 24 mo Quast et al. (1988)	Inhalation (whole-body exposure) 1,1,1-Trichloroethane, ~94% (5% stabilizers (butylene oxide, *tert*-amyl alcohol, methyl butynol, nitroethane, and nitromethane), and < 1% minor impurities) Air 0, 150, 500, 1500 ppm (equivalent to 0, 0.82, 2.73, or 8.19 mg/L in air); 6 h/day, 5 days/wk (except holidays) 50, 50, 50, 50 NR	*Lacrimal/Harderian gland* Adenoma 0/50, 0/50, 0/50, 1/50 Cystadenoma 3/50, 1/50, 2/50, 6/50 Adenoma or cystadenoma (combined) 3/50, 1/50, 2/50, 7/50	 NS NS $P < 0.05$, Cochran–Armitage trend test (one-sided)	Principal strengths: males and females used; adequate duration of exposure and observation; multiple doses used; well-conducted study; adequate number of mice per group Principal limitations: number of mice at study termination was not reported No effect of treatment on survival [range, 55–70% across groups; read from Figure]
Full carcinogenicity Mouse, B6C3F$_1$ (M) 5 wk 90 wk NTP (1977)	Oral administration (gavage) 1,1,1-Trichloroethane, technical grade, ~95% (3% *para*-dioxane [1,4-dioxane] and 2% minor impurities, probably 1,1-dichloroethane and 1,1 dichloroethylene) Corn oil 0, 2807, 5615 mg/kg bw (TWA) 5 days/wk for 78 wk 20, 50, 50 2, 15, 11	*Liver* Hepatocellular adenoma 0/15, 0/47, 3/49 Hepatocellular carcinoma 0/15, 0/47, 1/49 Hepatocellular adenoma, hepatocellular carcinoma, or neoplastic nodule (combined) 0/15, 0/47, 4/49	 NS NS $P < 0.05$, Cochran–Armitage trend test	Principal limitations: limited size of control group; decreased survival rate at the higher dose

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 5 wk 90–91 wk NTP (1977)	Oral administration (gavage) 1,1,1-Trichloroethane, technical grade, ~95% (3% *para*-dioxane [1,4-dioxane] and 2% minor impurities, probably 1,1-dichloroethane and 1,1 dichloroethylene) Corn oil 0, 2807, 5615 mg/kg bw (TWA) 5 days/wk for 78 wk 20, 50, 50 11, 23, 13	No significant increase in tumour incidence in treated animals		Principal limitations: limited size of control group; decreased survival rate at the higher dose Other comments: histopathological evaluation of 18 controls, 48 mice at the lower dose, and 50 at the higher dose
Full carcinogenicity Rat, F344/DuCrj (M) 6 wk 104 wk Ohnishi et al. (2013)	Inhalation (whole-body exposure) 1,1,1-Trichloroethane, > 95% (impurity, 1,4-dioxane ranging from 3.34% to 3.50%) Air 0, 200, 800, 3200 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 34, 36, 36, 28	*Peritoneum*: mesothelioma 1/50 (2%), 2/50 (4%), 1/50 (2%), 16/50 (32%)* *Lung*: bronchioloalveolar adenoma 0/50, 1/50 (2%), 7/50 (14%)*, 4/50 (8%)	$P < 0.01$, Peto trend test; *$P < 0.01$, Fisher exact test $P < 0.05$, Peto trend test; *$P < 0.05$, Fisher exact test.	Principal strengths: males and females used; multiple doses used; adequate duration of exposure and observation; well-conducted GLP study; adequate number of rats per group Historical controls: peritoneum mesothelioma, 17/649 (2.6%); range, 0–8%; bronchioloalveolar adenoma, 16/649 (2.5%); range, 0–6%
Full carcinogenicity Rat, F344/DuCrj (F) 6 wk 104 wk Ohnishi et al. (2013)	Inhalation (whole-body exposure) 1,1,1-Trichloroethane, > 95% (impurity, 1,4-dioxane ranging from 3.34% to 3.50%) Air 0, 200, 800, 3200 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 38, 38, 42, 38	No significant increase in tumour incidence in treated animals		Principal strengths: males and females used; multiple doses used; adequate duration of exposure and observation; well-conducted GLP study; adequate number of rats per group

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344 (M) 4–6 wk 24 mo Quast et al. (1988)	Inhalation (whole-body exposure) 1,1,1-Trichloroethane, ~94% (5% stabilizers (butylene oxide, *tert*-amyl alcohol, methyl butynol, nitroethane, and nitromethane), and < 1% minor impurities) Air 0, 150, 500, 1500 ppm (equivalent to 0, 0.82, 2.73, or 8.19 mg/L in air); 6 h/day, 5 days/wk (except holidays) 50, 50, 50, 50 NR	*Testis* Interstitial cell tumour, benign, unilateral 7/50, 11/50, 3/50, 4/50 Interstitial cell tumour, benign, bilateral 36/50, 30/50, 38/50, 45/50 Interstitial cell tumour, benign, unilateral or bilateral (combined) 43/50, 41/50, 41/50, 49/50	NS $P = 0.02$, Cochran–Armitage trend test (two-sided) NS	Principal strengths: males and females used; adequate duration of exposure and observation; multiple doses used; well-conducted study; adequate number of rats per group Principal limitations: number of rats at study termination was not reported Other comments: no effect of treatment on survival [range, 50–70% across groups, read from Figure]
Full carcinogenicity Rat, F344 (F) 4–6 wk 24 mo Quast et al. (1988)	Inhalation (whole-body exposure) 1,1,1-Trichloroethane, ~94% (5% stabilizers (butylene oxide, *tert*-amyl alcohol, methyl butynol, nitroethane, and nitromethane), and < 1% minor impurities) Air 0, 150, 500, 1500 ppm (equivalent to 0, 0.82, 2.73, or 8.19 mg/L in air); 6 h/day, 5 days/wk (except holidays) 50, 50, 50, 50 NR	No significant increase in tumour incidence in treated animals		Principal strengths: males and females used; adequate duration for exposure and observation; multiple doses used; well-conducted study; adequate number of rats per group Principal limitations: number of rats at study termination was not reported No effect of treatment on survival [range, 35–55% across groups, read from Figure]; 50 rats per group were evaluated histopathologically; the body weight of females at the intermediate and highest dose decreased compared with controls

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Osborne-Mendel (M) 7 wk 110 wk NTP (1977)	Oral administration (gavage) 1,1,1-Trichloroethane, technical grade, ~95% (3% *para*-dioxane [1,4-dioxane] and 2% minor impurities, probably 1,1-dichloroethane and 1,1 dichloroethylene) Corn oil 0, 750, 1500 mg/kg bw 5 days/wk for 78 wk 20, 50, 50 0, 0, 0	No significant increase in tumour incidence in treated animals		Principal limitations: limited size of control group; survival rate of both treated groups decreased compared with controls Histopathological evaluation of 20 controls, 49 rats at the lower dose, and 50 at the higher dose
Full carcinogenicity Rat, Osborne-Mendel (F) 7 wk 110 wk NTP (1977)	Oral administration (gavage) 1,1,1-Trichloroethane, technical grade, ~95% (3% *para*-dioxane [1,4-dioxane] and 2% minor impurities, probably 1,1-dichloroethane and 1,1 dichloroethylene) Corn oil 0, 750, 1500 mg/kg bw 5 days/wk for 78 wk 20, 50, 50 3, 2, 1	No significant increase in tumour incidence in treated animals		Principal limitations: limited size of control group; survival rate of both treated groups decreased compared with controls Other comments: histopathological evaluation of 20 control, 50 low-dose, and 50 high-dose animals

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) 7 wk ≤ 141 wk Maltoni et al. (1986)	Oral administration (gavage) 1,1,1-Trichloroethane, technical grade, ≥ 95% (stabilizers and impurities: 3.8% 1,4-dioxane, 0.47% 1,2-epoxybutane, 0.27% nitromethane, and < 1% minor impurities) Olive oil 0, 500 mg/kg bw 4–5 days/wk for 104 wk 50, 40 0, 0	*All organs*: "leukaemias" 3/50, 9/40*	* [$P < 0.05$, Fisher exact test]	Principal strengths: None Principal limitations: only one dose group. Other comments: all rats were allowed to survive until spontaneous death (≤ 141 wk); "leukaemias" included lymphoblastic lymphosarcomas, lymphoid leukaemias, immunoblastic lymphosarcomas and reticulohistiocytosarcomas
Full carcinogenicity Rat, Sprague-Dawley (F) 7 wk ≤ 141 wk Maltoni et al. (1986)	Oral administration (gavage) 1,1,1-Trichloroethane, technical grade, ≥ 95% (stabilizers and impurities: 3.8% 1,4-dioxane, 0.47% 1,2-epoxybutane, 0.27% nitromethane, and < 1% minor impurities) Olive oil 0, 500 mg/kg bw 4–5 days/wk for 104 wk 50, 40 0, 0	*All organs*: "leukaemias" 1/50, 4/40	[NS]	Principal limitations: only one dose group. Other comments: all rats were allowed to survive until spontaneous death (≤ 141 wk); "leukaemias" included lymphoblastic lymphosarcomas, lymphoid leukaemias, immunoblastic lymphosarcomas and reticulohistiocytosarcomas

bw, body weight; F, female; GLP, Good Laboratory Practice; M, male; mo, month; NR, not reported; NS, not significant; ppm, parts per million; TWA, time-weighted average; wk, week.

lower than that in the control group. The survival rate in all other groups of males exposed to 1,1,1-trichloroethane and all groups of exposed females was similar to that for their respective controls. At study termination, survival was 40/50, 34/50, 34/50, and 31/50 in males, and 29/50, 28/48, 29/50, and 29/49 in females, for the control group and the groups at the lowest, intermediate, and highest dose, respectively. The body weights of male and female mice exposed to 1,1,1-trichloroethane were similar to those of their respective controls. All mice underwent complete necropsy (except for two females at the lowest dose and one female at the highest dose). All organs and tissues from all the animals were sampled for histopathological examination.

In male mice, there was a significant positive trend in the incidence of bronchioloalveolar carcinoma and of bronchioloalveolar adenoma or carcinoma (combined) ($P < 0.01$ and $P < 0.05$, respectively, Peto test). There was a significant positive trend in the incidence of hepatocellular adenoma in male mice ($P < 0.05$, Peto test). A significant positive trend in the incidence of malignant lymphoma in the spleen ($P < 0.01$, Peto test) was observed: control, 3/50 (6%); lowest dose, 4/50 (8%); intermediate dose, 3/50 (6%); and highest dose, 9/50 (18%). The incidence of malignant lymphoma in the spleen in male mice at the highest dose exceeded the upper bound of the range observed in historical controls in this laboratory: 24/597 (4%); range, 2–8%. A significant positive trend in the incidence of Harderian gland adenoma ($P < 0.01$, Peto test) was also observed in male mice, with incidence being significantly increased at the highest dose – control, 1/50 (2%); lowest dose, 4/50 (8%); intermediate dose, 4/50 (8%); and highest dose, 8/50 (16%); $P < 0.05$, Fisher exact test – and exceeding the upper bound of the range observed in historical controls in this laboratory: 30/598 (5%); range, 2–10%.

In female mice, inhalation of 1,1,1-trichloroethane caused a significant positive trend in the incidence of bronchioloalveolar adenoma and bronchioloalveolar adenoma or carcinoma (combined) (both $P < 0.01$, Peto test). The incidence of bronchioloalveolar adenoma at the highest dose exceeded the upper bound of the range observed in historical controls in this laboratory (23/599, 3.8%; range, 0–10%). The incidence of bronchioloalveolar adenoma or carcinoma (combined) was significantly increased at the highest dose – control, 1/50 (2%); lowest dose, 3/48 (6%); intermediate dose, 1/50 (2%); and highest dose, 7/49 (14%) ($P < 0.05$, Fisher exact test) – exceeding the upper bound of the range observed in historical controls in this laboratory (40/599, 6.7%; range, 2–12%). A significant positive trend in the incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) (both $P < 0.01$, Peto test) was observed; with the incidence of hepatocellular adenoma being significantly increased at all doses (lowest dose, $P < 0.05$; and intermediate and highest dose, $P < 0.01$, Fisher exact test) and the incidence of hepatocellular adenoma or carcinoma (combined) being significantly increased at the intermediate and highest doses ($P < 0.05$ and $P < 0.01$, respectively; Fisher exact test). The incidence of hepatocellular adenoma – control, 2/50 (4%); lowest dose, 9/48 (18%); intermediate dose, 14/50 (28%); and highest dose, 19/49 (38%) – and of hepatocellular adenoma or carcinoma (combined) – control, 4/50 (8%); lowest dose, 10/48 (20%); and intermediate dose, 16/50 (32%); and highest dose, 20/49 (40%) – in all treated groups exceeded the upper bound of the range observed in historical controls in this laboratory: 29/599 (4.8%); range, 2–10%; and 40/599 (6.7%); range, 2–12%, respectively. No significant increase in the incidence of hepatocellular carcinoma was observed (control, 2/50; lowest dose, 1/48; intermediate dose, 2/50; highest dose, 1/49). [The Working Group noted the lack of a significant positive trend or a significant increase in the incidence of hepatocellular carcinoma in any of the treated groups compared with controls, making the contribution of the

hepatocellular carcinomas to the increased incidence of hepatocellular adenoma or carcinoma (combined) negligible.]

Regarding non-neoplastic lesions, none that were related to treatment with 1,1,1-trichloroethane were observed in males or females. [The Working Group noted that this was a well-conducted study that complied with GLP, males and females were used, the durations of exposure and observation were adequate, and an adequate number of animals per group and multiple doses were used.]

In another well-conducted study, groups of 50 male and 50 female B6C3F$_1$ mice (age, 5–6 weeks) were exposed by inhalation (whole-body exposure) to 1,1,1-trichloroethane (purity, ~94%; 5% stabilizers and < 1% minor impurities) at a concentration of 0, 150, 500, or 1500 ppm, for the control group and groups at the lowest, intermediate, and highest dose, respectively, for 6 hours per day, 5 days per week (except holidays), for 24 months (Quast et al., 1988). The survival rates of all groups of males and females exposed to 1,1,1-trichloroethane were similar to those of their respective control groups. [The Working Group noted that the number of animals at study termination was not reported.] The body weights of all groups of male and female mice exposed to 1,1,1-trichloroethane were similar to those of their respective controls. All mice underwent complete necropsy. Histopathological evaluation was performed on the main tissues and organs.

In female mice, a significant positive trend in the incidence of lacrimal/Harderian gland adenoma or cystadenoma (combined) was observed ($P < 0.05$, Cochran–Armitage trend test). In male mice, 1,1,1-trichloroethane had no significant effects on the incidence of tumours. Regarding non-neoplastic lesions, no effects related to exposure to 1,1,1-trichloroethane were observed in male or female mice. [The Working Group noted that this was a well-conducted study, males and females were used, the durations of exposure and observation were adequate, and an adequate number of animals per group and multiple doses were used.]

3.1.2 Oral administration (gavage)

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 5 weeks) were treated by gavage with two dose levels of 1,1,1-trichloroethane (purity, ~95%; with 3% *para*-dioxane [1,4-dioxane] and 2% minor impurities probably including 1,1-dichloroethane and 1,1-dichloroethylene) in corn oil for 5 days per week, for 78 weeks (NTP, 1977; also reported in Weisburger, 1977). At the lower dose level, male and female mice received 1,1,1-trichloroethane at 2000 mg/kg body weight (bw) per day for weeks 1–10, 2500 mg/kg bw per day for weeks 11–20, and 3000 mg/kg bw per day for weeks 21–78. At the higher dose level, male and female mice received 1,1,1-trichloroethane at 4000 mg/kg bw per day for weeks 1–10, 5000 mg/kg bw per day for weeks 11–20, and 6000 mg/kg bw per day for weeks 21–78. Time-weighted average (TWA) doses for the mice at the lower and higher doses were, respectively, 2807 and 5615 mg/kg bw. Control groups of 20 male and 20 female mice received corn oil alone for 78 weeks. After 78 weeks of treatment, all groups of mice were maintained without treatment until study termination 12–13 weeks later. At study termination, survival was: 2/20, 15/50, and 11/50 in males, and 11/20, 23/50, and 13/50 in females, for the control group and groups at the lower and higher dose, respectively. The survival rates of females treated with 1,1,1-trichloroethane were lower than that of the respective control group. [The Working Group noted that survival at 78 weeks was low: 6/20 (control), 21/50 (lower dose), and 14/50 (higher dose) in males; 12/20 (control), 28/50 (lower dose), and 14/50 (higher dose) in females.] In treated male and female mice, body-weight gain was lower than that of their respective controls over the course of the study. All mice underwent complete necropsy.

Histopathological evaluation was performed on the main tissues and organs.

In male mice, there was a significant positive trend in the incidence of hepatocellular adenoma, hepatocellular carcinoma, or neoplastic nodule (combined) of the liver ($P < 0.05$, Cochran–Armitage test). In female mice, the incidence of neoplasms of all organs and types was not affected by treatment with 1,1,1-trichloroethane.

[The Working Group noted that this study was limited by the small number of animals evaluated in the control groups of males and females, the low survival of control males, and the decreased survival of females at the highest dose. For this reason, the Working Group considered this study inadequate for the evaluation of the carcinogenicity of 1,1,1-trichloroethane in experimental animals.]

3.2 Rat

3.2.1 Inhalation

In a well-conducted chronic toxicity and carcinogenicity study that complied with GLP (Ohnishi et al., 2013), groups of 50 male and 50 female F344/DuCrj rats (age, 6 weeks) were exposed by inhalation (whole-body exposure) to 1,1,1-trichloroethane (purity, > 95%; one of the impurities was identified as 1,4-dioxane, present at concentrations ranging from 3.34% to 3.50%) at a concentration of 0, 200, 800, or 3200 ppm for the control group and the groups at the lowest, intermediate, and highest dose, respectively, for 6 hours per day, 5 days per week, for 104 weeks. The survival rate of males at the highest dose was slightly lower than that of controls; this was attributable to neoplasm-related deaths. The survival rates of all groups of females treated with 1,1,1-trichloroethane were similar to that of controls. At study termination, survival was 34/50, 36/50, 36/50, and 28/50 in males, and 38/50, 38/50, 42/50, and 38/50 in females, for the control group and the groups at the lowest, intermediate, and highest dose, respectively. The body weights of the groups of male and female rats exposed to 1,1,1-trichloroethane were similar to those of their respective controls. All rats underwent complete necropsy. All organs and tissues were sampled for histopathological examination in all the animals.

In male rats, there was a significant positive trend ($P < 0.01$, Peto test) in the incidence of peritoneal mesothelioma – control, 1/50 (2%); lowest dose, 2/50 (4%); intermediate dose, 1/50 (2%); and highest dose, 16/50 (32%) – with the incidence being significantly increased at the highest dose ($P < 0.01$, Fisher exact test), and exceeding the upper bound of the range observed in historical controls in this laboratory: 17/649 (2.6%); range, 0–8%. There was a significant positive trend ($P < 0.05$, Peto test) in the incidence of bronchioloalveolar adenoma – control, 0/50; lowest dose, 1/50 (2%); intermediate dose, 7/50 (14%); and highest dose, 4/50 (8%) – with the incidence being significantly increased at the intermediate dose ($P < 0.05$, Fisher exact test). The incidence of bronchioloalveolar adenoma in male rats at the intermediate and highest dose exceeded the upper bound of the range observed in historical controls in this laboratory: 16/649 (2.5%); range, 0–6%.

In female rats, there were no significant treatment-related effects on the incidence of any tumour.

Regarding non-neoplastic lesions, no effects related to treatment with 1,1,1-trichloroethane were observed in male or female rats. [The Working Group noted that this was a well-conducted study that complied with GLP, males and females were used, the durations of exposure and observation were adequate, and an adequate number of animals per group and multiple doses were used.]

In another well-conducted study, groups of 50 male and 50 female Fischer 344 rats (age, 4–6 weeks) were exposed by inhalation (whole-body exposure) to 1,1,1-trichloroethane (purity,

~94%; with 5% stabilizers and < 1% minor impurities) at a concentration of 0, 150, 500, or 1500 ppm for 6 hours per day, 5 days per week, for 24 months (Quast et al., 1988). The survival rates of all groups of 1,1,1-trichloroethane-exposed males and females were similar to those in the respective control groups. [The Working Group noted that the number of animals at study termination was not reported.] The body weights of female rats at 500 and 1500 ppm were lower than those of the controls. The body weights of all groups of male rats exposed to 1,1,1-trichloroethane were similar to those of the controls. All rats underwent complete necropsy. Histopathological evaluation was performed on main tissues and organs.

In male rats, a significant positive trend in the incidence of bilateral benign interstitial cell tumour of the testis (P = 0.02, Cochran–Armitage trend test) was observed. Exposure to 1,1,1-trichloroethane had no significant effect on the incidence of unilateral or bilateral (combined) benign interstitial cell tumours of the testis or on the incidence of unilateral benign interstitial cell tumours of the testis.

In female rats, exposure to 1,1,1-trichloroethane had no significant effect on the incidence of tumours. Regarding non-neoplastic lesions, no effects related to treatment with 1,1,1-trichloroethane were observed in male or female rats. [The Working Group noted that this was a well-conducted study, males and females were used, the durations of exposure and observation were adequate, and an adequate number of animals per group and multiple doses were used.]

3.2.2 Oral administration (gavage)

Groups of 50 male and 50 female Osborne-Mendel rats (age, 7 weeks) were treated by gavage with 1,1,1-trichloroethane (purity, ~95%; with approximately 3% *para*-dioxane [1,4-dioxane] and 2% minor impurities probably including 1,1-dichloroethane and 1,1-dichloroethylene) at 750 mg/kg bw (lower dose) or 1500 mg/kg bw (higher dose) in corn oil for 5 days per week, for 78 weeks, followed by study termination 32 weeks later (NTP, 1977; also reported in Weisburger, 1977). Control groups of 20 male and 20 female rats received corn oil alone. At study termination, survival was 0/20, 0/50, and 0/50 in males, and 3/20, 2/50, and 1/50 in females, for the control group and the groups at the lower and higher dose, respectively. The survival rates of all males and females exposed to 1,1,1-trichloroethane were lower than those of their respective control groups. [The Working Group noted that survival at 78 weeks was low: 7/20, 1/50, and 4/50 in males; 14/20, 9/50, and 12/50 in females, for the control group and the groups at the lower and higher dose, respectively.] The body weights of male and female rats exposed to 1,1,1-trichloroethane were lower than those of their respective controls. All rats underwent complete necropsy. Histopathological evaluation was performed on main tissues and organs.

The incidence of neoplasms of all organs and types in male and female rats treated with 1,1,1-trichloroethane was similar to that observed in their respective control groups. [The Working Group noted that this study was limited by the low number of animals evaluated in the male and female control groups and the decreased survival of rats treated with 1,1,1-trichloroethane. For this reason, the Working Group considered this study inadequate for the evaluation of the carcinogenicity of 1,1,1-trichloroethane in experimental animals.]

In another study, groups of 40 male and 40 female Sprague-Dawley rats (age, 7 weeks) were treated by gavage with 1,1,1-trichloroethane (purity, ≥ 95%; stabilizers: 1,4-dioxane, 3.8%; 1,2-epoxybutane, 0.47%; and nitromethane, 0.27%; and < 1% minor impurities) at a dose of 500 mg/kg bw in olive oil for 4–5 days per week, for 104 weeks (Maltoni et al., 1986). Control groups of 50 male and 50 female rats (same

strain and age) were treated with olive oil alone. All surviving animals at the end of the treatment period were maintained until spontaneous death (up to 141 weeks). The survival rates and body weights of male and female rats exposed to 1,1,1-trichloroethane were similar those of the controls. All rats underwent complete necropsy. Histopathological evaluation was performed on main tissues and organs.

In male rats, treatment with 1,1,1-trichloroethane significantly increased [$P < 0.05$, Fisher exact test] the incidence of all leukaemias (combination of various histological types) in a variety of organs and tissues; incidences being 3/50 (control), and 9/40 (500 mg/kg bw). No increase in the incidence of neoplasms of any organ or type was observed in female rats treated with 1,1,1-trichloroethane. [The Working Group noted that this study was limited by the use of only one dose level.]

3.3 Evidence synthesis for cancer in experimental animals

The carcinogenicity of 1,1,1-trichloroethane has been assessed in one well-conducted GLP study in male and female Crj:BDF_1 mice (Ohnishi et al., 2013), in one well-conducted GLP study in male and female F344/DuCrj rats (Ohnishi et al., 2013), in one well-conducted study in male and female $B6C3F_1$ mice (Quast et al., 1988), and in one well-conducted study in male and female Fischer 344 rats (Quast et al., 1988) treated by inhalation with whole-body exposure. The carcinogenicity of 1,1,1-trichloroethane in mice and rats was also evaluated in studies that did not comply with GLP. Specifically, there was one study in male and female $B6C3F_1$ mice (NTP, 1977), one study in male and female Osborne-Mendel rats (NTP, 1977), and one study in male and female Sprague-Dawley rats (Maltoni et al., 1986) treated by oral administration (gavage).

In the inhalation study that complied with GLP in male and female Crj:BDF_1 mice, there was a significant positive trend in the incidence of malignant lymphoma in the spleen and of Harderian gland adenoma in males; the incidence of Harderian gland adenoma was also significantly increased in males at the highest dose. There was a significant positive trend in the incidence of bronchioloalveolar carcinoma and bronchioloalveolar adenoma or carcinoma (combined) in males. There was a significant positive trend in the incidence of hepatocellular adenoma in male mice. In female mice, there was a significant positive trend in the incidence of bronchioloalveolar adenoma and bronchioloalveolar adenoma or carcinoma (combined). The incidence of bronchioloalveolar adenoma or carcinoma (combined) was also significantly increased at the highest dose in females. A significant positive trend in the incidence of hepatocellular adenoma was observed in females; with the incidence of hepatocellular adenoma being significantly increased at all doses (Ohnishi et al., 2013).

In the inhalation study that complied with GLP in male and female F344/DuCrj rats, there was a significant positive trend in the incidence of peritoneal mesothelioma in males, with the incidence being significantly increased at the highest dose. In males, there was a significant positive trend in the incidence of bronchioloalveolar adenoma, and the incidence was significantly increased at the intermediate dose. In female rats, there were no significant effects upon the incidence of neoplasms (Ohnishi et al., 2013).

In another well-conducted study in male and female $B6C3F_1$ mice exposed by inhalation, a significant positive trend in the incidence of lacrimal/Harderian gland adenoma or cystadenoma (combined) was observed in females. In male mice, there were no significant effects of treatment on the incidence of neoplasms (Quast et al., 1988).

In another well-conducted study in male and female Fischer 344 rats exposed by inhalation, a significant positive trend in the incidence of bilateral benign interstitial cell tumour of the testis was observed in males. In females, there was no significant effects of treatment on the incidence of neoplasms (Quast et al., 1988).

In the study in male and female Sprague-Dawley rats treated by oral administration (gavage), the incidence of all leukaemias (combination of various histological types) in a variety of organs and tissues was significantly increased in treated males. In female rats, there was no treatment-related effects. No increased incidence of neoplasms was observed in treated female rats (Maltoni et al., 1986).

Studies on oral administration of 1,1,1-trichloroethane administered by gavage to male and female B6C3F$_1$ mice and male and female Osborne-Mendel rats (NTP, 1977) were judged inadequate for the evaluation of the carcinogenicity of 1,1,1-trichloroethane in experimental animals.

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

(a) Absorption

Numerous studies have been published on the absorption of 1,1,1-trichloroethane in humans by either the dermal or inhalation routes of exposure. In general, all studies demonstrated rapid absorption, with many, especially the more recent studies, relating absorption to some measure of either 1,1,1-trichloroethane or one of its metabolites in either the urine or the blood. Dermal or percutaneous absorption is assessed either by direct application of 1,1,1-trichloroethane to the skin or by assessing dermal penetration of 1,1,1-trichloroethane vapours. Studies involving dermal absorption showed rapid absorption related to the type or condition of skin exposed, duration of exposure, and exposure concentration (Stewart & Dodd, 1964; Aitio et al., 1984; Poet et al., 2000). Several studies have been conducted on the percutaneous absorption of solvent vapours. Absorption was shown to be rapid for the vapour from several halogenated solvents, including 1,1,1-trichloroethane, with differences noted according to solvent lipid solubility, skin condition, and activity level of the participant (Riihimäki & Pfäffli, 1978; Wallace et al., 1989; Giardino et al., 1999). With volatile solvents such as 1,1,1-trichloroethane, absorption by the dermal route is very low when compared with inhalation (Giardino et al., 1999). Dermal absorption of 1,1,1-trichloroethane is considerably slower than that of other organic solvents, such as trichloroethylene, perchloroethylene [tetrachloroethylene], toluene, or xylene (Kezic et al., 2000, 2001).

Another focus of several studies on the absorption of 1,1,1-trichloroethane in humans exposed by inhalation has been to use measurements of 1,1,1-trichloroethane in exhaled breath, blood, or urine as surrogates for estimating the exposure dose. Droz et al. (1988) exposed participants to 1,1,1-trichloroethane at 200 ppm by inhalation for 6 hours and detected 1,1,1-trichloroethane in the breath up to 15 hours after exposure. Nagatoshi et al. (1994) monitored urinary excretion of various organic solvents, including 1,1,1-trichloroethane, and concluded that worker exposure was extremely small in factories that exercised proper control over toxic materials. [The Working Group noted that the nature of the controls, specifically whether protection against inhalation and dermal exposures was included, was unclear.] Nolan et al. (1984) used concentrations of 1,1,1-trichloroethane in both blood and exhaled air to validate inhalation exposure. They found that both measurements were

proportional to exposure and indicated that 25% of the administered 1,1,1-trichloroethane was absorbed during the 6-hour exposure. Tay et al. (1995) similarly found a good correlation between concentrations of 1,1,1-trichloroethane in end-of-shift exhaled air (r = 0.81) and venous blood samples (r = 0.88). Gill et al. (1991), Hajimiragha et al. (1986), and Monster & Houtkooper (1979) all found that blood concentrations of 1,1,1-trichloroethane provided an accurate assessment of inhalation exposure and absorption. Monster & Houtkooper (1979) directly compared the accuracy of measurements in the blood, urine, and exhaled air as an indication of exposure by inhalation to 1,1,1-trichloroethane, trichloroethylene, or perchloroethylene [tetrachloroethylene]. For all three solvents, blood concentrations of the parent compound gave the best estimates of exposure, although the advantages of using blood were very small compared with using exhaled air. Measuring solvent concentrations in the urine and exhaled air simultaneously did not significantly improve exposure estimates.

(b) Distribution

Much of the absorbed 1,1,1-trichloroethane in humans is rapidly excreted in exhaled air as the unmetabolized parent compound (Gamberale & Hultengren, 1973). Caplan et al. (1976) analysed the tissue distribution of 1,1,1-trichloroethane in an otherwise healthy woman aged 40 years who had been accidentally poisoned by 1,1,1-trichloroethane. The deceased woman was found in a closed and poorly ventilated room in which paint, paint thinner, and towels soaked in those materials were found. There were paint stains on areas of the skin, suggesting that exposure was both by inhalation and the dermal route. By far the highest concentration of 1,1,1-trichloroethane was found in the brain (36 mg/100 mL), with markedly lower concentrations found in the kidney, liver, lung, blood, and bile (12, 5, 1, 2, and < 1 mg/100 mL, respectively).

Hajimiragha et al. (1986) concluded that their data on human exposures to volatile halogenated hydrocarbons agreed with those of Monster (1979) in that blood concentrations of 1,1,1-trichloroethane are determined by a complex equilibrium involving uptake, exhalation, and tissue storage, especially in adipose tissue. From the tissues, 1,1,1-trichloroethane is redistributed into the blood, and from the blood it is redistributed into alveolar air or undergoes biotransformation. Tissue depletion occurs quickly, with the exception of adipose tissue, from which depletion begins once blood concentrations decrease below a certain level as determined by the fat:blood partition coefficient of 1,1,1-trichloroethane. Consistent with the conclusion that 1,1,1-trichloroethane is stored and gradually released after repeated exposures, Seki et al. (1975) found that in printing-factory workers exposed solely to 1,1,1-trichloroethane at concentrations of up to 53 ppm, there was a linear relationship between total trichloro-compounds in the urine and environmental vapour concentrations. Towards the end of the work week, however, increased levels of urinary metabolites were generally noted, consistent with potential accumulation of 1,1,1-trichloroethane over the course of the work week. [The Working Group noted that the variability of measurements of urinary metabolites, such as in the study by Monster & Houtkooper (1979), suggests that some caution is needed in making conclusions about the accumulation of 1,1,1-trichloroethane.] The rapid initial distribution of 1,1,1-trichloroethane from blood into tissues and subsequent elimination, however, results in a weak correlation between clinical toxicity and blood concentrations (Meredith et al., 1989).

(c) Metabolism

The metabolites of 1,1,1-trichloroethane are not unique to 1,1,1-trichloroethane and are also formed after exposure to trichloroethene [trichloroethylene] and tetrachloroethene

[tetrachloroethylene], although in different proportions (Fernández et al., 1977; Monster, 1986). Only a small fraction (< 10%) of the absorbed 1,1,1-trichloroethane is metabolized (ATSDR, 2006). Of the absorbed 1,1,1-trichloroethane, 2–5% is eliminated in the urine as trichloroethanol (half-life, 10–27 hours) and 1–2% as trichloroacetic acid (half-life, 70–85 hours), representing a minor elimination pathway (Humbert & Fernández, 1976; Monster, 1986; ATSDR, 2006). Nevertheless, urinary levels of trichloroethanol and trichloroacetic acid are well correlated with airborne exposures, indicating possibly useful biomarkers of current exposure (trichloroethanol) and weekly average exposure (trichloroacetic acid), in the absence of exposure to other chlorinated solvents (Imbriani et al., 1988; ATSDR, 2006).

As most of the pharmacokinetics data in humans for 1,1,1-trichloroethane show that only a limited amount of absorbed compound is metabolized (i.e. < 10%) (Monster, 1979), there is not an extensive amount of data available on rates of metabolism. Nonetheless, several studies in humans have demonstrated that trichloroethanol and trichloroacetic acid are the primary metabolites, with trichloroethanol being the more abundant one of the two (Nolan et al., 1984; Berode et al., 1990; Kawai et al., 1991; Pedrozo & Siqueira, 1996; Tomicic et al., 2011).

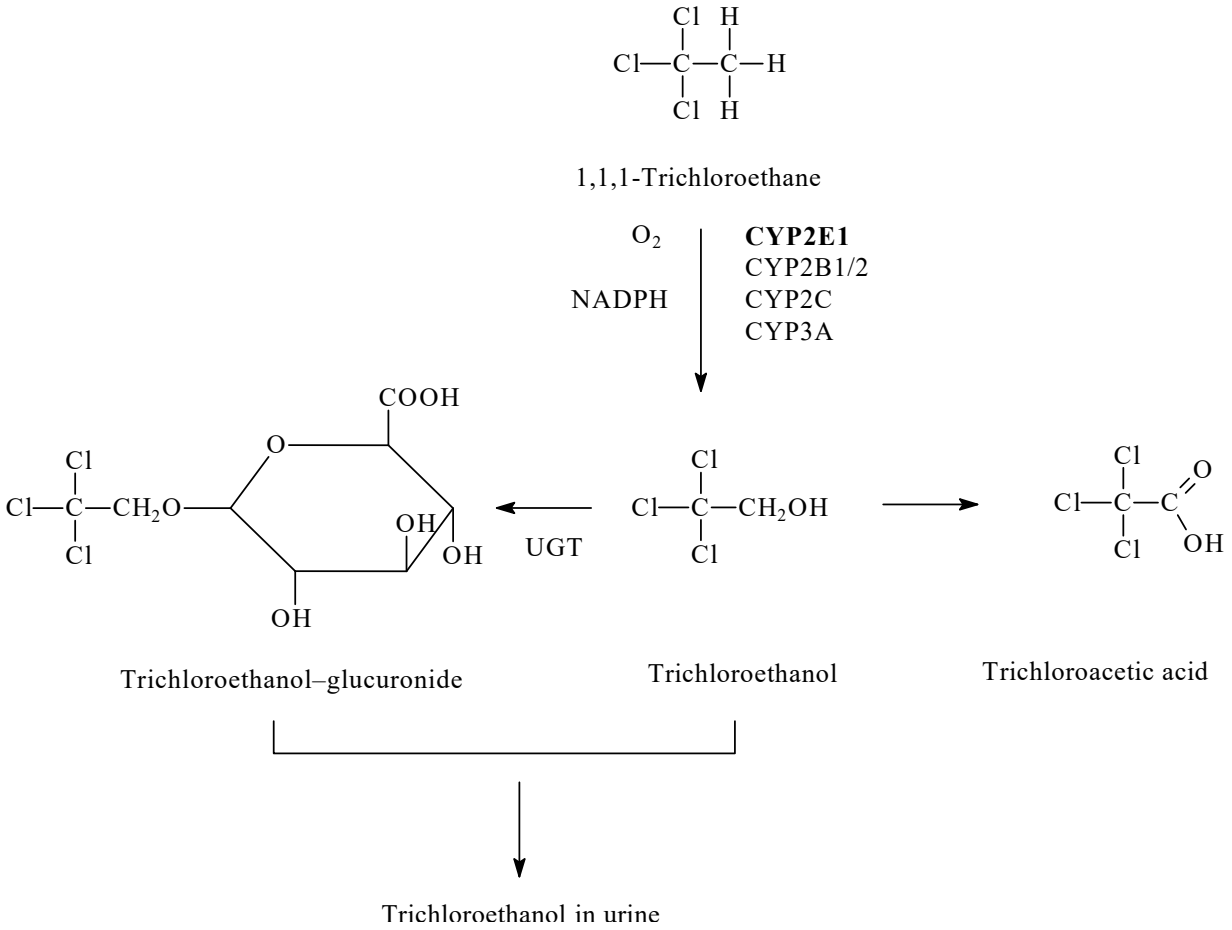
On the basis of similarities with the more widely studied solvent trichloroethylene and on experimental data from rodent studies (see Section 4.1.2(c)), Guengerich et al. (1991) concluded that the metabolism of 1,1,1-trichloroethane to trichloroethanol occurs primarily via human cytochrome P450 2E1 (CYP2E1). Supporting this suggestion are two studies that provided indirect evidence for the function of various CYP enzymes in the oxidation of 1,1,1-trichloroethane (Berode et al., 1990; Johns et al., 2006). These studies correlated the metabolism of 1,1,1-trichloroethane with that of other CYP2E1 substrates and showed that metabolism of 1,1,1-trichloroethane is increased by ethanol consumption.

The major pathways for 1,1,1-trichloroethane metabolism, according to data from both human and experimental animal studies, are illustrated in Fig. 4.1. 1,1,1-Trichloroethane is oxidized by one of several CYP enzymes to form trichloroethanol, which subsequently undergoes either oxidation to trichloroacetic acid, or glucuronidation to form the corresponding glucuronide conjugate trichloroethanol–glucuronide (TCOG). Both metabolites are recovered in the urine, with the majority being trichloroethanol. Most of the metabolic flux is to trichloroethanol rather than trichloroacetic acid (Kawai et al., 1991). Other minor metabolites, including carbon dioxide and acetylene excreted in the exhaled air, have also been described (Tomicic et al., 2011). The potential implications of formation of acetylene from 1,1,1-trichloroethane are discussed in Section 4.2.1. It has been proposed that acetylene is formed from 1,1,1-trichloroethane via multiple steps of reductive dehalogenation that also involve CYP enzymes. Similar studies in experimental animal models that could provide additional support for this pathway are not available. The proposed scheme for this reductive metabolic pathway is shown in Fig. 4.2. [The Working Group noted that although this reductive pathway provides a chemical mechanism that could explain some of the adverse effects of 1,1,1-trichloroethane, its quantitative significance, especially in humans, is unclear.]

(d) Excretion

Excretion of 1,1,1-trichloroethane absorbed either dermally or via inhalation occurs by one of two mechanisms: exhalation of unmetabolized 1,1,1-trichloroethane, or urinary excretion of either 1,1,1-trichloroethane or its metabolites. For the latter, the urinary metabolites are primarily trichloroethanol and trichloroacetic acid, with the former being the predominant form. Studies on workers exposed to 1,1,1-trichloroethane have

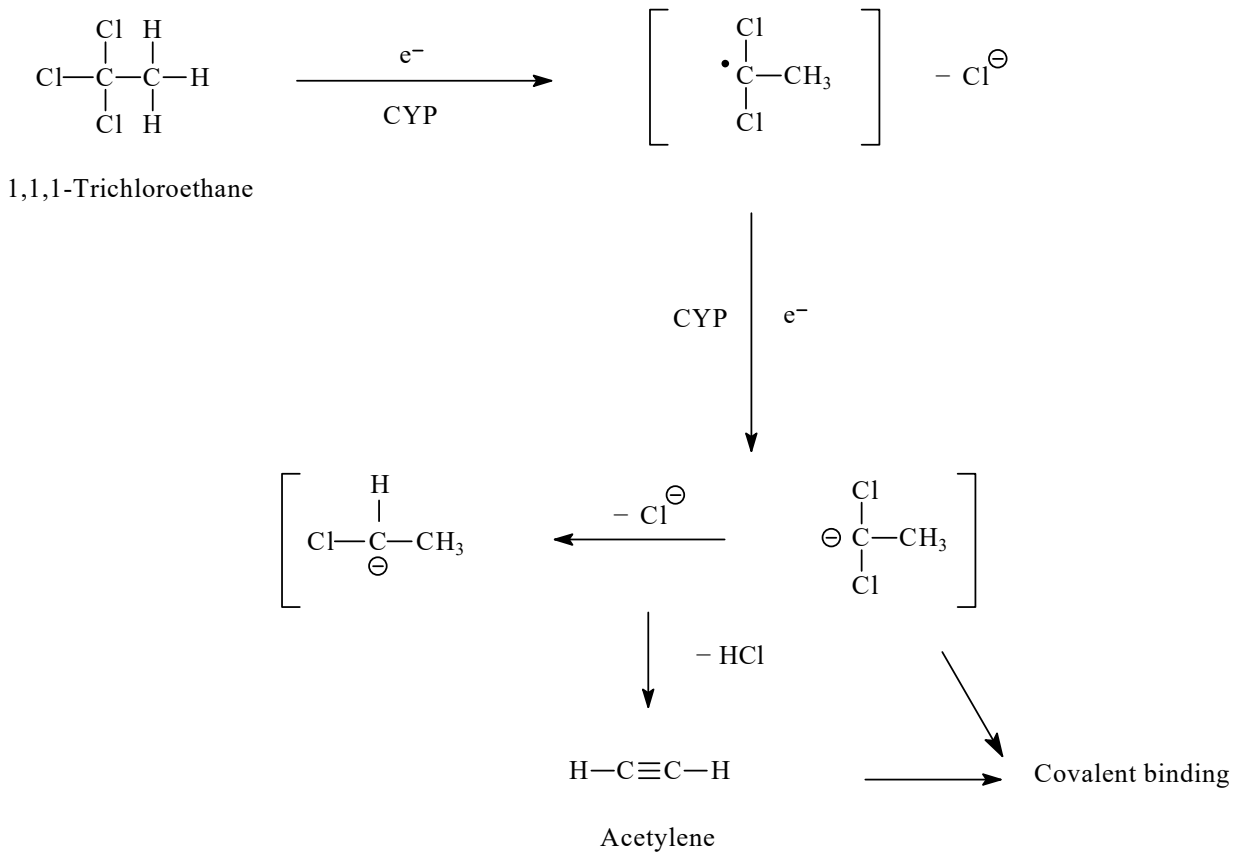
Fig. 4.1 Scheme for oxidative metabolism of 1,1,1-trichloroethane



CYP, cytochrome P450; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form; UGT, uridine 5′-diphosphoglucuronyltransferase.
The initial step in the oxidative metabolism of 1,1,1-trichloroethane is catalysed by one of several CYP enzymes, although most data suggest that CYP2E1 is the predominant active enzyme. The initial metabolite, trichloroethanol, has one of three fates: (1) further oxidation to trichloroacetic acid; (2) direct excretion into the urine; or (3) glucuronidation to form trichloroethanol–glucuronide. The glucuronide undergoes urinary excretion and is typically recovered as trichloroethanol.
Created by the Working Group.

focused for many years on validating measures that can be sensitive indicators or biomarkers of exposure. For example, Stewart et al. (1961) performed controlled human exposures to 1,1,1-trichloroethane vapour and showed an exponential decay curve for the concentration of 1,1,1-trichloroethane in exhaled air. Similar studies, such as those by Seki et al. (1975); Abe & Wakui (1984); Nolan et al. (1984); Hajimiragha et al. (1986); Imbriani et al. (1988); Gill et al. (1991); Kawai et al. (1991); Laparé et al. (1995); Mizunuma et al. (1995); Tay et al. (1995); and Tomicic et al. (2011) have all shown the predominance of exhalation of unmetabolized 1,1,1-trichloroethane in the excretion of inhaled or absorbed 1,1,1-trichloroethane. Moreover, two of these studies (Nolan et al., 1984; Laparé et al., 1995) concluded that measurement of 1,1,1-trichloroethane concentration in exhaled air is the most reliable indicator of exposure and that measurement of urinary

Fig. 4.2 Proposed scheme for reductive dehalogenation of 1,1,1-trichloroethane



CYP, cytochrome P450.
The presumed pathway occurs under hypoxic or anaerobic conditions and involves formation of several electrophilic intermediates that are shown in square brackets. Studies indicate that the first two dehalogenation steps are dependent on reduced nicotinamide adenine dinucleotide phosphate (NADPH) and probably catalysed by CYP enzymes. The second set of reactive intermediates can either form covalent adducts with cellular nucleophiles or spontaneously lose HCl to form acetylene. The latter has been detected and can also form covalent adducts with cellular nucleophiles.
Created by the Working Group.

metabolites is subject to error and has the potential for significant individual variation. If urine is selected for monitoring exposure, parent chemical or total trichloro-compounds rather than specific metabolites are recommended by most of these studies. [The Working Group noted that this conclusion would seem to be inconsistent with that of Monster & Houtkooper (1979) discussed above in Section 4.1.1(a), who suggested that blood values correlated best with exposure dose. As noted above, the strength of this conclusion was weak.]

4.1.2 Experimental systems

(a) Absorption

There were several studies on the absorption of 1,1,1-trichloroethane in animal models. For studies on dermal or percutaneous absorption, the guinea-pig is the most common model, whereas rats are primarily used for inhalation

studies. In addition to the characterization of chemical properties that facilitate absorption, the influence of occlusive agents, including gloves or barrier creams, has also been determined.

In a series of studies by Boman and colleagues (Boman et al., 1982, 1989, 1995; Boman & Wahlberg, 1986, 1989; Boman, 1989; Boman & Mellström, 1989; Mellström & Boman, 1992), the absorption of 1,1,1-trichloroethane through guinea-pig skin was characterized and compared with the absorption of other organic solvents, such as toluene or butanol. A key observation from all of these studies was that lipid solubility is a key determinant of the rate at which solvents are absorbed through the skin and that damage to the skin or the existence of barriers or occlusions can markedly affect the process of absorption. Morgan et al. (1991) studied dermal absorption in rats and concluded that absorption (as detected by the appearance of 1,1,1-trichloroethane in the blood) is rapid and can be significant even if only about 1% of the skin surface area is exposed.

Dallas et al. (1986, 1989) characterized the absorption of 1,1,1-trichloroethane in male Sprague-Dawley rats exposed to 1,1,1-trichloroethane at 50 or 500 ppm via inhalation. Absorption from the lungs was rapid, with substantial levels of 1,1,1-trichloroethane being detected in arterial blood within 2 minutes. Inhalation studies in mice exposed for 100 minutes to 1,1,1-trichloroethane at 3500 or 5000 ppm showed rapid uptake into the blood and brain, with near steady-state levels being reached after 40–60 minutes of exposure (You et al., 1994a). Accumulation of 1,1,1-trichloroethane in all tissues except fat was similar; maximal concentrations in fat were 20–30 times higher than those in other tissues (You et al., 1994b).

A study by Hobara et al. (1981) indicated systemic availability of 1,1,1-trichloroethane in dogs treated intravenously. 1,1,1-Trichloroethane was detected in exhaled breath within 1 minute, indicating rapid absorption.

(b) Distribution

As with studies in humans, assessments of the tissue distribution of 1,1,1-trichloroethane in experimental animals (rats, mice, and dogs) show accumulation predominantly in fat (Savolainen et al., 1977; Vainio et al., 1978; Savolainen, 1981). Schumann et al. (1982a) exposed male Fischer 344 rats and B6C3F$_1$ mice to [^{14}C]-labelled 1,1,1-trichloroethane at 150 or 1500 ppm for 6 hours and found a higher recovery of radiolabel in fat than in either liver or kidney. They noted, however, that in both species, < 2% of the initial radiolabel remained after 24 hours, suggesting rapid excretion and little potential for bioaccumulation.

Besides the predominant, early accumulation of 1,1,1-trichloroethane in fat, other studies in rats (Westerberg & Larsson, 1982; Warren et al., 1998; and mice (Warren et al., 2000) have focused on distribution into the blood and brain. These studies showed rapid and concentration-dependent increases in 1,1,1-trichloroethane concentrations in both blood and brain after inhalation exposure, with concentrations in the brain being roughly twice those in the blood. In one study, You et al. (1994a) similarly found rapid distribution of 1,1,1-trichloroethane to the blood and brain. In another study, You et al. (1994b) also showed rapid distribution of 1,1,1-trichloroethane to the blood and several tissues besides brain. You & Dallas (1998) also noted that mice exhibited a greater capacity for 1,1,1-trichloroethane accumulation than did rats.

(c) Metabolism

(i) Non-human mammals in vivo

As noted in Section 4.1.1(c) and illustrated in Fig. 4.1, oxidative metabolism of 1,1,1-trichloroethane appears to be mediated by several CYP enzymes, although primarily by CYP2E1. 1,1,1-Trichloroethane has long been considered to be a relatively poor substrate for CYPs (Hake et al., 1960), especially compared with solvents

such as trichloroethylene (Dobrev et al., 2001) or *meta*-xylene (Tardif & Charest-Tardif, 1999). Despite a number of studies that conclude that metabolism plays a very minor role in the overall handling and disposition of 1,1,1-trichloroethane, several observations in rodents are consistent with a role for CYP-dependent metabolism, especially under certain conditions. For example, Blohm et al. (1985) exposed rats to 1,1,1-trichloroethane at 200 or 2000 ppm for several hours per day for nearly 3 months and found an increase in liver microsomal protein content and monooxygenase activity, indicating an increase in liver endoplasmic reticulum content. Kaneko et al. (1994) examined the effects of ethanol on the metabolism of either 1,1,1-trichloroethane or trichloroethylene to compare a "poorly metabolized" with a "highly metabolized" substance. Increases in the rate of metabolism of 1,1,1-trichloroethane to trichloroethanol were observed in ethanol-exposed rats, providing indirect evidence for the role of CYPs, particularly CYP2E1, in the metabolism of 1,1,1-trichloroethane.

Other studies have also provided indirect data supporting the role of CYPs in the metabolism of 1,1,1-trichloroethane. For example, Carlson (1981) exposed rabbits to 1,1,1-trichloroethane at 5600 ppm by inhalation and looked at the impact of pre-treatment with either phenobarbital (which induces multiple CYPs) or two broad CYP inhibitors on the oxidative metabolism of 1,1,1-trichloroethane. Pre-treatment with phenobarbital had a small effect in decreasing blood concentrations of 1,1,1-trichloroethane, whereas pre-treatment with the two CYP inhibitors decreased the metabolism of 1,1,1-trichloroethane, thus increasing blood concentrations of 1,1,1-trichloroethane. Bruckner et al. (2001) exposed male Sprague-Dawley rats to 1,1,1-trichloroethane at a range of doses by oral administration (gavage) and assessed the activities and expression of various CYPs. Induction of both CYP2E1 and CYP2B1/2 was observed. The metabolism of 1,1,1-trichloroethane was enhanced by pre-treatment with phenobarbital or ethanol, or by fasting. From these more direct data, the authors concluded that both CYP2E1 and CYP2B1/2 are involved in 1,1,1-trichloroethane metabolism.

Despite the various rodent studies with positive results that are consistent with a role for CYPs in the metabolism of 1,1,1-trichloroethane, there are a few studies in which the results are less clear. Savolainen et al. (1977) found that exposure to 1,1,1-trichloroethane for 5 days decreased the microsomal CYP content of rat liver, whereas exposure to trichloroethylene (for which metabolism by CYPs is much better characterized) increased the microsomal CYP content of rat liver. Toftgård et al. (1981) found that 1,1,1-trichloroethane had very modest or no effects on total CYP levels or activities, whereas other organic solvents, such as xylene, produced clearly significant increases. Wang et al. (1996) exposed rats to one of four solvents (including 1,1,1-trichloroethane) for 6 hours and assessed metabolic effects in the liver. Toluene, trichloroethylene, and benzene had marked effects on the activity of CYP-dependent enzymes and the expression of several CYP enzymes, whereas 1,1,1-trichloroethane had no effect on these processes. [The Working Group noted that the 6-hour exposure time was probably insufficient to observe all potential induction of CYPs or other drug-metabolizing enzymes, thus conclusions about the ability of 1,1,1-trichloroethane to induce CYP expression in this study would only be preliminary and based on a short exposure time.]

The metabolism of 1,1,1-trichloroethane has been compared to that of its isomer 1,1,2-trichloroethane and of trichloroethylene. Ikeda & Otsuji (1972) compared the excretion of trichloroethanol and trichloroacetic acid in rats or mice exposed by inhalation to 1,1,1-trichloroethane, 1,1,2-trichloroethane, 1,1,1,2-tetrachloroethane, or 1,1,2,2-tetrachloroethane. All compounds except 1,1,2-trichloroethane generated significant amounts of urinary trichloroethanol and

trichloroacetic acid. [The Working Group noted that this finding would seem to contradict those of other studies that showed metabolism of 1,1,2-trichloroethane to be much faster than that of 1,1,1-trichloroethane.] Similar comparisons of the metabolism or effects on metabolism of 1,1,1-trichloroethane and *meta*-xylene (Tardif & Charest-Tardif, 1999) also supported the findings of relatively poor metabolism of 1,1,1-trichloroethane. In male Sprague-Dawley rats, co-exposure to both 1,1,1-trichloroethane and *meta*-xylene resulted in markedly lower excretion of urinary metabolites of 1,1,1-trichloroethane (i.e. trichloroethanol and trichloroacetic acid) than did exposure to 1,1,1-trichloroethane only.

Koizumi et al. (1983) exposed rats to 1,1,1-trichloroethane at 200, 400, or 800 ppm for 10 days and followed the conversion of 1,1,1-trichloroethane to trichloroethanol. While the amount of trichloroethanol produced increased markedly between 200 ppm and 400 ppm, the increase between 400 ppm and 800 ppm was much smaller, suggesting saturation of metabolism. In terms of species-dependent differences, it is estimated that the metabolism of 1,1,1-trichloroethane in mice is 2- to 3-fold that in rats on a body-weight basis (Schumann et al., 1982a, 1982b). Other studies, such as those conducted by Yoshida et al. (1998), further emphasize the modest role of metabolism versus excretion of unmetabolized 1,1,1-trichloroethane in overall disposition.

(ii) Non-human mammalian systems in vitro

Lal et al. (1969) reported that 1,1,1-trichloroethane increased the hepatic oxidative metabolism of CYP substrates in vitro. [The Working Group noted that in this abstract no details were provided about the nature of CYP activities affected or the type of in vitro hepatic system used.] A study by Takano et al. (1988) also supported a role, albeit modest, for CYP in the metabolism of 1,1,1-trichloroethane. For example, although 1,1,1-trichloroethane increased the rate of oxygen (O_2) consumption and hydrogen peroxide (H_2O_2) production in rat liver microsomes, the ratio of metabolism rate to O_2 consumption rate was very small (i.e. 0.011).

Van Dyke & Wineman (1971) examined the dechlorination of various chloroethanes and chloropropanes by hepatic microsomes from rat, rabbit, and guinea-pig. The rate of dechlorination of 1,1,2-trichloroethane by rat liver microsomes was about 20-fold that of 1,1,1-trichloroethane. While Takano et al. (1985) found CYP-dependent metabolism of 1,1,2-trichloroethane to be much faster than that of 1,1,1-trichloroethane, they emphasized that 1,1,1-trichloroethane should not be considered inert towards the mixed function oxidase system; they concluded that 1,1,1-trichloroethane binds to CYP, although only a small proportion of the bound molecules are metabolized.

While few studies are available in which a detailed analysis of the kinetics of CYP-dependent metabolism of haloalkanes was conducted, one study by Salmon et al. (1981) examined the microsomal de-chlorination of several chloroethanes, including 1,1,1-trichloroethane, 1,1,2-trichloroethane, 1,1,2,2-tetrachloroethane, 1,1-dichloroethane, 1,2-dichloroethane, 1,1,1-trifluoro-2-chloroethane, and hexachloroethane. Of these seven compounds, 1,1,1-trichloroethane exhibited by far the lowest V_{max} (0.2 nmol/min per mg protein) with a K_m of 0.27 mM.

As noted in Section 4.1.1(c) and illustrated in Fig. 4.2, in addition to oxidative metabolism, 1,1,1-trichloroethane may also undergo reductive metabolism in hepatic microsomes to yield 1,1-dichloroethane (Thompson et al., 1985). The reaction was dependent on reduced nicotinamide adenine dinucleotide phosphate (NADPH) and occurred only under anaerobic conditions. [Thus, the role of reductive metabolism under most exposure conditions will probably be very minor.]

(d) Excretion

The scientific literature on excretion of 1,1,1-trichloroethane for experimental animals resembles that for humans in terms of the number of published studies, major findings, and conclusions. For example, most of the absorbed 1,1,1-trichloroethane (94–98% in rats and 87–97% in mice) is recovered in exhaled air within 24 hours as unmetabolized 1,1,1-trichloroethane, with excretion of 1,1,1-trichloroethane being more rapid in mice than in rats (Schumann et al., 1982a, 1982b). Andoh et al. (1977) (cited in Yoshida et al, 1998) also reported that about 90% of the absorbed 1,1,1-trichloroethane was excreted by rats in exhaled air as the unchanged parent compound within 8 hours after intraperitoneal injection of 1,1,1-trichloroethane at 200 mg/kg bw.

Urinary excretion of metabolites (i.e. trichloroethanol and trichloroacetic acid) has also been assessed. Caperos et al. (1982) conducted a modelling study and concluded that urinary trichloroethanol level is a more sensitive indicator of exposure to 1,1,1-trichloroethane than is 1,1,1-trichloroethane level in the breath. They further noted that urinary trichloroacetic acid level is not a sufficiently sensitive or accurate indicator of exposure to 1,1,1-trichloroethane owing to the potential for variation with exposure concentrations.

Dallas et al. (1989), in their study on inhalation of 1,1,1-trichloroethane at 50 or 500 ppm for 2 hours in male Sprague-Dawley rats, found that concentrations of 1,1,1-trichloroethane in both blood and exhaled breath were directly proportional to exposure dose. By the end of the exposure period, one third to one half of the absorbed 1,1,1-trichloroethane was eliminated.

Hobara et al. (1981, 1982) investigated the toxicokinetics of 1,1,1-trichloroethane in one study and both 1,1,1-trichloroethane and 1,1,2-trichloroethane in a second study in dogs exposed by intravenous injection. Similar to findings in humans, mice, and rats, both compounds were rapidly available systemically and were detected in exhaled air within 1 minute.

Jakobson et al. (1982) dermally exposed anaesthetized guinea-pigs to a series of solvents and showed that elimination curves were non-linear in all cases and corresponded to a kinetic model involving at least two compartments for 1,1,1-trichloroethane and the other solvents. [The Working Group noted that this contrasted with the simpler, linear relationships for elimination described for humans and rodents exposed by inhalation or intravenous injection. The complexities of cutaneous absorption and transient storage of solvent in fat may explain these differences.] Mortuza et al. (2018) analysed the toxicokinetics and elimination of trichloroethylene and 1,1,1-trichloroethane in male Sprague-Dawley rats exposed by gavage. While trichloroethylene exhibited nonlinear toxicokinetics, those for 1,1,1-trichloroethane were nearly linear.

In a study by Mitoma et al. (1985), male B6C3F$_1$ mice and Osborne-Mendel rats were exposed orally to 1,1,1-trichloroethane at two doses, the maximum tolerated dose (MTD) and ¼ MTD (rats, 3000 or 750 mg/kg bw, equal to 22.5 or 5.6 mmol/kg bw; and mice, 4000 or 1000 mg/kg bw, equal to 30.0 or 7.5 mmol/kg bw). 1,1,1-Trichloroethane was mostly eliminated as the parent compound in exhaled air (85–93% of the total administered dose) and metabolism only accounted for 4% or 6% of the total dose in rats and mice, respectively. Urinary metabolite profiles (for trichloroethanol and trichloroacetic acid) were similar in rats and mice.

4.2 Evidence relevant to key characteristics of carcinogens

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

(a) Humans

(i) Exposed humans

No studies on DNA adducts or protein adducts were available to the Working Group.

In a study in aircraft-maintenance personnel exposed to solvents that included 1,1,1-trichloroethane, Lemasters et al. (1999a) measured concentrations of parent 1,1,1-trichloroethane in the blood, urine, and exhaled breath, together with micronucleus formation and sister-chromatid exchange in peripheral blood lymphocytes over the course of 30 weeks of exposure. In participants who worked in the sheet metal shop, the frequency of sister-chromatid exchange was significantly higher after 30 weeks when compared with baseline levels. Micronucleus counts also increased significantly from 12 to 19.8 by 15 weeks but then decreased to near baseline by 30 weeks (see also Section 4.2.2). [The Working Group noted that although this study did not address the question of whether electrophilic intermediates are formed during 1,1,1-trichloroethane metabolism, the finding of an increased frequency of sister-chromatid exchange is consistent with such intermediates being formed. The Working Group also noted that this was a co-exposure to multiple solvents, and exposure characterization of the individual solvents was not presented for the participants undergoing genotoxicity assessments, thus a conclusion cannot be made regarding the genotoxic effects of only 1,1,1-trichloroethane in this study.]

(ii) Human cells in vitro

One study in human-derived cells indirectly addressed the question of the potential for formation of electrophilic metabolites from 1,1,1-trichloroethane (Doherty et al., 1996) (see also Section 4.2.2 on genotoxicity). In this comprehensive study, the authors investigated the ability of 13 chlorinated hydrocarbons, toluene, and *n*-hexane to induce micronucleus formation in the cytochalasin B-blocked micronucleus assay. Genetically engineered cell lines were used: (i) AHH-1 cells, a human lymphoblastoid cell line that natively possesses a relatively low level of CYP1A1 activity; (ii) h2E1 cells, a human lymphoblastoid cell line that possesses native CYP1A1 and contains a cDNA for CYP2E1; and (iii) MCL-5 cells, an AHH-1-derived cell line that stably expresses cDNAs encoding human CYP1A2, CYP2A6, CYP3A4, CYP2E1, and microsomal epoxide hydrolase and contains relatively high levels of native CYP1A1. Each cell line was exposed to three concentrations of each chemical. 1,1,1-Trichloroethane caused a relatively large increase in the ratio of mononucleated:binucleated cells in the two cell lines (h2E1 and MCL-5) that express high activities of CYP2E1. [The Working Group noted that, on the basis of these in vitro genotoxicity assays, 1,1,1-trichloroethane would be presumed to form an electrophilic metabolite. Cautions or limitations for this conclusion include the relatively high concentrations of 1,1,1-trichloroethane and other chemicals to which the cell lines were exposed, and the absence of any direct evidence showing formation of specific electrophilic and reactive intermediates.]

(b) Experimental systems

Compared with studies in humans or human-derived cells or tissues, there is not much evidence in experimental systems regarding the potential for the formation of electrophilic intermediates from 1,1,1-trichloroethane, although there are some studies that address this question more directly.

(i) Non-human mammals in vivo

Filser et al. (1982) exposed rats to various halogenated hydrocarbons under conditions of saturated metabolism and measured concentrations of the parent compound and acetone in exhaled breath. The authors proposed that acetonaemia was due to metabolism of the halogenated compounds to reactive epoxides. These epoxides are proposed to alkylate coenzyme A and thereby block the citric acid cycle. Exposure to many of the compounds studied, including vinyl chloride, vinyl bromide, vinyl fluoride, vinylidene fluoride, *cis*- and *trans*-1,2-dichloroethylene, trichloroethylene, perchloroethylene [tetrachloroethylene], methylene chloride [dichloromethane], chloroform, carbon tetrachloride, and 1,1,2-trichloroethane was associated with increased excretion of acetone. In contrast, no significant effect on acetone excretion was observed in rats exposed to either 1,1,1-trichloroethane or *n*-hexane. [The Working Group noted that neither 1,1,1-trichloroethane nor *n*-hexane form significant amounts of epoxides during their metabolism.]

In a study by Mitoma et al. (1985), male B6C3F$_1$ mice and Osborne-Mendel rats were exposed orally to 1,1,1-trichloroethane at two doses, the MTD and ¼ MTD (rats, 3000 or 750 mg/kg bw, equal to 22.5 or 5.6 mmol/kg bw; mice, 4000 or 1000 mg/kg bw, equal to 30.0 or 7.5 mmol/kg bw). In addition to assessing excretion and overall metabolism, dose-dependent liver protein binding was also demonstrated. This binding was detected at slightly greater levels in rats than in mice, indicating some formation of reactive electrophiles.

Turina et al. (1986) measured radiolabelling of DNA, RNA, and protein in various tissues from rats and mice exposed to [^{14}C]-labelled 1,1,1-trichloroethane. A low level of DNA radiolabelling was detected in the liver. [The Working Group noted that the binding is typical of weak initiators.]

(ii) Non-human mammalian systems in vitro

Some evidence for the formation of electrophilic metabolites from 1,1,1-trichloroethane was provided in a study by Casciola & Ivanetich (1984), who assessed and compared the metabolism of multiple chloroethanes by rat hepatic nuclear CYP and by hepatic microsomes. [The Working Group noted that chloral hydrate is formed from the incubation of rat liver nuclei with 1,1,1-trichloroethane in the presence of NADPH, unlike in the main system in the endoplasmic reticulum. This would suggest the potential intermediate formation of an epoxide, as is the case for trichloroethylene; the quantitative significance of this pathway is unclear but is not likely to be very large.]

Maiorino et al. (1982) isolated liver microsomes from phenobarbital-induced rats and incubated them under a nitrogen atmosphere with 2 µmol of radiolabelled 1,1,1-trichloroethane and an NADPH-generating system. A low amount of protein binding (1.5 ± 0.7 nmol/mg protein) with 1,1,1-trichloroethane was detected. In comparison, protein binding (18.9 nmol/mg protein) at the same dose of 1,1,2-trichloroethane was more than 10-fold higher that for 1,1,1-trichloroethane. [The Working Group noted that these data indicate that although 1,1,1-trichloroethane can form electrophilic metabolites, its ability to do so is very modest compared with that of other, similar halogenated compounds.]

Takano et al. (1988) provided evidence for a low rate of CYP-dependent metabolism of 1,1,1-trichloroethane in rat liver microsomes. There was no detectable increase in the formation of malondialdehyde in incubations of 1,1,1-trichloroethane with rat liver microsomes (see Section 4.2.3), suggesting little in the way of formation of electrophilic or oxidizing metabolites.

As described in Sections 4.1.1(c) and 4.1.2(c) and illustrated in Fig. 4.2, reductive de-chlorination of 1,1,1-trichloroethane is expected to yield

multiple electrophilic and reactive intermediates and ultimately to produce acetylene (Thompson et al., 1985). [The Working Group noted that such a reaction, however, should occur under severely hypoxia or anaerobic conditions. Hence, this is not likely to be a quantitatively significant pathway under most conditions.]

Turina et al. (1986) also detected covalent binding of [^{14}C]-labelled 1,1,1-trichloroethane in microsomes from various tissues isolated from rats and mice. Like for the in vivo exposures described above, labelling of microsomal proteins was low, although CYP-dependent binding was shown for liver microsomes and was less clear for lung microsomes.

4.2.2 Is genotoxic

(a) Humans

(i) Exposed humans

See Table 4.1.

A study on aircraft-maintenance workers at a United States Air Force base investigated the correlation between measurements of the internal dose (i.e. in breath, blood, and urine) of solvents including 1,1,1-trichloroethane, and genotoxic effects in peripheral blood lymphocytes (Lemasters et al., 1999a). The results of the preliminary exposure assessment (pilot study) of industrial hygiene air samples and internal dose measurements in eight existing employees indicated that, of the solvents measured, 1,1,1-trichloroethane was present at the highest breath concentrations, specifically in the two sheet metal workers tested, in whom 1,1,1-trichloroethane was measured at 8.9 and 23.0 ppb in exhaled breath. The results of the subsequent genotoxicity assessment in a separate cohort of new hires in the sheet metal shop indicated small, but statistically significant increases (P = 0.003) in the frequency of sister-chromatid exchange after 30 weeks of exposure. There were significant increases (P = 0.03) in the frequency of micronucleus formation after 15 weeks of exposure compared with unexposed individuals; however, there was no significant difference at 30 weeks. [The Working Group noted that this was a co-exposure to multiple solvents, and exposure characterization of the individual solvents was not presented for the participants undergoing genotoxicity assessments; thus, a conclusion could not be made regarding the genotoxic effects of only 1,1,1-trichloroethane in this study.]

(ii) Human cells in vitro

See Table 4.2.

1,1,1-Trichloroethane induced an increase in DNA damage as assessed by the comet assay in erythroid progenitor cells derived from human umbilical cord blood (Irvin-Barnwell et al., 2021).

1,1,1-Trichloroethane did not induce unscheduled DNA synthesis in HeLa cells in either the presence or absence of metabolic activation (Martin & McDermid, 1981).

An investigation into the genotoxicity of chlorinated hydrocarbons in metabolically competent human cells reported that 1,1,1-trichloroethane induced a significant increase in the frequency of both kinetochore-positive and kinetochore-negative micronuclei in AHH-1, h2E1, and MCL-5 cells (Doherty et al., 1996) (see Section 4.2.1). [The results indicate that 1,1,1-trichloroethane has both clastogenic and aneugenic activity.]

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3.

In a multigenerational study, mice in the F_0 generation were given drinking-water containing 1,1,1-trichloroethane, then mated to produce the F_1 generation. Some F_1 treated animals were also given drinking-water containing 1,1,1-trichloroethane and mated to produce the F_2 generation. Untreated F_1 and F_2 generation males were used for a dominant lethal study. No evidence of dominant lethal mutations was observed in either the F_1 or the F_2 generation (Lane et al., 1982).

Table 4.1 Genetic and related effects of 1,1,1-trichloroethane in exposed humans

End-point	Biosample type	Location, setting, study design	Exposure level and number of exposed and controls	Results[a]	Covariates controlled	Comments	Reference
Micronucleus formation	Peripheral blood lymphocytes	USA, Air Force base/cross-sectional	6 exposed (exposure not measured), 8 controls	(+)	Smoking, number of caffeinated beverages per day	No exposure characterization in participants undergoing genotoxicity assessment; small sample size; study participants exposed to mixture of solvents and fuel fumes	Lemasters et al. (1999a)
Sister-chromatid exchange	Peripheral blood lymphocytes	USA, Air Force base/cross-sectional	6 exposed (exposure not measured), 8 controls	(+)	Smoking, number of caffeinated beverages per day	No exposure characterization in participants undergoing genotoxicity assessment; small sample size; study participants exposed to mixture of solvents and fuel fumes	Lemasters et al. (1999a)

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

Table 4.2 Genetic and related effects of 1,1,1-trichloroethane in human cells in vitro

End-point	Tissue, cell type	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA strand breaks (comet assay)	Cord blood, erythroid progenitor cells	+	NT	10 nM [1.335 ng/mL]	Purity, NR	Irvin-Barnwell et al. (2021)
Unscheduled DNA synthesis	HeLa S3 cells	–	–	100 µg/mL (–S9); 100 µg/mL (+ phenobarbital-induced rat S9)	Purity, NR	Martin & McDermid (1981)
		NT	–	100 µg/mL (+ 3-methylcholanthrene-induced rat S9)		
Micronucleus formation	AHH-1 cells h2E1 cells MCL-5 cells	+ + +	NT NT NT	2.5 mM [333.5 µg/mL]	Purity, NR	Doherty et al. (1996)

AHH-1, a human lymphoblastoid cell line; h2E1, a human lymphoblastoid cell line which possesses native CYP1A1 and contains a cDNA for CYP2E1; HIC, highest ineffective concentration; LEC, lowest effective concentration; MCL-5, an AHH-1-derived cell line that stably expresses cDNAs encoding human CYP1A2, CYP2A6, CYP3A4, CYP2E1, and microsomal epoxide hydrolase, and contains relatively high levels of native CYP1A1; NR, not reported; NT, not tested; S9, 9000 × *g* supernatant.

[a] +, positive; –, negative.

Table 4.3 Genetic and related effects of 1,1,1-trichloroethane in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Dominant lethal mutations	Mouse, ICR Swiss	F_1 mating generation F_2 mating generation	– –	5.83 mg/mL (1000 mg/kg bw per day) (F_0 generation) 5.83 mg/mL (1000 mg/kg bw per day) (F_1 generation)	F_0 generation exposed in drinking-water for 5 wk F_1 generation exposed in drinking-water for 11 wk	Purity, 97% (3% *para*-dioxane)	Lane et al. (1982)
Micronucleus formation	Mouse, B6C3F_1 (M, F)	Peripheral blood normochromatic erythrocytes	+/– –	80 000 ppm	13 wk oral exposure with feed containing microencapsulated 1,1,1-trichloroethane	Purity, > 99%; Positive trend test, but no significance relative to controls in males; $n = 5$ per group	NTP (2000)
Micronucleus formation	Mouse, NMRI (M, F)	Bone marrow polychromatic erythrocytes	(–)	2000 mg/kg bw	Intraperitoneal ×2 (at 0 and 24 h)	Purity, NR; 2 males and 2 females per group; bone marrow exposure not determined	Gocke et al. (1981)
Micronucleus formation	Mouse, B6C3F_1 (NR)	Bone marrow polychromatic erythrocytes	(+) (–) (–)	80% $LD_{50/7}$ (sampled at 48 and 72 h) 80% $LD_{50/7}$ (sampled at 72 h) 80% $LD_{50/7}$ (sampled at 36, 48, and 60 h)	Intraperitoneal ×2 (at 0 and 24 h) or ×1 (last test only)	Purity, NR; $n = 4–5$ per group; doses, NR; described as percentage of $LD_{50/7}$ (i.e. the dose required to kill 50% of animals within 7 days); statistical method based on historical control data, not on concurrent control; positive response only observed at 72 h time point in first experiment; bone marrow exposure not determined	Salamone et al. (1981)
Micronucleus formation	Mouse, CD-1 (M, F)	Bone marrow polychromatic erythrocytes	(–)	0.032 mg/kg bw	Intraperitoneal ×2 (at 0 and 24 h)	Purity, NR; 2 males and 2 females; bone marrow exposure not determined	Tsuchimoto et al. (1981)

bw, body weight; F, female; LD, lethal dose; LED, lowest effective dose; HID, highest ineffective dose; M, male; NR, not reported; ppm, parts per million; wk, week.

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

1,1,1-Trichloroethane elicited equivocal and negative responses for micronucleus formation in peripheral blood lymphocyes in male and female mice, respectively, after exposure to feed containing 1,1,1-trichloroethane for 13 weeks (NTP, 2000).

Negative responses and one weak positive response were observed in the bone marrow micronucleus assay in mice (Gocke et al., 1981; Salamone et al., 1981; Tsuchimoto et al., 1981).

Evidence of oxidative stress-induced DNA damage in mice treated with 1,1,1-trichloroethane was also observed and is discussed in more detail in Section 4.2.3 (Al-Griw et al., 2016).

(ii) Non-human mammalian cells in vitro

See Table 4.4.

1,1,1-Trichloroethane in either the liquid or vapour phase did not induce unscheduled DNA synthesis in cultured hepatocytes from rats (Althaus et al., 1982; Shimada et al., 1985; Milman et al., 1988). 1,1,1-Trichloroethane induced unscheduled DNA synthesis in cultured hepatocytes from male mice (Milman et al., 1988).

1,1,1-Trichloroethane exposure yielded five negative gene-mutation responses in the mouse lymphoma assay in the absence of metabolic activation and three positive and four negative responses in the presence of metabolic activation (Mitchell et al., 1988; Myhr & Caspary, 1988). [The Working Group noted that the positive responses in the presence of metabolic activation were inconsistent.]

1,1,1-Trichloroethane induced chromosomal aberrations in Chinese hamster ovary (CHO) cells in the absence of metabolic activation, but not in the presence of metabolic activation (Galloway et al., 1987). Equivocal responses were observed in the chromosomal aberration assay in Chinese hamster lung fibroblast (CHL/IU) cells (JETOC, 2005).

One negative response and one equivocal response were observed for sister-chromatid exchange induction in CHO cells in the absence of metabolic activation and an equivocal response and a negative response were observed in the presence of metabolic activation (Perry & Thomson, 1981; Galloway et al., 1987).

(iii) Non-mammalian experimental systems

See Table 4.5.

1,1,1-Trichloroethane elicited a negative response in the sex-linked recessive lethal *Basc* test in *Drosophila melanogaster* after the assessment of three successive broods (Gocke et al., 1981).

The genotoxicity of 1,1,1-trichloroethane was assessed in two plant systems. Chromosome aberrations were significantly induced in onion (*Allium cepa*) root tip cells in the *Allium* anaphase-telophase test (Rank & Nielsen, 1994). Conversely, 1,1,1-trichloroethane in the vapour phase did not yield a significant mutagenic effect in the *Tradescantia* stamen hair bioassay (Schairer & Sautkulis, 1982).

1,1,1-Trichloroethane did not induce reverse mutations in *Saccharomyces cerevisiae* (Mehta & von Borstel, 1981). 1,1,1-Trichloroethane (US EPA standard, free of epoxide preservative) weakly induced deletions at the highest concentration tested in the deletion recombination assay; however, 1,1,1-trichloroethane containing 0.05% 1,2-epoxybutane as a stabilizer elicited a stronger response (Brennan & Schiestl, 1998). 1,1,1-Trichloroethane did not induce mitotic gene conversion in *S. cerevisiae* in strains JD1 or D7 (Sharp & Parry, 1981a; Zimmermann & Scheel, 1981). 1,1,1-Trichloroethane did not induce mitotic crossing-over in *S. cerevisiae* in strains T1 or T2, or in the rep-test in strains T4 and T5 (analagous to the rec-test in *B. subtilis*) (Kassinova et al., 1981). A repair assay using wild-type and *rad* strains of *S. cerevisiae* demonstrated that 1,1,1-trichloroethane does not cause relative growth inhibition in repair-deficient yeast, thus indicating a lack of genotoxicity (Sharp & Parry, 1981b). 1,1,1-Trichloroethane did not induce

Table 4.4 Genetic and related effects of 1,1,1-trichloroethane in non-human mammalian cells in vitro

End-point	Species, cell type	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Unscheduled DNA synthesis	Rat, primary hepatocytes	–	NT	7.5 µM (– pyridines)	Purity, NR	Althaus et al. (1982)
		–	NT	7.5 µM (+ pyridines)		
Unscheduled DNA synthesis	Rat, primary hepatocytes	–	NT	0.1% in air (non-stabilized)	Modified vapour-phase exposure performed in an exposure chamber; both non-stabilized (purity, 99.8%) and stabilized (purity, 94.10%, with 5.65% stabilizer mixture containing butylene oxide) 1,1,1-trichloroethane were tested	Shimada et al. (1985)
		–	NT	0.1% in air (stabilized)		
Unscheduled DNA synthesis	Rat, primary hepatocytes	–	NT	NR	Modified vapour-phase exposure performed in an exposure chamber	Milman et al. (1988)
Unscheduled DNA synthesis	Mouse, primary hepatocytes	+	NT	NR	Modified vapour-phase exposure performed in an exposure chamber	Milman et al. (1988)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/ *Tk*$^{+/-}$ lymphoma cells	–	+	400 nL/mL (–S9); 31.3 nL/mL (+Aroclor 1254-induced rat S9)	Purity, NR	Myhr & Caspary (1988)
		–	+	400 nL/mL (–S9); 200 nL/mL (+Aroclor 1254-induced rat S9)		
		–	–	400 nL/mL (–S9); 400 nL/mL (+Aroclor 1254-induced rat S9)		
		NT	–	400 nL/mL (+ uninduced rat S9)		
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/ *Tk*$^{+/-}$ lymphoma cells	–	(+)	0.51 µL/mL (–S9); 0.64 µL/mL (+Aroclor 1254-induced rat S9)	Purity, NR	Mitchell et al. (1988)
		–	–	0.51 µL/mL (–S9); 0.51 µL/mL (+Aroclor 1254-induced rat S9)		
		NT	–	0.51 µL/mL (+Aroclor 1254-induced rat S9)		
Chromosomal aberrations	Chinese hamster, ovary cells (CHO)	+	–	160 µg/mL (–S9); 5000 µg/mL (+Aroclor 1254-induced rat S9)	Purity, NR	Galloway et al. (1987)

Table 4.4 (continued)

End-point	Species, cell type	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Chromosomal aberrations	Chinese hamster, lung fibroblast cells (CHL/IU)	+/–	+/–	6 h exposure: 0.80 mg/mL (–S9); 0.75 mg/mL (+S9)	Increases in chromosomal aberrations only seen at cytotoxic and precipitating concentrations; purity, 99.4%	JETOC (2005)
		+/–	NT	24 h exposure: 0.70 mg/mL		
		–	NT	48 h exposure: 0.60 mg/mL		
Sister-chromatid exchange	Chinese hamster, ovary cells	+/–	+/–	500 µg/mL (–S9); 500 µg/mL (+Aroclor 1254-induced rat S9)	Purity, NR	Galloway et al. (1987)
		–	NT	1000 µg/mL (–S9)		
Sister-chromatid exchange	Chinese hamster, ovary cells	NT	(–)	10.0 µg/mL (+Aroclor 1254-induced rat S9)	Statistical analysis not performed; response deemed negative because < 1.5-fold increase over control; purity, NR	Perry & Thomson (1981)

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

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

Table 4.5 Genetic and related effects of 1,1,1-trichloroethane in non-mammalian experimental systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Drosophila melanogaster, Berlin K (wildtype and *Basc*)	Sex-linked recessive lethal mutations	–	NT	25 mM [3335 µg/mL]	Purity, NR	Gocke et al. (1981)
Allium cepa	Chromosome aberrations	+	NT	175 µM [23.3 µg/mL]	24 h exposure; purity, NR	Rank & Nielsen (1994)
Tradescantia, clone 4430	Forward mutation	–	NT	5170 ppm [28 × 103 mg/m^3]	Vapour-phase-exposure; 6 h exposure; purity, NR	Schairer& Sautkulis (1982)
Saccharomyces cerevisiae, T4/T5	DNA damage	–	NT	Concentration, NR (rep-test with strains T4 and T5)	Purity, NR	Kassinova et al. (1981)
Saccharomyces cerevisiae, 197/2d (wildtype and *rad*)	DNA damage	–	–	750 µg/mL (–S9); 750 µg/mL (+Aroclor 1254-induced rat S9)	Performed in stationary cells; purity, NR	Sharp & Parry (1981b)
Saccharomyces cerevisiae XV185-14C	Reverse mutation	–	–	1111 µL/mL (–S9); 1111 µL/mL (+S9)	Purity, NR	Mehta & von Borstel (1981)
Saccharomyces cerevisiae, RS112	Deletion	(+)	NT	5.35 mg/mL (US EPA standard free of epoxide preservative)	Purity, NR	Brennan & Schiestl 1998
		+	NT	4.01 mg/mL (stabilized with 0.05% 1,2-epoxybutane)		
Saccharomyces cerevisiae, JD1	Mitotic gene conversion	–	–	750 µg/mL (–S9); 750 µg/mL (+Aroclor 1254-induced rat S9)	Purity, NR	Sharp & Parry (1981a)
Saccharomyces cerevisiae, D7	Mitotic gene conversion	–	–	2 µL/mL [2600 µg/mL] (–S9); 2 µL/mL (+S9)	One concentration tested; purity, NR	Zimmermann & Scheel (1981)
Saccharomyces cerevisiae, T1 and T2	Mitotic crossing-over	–	–	100 µg/mL (–S9); 1000 µg/mL (+Aroclor 1254-induced rat S9)	Purity, NR	Kassinova et al. (1981)
Saccharomyces cerevisiae, D61.M	Aneuploidy	–	NT	5330 µg/mL (cold interruption)	Purity, 99%	Whittaker et al. (1990)
	Aneuploidy	–	NT	5990 µg/mL (standard incubation)		
Saccharomyces cerevisiae, D6	Aneuploidy	–	–	750 µg/mL (–S9); 750 µg/mL (+Aroclor 1254-induced rat S9)	Purity, NR	Parry & Sharp (1981)
Aspergillus nidulans, P1	Mitotic malsegregation	–	NT	0.1% v/v in medium (~1320 µg/mL)	Purity, > 99%	Crebelli et al. (1988)
Salmonella typhimurium, BA13 and BAL13	Forward mutation	–	–	74.96 µM (–S9); 74.96 µM (+Aroclor 1254-induced rat S9)	Pre-incubation protocol; purity, 97%	Roldán-Arjona et al. (1991)

Table 4.5 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium, TM677	Forward mutation	–	–	1000 µg/mL (–S9); 1000 µg/mL (+ phenobarbital-induced rat S9)	Purity, NR	Skopek et al. (1981)
		NT	–	1000 µg/mL (+Aroclor 1254-induced rat S9)		
Salmonella typhimurium, TA92, TA98, TA100, TA1535, TA1537, and TA1538	Reverse mutation	(–)	(–)	2000 µg/plate (–S9); 2000 µg/plate (+Aroclor 1254-induced rat S9)	Not vapour-phase exposure; pre-incubation protocol; purity, NR	Brooks & Dean (1981)
Salmonella typhimurium, TA97 and TA98	Reverse mutation	(+)	(+)	10 µg/plate (–S9); 10 µg/plate (+Aroclor 1254-induced rat S9)	Not vapour-phase exposure; standard plate incorporation; purity, NR	Strobel & Grummt (1987)
Salmonella typhimurium, TA98, TA1537 and TA1538	Reverse mutation	–	–	10% in air (–S9); 10% in air (+Aroclor 1254-induced rat S9) (non-stabilized)	Plate incorporation assay, modified vapour-phase exposure	Shimada et al. (1985)
		–	–	10% in air (–S9); 10% in air (+Aroclor 1254-induced rat S9) (stabilized)	Non-stabilized, purity, 99.8%; stabilized, purity, 94.1% (5.65% stabilizer)	
Salmonella typhimurium, TA98	Reverse mutation	–	–	1.0 mL in desiccator (–S9); 1.0 mL in desiccator (+Aroclor 1254-induced rat S9)	Plate incorporation assay modified in sealed desiccators to allow vapour-phase exposure; purity, NR	Nestmann et al. (1980)
Salmonella typhimurium, TA98 and TA1537	Reverse mutation	–	–	5% in air for 24 h (–S9); 5% in air for 24 h (+S9)	Vapour-phase exposure; purity, 99%; results observed in 2 replicate experiments	JETOC (2005)
Salmonella typhimurium, TA98, TA100, TA1535, and TA1537	Reverse mutation	(–)	(–)	Concentrations, NR (–S9; + rat S9)	Plate incorporation assay modified in sealed desiccators to allow vapour-phase exposure; purity, 97–99%	Milman et al. (1988)
		NT	(–)	Concentrations, NR (–S9; + mouse S9)		
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, and TA1538	Reverse mutation	(–)	(–)	2000 µg/plate (–S9); 2000 µg/plate (+Aroclor 1254-induced rat S9)	Not vapour-phase exposure; standard plate incorporation; purity, NR	Rowland & Severn (1981)
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, and TA1538	Reverse mutation	(–)	(–)	1000 µg/mL (–S9); 1000 µg/mL (+Aroclor 1254-induced rat S9)	Not vapour-phase exposure; standard plate incorporation; purity, NR	Falck et al. (1985)

Table 4.5 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium, TA98, TA100, and TA1537	Reverse mutation	(–)	(–)	5000 µg/plate (–S9); 5000 µg/plate (+Aroclor 1254-induced mouse S9)	Not vapour-phase exposure; standard plate incorporation; purity, NR	MacDonald (1981)
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, and TA1538	Reverse mutation	(–)	(–)	2500 µg/plate (–S9); 2500 µg/plate (+Aroclor 1254-induced rat S9)	Not vapour-phase exposure; standard plate incorporation; purity, NR	Trueman (1981)
Salmonella typhimurium, TA98, TA100, TA1535, and TA1537	Reverse mutation	(–) NT (–) NT	(–) (–) (–) (–)	10 000 µg/plate (–S9); 10 000 µg/plate (+ rat S9); 3333 µg/plate (+ hamster S9) 3333 µg/plate (–S9); 3333 µg/plate (+ rat S9); 3333 µg/plate (+ hamster S9)	Not vapour-phase exposure; pre-incubation protocol; purity, NR	Haworth et al. (1983)
Salmonella typhimurium, TA98	Reverse mutation	(–) NT (–) NT	(–) (–) (–) (–)	1000 µg/plate (–S9); 3333 µg/plate (+ rat S9); 1000 µg/plate (+ hamster S9) 10 000 µg/plate (–S9); 10 000 µg/plate (+ rat S9); 10 000 µg/plate (+ hamster S9)	Not vapour-phase exposure; pre-incubation protocol; purity, NR	NTP (2000)
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, and TA1538	Reverse mutation	(–)	(–)	1000 µg/mL(–S9); 1000 µg/mL (+Aroclor 1254-induced rat S9)	Not vapour-phase exposure; Mutascreen test; purity, NR	Falck et al. (1985)
Salmonella typhimurium, TA98, TA1535, and TA1537	Reverse mutation	(–)	(–)	500 µg/mL (–S9); 500 µg/mL (+Aroclor 1254-induced rat S9)	Not vapour-phase exposure; microtiter fluctuation test; purity, NR	Gatehouse (1981)
Salmonella typhimurium, TA100	Reverse mutation	+	+	1000 µL in desiccator (–S9); 1000 µL in desiccator (+Aroclor 1254-induced rat S9)	Plate incorporation assay modified in sealed desiccators to allow vapour-phase exposure; purity, NR	Simmon et al. (1977)
Salmonella typhimurium, TA100	Reverse mutation	+ –	+ NT	150 mg/L in air (–S9); 150 mg/L in air (+Aroclor 1254-induced rat S9) (Fisher Co.); 210 mg/L in air (Aldrich Co.)	Plate incorporation assay modified in sealed desiccators to allow vapour-phase exposure; purity, 97%	Nestmann et al. (1984)

Table 4.5 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium, TA100	Reverse mutation	+	+	10% in air (–S9); 10% in air (+Aroclor 1254-induced rat S9) (non-stabilized)	Plate incorporation assay modified vapour-phase exposure; non-stabilized, purity, 99.8%; stabilized, purity, 94.1% (5.65% stabilizer)	Shimada et al. (1985)
		+	+	2.5% in air (–S9); 10% in air (+Aroclor 1254-induced rat S9) (stabilized)		
Salmonella typhimurium, TA100	Reverse mutation	+	+	5% in air for 24 h (–S9); 5% in air for 24 h (+S9)	Vapour-phase exposure; purity, 99%; results observed in 2 replicate experiments	JETOC (2005)
Salmonella typhimurium, TA100 and TA1535	Reverse mutation	+	+	1.0 mL in desiccator (–S9); 1.0 mL in desiccator (+Aroclor 1254-induced rat S9)	Plate incorporation assay modified in sealed desiccators to allow vapour-phase exposure; purity, NR	Nestmann et al. (1980)
Salmonella typhimurium, TA100 and TA1535	Reverse mutation	+	+	2000 µL in desiccator (–S9); 2000 µL in desiccator (+Aroclor 1254-induced rat S9)	Plate incorporation assay modified in sealed desiccators to allow vapour-phase exposure; purity, NR	Gocke et al. (1981)
Salmonella typhimurium, TA100	Reverse mutation	(+)	(–)	1000 µg/mL (–S9); 1000 µg/mL (+Aroclor 1254-induced rat S9)	Not vapour-phase exposure; standard plate incorporation; purity, NR	Strobel & Grummt (1987)
Salmonella typhimurium, TA100	Reverse mutation	(–) NT	(–) (–)	10 000 µg/plate (–S9); 10 000 µg/plate (+ rat S9); 10 000 µg/plate (+ hamster S9)	Not vapour-phase exposure; pre-incubation protocol; purity, NR	Haworth et al. (1983)
		(–) NT	(–) (–)	3333 µg/plate (–S9); 3333 µg/plate (+ rat S9); 1000 µg/plate (+ hamster S9)		
Salmonella typhimurium, TA100	Reverse mutation	(–) NT	(–) (–)	1000 µg/plate (–S9); 3333 µg/plate (+ rat S9); 1000 µg/plate (+ hamster S9)	Not vapour-phase exposure; pre-incubation protocol; purity, NR	NTP (2000)
		(–) NT	(–) (–)	10 000 µg/plate (–S9); 10 000 µg/plate (+ rat S9); 10 000 µg/plate (+ hamster S9)		
Salmonella typhimurium, TA104	Reverse mutation	(–)	(+)	1000 µg/mL (–S9); 10 µg/mL (+Aroclor 1254-induced rat S9)	Not vapour-phase exposure; standard plate incorporation; purity, NR	Strobel & Grummt (1987)

Table 4.5 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium, TA1535	Reverse mutation	+	+	80 mg/L in air (–S9); 80 mg/L in air (+Aroclor 1254-induced rat S9) (Fisher Co.)	Plate incorporation assay modified in sealed desiccators to allow vapour-phase exposure; purity, 97%	Nestmann et al. (1984)
		+	NT	210 mg/L in air (Aldrich Co.)		
Salmonella typhimurium, TA1535	Reverse mutation	+	+	10% in air (–S9); 10% in air (+Aroclor 1254-induced rat S9) (non-stabilized)	Plate incorporation assay in sealed dessicator to allow modified vapour-phase exposure; non-stabilized, purity, 99.8%; stabilized, purity, 94.1% (5.65% stabilizer)	Shimada et al. (1985)
		+	+	2.5% in air (–S9); 2.5% in air (+Aroclor 1254-induced rat S9) (stabilized)		
Salmonella typhimurium, TA1535	Reverse mutation	+	+	0.1% in air for 24 h (–S9); 0.5% in air for 24 h (+S9)	Vapour-phase exposure; purity, 99%; results of two replicate experiments	JETOC (2005)
		+	+	0.5% in air for 24 h (–S9); 0.5% in air for 24 h (+S9)		
Salmonella typhimurium, TA1535, TA1537, and TA1538	Reverse mutation	(–)	(–)	10 000 µg/plate (–S9); 10 000 µg/plate (+Aroclor 1254-induced rat S9)	Not vapour-phase exposure; standard plate incorporation; purity, NR	Richold & Jones (1981)
Salmonella typhimurium, TA1535 and TA1537	Reverse mutation	(–) NT	(–) (–)	10 000 µg/plate (–S9); 10 000 µg/plate (+ rat S9); 10 000 µg/plate (+ hamster S9)	Not vapour-phase exposure; pre-incubation protocol; purity, NR	Haworth et al. (1983)
		(–) NT	(–) (–)	1000 µg/plate (–S9); 3333 µg/plate (+ rat S9); 3333 µg/plate (+ hamster S9)		
Salmonella typhimurium, TA1535 and TA1537	Reverse mutation	(–) NT	(–) (–)	1000 µg/plate (–S9); 3333 µg/plate (+ rat S9); 1000 µg/plate (+ hamster S9)	Not vapour-phase exposure; pre-incubation protocol; purity, NR	NTP (2000)
		(–) NT	(–) (–)	10 000 µg/plate (–S9); 10 000 µg/plate (+ rat S9); 10 000 µg/plate (+ hamster S9)		
Salmonella typhimurium, TA1535/pSK1002	DNA damage SOS *(umu)* induction assay	–	–	666 µg/mL		Nakamura et al. (1987)
Escherichia coli, WP2 *uvrA*/pKM101	Reverse mutation	–	–	5% in air for 24 h (–S9); 5% in air for 24 h (+S9)	Vapour-phase exposure; purity, 99%; results observed in 2 replicate experiments	JETOC (2005)

Table 4.5 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Escherichia coli, WP2 *uvrA*	Reverse mutation	(–)	(–)	1000 µg/mL (–S9); 1000 µg/mL (+Aroclor 1254-induced rat S9)	Not vapour-phase exposure; microtiter fluctuation test; purity, NR	Gatehouse (1981)
Escherichia coli, WP2 *uvrA*	Reverse mutation	(–)	(–)	1000 µg/mL (–S9); 1000 µg/mL (+Aroclor 1254-induced rat S9)	Not vapour-phase exposure; standard plate incorporation; purity, NR	Falck et al. (1985)
Escherichia coli, WP2 *uvrA*	Reverse mutation	(–)	(–)	1000 µg/mL (–S9); 1000 µg/mL (+Aroclor 1254-induced rat S9)	Not vapour-phase exposure; Mutascreen test; purity, NR	Falck et al. (1985)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; ppm, parts per million; S9, 9000 × *g* supernatant from liver; US EPA, United States Environmental Protection Agency.

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

chromosome loss (Whittaker et al., 1990) or aneuploidy in *S. cerevisiae* (Parry & Sharp, 1981).

1,1,1-Trichloroethane did not induce mitotic malsegregation in *Aspergillus nidulans* strain P1 (Crebelli et al., 1988).

1,1,1-Trichloroethane gave negative results in the L-arabinose resistance (Ara) forward-mutation assay in *Salmonella typhimurium* both in the presence and absence of metabolic activation (Roldán-Arjona et al., 1991). A negative response was also observed in the 8-azaguanine resistance forward-mutation assay in *S. typhimurium* in both the presence and absence of metabolic activation (Skopek et al., 1981).

With a modified vapour-phase exposure protocol in a sealed exposure chamber, 1,1,1-trichloroethane induced reverse mutations in *S. typhimurium* strains TA100 and TA1535, but not in TA98, TA1537, or TA1538 (Shimada et al., 1985). In this study, it was observed that 1,1,1-trichloroethane stabilized with 5.65% butylene oxide yielded a higher potency and magnitude of response than did 1,1,1-trichloroethane with a purity of 99.8%. [The Working Group noted that this study highlights the confounding nature of contaminating "stabilizer" additives.] Similarly, mostly positive responses in TA100 and TA1535 were observed in studies that used a modified vapour-phase exposure in a sealed desiccator to assess mutagenicity in *S. typhimurium* (Simmon et al., 1977; Nestmann et al., 1980; Gocke et al., 1981; Nestmann et al., 1984; JETOC, 2005). 1,1,1-Trichloroethane induced reverse mutations in TA100 in both the presence and absence of metabolic activation (Simmon et al., 1977). Using the vapour-phase exposure approach, 1,1,1-trichloroethane did not induce reverse mutations in TA98 but did induce reverse mutations in TA100 and TA1535 (Nestmann et al., 1980). In another vapour-phase exposure study, positive responses were observed in *S. typhimurium* strains TA100 and TA1535 with 1,1,1-trichloroethane obtained from one source, whereas a negative response and a less potent positive response were observed in TA100 and TA1535, respectively, with 1,1,1-trichloroethane from a different source (Nestmann et al., 1984). [The Working Group noted that these differences may be attributed to minute, undetermined differences in chemical composition between the two sources of 1,1,1-trichloroethane.] A positive response in TA100 and TA1535 was observed after vapour-phase exposure in a sealed desiccator (Gocke et al., 1981). In another study using vapour-phase exposures, positive responses were observed for *S. typhimurium* strains TA100 and TA1535 both with and without metabolic activation, whereas negative responses were observed for *Escherichia coli* strain WP2 *uvrA*/pKM101 and *S. typhimurium* strains TA98 and TA1537, both with and without metabolic activation (JETOC, 2005). One bacterial reverse-mutation study employing a sealed desiccator for vapour-phase exposure reported negative responses in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, both with and without metabolic activation (Milman et al., 1988). [The Working Group noted that these studies demonstrate the importance of using modified vapour-phase exposure to assess the mutagenicity of 1,1,1-trichloroethane. The Working Group also noted that *S. typhimurium* strains TA100 and TA1535, which are both used to measure base substitution, are the strains that are most sensitive to 1,1,1-trichloroethane-induced mutagenicity.]

1,1,1-Trichloroethane generally did not induce reverse mutations in the standard plate-incorporation assay. Negative results were observed in *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538, and in *E. coli* strain WP2 *uvrA* when following the standard plate-incorporation protocol (MacDonald, 1981; Richold & Jones, 1981; Rowland & Severn, 1981; Trueman, 1981; Falck et al., 1985). One study, however, yielded positive responses in *S. typhimurium* strains TA97, TA98, TA100, and TA104 following the standard plate-incorporation protocol (Strobel & Grummt, 1987). Similarly,

1,1,1-trichloroethane did not induce reverse mutations in the pre-incubation assay in *S. typhimurium* strains TA92, TA98, TA100, TA1535, TA1537, and TA1538, either with or without metabolic activation (Brooks & Dean, 1981; Haworth et al., 1983; NTP, 2000). 1,1,1-Trichloroethane also did not induce reverse mutations in the Mutascreen automated assay in *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538, and in *E. coli* strain WP2 *uvrA* (Falck et al., 1985). 1,1,1-Trichloroethane gave negative results in the microtiter fluctuation test in *S. typhimurium* strains TA98, TA1535, and TA1537, and in *E. coli* strain WP2 *uvrA* (Gatehouse, 1981).

1,1,1-Trichloroethane did not induce *umu* gene expression in *S. typhimurium* strain TA1535/pSK1002 in either the presence or absence of metabolic activation (Nakamura et al., 1987).

4.2.3 Induces oxidative stress

(a) Humans

No studies were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

A study analysing the relationship between hepatotoxicity and free radical production found that, while 1,1,1-trichloroethane at a dose of 5 mmol/kg bw administered orally to rats caused mild hepatotoxicity, as measured by a weak, but significant increase in serum glutamic--pyruvic transaminase (GPT) activity, it did not lead to an increase in free radical concentrations (Xia & Yu, 1992).

Tabatabaei & Abbott (1999) measured the generation of 2,3-dihydroxybenzoic acid (2,3-DHBA) as a marker of oxidative stress, since 2,3-DHBA is generated after hydroxyl radical attack on salicylate and can be measured with high sensitivity by liquid chromatography-mass spectrometry. They found that in rats pre-treated with salicylate then given 1,1,1-trichloroethane at 700 mg/kg bw via intraperitoneal injection, mean maximal plasma 2,3-DHBA concentrations increased 6.4-fold compared with saline-treated controls (Tabatabaei & Abbott, 1999).

Hepatotoxicity, as demonstrated by a heavily congested central vein, blood sinusoids, leukocytic infiltration, and hepatocellular apoptosis, was observed in young (i.e. age 3–5 weeks) Swiss albino mice given 1,1,1-trichloroethane at 100 and 400 µg/kg bw by intraperitoneal injection twice per week for 3 weeks. Internucleosomal DNA fragmentation was identified by histopathology, and increased levels of lipid peroxidation were measured by the quantification of thiobarbituric acid-reactive substances (TBARS), thus indicating DNA damage caused by oxidative stress (Al-Griw et al., 2016).

In one study on the transgenerational hepatic effects of 1,1,1-trichloroethane in Swiss albino mice, young (i.e. age ~3 weeks) females in the F_0 generation were given 1,1,1-trichloroethane at 100 µg/kg bw by intraperitoneal injection twice per week for 3 weeks and bred at age 10 weeks. An increase in adult-onset liver abnormalities was observed in both F_0 female mice and F_1 (offspring) mice, increased signs of lipid peroxidation, as measured by TBARS, in the livers of both the F_0 and F_1 mice, and increased nitric oxide and protein carbonyl content (i.e biomarkers of oxidative stress) in the livers of F_1 mice (Al-Griw et al., 2017).

(ii) Non-human mammalian cells in vitro

A study in cardiac myocytes isolated from neonatal rats did not find any evidence that 1,1,1-trichloroethane at up to 1 and 4 mM enhanced H_2O_2-induced oxidative injury as measured by release of TBARS during lipid peroxidation and loss of lactate dehydrogenase through damaged sarcolemma membranes, respectively (Toraason et al., 1994).

Electron spin resonance spectroscopy coupled to the spin trapping technique was used to

investigate the formation of free radicals in cultured primary hepatocytes from rats pre-treated with phenobarbital. The results revealed that 1,1,1-trichloroethane at 2.5 μL/mL induced the formation of free radicals in cultured primary rat hepatocytes under both normoxic and hypoxic conditions (Tomasi et al., 1984).

1,1,1-Trichloroethane did not induce lipid peroxidation, as measured by TBARS, in either bovine pulmonary arterial endothelial cells or in rabbit aortic smooth muscle cells at concentrations ranging from 0.6% to 4% v/v. An increase in lipid peroxidation was observed in endothelial cells treated with both 1,1,1-trichloroethane and Fe(III)ADP and in smooth muscle cells treated with 1,1,1-trichloroethane and either Fe(III) ADP or Fe(II)ADP. These increases significantly exceeded the effects observed in cells treated with Fe(III)ADP or Fe(II)ADP alone (Tse et al., 1990). [The Working Group noted that this study indicated evidence of synergistic oxidative-stress activity with iron and 1,1,1-trichloroethane.]

(iii) Acellular systems in vitro

The effects of 1,1,1-trichloroethane on CYP-dependent mixed-function oxidation by rat liver microsomes was studied by determination of the rates of O_2 consumption, H_2O_2 production, 1,1,1-trichloroethane metabolism, and spectral change in CYP. After incubation with phenobarbital-induced rat liver microsomes, 1,1,1-trichloroethane increased rates of O_2 consumption and H_2O_2 production, but metabolism was minimal. 1,1,1-Trichloroethane bound to CYP caused a type I spectral change. No increase in TBARS was observed. Together, the results indicate that 1,1,1-trichloroethane is not metabolized by CYP-dependent mixed-function oxidation, but rather that it has an uncoupling effect on the enzymes and causes futile O_2 consumption and H_2O_2 production (Takano et al., 1988). [The Working Group noted that this study provides a potential mechanism for the induction of oxidative stress by 1,1,1-trichloroethane.]

4.2.4 Induces chronic inflammation

(a) Humans

(i) Exposed humans

Muttray et al. (1999) exposed 12 healthy, non-smoking students to 1,1,1-trichloroethane at 20 and 200 ppm for 4 hours in an exposure chamber, using a crossover study design. Concentrations of interleukin (IL) 1β, IL6, and IL8 were significantly elevated, and prostaglandin E_2 was unchanged in nasal secretions after exposure to 1,1,1-trichloroethane at 200 ppm, indicating the initiation of a subclinical inflammatory response. [The Working Group noted that the results presented in this study represent an acute inflammatory response.]

(ii) Human cells in vitro

No studies were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

Chronic inflammation was observed in the kidneys of male rats exposed to feed containing microencapsulated 1,1,1-trichloroethane at 10 000 ppm or more for 13 weeks (NTP, 2000).

In an investigation into the relative effects of 1,1,1-trichloroethane on liver and kidney function in Swiss-Webster mice exposed via a single intraperitoneal injection, liver changes consistent with inflammation were reported; these are described in more detail in Section 4.3 (Klaassen & Plaa, 1966). [The Working Group noted that the results presented in this study represent an acute inflammatory response.]

(ii) Non-human mammalian cells in vitro

In an in vitro study in mouse embryo fibroblasts, 1,1,1-trichloroethane at up to 100 μM did not have any effect on the induction of interferon α or β (Sonnenfeld et al., 1983). [The Working Group noted that the results presented in this

study may not be relevant to a chronic inflammatory response.]

4.2.5 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

No studies were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

In a chronic study, groups of 50 male and 50 female Osborne-Mendel rats and $B6C3F_1$ mice were given 1,1,1-trichloroethane in corn oil by oral administration at two dose levels on 5 days per week for 78 weeks. Rats received doses of 750 or 1500 mg/kg bw per day, and mice were given time-weighted average doses of 2807 or 5615 mg/kg bw per day. The 1,1,1-trichloroethane used had a purity of 95% with 3% *para*-dioxane [1,4-dioxane]. No signs of altered cell proliferation, cell death, or nutrient supply were observed (NTP, 1977).

In a chronic inhalation study, cortical hyperplasia was observed in the adrenal glands at slightly increased incidence in female Fischer 344 rats exposed to 1,1,1-trichloroethane at 3200 ppm compared with the controls. Urine analysis in the last week of the 2-year exposure period demonstrated increased frequency of ketone bodies in male mice at 3200 ppm (Ohnishi et al., 2013). [The Working Group noted that the results of this study provide evidence of increased cellular proliferation (e.g. hyperplasia) and altered nutrient supply (e.g. the presence of ketone bodies in the urine of male mice).]

Two studies noted that 1,1,1-trichloroethane did not induce significant effects on either the initiation (at 9.9 mmol/kg bw, the MTD) or promotion (at 7.4 mmol/kg bw) of liver foci in Osborne-Mendel rats when increased γ-glutamyltranspeptidase activity was used as a marker for putative preneoplastic lesions (Story et al., 1986; Milman et al., 1988).

(ii) Non-human mammalian cells in vitro

No studies were available to the Working Group.

4.2.6 Evidence relevant to other key characteristics

(a) Humans

Regarding immunosuppression, the effects of 1,1,1-trichloroethane on the immune function of natural killer, natural cytotoxic, and natural P815 killer cells isolated from human liver were assessed in vitro by measuring the tumoricidal activity of the exposed immune cells against K562 human erythroleukaemia, WEHI-164 mouse fibrosarcoma, and P815 mouse mastocytoma cells, respectively. 1,1,1-Trichloroethane had no significant effect on the immune function of the natural killer, natural cytotoxic, and natural P815 killer cells (Wright et al., 1994).

(b) Experimental systems

Regarding immunosuppression, the effects of single and multiple 3-hour exposures to 1,1,1-trichloroethane at 350 ppm were evaluated in CD-1 mice by monitoring changes in their susceptibility to experimentally induced *Streptococcus* aerosol infection and pulmonary bactericidal activity against inhaled *Klebsiella pneumoniae*. Neither single nor 5 day repeated exposures to 1,1,1-trichloroethane had any effect on mortality or bactericidal activity (Aranyi et al., 1986).

Regarding the modulation of receptor-mediated effects, increased butyrylcholinesterase activity is associated with depleted testosterone. After continuous exposure by inhalation at 625 ppm for 30 days, 1,1,1-trichloroethane did not increase plasma butyrylcholinesterase levels in male NMRI mice, thus no evidence of receptor-mediated effects was observed (Kjellstrand

et al., 1985). Inhalation of 1,1,1-trichloroethane at 3500 ppm for 30 minutes led to decreased plasma levels of corticosterone and increased hypothalamic corticotropin-releasing factor in male Sprague-Dawley rats. Inhalation of 5000 ppm 1,1,1-trichloroethane for 30 minutes led to decreased plasma corticosterone and plasma adrenocorticotropic hormone levels, but no change in adrenocorticotropic hormone or corticotropin-releasing factor levels in the hypothalamus, hippocampus, or frontal cortex in rats. These results indicate a suppression of hypothalamic-pituitary-adrenal axis activity (Pise et al., 1998).

Regarding immortalization, 1,1,1-trichloroethane at 99 and 990 µM induced transformation in Fischer rat embryo cells (F1706). These transformed cells produced undifferentiated fibrosarcomas in inoculated newborn Fischer rats (Price et al., 1978). Syrian hamster embryo cells exposed to 1,1,1-trichloroethane at vapour concentrations of 8–23 µg/cm^3 for 20 hours experienced significantly enhanced transformation by SA7 adenovirus. Conversely, exposure to the liquid did not enhance transformation (Hatch et al., 1983). 1,1,1-Trichloroethane induced a positive dose-dependent transformation response in BALB/c-3T3 cells exposed in sealed glass chambers in two separate studies (Tu et al., 1985; Milman et al., 1988). [The Working Group noted that the available studies suggest that 1,1,1-trichloroethane is capable of immortalizing cells in vitro.]

4.2.7 High-throughput in vitro toxicity screening data evaluation

The analysis of the in vitro bioactivity of the agents reviewed in *IARC Monographs* Volume 130 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). 1,1,1-Trichloroethane was one of thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes of the US EPA and the United States National Institutes of Health. Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2021d). A supplementary table (Annex 2, Supplementary material for Section 4, Mechanistic Evidence, web only; available from: https://publications.iarc.fr/611) contains a summary of the findings (including the assay name, the corresponding key characteristic, the resulting "hit calls" both positive and negative, and any reported caution flags) for 1,1,1-trichloroethane. The results were generated with the software "kc-hits" (key characteristics of carcinogens – high-throughput screening discovery tool) (available from: https://gitlab.com/i1650/kc-hits) using the US EPA ToxCast and Tox21 assay data and the curated mapping of key characteristics to assays available at the time of the evaluations performed for the present monograph. Findings and interpretations from these high-throughput assays for 1,1,1-trichloroethane are discussed below.

After mapping against the key characteristics of carcinogens, the ToxCast/Tox21 database contained 111 assays in which 1,1,1-trichloroethane was tested. Of these, it was found to be active in only one assay corresponding to a loss of HEK293 cell viability, in which it exhibited a half-maximal activity concentration (AC_{50}) of 57.50 µM. However, this assay was reported with a caution flag: less than 50% active and borderline activity (US EPA, 2021d). [The Working Group noted that this result is not relevant to the carcinogenicity of the chemical.]

4.3 Other relevant evidence

Groups of 10 male and 10 female F344/N rats and B6C3F$_1$ mice were given feed containing microencapsulated 1,1,1-trichloroethane at a

concentration of 5000, 10 000, 20 000, 40 000, or 80 000 ppm for 13 weeks. Relative and absolute liver weights in female rats were decreased at the highest dose. Male rats at 10 000 ppm or greater exhibited a spectrum of non-neoplastic kidney lesions, including renal tubule casts and renal tubule degeneration, consistent with hyaline droplet nephropathy (NTP, 2000).

In chronic inhalation studies, male and female Fischer 344 rats and B6C3F$_1$ mice were exposed to 1,1,1-trichloroethane at 150, 500, or 1500 ppm for 6 hours per day, 5 days per week, for 2 years. Very slight microscopic hepatic effects, such as an accentuation of the normal hepatic lobular pattern and smaller hepatocytes with altered cytoplasmic staining around the portal vein, were seen in the liver of male and female rats at 1500 ppm and necropsied at 6, 12, and 18 months (Quast et al., 1988).

In a short-term renal toxicity study in male F344/N rats, 1,1,1-trichloroethane administered by gavage at a dose of 0.62 or 1.24 mmol/kg bw per day once daily for 21 days did not lead to hyaline droplet nephropathy, although clinical pathology suggested renal injury, and urinary protein output and aspartate aminotransferase activity were higher than in the controls (NTP, 1996).

An investigation into the relative effects of chlorinated hydrocarbons on liver and kidney function in Swiss-Webster mice exposed via a single intraperitoneal injection noted that 1,1,1-trichloroethane caused less severe liver dysfunction than did the other chlorinated hydrocarbons tested, as measured by sulfobromophthalein retention and serum GPT determination, and did not cause renal dysfunction, as measured by phenolsulfonephtalein excretion. Enlargement of hepatocytes with portal lymphocytic infiltration and vacuolation and slight necrosis were noted in the livers of animals treated with lethal concentrations of 1,1,1-trichloroethane. No microscopic changes were observed in the kidneys (Klaassen & Plaa, 1966).

5. Summary of Data Reported

5.1 Exposure characterization

1,1,1-Trichloroethane is a High Production Volume chlorinated hydrocarbon that was widely used in the 1970s and 1980s for cold cleaning and vapour degreasing of metal parts and machinery such as printing presses, printed circuit boards, plastic moulds, and many other appliances in a variety of industries including metalworking, printing, chemicals, plastics, and in numerous workplaces, such as garages. 1,1,1-Trichloroethane was also used in various other applications and products, including aerosol products, adhesives, coatings and inks, and textiles. Starting in the late 1990s, use of 1,1,1-trichloroethane was gradually phased out because of its capacity to deplete stratospheric ozone; however, it continued to be a major feedstock material for other hydrochlorofluorocarbon products, and had more minor but essential uses, such as for medical devices and aviation safety testing.

1,1,1-Trichloroethane is readily released into the environment from fugitive air emissions, and to surface water and soil, and leachates from landfills, during the production and use of both industrial and consumer products. Once in the environment, 1,1,1-trichloroethane can migrate far from its source of origin because of its long half-life, and has been measured at varying levels in urban, rural, and indoor air samples; in surface water and groundwater samples; and in soil, and waste samples. Historically, it was also present in a variety of food products, drinking-water, and many household products.

Occupational exposure to 1,1,1-trichloroethane may occur during its manufacture and during its use in a variety of industries. In these diverse workplaces, 1,1,1-trichloroethane is taken up via all routes, but inhalation is the major route of exposure. 1,1,1-Trichloroethane can be quantified in biological samples, and its metabolites

trichloroethanol and trichloroacetic acid have been quantified in blood, end-exhaled air, and urine samples from exposed humans. The number of exposed workers, however, is likely to be substantially lower now than in the 1970s to 1990s.

The general population was also probably exposed to low levels of 1,1,1-trichloroethane in the 1970s to 1990s because of widespread use. 1,1,1-Trichloroethane was present in blood samples of participants in earlier National Health and Nutrition Examination Surveys (1988–1994, NHANES III); however, more recent surveys since 2005 have not detected 1,1,1-trichloroethane in the blood, indicating diminished exposures. Implementation of the Montreal Protocol has resulted in significant decline in the production and use of 1,1,1-trichloroethane, which has caused reduction in environmental contamination and significant reduction in human exposure.

5.2 Cancer in humans

The available evidence on cancer in humans consisted of two cohort studies, five nested case–control studies, and sixteen population-based case–control studies, with most of these having been published since the previous evaluation of 1,1,1-trichloroethane by the *IARC Monographs* programme. These studies examined occupational exposure to 1,1,1-trichloroethane and the risk of lymphatic and haematopoietic malignancies, cancers of the kidney and urinary bladder, breast, and brain and nervous system, as well as melanoma of the skin and cancers of the digestive tract, bone, lung, cervix, prostate, and testis. There were also two case studies on cholangiocarcinoma and ampullary carcinoma.

Among the studies on multiple myeloma, some statistically significant positive, although imprecise, associations with ever-exposure to 1,1,1-trichloroethane were observed in two cohort studies with very small numbers of exposed cases; in one of the studies, the positive finding was observed among female but not male cohort members. There was also a statistically significant positive association with ever-exposure to 1,1,1-trichloroethane in a case–control study, based on 36 exposed cases. The association remained in sensitivity analysis reassigning jobs with low confidence in the assessment to the unexposed category. Odds ratios were elevated across most categories of exposure duration, unlagged cumulative exposure, and cumulative exposure with a 10-year lag, although no evidence of a positive trend with increasing exposure category was observed. Overall, the Working Group considered that a positive association between exposure to 1,1,1-trichloroethane and multiple myeloma was credible; however, in view of the small numbers of exposed participants, potential misclassification in exposure assessment, and potential selection bias, systematic or random errors could not be ruled out with reasonable confidence.

Among studies on other cancer types, there were few positive findings and the available studies in humans were not sufficiently informative to permit a conclusion to be drawn about the presence or absence of a causal association owing to the small numbers of exposed participants (particularly for highly exposed participants), potential misclassification in exposure assessment, and potential selection bias, information bias, or other methodological sources of bias.

5.3 Cancer in experimental animals

Treatment with 1,1,1-trichloroethane caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in two species.

1,1,1-Trichloroethane was administered by inhalation in one study in male and female Crj:BDF_1 mice. In males, 1,1,1-trichloroethane caused an increase in the incidence of malignant lymphoma in the spleen, bronchioloalveolar carcinoma, and bronchioloalveolar adenoma or

carcinoma (combined). In females, 1,1,1-trichloroethane caused an increase in the incidence of bronchioloalveolar adenoma or carcinoma (combined).

1,1,1-Trichloroethane was administered by inhalation in one study in F344/DuCrj rats. In males, 1,1,1-trichloroethane caused an increase in the incidence of peritoneal mesothelioma.

1,1,1-Trichloroethane was administered by oral administration (gavage) in one study in Sprague-Dawley rats. In males, 1,1,1-trichloroethane caused an increase in the incidence of leukaemia (the combination of various histological types) in a variety of organs and tissues.

5.4 Mechanistic evidence

1,1,1-Trichloroethane is rapidly absorbed in humans after either dermal/percutaneous exposure or inhalation, as confirmed by measurements of 1,1,1-trichloroethane in blood or exhaled air. Once absorbed, 1,1,1-trichloroethane is distributed primarily into the brain and adipose tissue, with significantly lower amounts in other tissues. Most pharmacokinetic data in humans indicate that < 10% of absorbed 1,1,1-trichloroethane is metabolized. Multiple cytochrome P450s (CYPs) can metabolize 1,1,1-trichloroethane to trichloroethanol and trichloroacetic acid, although CYP2E1 is believed to be the primary enzyme involved. 1,1,1-Trichloroethane is a relatively poor substrate for CYP-dependent oxidative metabolism compared with other organic solvents. Elimination of 1,1,1-trichloroethane occurs by either exhalation of unmetabolized 1,1,1-trichloroethane in the breath, or excretion of either unmetabolized 1,1,1-trichloroethane or the metabolites trichloroethanol or trichloroacetic acid in the urine. Most of the absorbed 1,1,1-trichloroethane (~90% in humans, ~95% in rats) is excreted as unmetabolized 1,1,1-trichloroethane rather than as metabolites. Studies on the absorption, distribution, metabolism, and excretion of 1,1,1-trichloroethane in experimental systems (including in rats, mice, guinea-pigs, and dogs) generally support the findings in humans and human-derived cells or tissues, although rates are faster in these systems than in humans.

Overall, the mechanistic evidence for 1,1,1-trichloroethane regarding the key characteristics of carcinogens ("is electrophilic or metabolically activated", "is genotoxic", "induces oxidative stress", "induces chronic inflammation", "modulates receptor-mediated effects" "causes immortalization", and "alters cell proliferation, cell death, or nutrient supply") is suggestive but incoherent across different experimental systems. There were no studies in humans with exposure specifically attributable to 1,1,1-trichloroethane.

There is suggestive indirect evidence for the formation of electrophilic metabolites from 1,1,1-trichloroethane in human cells in vitro. In experimental systems, there is suggestive evidence for DNA and protein binding. Consequences suggesting the formation of an electrophilic intermediate from 1,1,1-trichloroethane occur at exposure levels that are lower than for most other characterized halogenated solvents. There is suggestive evidence indicating that 1,1,1-trichloroethane is genotoxic under specific test conditions. Positive responses were obtained in comet and micronucleus-formation assays in human cells in vitro, but results were generally negative in non-human mammalian systems in vivo, and incoherent across other experimental systems in vitro. Positive responses were observed in 2 out of 10 genotoxicity studies in non-human mammalian cells in vitro, with the remaining studies yielding negative or equivocal results. Using a modified vapour-phase exposure protocol, 1,1,1-trichloroethane gave positive results for mutagenicity in two strains of *Salmonella typhimurium*.

Regarding the key characteristics "induces oxidative stress", "induces chronic inflammation", "modulates receptor-mediated effects", and "causes immortalization", there is suggestive

mechanistic evidence. 1,1,1-Trichloroethane induced oxidative stress in rodents and in mammalian experimental systems in vitro. 1,1,1-Trichloroethane also induced chronic inflammation in the kidney of rats. The results of one study indicated that 1,1,1-trichloroethane suppressed hypothalamic–pituitary–adrenal axis activity in rats, but no receptor-mediated effects were observed in another study in mice. Four studies indicated that 1,1,1-trichloroethane was capable of immortalizing rodent cells in vitro. Regarding the key characteristic "alters cell proliferation, cell death, or nutrient supply", there is suggestive but incoherent mechanistic evidence for 1,1,1-trichloroethane. In one study, exposure to 1,1,1-trichloroethane induced cortical hyperplasia in the adrenal gland of female rats and an increase in the frequency of ketone bodies in the urine of male mice, but in another chronic study in rodents, no alterations in cell proliferation, cell death, or nutrient supply were observed. 1,1,1-Trichloroethane also had no effect on either the initiation or promotion of rat liver foci in two studies. Regarding the key characteristic "is immunosuppressive", 1,1,1-trichloroethane had no effects in two studies. 1,1,1-Trichloroethane 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 *limited evidence* in humans for the carcinogenicity of 1,1,1-trichloroethane. Positive associations have been observed between exposure to 1,1,1-trichloroethane and multiple myeloma.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,1,1-trichloroethane.

6.3 Mechanistic evidence

There is *limited mechanistic evidence*.

6.4 Overall evaluation

1,1,1-Trichloroethane is *probably carcinogenic to humans (Group 2A)*.

6.5 Rationale

The Group 2A evaluation for 1,1,1-trichloroethane is based on *limited evidence* for cancer in humans and *sufficient evidence* for cancer in experimental animals.

The evidence was *limited* that exposure to 1,1,1-trichloroethane causes multiple myeloma in humans. There were some statistically significant positive, although imprecise, associations between ever-exposure to 1,1,1-trichloroethane and multiple myeloma observed in two cohort studies with very small numbers of exposed cases. There was also a statistically significant positive association between ever-exposure to 1,1,1-trichloroethane and multiple myeloma in a case–control study. Odds ratios were elevated across most categories of exposure duration and cumulative exposure, but no evidence of a positive trend with increasing exposure category was observed. While positive associations were seen in the body of evidence, the small numbers of exposed participants, and concerns regarding potential misclassification in exposure assessment and potential selection bias meant that chance and bias could not be ruled out with reasonable confidence. The evidence for cancer at other sites in humans was *inadequate*: there

were few positive findings and the available studies were not sufficiently informative to permit a conclusion to be drawn about the presence or absence of a causal association.

The *sufficient evidence* for cancer in experimental animals is based on an increased incidence of either malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in two species.

The mechanistic evidence was *limited* as the findings regarding the key characteristics of carcinogens across experimental systems, including in some studies using human cells in vitro, were suggestive, but incoherent.

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1,2-DIPHENYLHYDRAZINE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 122-66-7

EC/List No.: 204-563-5

Chem. Abstr. Serv. name: 1,2-diphenylhydrazine

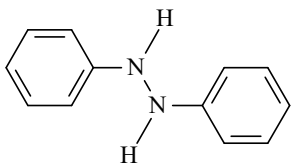
IUPAC systematic name: 1,2-diphenylhydrazine

Synonyms: hydrazobenzene; *N*,*N*′-diphenylhydrazine; hydrazodibenzene; *N*,*N*′-bianiline; 1,2-diphenyldiazane; symmetrical diphenylhydrazine; and other depositor-supplied synonyms and acronyms (OEHHA, 2021; NCBI, 2021)

1.1.2 Structural and molecular information

Relative molecular mass: 184.24 (IFA, 2021)

Chemical structure:



Molecular formula: $C_{12}H_{12}N_2$

1.1.3 Chemical and physical properties

Description: colourless crystalline solid or powder, colourless in solution; the compound colour may change to yellow or orange owing to the oxidative formation of azobenzene (ATSDR, 2020; IFA, 2021; NCBI, 2021)

Melting point: 123–126 °C (IFA, 2021)

Boiling point: 309 °C, decomposes (NCBI, 2021)

Relative density: 1.16 at 16 °C (IFA, 2021)

Lower explosion limit: 15 g/m³ (IFA, 2021)

Vapour pressure: 4.4×10^{-4} hPa at 25 °C (NCBI, 2021)

Solubility: poorly soluble in water (221 mg/L at 25 °C) (Kühne et al.,1995; IFA, 2021); insoluble in acetic acid, slightly soluble in benzene and dimethyl sulfoxide, very soluble in ethanol (NCBI, 2021)

Octanol/water partition coefficient (P): log K_{ow} = 2.94 (IFA, 2021)

Decomposition temperature: 131 °C (IFA, 2021)

Reactivity: decomposes to aniline and azobenzene at its melting point and above; readily and dangerously reacts with strong oxidizing agents and acids, acid chlorides, and acid anhydrides; autoxidizes in air; rearranges to

benzidine in strong mineral acid (IFA, 2021; NCBI, 2021).

1.1.4 Impurities

Technical-grade 1,2-diphenylhydrazine has considerable levels of impurities and may contain benzidine as a contaminant. In 1 of 16 samples from a manufacturer's continuous production, 25 µg of benzidine per 1 g of 1,2-diphenylhydrazine [equals 25 ppm] was reported (NCBI, 2021).

1.2 Production and use

1.2.1 Production process

1,2-Diphenylhydrazine is produced by the chemical reduction of nitrobenzene in an alkaline medium. Various reducing agents may be used in the process, the most common being iron or zinc metal powders (ATSDR, 2020; NCBI, 2021). After synthesis, separation of 1,2-diphenylhydrazine may be performed with solvent extraction or crystallization in an alcoholic solution (Hallie, 1949; ATSDR, 2020).

1.2.2 Production volume

In 1977, the USA produced at least 450 000 kg of 1,2-diphenylhydrazine and imported 72 100 kg. In 1982, the USA imported 23 200 kg of 1,2-diphenylhydrazine (NTP, 2016). [The Working Group noted that no information was available on current production in the USA or European Union (EU).] The substance is not registered under the REACH Regulation (Registration, Evaluation and Authorization of Chemicals of the European Union), suggesting that less than 1 tonne is manufactured in and/or imported to the European Economic Area (ECHA, 2021). The ChemicalBook database lists more than 100 global suppliers of 1,2-diphenylhydrazine and 3 manufacturers in China (ChemicalBook, 2017) [but it is unknown whether production is continuous or on-demand].

1.2.3 Uses

Historically, 1,2-diphenylhydrazine was widely used globally as a chemical precursor to produce benzidine-based dyes, which were mostly used in the textile industry (NTP, 2016; ATSDR, 2020). Starting from the late 1970s, countries began limiting the manufacture and use of benzidine-based dyes owing to reports of increased incidence of bladder cancer associated with exposure to benzidine (Dapson, 2009). Also in the late 1970s, major dye manufacturers in the USA began phasing out benzidine-based dyes, and production in the USA ceased by 1988 (Dapson, 2009). [The Working Group noted that in developing countries such as China, 1,2-diphenylhydrazine use may be ongoing in some dye- and textile-manufacturing facilities. Textile products imported to Europe occasionally contain benzidine or its derivatives (Piccinini et al., 2008), which are prohibited in the EU (Council Directive 2002/61/EC; European Council, 2002). These chemicals originate from benzidine-based dyes, which can be produced using 1,2-diphenylhydrazine.]

1,2-Diphenylhydrazine has additional uses in the pharmaceutical industry as a chemical intermediate in the manufacture of phenylbutazone (an anti-inflammatory medication) and sulfinpyrazone (a uricosuric medication) (ATSDR, 2020). Although both drugs are now very rarely used in humans in the USA and other developed countries, phenylbutazone is frequently used in veterinary medicine, particularly for treating lameness in horses (Worboys & Toon, 2018).

1.3 Detection and quantification

1.3.1 Air, water, soil, sediment, and other media

No standardized sampling and analytic protocols are available for 1,2-diphenylhydrazine in air, water, soil, sediment, and other media. United States Environmental Protection Agency (US EPA) Method 625.1 is the standard protocol for detecting benzidine and other semi-volatile organic pollutants qualitatively and quantitatively in environmental samples (US EPA, 2016). US EPA Method 625.1 lists 1,2-diphenylhydrazine as a potential "additional extractable analyte", but noted that quantitative determination may be difficult for chemicals in this category. In this method and other published works, 1,2-diphenylhydrazine is extracted from an environmental or product sample with a solvent, and then detection and quantification are carried out using chromatographic and mass spectrometry methods (US EPA, 2016; ATSDR, 2020).

1.3.2 Biological specimens

No specific human biomarker has been identified for detecting and quantifying exposure to 1,2-diphenylhydrazine.

1.4 Occurrence and exposure

1.4.1 Occurrence in the environment, food, and consumer products

The US EPA Toxics Release Inventory (TRI) documented small quantities of 1,2-diphenylhydrazine released into air, surface water, and landfills. Total annual releases from 1998 to 2019 were mostly less than 25 pounds [11.3 kg], except for a landfill release of 260 pounds [118 kg] in 2001 and an air release of 48 pounds [22 kg] in 2017 (US EPA, 2021a).

According to water quality data from the United States National Water Quality Monitoring Council (NWQMC) and summarized by the Agency for Toxic Substances and Disease Registry (ATSDR), 1,2-diphenylhydrazine was detected in 92 of 2409 groundwater samples collected between 1990 and 2020 at concentrations ranging from 0.12 to 21 ppb [µg/L]. Over the same period, 1,2-diphenylhydrazine was detected in 14 of 3286 samples of surface water, with detected concentrations ranging from < 0.2 to 260 ppb [µg/L], and in 3 of 1238 sediment samples, with detected concentrations ranging from < 340 to < 1700 ppb [µg/L] (ATSDR, 2020).

In a national wastewater survey published in 1985, 1,2-diphenylhydrazine was detected in 1.2% of 1205 effluent samples, with a median concentration of < 10 µg/L (Staples et al., 1985). According to a survey of 50 public water-treatment plants by the US EPA and published in 1982, 1,2-diphenylhydrazine was detected in 10 of 347 samples of influent wastewater, with a concentration range of 1–50 µg/L, and in 5 of 362 effluent samples, with a concentration range of 1–2 µg/L (US EPA, 1982).

In other media, 1,2-diphenylhydrazine was screened for but not detected in two studies screening for pollutants in fish caught in the Great Lakes or nearby tributaries, USA (De Vault, 1985; Camanzo et al., 1987). Medications produced using 1,2-diphenylhydrazine (i.e. phenylbutazone and sulfinpyrazone) may contain trace concentrations of the compound (Matsui et al., 1983); however, in the USA and other developed countries, use of these drugs in human medicine has either been discontinued or is very limited (Worboys & Toon, 2018).

[The Working Group noted that 1,2-diphenylhydrazine is difficult to detect and quantify in environmental samples owing to its rapid oxidation during both sample storage and analysis (ATSDR, 2020). Thus, reported concentrations may include azobenzene or 1,2-diphenylhydrazine as degradation products from

other compounds. It was further noted that no data were available on environmental, dietary, or exposure to consumer products outside of the USA.]

1.4.2 Occupational exposure

Workers involved in the manufacture of 1,2-diphenylhydrazine, benzidine-based dyes, phenylbutazone, and sulfinpyrazone may be exposed via ingestion and inhalation of 1,2-diphenylhydrazine. The 1983 United States National Occupational Exposure Survey estimated that 977 workers, including 154 women, were potentially exposed to 1,2-diphenylhydrazine in seven facilities (NIOSH, 2018). [The Working Group noted that the National Occupational Exposure Survey was performed nearly 40 years ago and the estimates reported may not be representative of current exposures.] In the Finnish national register of workers exposed to carcinogenic substances and processes, one chemist was identified as having exposure to 1,2-diphenylhydrazine in 2013 and 2014 (Saalo et al., 2016a, b). No other published studies and reports were available to assess current and historical occupational exposures to 1,2-diphenylhydrazine.

1.4.3 Exposure of the general population

Owing to limited use and release as well as relatively rapid environmental degradation, general population exposure to 1,2-diphenylhydrazine is expected to be low. [The Working Group noted that exposure may have been higher historically for populations living near dye- and textile-production sites when 1,2-diphenylhydrazine was more widely used, or for patients who used phenylbutazone.] However, no specific studies or reports were available to further characterize current and historical exposures in the general population.

1.5 Regulations and guidelines

1.5.1 Exposure limits and guidelines

The United States National Recommended Water Quality Criteria contain two guideline values for 1,2-diphenylhydrazine: 0.03 µg/L for human health, for the consumption of water and aquatic organisms; and 0.2 µg/L for human health, for the consumption of aquatic organisms only (US EPA, 2021b). The Australian and New Zealand Guidelines for Fresh and Marine Water Quality recommended 2 µg/L in fresh and marine water as a toxicant default guideline value for protecting aquatic ecosystems (Water Quality Australia, 2020).

The United States ATSDR derived an intermediate duration minimal risk level (MRL) of 0.05 mg/kg per day for oral intake of 1,2-diphenylhydrazine on the basis of hepatic toxicity in rats (ATSDR, 2020). No other MRLs were derived for acute or chronic oral intake, or for other exposure routes owing to limited data.

According to the harmonized classification and labelling implemented in the European Union (Classification, Labelling and Packaging Regulation, 1272/2008/EC), 1,2-diphenylhydrazine has the following classification: carcinogen, category 1B; acute toxicity, category 4; acute aquatic toxicity, category 1; and chronic aquatic toxicity, category 1. Because of this classification, this substance is subject to several other European Union regulations (e.g. general product safety, food contact, medical devices) (ECHA, 2021). Employers are obliged under the Classification, Labelling and Packaging Regulation to minimize worker exposure to 1,2-diphenylhydrazine and must arrange for medical surveillance of exposed workers (Council Directive 98/24/EC; European Council, 1998). European Union Council Directives state that pregnant or breastfeeding workers and persons under age 18 years may not be occupationally exposed to 1,2-diphenylhydrazine (Council directive 92/85/EEC;

Council directive 94/33/EC; European Council, 1992, 1994).

1.5.2 Reference values for biological monitoring of exposure

No reference value was available for biological monitoring of exposure to 1,2-diphenylhydrazine in humans.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1.

3.1 Mouse

3.1.1 Oral administration (feed)

In a well-conducted study by the United States National Cancer Institute (NCI), groups of 50 male and 47–50 female B6C3F$_1$ mice (age, 6 weeks) were given feed containing technical-grade hydrazobenzene [1,2-diphenylhydrazine] [purity not reported; one unidentified impurity] (NTP, 1978). The experiments at the higher and lower doses were conducted separately, each dose group having its own controls for males and females (50 males and 50 females per group). The time-weighted average (TWA) dietary concentrations used were 0%, 0.008%, and 0.04% for males and 0%, 0.004%, and 0.04% for females in the control groups and at the lower and higher doses, respectively. After 78 weeks of treatment with 1,2-diphenylhydrazine, observation of the mice continued for an additional 17 weeks (lower dose) or 18 weeks (higher dose). The study was terminated at week 95–96. Survival of male and female mice was significantly lower for the group at the higher dose than for the controls. However, there were adequate numbers of animals at risk for late-developing tumours, with survival at study termination being 66% (33/50) for males at the higher dose compared with 78% (39/50) for the respective control group, and 52% (26/50) for females at the higher dose compared with 76% (38/50) for the respective control group. In mice at the lower dose, survival at study termination was 88% (44/50) for males compared with 86% (43/50) for males in the respective control group, and 79% (37/47) for females compared with 72% (36/50) for females in the respective control group. No distinct pattern of mean body-weight change was evident in groups of males and females at the lower dose. After week 28, a decrease in mean body weight compared with controls was observed for male and female mice at the higher dose [read from the figure, the decreases reached approximately 30% at week 78 of administration]. All mice underwent complete necropsy, and histopathology was performed on major tissues, organs, and gross lesions taken from killed animals and, whenever possible, from animals found dead.

In female mice, there was a significant increase in the incidence of hepatocellular carcinoma ($P < 0.001$, Fisher exact test) at the higher dose but not at the lower dose (higher dose, 20/43 versus 1/50 in controls; lower dose, 4/39 versus 2/47 in controls). The incidence of hepatocellular adenoma or carcinoma (combined) was significantly increased ($P < 0.001$, Fisher exact test) at the higher dose (22/43 versus 1/50 in controls), but not at the lower dose (4/39 versus 2/47 in controls). 1,2-Diphenylhydrazine did not significantly increase the incidence of any tumours in treated male mice. [The Working Group noted that this was a well-described and well-conducted study, using two doses in both males and females (with respective control groups), with an adequate number of animals per group. The lack of data confirming the purity and stability of 1,2-diphenylhydrazine in the dosed feed was considered a

Table 3.1 Studies of carcinogenicity in experimental animals exposed to 1,2-diphenylhydrazine

Study design Species, strain (sex) Age at start Duration Reference	Route, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence or multiplicity	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) ~6 wk 95 wk NTP (1978)	Oral administration (feed) Purity, NR (technical grade) Feed 0%, 0.008% diet, ad libitum for 78 wk 50, 50 43, 44	No significant increase in tumour incidence in treated animals		Principal strengths: males and females used; well-conducted study; adequate number of mice per group Principal limitations: lack of data confirming the stability of 1,2-diphenylhydrazine Other comments: concentrations are TWA
Full carcinogenicity Mouse, B6C3F$_1$ (M) ~6 wk 95–96 wk NTP (1978)	Oral administration (feed) Purity, NR (technical grade) Feed 0%, 0.04% diet, ad libitum for 78 wk 50, 50 39, 33	No significant increase in tumour incidence in treated animals		Principal strengths: males and females used; well-conducted study; adequate number of mice per group Principal limitations: lack of data confirming the stability of 1,2-diphenylhydrazine Other comments: concentrations are TWA
Full carcinogenicity Mouse, B6C3F$_1$ (F) ~6 wk 95–96 wk NTP (1978)	Oral administration (feed) Purity, NR (technical grade) Feed 0%, 0.004% diet, ad libitum for 78 wk 50, 47 36, 37	No significant increase in tumour incidence in treated animals		Principal strengths: males and females used; well-conducted study; adequate number of mice per group Principal limitations: lack of data confirming the stability of 1,2-diphenylhydrazine Other comments: concentrations are TWA
Full carcinogenicity Mouse, B6C3F$_1$ (F) ~6 wk 96 wk NTP (1978)	Oral administration (feed) Purity, NR (technical grade) Feed 0%, 0.04% diet, ad libitum for 78 wk 50, 50 38, 26	*Liver* Hepatocellular carcinoma Tumour incidence: 1/50, 20/43* Hepatocellular adenoma or carcinoma (combined) Tumour incidence: 1/50, 22/43*	*$P < 0.001$, one-tailed Fisher exact test *$P < 0.001$, one-tailed Fisher exact test	Principal strengths: males and females used; well-conducted study; adequate number of mice per group Principal limitations: lack of data confirming the stability of 1,2-diphenylhydrazine Other comments: concentrations are TWA

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	Tumour incidence or multiplicity	Significance	Comments
Full carcinogenicity Mouse, strain A (M) 6–8 wk 24 wk Maronpot et al. (1986)	Intraperitoneal injection Purity, NR Tricaprylin 0, 50, 100, 200 mg/kg bw 3×/wk for 8 wk 60, 10, 10, 10 54, 10, 8, 9	*Lung*: pulmonary tumours Tumour incidence: 7/54, 1/10, 0/8, 6/9* Tumour multiplicity: 0.167, 0.10, 0.00, 0.89*	*$P < 0.05$, Fisher exact test *$P < 0.05$, *t*-test	Principal strengths: used males and females. Other comments: histological characterization of "pulmonary tumours" was not reported
Full carcinogenicity Mouse, strain A (F) 6–8 wk 24 wk Maronpot et al. (1986)	Intraperitoneal injection Purity, NR Tricaprylin 0, 50, 100, 200 mg/kg bw 3×/wk for 8 wk 60, 10, 10, 10 54, 10, 10, 9	*Lung*: pulmonary tumours Tumour incidence: 6/54, 2/10, 2/10, 3/9 Tumour multiplicity: 0.110, 0.30, 0.30, 0.56*	NS *$P < 0.05$, *t*-test	Principal strengths: used males and females. Other comments: histological characterization of "pulmonary tumours" was not reported
Full carcinogenicity Rat, F344 (M) ~6 wk 107–108 wk NTP (1978)	Oral administration (feed) Purity, NR (technical grade) Feed 0%, 0.008% diet, ad libitum for 78 wk 50, 50 35, 39	*Liver* Hepatocellular carcinoma Tumour incidence: 0/47, 5/49* Neoplastic nodules or hepatocellular carcinoma (combined) Tumour incidence: 5/47, 13/49*	*$P = 0.031$, one-tailed Fisher exact test *$P = 0.040$, one-tailed Fisher exact test	Principal strengths: used males and females; well-conducted study; adequate number of rats per group Principal limitations: lack of data confirming the stability of 1,2-diphenylhydrazine Other comments: concentrations are TWA

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	Tumour incidence or multiplicity	Significance	Comments
Full carcinogenicity Rat, F344 (M) ~6 wk 106–109 wk NTP (1978)	Oral administration (feed) Purity, NR (technical grade) Feed 0%, 0.03% diet, ad libitum for 78 wk 49, 50 36, 32	*Liver* Hepatocellular carcinoma Tumour incidence: 1/48, 31/49*	*$P < 0.001$, one-tailed Fisher exact test	Principal strengths: used males and females; well-conducted study; adequate number of rats per group Principal limitations: lack of data confirming the stability of 1,2-diphenylhydrazine Other comments: concentrations are TWA
		Neoplastic nodules or hepatocellular carcinoma (combined) Tumour incidence: 1/48, 37/49*	*$P < 0.001$, one-tailed Fisher exact test	
		Adrenal gland: pheochromocytoma or malignant pheochromocytoma (combined) Tumour incidence: 8/47, 16/46*	*$P = 0.042$, one-tailed Fisher exact test	
		Zymbal gland: squamous cell carcinoma Tumour incidence: 0/48, 5/49*	*$P = 0.030$, one-tailed Fisher exact test	
		Ear canal, Zymbal gland, or skin of the ear: squamous cell carcinoma or squamous cell papilloma (combined) Tumour incidence: 0/48, 7/49*	*$P = 0.007$, one-tailed Fisher exact test	
Full carcinogenicity Rat, F344 (F) ~6 wk 108–109 wk NTP (1978)	Oral administration (feed) Purity, NR (technical grade) Feed 0%, 0.004% diet, ad libitum for 78 wk 50, 50 39, 37	No significant increase in tumour incidence in treated animals		Principal strengths: used males and females; well-conducted study; adequate number of rats per group Principal limitations: lack of data confirming the stability of 1,2-diphenylhydrazine Other comments: concentrations are TWA

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	Tumour incidence or multiplicity	Significance	Comments
Full carcinogenicity Rat, F344 (F) ~6 wk 107–109 wk NTP (1978)	Oral administration (feed) Purity, NR (technical grade) Feed 0%, 0.01% diet, ad libitum for 78 wk 50, 50 43, 25	*Liver*: neoplastic nodules Tumour incidence: 0/50, 6/50* *Mammary gland*: adenocarcinoma, NOS Tumour incidence: 0/50, 6/50*	*P = 0.013, one-tailed Fisher exact test *P = 0.013, one-tailed Fisher exact test	Principal strengths: used males and females; well-conducted study; adequate number of rats per group Principal limitations: lack of data confirming the stability of 1,2-diphenylhydrazine Other comments: concentrations are TWA; historical controls – mammary gland adenocarcinoma: 8/585 (1.4%)

bw, body weight; F, female; M, male; NOS, not otherwise specified; NR, not reported; NS, not significant; TWA, time-weighted average; wk, week.

limitation of this study. The Working Group also noted possible contamination of technical-grade 1,2-diphenylhydrazine with benzidine at concentrations up to 25 ppm (see Section 1.1.4). Although oral administration of benzidine (in the feed) has been shown to cause hepatocellular neoplasms in male and female B6C3F$_1$ mice (IARC, 2012), the dose level at which benzidine was carcinogenic was considerably higher than that reported in the present study (NTP, 1978) had the carcinogenic response been attributable to benzidine as a contaminant of 1,2-diphenylhydrazine. A difference between 1,2-diphenylhydrazine and benzidine in terms of carcinogenic response elicited was that 1,2-diphenylhydrazine caused hepatocellular neoplasms only in female B6C3F$_1$ mice.]

3.1.2 Skin application

In a lifetime carcinogenicity study in CC57 brown mice (Pliss, 1974), 2 mg of 1,2-diphenylhydrazine [purity not reported] dissolved in 0.05 mL of benzene was applied to the skin (of the interscapular region) of 50 male and female mice (age, 2 months) [sex distribution not reported], three times per week for 63 weeks. The total dose of 1,2-diphenylhydrazine per mouse was 360 mg. At 25 weeks, when the first tumour (a squamous cell carcinoma of the skin) was observed, survival was 41/50 (12 females and 29 males). Survival was 16/50 (3 females and 13 males) after 1 year, and only 4/50 (all males) after 1.5 years. All mice underwent complete necropsy, and main organs and tissues [Working Group assumption] were evaluated by histopathology.

No tumours developed at the site of application, but nine mice developed systemic tumours: one mouse developed a squamous cell carcinoma of the skin, two developed liver haemangioma, three developed leukaemia, and three developed lung adenoma. Two mice developed two tumours simultaneously: the first mouse developed liver adenoma and lung adenoma, and the second mouse developed liver adenoma and leukaemia. [The Working Group noted the lack of controls, the use of benzene as the vehicle, the lack of information on body weight, the small number of animals per group, and the fact that the number of animals per sex was not reported; overall, the study was considered inadequate for the evaluation of the carcinogenicity of 1,2-diphenylhydrazine in experimental animals.]

3.1.3 Intraperitoneal injection

In a carcinogenicity study involving blind testing of 65 chemicals (Maronpot et al., 1986), groups of 10 male and 10 female strain A mice (age, 6–8 weeks) were treated with 1,2-diphenylhydrazine ([purity not reported]; dissolved in tricaprylin) at a dose of 50, 100, or 200 mg per kg body weight (bw) for the groups at the lowest, intermediate, and highest dose, respectively, by intraperitoneal injection (0.1 mL), three times per week for 8 weeks. [The Working Group considered that the short study duration and low number of animals per group were adequate for this strain of mice, which has a high incidence of spontaneous pulmonary neoplasms.] Groups of 60 male and 60 female tricaprylin-injected (0.1 mL) mice served as vehicle controls. After 8 weeks, the mice were held for 16 weeks until the end of the experiment. The highest dose level of 1,2-diphenylhydrazine used was chosen because this dose did not cause death, growth retardation, or overt toxicity in a preliminary dose-setting study.

A significant increase in the incidence of pulmonary tumours ($P < 0.05$, Fisher exact test) was observed in males at the highest dose compared with vehicle controls, and a significant increase in the multiplicity (ratio of tumours per mouse) of pulmonary tumours ($P < 0.05$, *t*-test) was observed in males and females at the highest dose compared with their respective controls. In these groups at the highest dose, the proportion of male survivors with tumours was 67% [6/9],

and tumour multiplicity was 0.89, while the proportion of female survivors with tumours was 33% [3/9] and tumour multiplicity was 0.56. In each of the groups of females at the lowest and intermediate dose, which each included 10 survivors, two mice developed pulmonary tumours; while in the group of males at the lowest dose, which included 10 survivors, one mouse developed pulmonary tumours; however, the differences were not significant compared with vehicle controls. The incidence and multiplicity of pulmonary tumours in mice in the vehicle-control groups were 7/54 and 0.167, respectively, in males, and 6/54 and 0.110, respectively, in females. [The Working Group noted the use of males and females, and that the histological characterization of "pulmonary tumours" was not reported, hence, the designation of the tumours as benign (adenoma) or malignant (carcinoma) was not possible.]

3.1.4 Subcutaneous injection

In a lifetime carcinogenicity study, 60 CC57 brown mice (age, 2 months; [sex distribution not reported]) were subcutaneously injected with a suspension containing 5 mg of 1,2-diphenylhydrazine [purity not reported] in 0.2 mL of sunflower oil, once per week for 73 weeks (Pliss, 1974). The first tumour (a rhabdomyosarcoma at the site of injection) was observed at week 38, when 29 males and 1 female were still alive. At 1 year after the first injection, 22 mice survived. Only 3 mice were alive at 1.5 years. All mice underwent complete necropsy, and main organs and tissues [Working Group assumption] were evaluated by histopathology.

Overall, 11 mice developed tumours (2 mice developed rhabdomyosarcoma at the site of injection, 5 mice developed lung adenoma, 1 mouse developed liver adenoma, 2 mice developed liver haemangioma, and 1 mouse developed leukaemia). One mouse developed both a rhabdomyosarcoma and a liver haemangioendothelioma; another mouse developed both a lung adenoma and a liver haemangioma. Thus, 36.7% (11/30) of mice treated with 1,2-diphenylhydrazine by subcutaneous infection developed tumours. In the control group [not further specified], only 17% of the animals developed spontaneous tumours, which the authors stated were of a type and morphology analogous to the tumours induced by 1,2-diphenylhydrazine. [The Working Group noted that tumours in the control group were unspecified, and no information on body weight was provided; overall, the study was considered inadequate for the evaluation of the carcinogenicity of 1,2-diphenylhydrazine in experimental animals.]

3.2 Rat

3.2.1 Oral administration (feed)

In a lifetime carcinogenicity study, 20 male Wistar rats (age, 6–8 weeks) were given feed ("Larsen's diet", a synthetic feed) containing 1,2-diphenylhydrazine [purity not reported] at a TWA dose of 2 mg per day until spontaneous death occurred (Marhold et al., 1968). An untreated control group of 50 male Wistar rats received feed only. Average duration of survival and average body weight at the end of the experiment were lower in the group treated with 1,2-diphenylhydrazine (288 days and 121 g, respectively) than in the control group (378 days and 141 g, respectively). No tumours were observed in either the group treated with 1,2-diphenylhydrazine or in the control group. [The Working Group noted the small number of treated animals and the limited reporting of experimental details, including information on postmortem examination and histopathological evaluation; overall, the study was considered inadequate for the evaluation of the carcinogenicity of 1,2-diphenylhydrazine in experimental animals.]

In a lifetime carcinogenicity study, 72 male and female outbred rats [age at start and sex distribution not reported], were given feed supplemented with 30 mg of 1,2-diphenylhydrazine [purity not reported] in 0.5 mL of sunflower oil, 5 times per week for 84 weeks (Pliss, 1974). The mean total dose of 1,2-diphenylhydrazine was 12.57 g per rat. After 1 year, 42 rats survived (25 females and 17 males). All mice underwent complete necropsy, and main organs and tissues [Working Group assumption] were evaluated by histopathology.

Overall, 21 rats (16 females and 5 males) developed tumours: 5 rats developed tumours of the Zymbal gland (malignant in two cases, and bilateral in one case); 6 rats developed liver tumours (including 4 hepatic adenomas, 1 cholangioma, and 1 lymphangioma); 4 rats developed mammary gland tumours (2 adenocarcinomas and 2 fibroadenomas); 3 rats developed uterine tumours (1 adenocarcinoma, 1 lymphangioma, and 1 lymphangiosarcoma); 2 rats developed kidney tumours (1 multiple papillary adenoma, and 1 bilateral tubulo-papillary adenocarcinoma associated with hepatocellular carcinoma and skin fibroma); and 1 rat developed a lymphoid leukaemia (Pliss, 1974). [The Working Group noted the lack of a control group, and the fact that sex distribution at start and bodyweight data were not reported; overall, the study was considered inadequate for the evaluation of the carcinogenicity of 1,2-diphenylhydrazine in experimental animals.]

In a well-conducted study by the NCI, groups of 49–50 male and 50 female Fischer 344 rats (age, 6 weeks) were given feed containing technical-grade hydrazobenzene [1,2-diphenylhydrazine] [purity not reported; one unidentified impurity] (NTP, 1978). The experiments at the higher dose and at the lower dose were conducted separately, each dose group having its own controls for males and females (49–50 males and 49–50 females per group). The TWA dietary concentrations used were 0%, 0.008%, and 0.03% for males, and 0%, 0.004% and 0.01% for females in the control groups and at the lower and higher doses, respectively. After 78 weeks of treatment with 1,2-diphenylhydrazine, observation of the rats continued for an additional 28–30 weeks. The study was terminated at week 106–109. Survival of female rats was significantly lower in the group at the higher dose than in the controls, but survival of male rats was not influenced by treatment with 1,2-diphenylhydrazine. For rats at the higher dose, survival at study termination was 64% (32/50) for males compared with 73% (36/49) for males in the respective control group, and 50% (25/50) for females compared with 86% (43/50) for females in the respective control group. For rats at the lower dose, survival at study termination was 78% (39/50) for males compared with 70% (35/50) for males in the respective control group, and 74% (37/50) for females compared with 78% (39/50) for females in the respective control group. A slight decrease in mean body weight relative to controls was apparent for males at the higher dose, but not for males at the lower dose. A slight decrease in mean body weight was also observed for females at the lower dose after week 46, and for females at the higher dose after week 22. All rats underwent complete necropsy, and full histopathology was performed on major tissues, organs, and gross lesions taken from killed animals and, whenever possible, from animals found dead.

The incidence of hepatocellular carcinoma was significantly increased in males at the lower dose (5/49; $P = 0.031$, Fisher exact test) and at the higher dose (31/49; $P < 0.001$, Fisher exact test), compared with their control groups (0/47 and 1/48, respectively). There was also a significant increase in the incidence of liver neoplastic nodules or hepatocellular carcinoma (combined) in males at the lower dose (13/49; $P = 0.040$, Fisher exact test) and at the higher dose (37/49; $P < 0.001$, Fisher exact test), compared with their control groups (5/47 and 1/48, respectively). A significant increase in the incidence of pheochromocytoma

or malignant pheochromocytoma (combined) of the adrenal gland (16/46; $P = 0.042$, Fisher exact test) was also observed in males at the higher dose compared with males in the respective control group (8/47). There were significant increases in the incidence of squamous cell carcinoma of the Zymbal gland (5/49; $P = 0.030$, Fisher exact test), and of squamous cell papilloma or carcinoma (combined) of the ear canal, Zymbal gland or skin of the ear (combined) (7/4; $P = 0.007$, Fisher exact test) in males at the higher dose compared with males in the respective control group (0/48 and 0/48, respectively).

In females, there was a significant increase in the incidence of mammary gland adenocarcinoma (not otherwise specified) (6/50 for the group at the higher dose versus 0/50 for the respective control group; $P = 0.013$, Fisher exact test), and in the incidence of neoplastic nodules in the liver (6/50 for the group at the higher dose versus 0/50 for the respective control group; $P = 0.013$, Fisher exact test). [The Working Group noted that this was a well-described and well-conducted study, using two doses in both males and females (with respective control groups), with an adequate number of animals per group. The lack of data confirming the stability of 1,2-diphenylhydrazine in dosed feed was a limitation of this study. The Working Group also noted possible contamination of technical-grade 1,2-diphenylhydrazine with benzidine at concentrations up to 25 ppm (see Section 1.1.4). Although oral administration (by gavage) of benzidine has also been shown to cause mammary gland carcinomas in female Sprague-Dawley rats (IARC, 2012), no hepatocellular neoplasms were observed, unlike the carcinogenic response in this study with 1,2-diphenylhydrazine (NTP, 1978).]

3.2.2 Subcutaneous injection

In a lifetime carcinogenicity study, a group of 52 Sherman female rats (age, ~10 weeks) was treated with 60 mg of technical-grade 1,2-diphenylhydrazine [purity not reported] in olive oil (1 mL) by subcutaneous injection, once per week, for life (Spitz et al., 1950). A control group of 50 rats was treated with olive oil (1 mL) only. The rats were killed if they showed dramatic loss of weight or obvious illness. The survival rate of rats treated with 1,2-diphenylhydrazine was the same as that of the control group. Rats in both the treated group and the control group (22%) died during the first 200 days of the experiment during a period of hot weather when the rooms were not air-conditioned. Rats in the control group additionally died from tracheobronchitis with associated abscesses of the lung, otitis, meningitis, and brain abscess. The body weights of control and treated rats were similar and reached a maximum of 250–280 g. All rats underwent complete necropsy.

No hepatic tumours developed in treated rats. One keratinized squamous cell carcinoma (1/52) occurred on the skin of the external auditory canal of a treated rat. [The Working Group noted the inadequacy of the conditions of animal maintenance, the lack of reporting results for controls, and the lack of details on postmortem examination and histopathological evaluation; overall, the study was considered inadequate for the evaluation of the carcinogenicity of 1,2-diphenylhydrazine in experimental animals.]

In a lifetime carcinogenicity study, 91 male and female outbred rats [age at start and sex distribution not reported] were given 60 mg of 1,2-diphenylhydrazine [purity not reported] in 0.3–0.5 mL of sunflower oil by subcutaneous injection, once per week, for 10 weeks. The dose was then lowered to 40 mg per week until the end of the exposure (84 weeks) (Pliss, 1974). All rats underwent complete necropsy, and main organs and tissues [Working Group assumption] were evaluated by histopathology.

The first tumour (Zymbal gland carcinoma) appeared at week 27, when 29 females and 24 males were still alive. After 1 year, 22 females and 15 males were still alive. The last rat with

a tumour (liver adenoma) died 98 weeks after the beginning of the experiment. Mean tumour latency was 74 weeks, and tumours developed in 12 rats: three rats developed mammary gland tumours (two with microfollicular cancers, and one with adenoma and fibroadenoma); three rats developed liver tumours (including one malignant adenoma [carcinoma] and one adenoma associated with a pulmonary lymphosarcoma); three rats developed Zymbal gland tumours (one basalioma [basal cell carcinoma], one carcinoma, one squamous cell carcinoma); one rat developed a uterine adenocarcinoma, one rat developed a haemangioma of the spleen, and one rat developed reticulosis. [The Working Group noted the lack of controls and that the number of animals per sex was not reported; therefore, the study was considered inadequate for the evaluation of the carcinogenicity of 1,2-diphenylhydrazine in experimental animals.]

In a lifetime carcinogenicity study, a group of 50 outbred rats (25 males and 25 females) ([age at start not reported]; body weight, 100–120 g), were exposed by subcutaneous injection to 20 mg of a paste that contained 55% 1,2-diphenylhydrazine [purity not reported], 15% zinc compounds, and 30% water (dissolved in 0.5 mL of sunflower oil), once per week for 1 year (Genin at al., 1975; Shabad & Genin, 1975; also reported in Kurliandskiĭ et al., 1976). A group of 50 control animals (25 males and 25 females) received sunflower oil only. All rats underwent complete necropsy and main organs and tissues [Working Group assumption] were evaluated by histopathology.

The survival rate until the time of onset of the first tumour (~86 weeks) in the group treated with 1,2-diphenylhydrazine was almost half that of controls (19/50 compared with 36/50). No hepatic tumours were observed in treated rats, but there was one polymorphic cell sarcoma at the site of injection, one squamous cell carcinoma of the Zymbal gland, one endometrial polyposis, and one anaplastic (embryonal) kidney cancer. Mean tumour latency in the group treated with 1,2-diphenylhydrazine was 86 ± 6 weeks. In the control group, one fibroadenoma of the mammary gland was detected, with a latent period of 90 weeks. [The Working Group noted that the body weights of control and treated animals, the composition of the feed, and rates of food consumption were not reported, and the time of occurrence of tumours was unspecified; overall, the study was considered inadequate for the evaluation of the carcinogenicity of 1,2-diphenylhydrazine in experimental animals.]

3.3 Evidence synthesis for cancer in experimental animals

The carcinogenicity of 1,2-diphenylhydrazine has been assessed in one well-conducted study in male and female B6C3F$_1$ mice and one well-conducted study in male and female Fischer 344 rats treated by oral administration (in the feed) (NTP, 1978), in two additional studies in male Wistar rats (Marhold et al., 1968) and in male and female outbred rats (Pliss, 1974) also treated by oral administration (in the feed); in male and female strain A mice treated by intraperitoneal injection (Maronpot et al., 1986); in male and female CC57 brown mice treated by skin application (Pliss, 1974); and in three studies in male and female CC57 brown mice (Pliss, 1974), in female Sherman rats (Spitz et al., 1950), and in male and female outbred rats (Pliss, 1974; Genin et al., 1975) treated by subcutaneous injection.

In the well-conducted study in male and female B6C3F$_1$ mice treated by oral administration, there was a significant increase in the incidence of hepatocellular carcinoma and of hepatocellular adenoma or carcinoma (combined) in female mice at the higher dose, but not at the lower dose, compared with their respective controls. There were no significant effects upon the incidence of neoplasms in treated male mice (NTP, 1978).

In the well-conducted study in male and female Fischer 344 rats treated by oral administration, the incidence of hepatocellular carcinoma was significantly increased in males at the lower and higher dose, compared with their respective controls. In male rats, there was also a significant increase in the incidence of liver neoplastic nodules or hepatocellular carcinoma (combined) at the lower and higher dose, compared with their respective controls. A significant increase in the incidence of pheochromocytoma or malignant pheochromocytoma (combined) of the adrenal gland was also observed in males at the higher dose compared with controls. There were significant increases in the incidence of squamous cell carcinoma of the Zymbal gland, and of squamous cell papilloma or carcinoma (combined) of the ear canal, Zymbal gland or skin of the ear (combined) in males at the higher dose compared with controls. In female rats, there was a significant increase in the incidence of mammary gland adenocarcinoma (not otherwise specified), and in the incidence of neoplastic nodules in the liver, both at the higher dose (NTP, 1978).

In the study in male and female strain A mice treated by intraperitoneal injection, a significant increase in the incidence of pulmonary tumours was observed in male mice at the higher dose, and a significant increase in the multiplicity (tumour per mouse ratio) of pulmonary tumours was observed in male and female mice at the higher dose compared with their respective controls (Maronpot et al., 1986).

Two studies in male and female Wistar rats (Marhold et al., 1968) and in male and female outbred rats (Pliss, 1974) treated by oral administration (in the feed), one study in male and female CC57 brown mice treated by skin application (Pliss, 1974), one study in male and female CC57 brown mice treated by intraperitoneal injection (Pliss, 1974), and three studies in male and female CC57 brown mice (Pliss, 1974), in female Sherman rats (Spitz et al., 1950), and in male and female outbred rats (Pliss, 1974; Genin et al., 1975) treated by subcutaneous injection were judged to be inadequate for the evaluation of the carcinogenicity of 1,2-diphenylhydrazine in experimental animals.

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) Absorption, distribution, and excretion

The only data available on the absorption, distribution, and excretion of 1,2-diphenylhydrazine in laboratory animals were those of Dodd et al. (2012) and Dutkiewicz & Szymanska (1973).

Dodd et al. (2012) detected 1,2-diphenylhydrazine in the blood of male Fischer 344 rats given feed containing 1,2-diphenylhydrazine at 200 or 300 ppm for 13 weeks; mean blood concentrations of 1,2-diphenylhydrazine ranged from 0.002 to 0.006 µg/mL. In rats exposed at ≤ 80 ppm, 1,2-diphenylhydrazine blood concentrations were below the limit of quantitation (approximately 0.001 µg/mL) throughout the study.

In a study by Dutkiewicz & Szymanska (1973) in Wistar rats, 1,2-diphenylhydrazine was administered as a single oral (200 or 400 mg/kg bw), intraperitoneal (100 or 200 mg/kg bw), intravenous (4 or 8 mg/kg bw), or intratracheal (5 or 10 mg/kg bw) dose and urinary metabolites were analysed. Dutkiewicz & Szymanska (1973) noted unchanged 1,2-diphenylhydrazine in the urine in rats treated by any route. In addition, there was an unidentified metabolite in the urine of rats treated by intratracheal and oral administration,

suggesting that some urinary excretion had occurred. [The Working Group noted that it was not clear whether this metabolite was associated with exposure to 1,2-diphenylhydrazine, although this was plausible.]

No data were available regarding the distribution of 1,2-diphenylhydrazine in laboratory animals.

(b) Metabolism

In the study by Dutkiewicz & Szymanska (1973), benzidine and aniline were reported to be metabolites in the urine of Wistar rats exposed to 1,2-diphenylhydrazine. Other metabolites included two unspecified hydroxy derivatives of benzidine, 2-aminophenol and 4-aminophenol, and unidentified compounds. [The Working Group noted that the analytical method used in this study was thin-layer chromatography, which may have produced degradation products that were identified as unchanged 1,2-diphenylhydrazine or metabolites.]

With the exception of the unspecified hydroxy derivatives noted above, chemical metabolites were also noted in several other studies in experimental systems (Williams, 1959; National Research Council, 1981; IARC, 1972). Bolton & Griffiths reported the metabolism of 1,2-diphenylhydrazine to aniline by isolated bacterial microflora from rats (Bolton & Griffiths, 1978). These findings are consistent with the metabolic scheme shown in Fig. 4.1, which is based on data for azobenzene and aniline. [The Working Group noted that the enzymes implicated in the metabolism of 1,2-diphenylhydrazine in rodents have not been identified. The Working Group also noted that, based on the available evidence, the metabolism of 1,2-diphenylhydrazine to aniline and benzidine is plausible but uncertain.]

4.2 Evidence relevant to key characteristics of carcinogens

4.2.1 Is genotoxic

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.1.

1,2-Diphenylhydrazine (100 mg/kg bw; [purity not reported]) administered intraperitoneally to male mice induced DNA damage as measured by inhibition of testicular DNA synthesis (thymidine incorporation) (Seiler, 1977), but did not induce hepatic DNA strand breaks, as measured by alkaline elution, in female Sprague-Dawley rats treated with 1,2-diphenylhydrazine at 60 and 130 mg/kg bw by gavage (Kitchin et al., 1994).

(ii) Non-human mammalian cells in vitro

See Table 4.2.

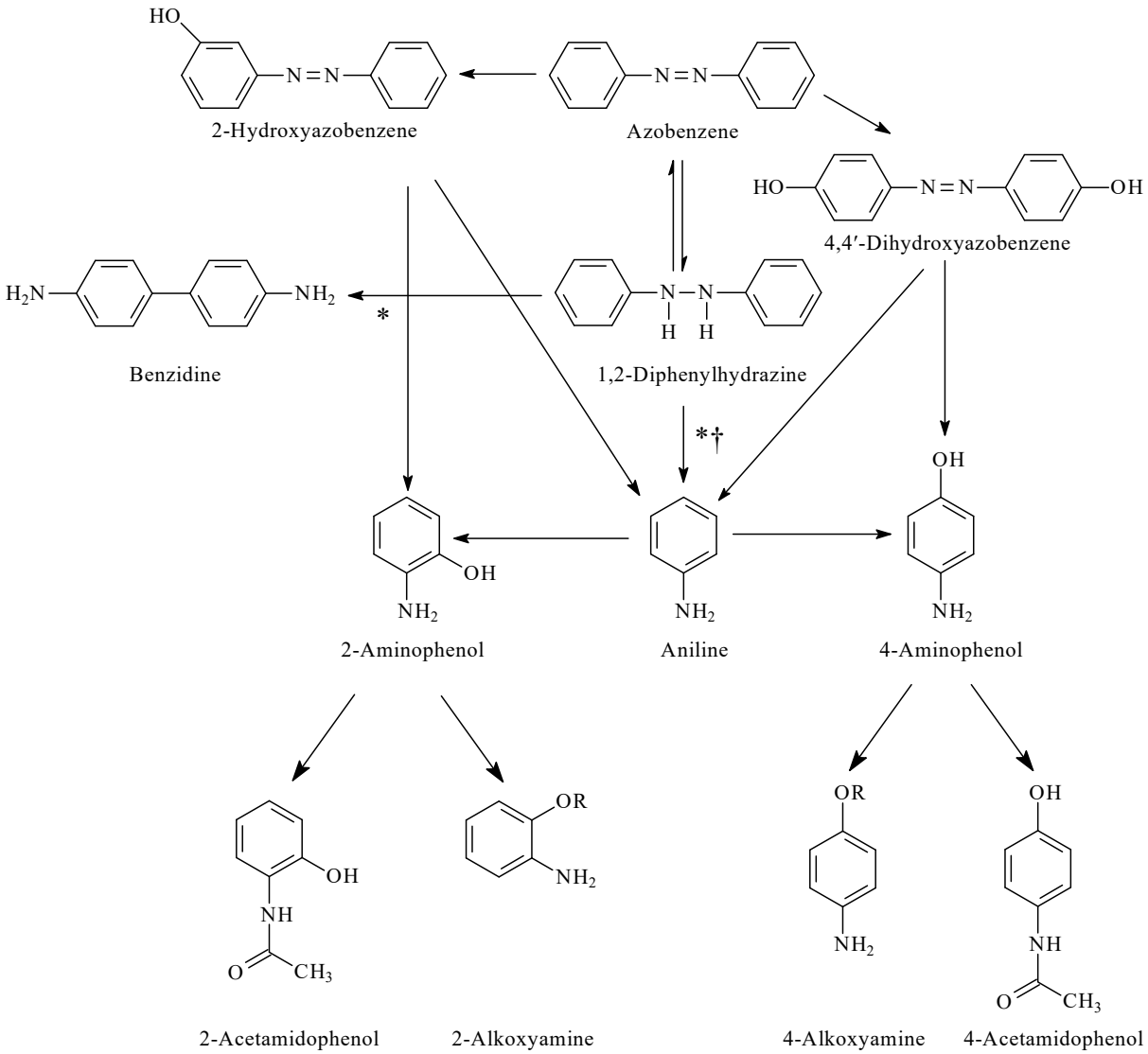
1,2-Diphenylhydrazine induced chromosomal aberrations and sister-chromatid exchanges in the presence of metabolic activation in Chinese hamster ovary (CHO) cells. The response was equivocal for chromosomal aberrations in the absence of metabolic activation (Galloway et al., 1987).

(iii) Non-mammalian experimental systems

See Table 4.3.

1,2-Diphenylhydrazine did not cause sex-linked recessive lethal mutations in *Drosophila melanogaster* (Yoon et al., 1985). 1,2-Diphenylhydrazine induced mutations in *Salmonella typhimurium* strain TA100 with metabolic activation but did not induce mutations in strains TA98, TA1535, TA1537, and TA1538 (Dunkel et al., 1985; Haworth et al., 1983), and gave negative results in the *Escherichia coli* WP2 *uvrA* (Dunkel et al., 1985). [The Working Group

Fig. 4.1 Proposed metabolic scheme for 1,2-diphenylhydrazine



R = SO_3H or $C_6H_9O_6$

* Reported in studies in rats treated with 1,2-diphenylhydrazine (Dutkiewicz & Szymanska, 1973) [The Working Group noted that the analytical method used in this study was thin-layer chromatography, which may have produced degradation products that were identified as unchanged 1,2-diphenylhydrazine or metabolites.]

† Reported in studies using the isolated microflora of rats (Bolton & Griffiths, 1978).

Created by the Working Group.

Table 4.1 Genetic and related effects of 1,2-diphenylhydrazine in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks, alkaline elution	Rat, Sprague-Dawley (F)	Liver	–	60 mg/kg bw, 130 mg/kg bw	Oral (gavage), first dose of 60 mg/kg bw given to one group of rats 21 h before killing; second dose of 130 mg/kg bw given to another group of rats 4 h before killing		Kitchin et al. (1994)
Inhibition of testicular DNA synthesis	Mouse, Swiss (M)	Testes	+	100 mg/kg bw	Single intraperitoneal dose, mice killed 3.5 h later	Purity, NR The assay used has low sensitivity	Seiler (1977)

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

[a] +, positive; –, negative.

Table 4.2 Genetic and related effects of 1,2-diphenylhydrazine in non-human mammalian cells in vitro

End-point	Species, cell type	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Chromosome aberrations	Chinese hamster, ovary (CHO) cells	+/–	+	42 µg/mL –S9, 14 µg/mL +S9		Galloway et al. (1987)
Sister-chromatid exchanges	Chinese hamster, ovary (CHO) cells	–	+	14 µg/mL –S9, 14 µg/mL +S9 in one trial (weak positive), 5 µg/mL +S9 in another trial (positive)		Galloway et al. (1987)

HIC, highest ineffective concentration; LEC, lowest effective concentration; S9, 9000 × *g* supernatant.

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

Table 4.3 Genetic and related effects of 1,2-diphenylhydrazine in non-mammalian experimental and acellular systems

Test system species, strain	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Drosophila melanogaster	Sex-linked recessive lethal mutations	NT	– –	50 ppm (oral, 3 days) 80 ppm (injection)		Yoon et al. (1985)
Salmonella typhimurium, TA100	Reverse mutation base-pair substitution	–	+	100 µg/plate –S9, 33.3 µg/plate + RLI S9 and 100 µg/plate + HLI S9	Purity, NR	Haworth et al. (1983)
Salmonella typhimurium, TA98, TA1535, and TA1537	Reverse mutation	–	–	100 µg/plate	Purity, NR	Haworth et al. (1983)
Salmonella typhimurium, TA100	Reverse mutation	–	+	333 µg/plate –S9, 100 µg/plate +S9	Results from four independent laboratories; metabolic activation compared across several systems: rat S9; RLI S9; mouse S9; MLI S9; hamster S9; HLI S9	Dunkel et al. (1985)
Salmonella typhimurium, TA98, TA1535, TA1537, and TA1538	Reverse mutation	–	–	333 µg/plate		
Escherichia coli, WP2 *uvrA*	Reverse mutation	–	–	3333 µg/plate		
DNA fragment from the human c-Ha-*RAS*-1 protooncogene and *TP53* tumour suppressor gene (acellular system)	DNA damage in the presence of copper(II)	+	NT	20 µM [3.7 µg/mL]	Four concentrations tested (20, 30, 40, and 50 µM) with increasing dose–response relationship	Ohnishi et al. (2000)

HIC, highest ineffective concentration; HLI, hamster liver (Aroclor 1254)-induced; LEC, lowest effective concentration; MILI, mouse liver (Aroclor 1254)-induced; NR, not reported; NT, not tested; ppm, parts per million; RLI, rat liver (Aroclor 1254)-induced; S9, 9000 × *g* supernatant.

[a] +, positive; –, negative.

noted that the purity of 1,2-diphenylhydrazine was not reported for the study by Haworth et al. (1983).] Ohnishi et al. (2000) reported that 1,2-diphenylhydrazine induced DNA damage at thymidine residues, in a study using [^{32}P]-5′-end-labelled DNA fragments obtained from the human c-Ha-*RAS*-1 protooncogene and the *TP53* tumour suppressor gene. They reported that the DNA damage was caused by 1,2-diphenylhydrazine in the presence of copper(II), and this was significantly enhanced by treatment with piperidine, suggesting that 1,2-diphenylhydrazine caused base modification and liberation. [The Working Group noted that this genotoxic effect required the presence of copper in an acellular system.]

4.2.2 Evidence relevant to other key characteristics

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Regarding oxidative stress, Ohnishi et al. (2000) reported that 1,2-diphenylhydrazine in the presence of copper(II) caused an increase in the formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine, a biomarker of oxidative DNA damage, in calf thymus DNA (and formation of H_2O_2), which suggests that 1,2-diphenylhydrazine induces oxidative stress.

Regarding chronic inflammation, in a study by the NCI (NTP, 1978), groups of male and female Fischer 344 rats and B6C3F$_1$ mice were given feed containing 1,2-diphenylhydrazine at a lower or higher dose for 78 weeks. The experiments at the lower and higher doses were conducted separately, each dose group having its own control group for males and females. In male and female rats at the lower dose (dietary concentration, 0.008% and 0.004%, respectively), there was an increased incidence of inflammation in several tissues, including the myocardium, lung, and pancreas, relative to the respective control groups. Similarly, in male and female mice at the lower dose (same doses as in rats), there was an increased incidence of inflammation in several tissues, including the lymph nodes and kidney, relative to the respective control groups. [The Working Group noted that in both sexes and species, the results for the higher-dose studies were often inconsistent with those from the lower-dose studies. In addition, the incidence of inflammation was often higher in the controls for the higher dose than in the controls for the lower dose.]

Regarding alterations in cell proliferation, cell death, or nutrient supply, in the abovementioned study by the NCI (NTP, 1978), exposure to 1,2-diphenylhydrazine in male and female rats at the lower dose increased hyperplasia in a few tissues, including the liver, relative to the respective controls. Exposure to 1,2-diphenylhydrazine in male and female mice at the lower dose led to an increase in hyperplasia, notably in the spleen, relative to the respective controls. [The Working Group noted that in both sexes and species, the results for the higher-dose studies were often inconsistent with those from the lower-dose studies. In addition, the incidence of hyperplasia was often higher in the controls for the higher dose than in the controls for the lower dose. Concerning the chronic inflammation and hyperplasia observed in the NCI study (NTP, 1978), the Working Group noted that none of the non-neoplastic lesions in rats or mice appeared to be treatment-related.] Kitchin et al. (1994) reported that rats treated with 1,2-diphenylhydrazine at a dose of 60 or 130 mg/kg bw by gavage had elevated levels of hepatic ornithine decarboxylase activity, indicative of increased cell proliferation.

4.2.3 High-throughput in vitro toxicity screening data evaluation

The analysis of the in vitro bioactivity of the agents reviewed in *IARC Monographs* Volume 130 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). 1,2-Diphenylhydrazine was one of thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes. Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available. A supplementary table (Annex 2, Supplementary material for Section 4, Mechanistic Evidence, web only; available from: https://publications.iarc.fr/611) provides a summary of the findings (including the assay name, the corresponding key characteristic, the resulting "hit calls" both positive and negative, and any reported caution flags for 1,2-diphenylhydrazine (US EPA, 2021c). The results were generated with the software "kc-hits" (key characteristics of carcinogens – high-throughput screening discovery tool) (available from: https://gitlab.com/i1650/kc-hits) using the US EPA ToxCast and Tox21 assay data and the curated mapping of key characteristics to assays available at the time of the evaluations performed for the present monograph. Findings and interpretations from these high-throughput assays for 1,2-diphenylhydrazine are discussed below.

After mapping against the key characteristics of carcinogens, the ToxCast/Tox21 database contained 290 assays in which 1,2-diphenylhydrazine was tested. Of these, it was found to be active and without caution flags in 24 assays relevant to the key characteristics of carcinogens [The Working Group noted that the cytotoxic limit for 1,2-diphenylhydrazine is 11.17 μM.]

1,2-Diphenylhydrazine was active in four assays mapped to key characteristic 2 (KC2), "is genotoxic". Two of these assays were conducted in chicken lymphoblasts and the half-maximal activity concentration (AC_{50}) was between 22.52 and 24.58 μM; two other assays were conducted in HepG2 cells and the AC_{50} was 104.2 μM.

1,2-Diphenylhydrazine was active in four assays mapped to KC5, "induces oxidative stress". In HepG2 cells, 1,2-diphenylhydrazine was active in one assay that measures nuclear factor erythroid 2-related factor 2 (NRF2) activity, at an AC_{50} of 32.7 μM, and in one assay that measures the level of phosphorylated H2A histone family, at an AC_{50} of 99.1 μM. 1,2-Diphenylhydrazine was active in one assay that measures the activity of cyclooxygenase in sheep testis, at an AC_{50} of 2.68 μM, and in one assay that measures monooxygenase activity in *E. coli*, at an AC_{50} of 4.26 μM.

The chemical was active in seven assays mapped to KC8, "modulates receptor-mediated effects". In HepG2 cells, 1,2-diphenylhydrazine activated nuclear receptors estrogen receptor α (ERα) and peroxisome proliferator-activated receptor gamma (PPARγ) at an AC_{50} of 60.1 and 77.4 μM, respectively. In addition, 1,2-diphenylhydrazine activated ERα at an AC_{50} of 60.1 μM in the human ovary cell line, VM7, and the thyroid hormone receptor α (THRA) and thyroid hormone receptor β (THRB) at an AC_{50} of 50.6 μM in the rat pituitary gland cell line, GH3. In addition, it was active in three assays that measured changes in the expression of the transcription factors for CYP1A1, CYP1A2, and CYP2B6 in metabolically competent HepaRG liver cells, at an AC_{50} of between 5.08 and 35.7 μM.

Finally, 1,2-diphenylhydrazine was active in nine assays mapped to KC10, "alters cell proliferation, cell death, or nutrient supply". However, only one assay indicated an increase in cell proliferation in HepG2 cells, at an AC_{50} of 112.5 μM. The other eight assays showed a loss of cell viability.

5. Summary of Data Reported

5.1 Exposure characterization

1,2-Diphenylhydrazine was used widely as a chemical precursor for the production of benzidine-based dyes until the late 1970s, after which its use declined significantly due to the phasing out of benzidine-based dyes in many countries. 1,2-Diphenylhydrazine has an additional use as a chemical intermediate in the manufacture of some anti-inflammatory and uricosuric medications, but this application is now also less common owing to discontinued or very limited use of these drugs in human medicine.

1,2-Diphenylhydrazine is occasionally detected in groundwater, surface water, sediment, and waste-water samples. Very few reports and publications were available to assess current and historical occupational and environmental exposure to 1,2-diphenylhydrazine. Because of limited use and relatively rapid degradation in most environmental media, current occupational and environmental exposure to 1,2-diphenylhydrazine is expected to be low.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

Treatment with 1,2-diphenylhydrazine caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in two species.

1,2-Diphenylhydrazine was administered by oral administration (in the feed) in one study in $B6C3F_1$ mice. In females, 1,2-diphenylhydrazine caused an increase in the incidence of hepatocellular carcinoma and of hepatocellular adenoma or carcinoma (combined).

1,2-Diphenylhydrazine was administered by oral administration (in the feed) in two concurrent studies in Fischer 344 rats. In males, 1,2-diphenylhydrazine caused an increase in the incidence of hepatocellular carcinoma, and of benign and malignant liver tumours (neoplastic nodule or hepatocellular carcinoma, combined) in both studies; and an increase in the incidence of squamous cell carcinoma of the Zymbal gland, squamous cell papilloma or carcinoma (combined) of the ear canal, Zymbal gland or skin of the ear (combined), and benign or malignant (combined) pheochromocytoma of the adrenal gland in one study. In females, 1,2-diphenylhydrazine caused an increase in the incidence of mammary gland adenocarcinoma (not otherwise specified) in one study.

1,2-Diphenylhydrazine was administered by intraperitoneal injection in one study in strain A mice. In males, 1,2-diphenylhydrazine caused an increase in the incidence and multiplicity of pulmonary tumours (not specified as benign or malignant). In females, 1,2-diphenylhydrazine caused an increase in the multiplicity of pulmonary tumours.

5.4 Mechanistic evidence

No data on absorption, distribution, metabolism, and excretion in humans exposed to 1,2-diphenylhydrazine were available to the Working Group. In rodents, two studies demonstrated that 1,2-diphenylhydrazine can be absorbed via multiple routes of exposure and is excreted as parent compound and/or metabolites in the urine. One of these studies reported that aniline, benzidine, and several unidentified metabolites were found in the urine; however, the evidence for the formation of these metabolites is suggestive but inconclusive.

There was no mechanistic evidence available for 1,2-diphenylhydrazine regarding the key characteristics of carcinogens in exposed humans or human cells in vitro. Overall, the

mechanistic evidence regarding the key characteristics of carcinogens ("is genotoxic", "induces oxidative stress", and "alters cell proliferation, cell death, or nutrient supply") is suggestive but incoherent across different experimental systems. 1,2-Diphenylhydrazine was shown in rodents to inhibit testicular DNA synthesis in one study but did not cause hepatic DNA strand breaks in another. The chemical caused chromosome aberration and sister-chromatid exchange in Chinese hamster ovary cells in one study but only in the presence of metabolic activation. 1,2-Diphenylhydrazine was mutagenic in two studies in one strain of bacteria with and without metabolic activation but not in multiple other strains. The chemical gave negative results for mutagenicity in *Drosophila melanogaster*, but positive results for DNA damage and oxidative stress in the presence of copper(II) in an acellular system. For the key characteristic "alters cell proliferation, cell death, or nutrient supply", there was a paucity of available data. In one study in rodents, 1,2-diphenylhydrazine caused an elevation in hepatic ornithine decarboxylase activity, indicative of increased cell proliferation.

1,2-Diphenylhydrazine 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 1,2-diphenylhydrazine.

6.2 Cancer in experimental animals

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

6.3 Mechanistic evidence

There is *limited mechanistic evidence*.

6.4 Overall evaluation

1,2-Diphenylhydrazine is *possibly carcinogenic to humans (Group 2B)*.

6.5 Rationale

The Group 2B evaluation for 1,2-diphenylhydrazine is based on *sufficient evidence* for cancer in experimental animals. The *sufficient evidence* in experimental animals is based on an increased incidence of either malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in two species. The evidence regarding cancer in humans is *inadequate* because no studies were available. The mechanistic evidence was *limited* as the findings regarding key characteristics of carcinogens across experimental systems were suggestive, but incoherent.

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DIPHENYLAMINE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 122-39-4

EC/List No.: 204-539-4

Chem. Abstr. Serv. name: piphenylamine

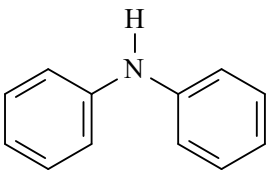
IUPAC systematic name: *N*-phenylaniline

Synonyms: *N*-phenylaniline, diphenylazane, *N*-phenylbenzenamine, anilinobenzene, (phenylamino) benzene, *N*,*N*-diphenylamine, and other depositor-supplied synonyms and acronyms (NCBI, 2021).

1.1.2 Structural and molecular information

Relative molecular mass: 169.22 (NCBI, 2021)

Chemical structure:



Molecular formula: $C_{12}H_{11}N$

1.1.3 Chemical and physical properties

Description: colourless, tan, amber, or brown crystalline solid, with a pleasant, floral odour; sinks in water (NCBI, 2021); forms lamellar crystals (IFA, 2021)

Melting point: 53 °C (IFA, 2021), 54–55 °C (NCBI, 2021)

Boiling point: 302 °C (IFA, 2021)

Density: 1.16 g/cm^3 at 20 °C (IFA, 2021)

Relative vapour density: 5.82 (air = 1) (NCBI, 2021)

Flash point: 153 °C (IFA, 2021)

Auto-ignition temperature: 630 °C (IFA, 2021), 634–635 °C (NCBI, 2021)

Vapour pressure: 6.70×10^{-4} hPa at 25 °C (NCBI, 2021)

Viscosity: 262 cP at 20 °C (NCBI, 2021)

Solubility: practically insoluble in water (50 mg/L at 25 °C) (IFA, 2021); soluble in oxygenated and aromatic solvents, i.e. very soluble in ethanol, propyl alcohol, acetone, benzene, carbon tetrachloride, pyridine, and ethyl acetate; soluble in ether, glacial acetic acid; slightly soluble in chloroform (NCBI, 2021)

Octanol/water partition coefficient (P): log K_{ow} = 3.50 (IFA, 2021, NCBI, 2021)

Odour threshold: 0.05 mg/L (NCBI, 2021)

Dissociation constants (of the conjugated acid BH^+): pK_a = 0.28 at 24 °C (Sangster, 1989)

Reactivity: risk of explosion in contact with oxidizing agents; the substance can react dangerously with strong acids and trichloromelamine; when heated to decomposition, the substance emits fumes of nitrogen oxides; dust explosion possible if in powder or dust form and mixed with air (IFA, 2021; NCBI, 2021).

1.1.4 Impurities

Several primary and secondary amines, including the carcinogen 4-aminobiphenyl (*carcinogenic to humans*, IARC Group 1), may be present as impurities in commercial diphenylamine (Babish et al., 1983). For example, 4-aminobiphenyl was quantified at up to 94 ppm in four out of six commercial brands of diphenylamine (Safe et al., 1977). In addition, 2-aminobiphenyl and *ortho*-cyclohexylaniline were quantified at up to 32 and 93 ppm in several of the six brands, respectively. A single brand (of the six) also contained *para*-cyclohexylaniline as an impurity [no concentration provided]. [The Working Group noted that, on the basis of the age of the studies, the impurities noted above do not necessarily reflect those of current commercial batches.]

1.2 Production and use

1.2.1 Production process

Diphenylamine is an aniline dimer made by heating the parent monomer in the presence of aniline hydrochloride or in the presence of phenol with an acid catalyst at high temperatures (NCBI, 2021).

1.2.2 Production volume

According to Drzyzga (2003), the global annual volume of production of diphenylamine in the 1980s was 40 000 tonnes.

In the European Union (EU) market in 1992–1993, the approximate total volume of production of diphenylamine was 10 000 tonnes (i.e. ~9000 tonnes of production and ~1000 tonnes of imports) (European Commission, 2008). According to the website of the European Chemicals Agency (ECHA) in 2021, diphenylamine is currently manufactured and/or imported in Europe at a volume of ≥ 10 to < 100 tonnes per year (ECHA, 2021). Many companies no longer produce diphenylamine; only four large companies manufacture diphenylamine in North America, Asia, and Europe (Industry Research, 2020). In 2008, diphenylamine was only produced by two companies in the EU and was mostly processed as a chemical intermediate (approximately 97.5%) (European Commission, 2008). In 2020, worldwide sales of diphenylamine reached 1.02 million tonnes (Chemanalyst, 2021). Asia and Pacific regions represented the largest market share (55.2%) in 2018 owing to industrial development and automobile manufacturing (Industry Research, 2020).

During 2000–2019, the annual use [or production and use] of diphenylamine in the Nordic countries (Denmark, Finland, Norway, Sweden) varied between 11 and 1759 tonnes (mean, 172 tonnes; median, 26 tonnes) (SPIN, 2021). [The values were calculated by the Working Group.]

In Chile, sales of diphenylamine for agricultural use reached 2496 kg and/or 2496 L in 2012 (Servicio Agrícola y Ganadero, 2012). According to this report, the sale was made only in the Maule Region (Servicio Agrícola y Ganadero, 2012), the leading apple-producing region of Chile (ODEPA, 2013).

1.2.3 Uses

(a) Main uses

Diphenylamine is predominantly used in lubricants and greases, hydraulic fluids, metal-working fluids, dyes, and textile treatment products including leather and fur (ECHA, 2021). Diphenylamine is also used as an intermediate and, considering the information reported by industries in the EU, the most common uses were in the production of: antioxidants widely used in the rubber industry and for lubricants; antiozonants used in the rubber industry; and phenothiazine used as stabilizer for plastics and for the preparation of several dyestuffs (Drzyzga, 2003; European Commission, 2008). In 2016, the market share for diphenylamine-derived lubricant and rubber antioxidants combined was 66% (Industry Research, 2020).

(b) Minor uses

Minor uses for diphenylamine include its function as a stabilizer for single- or multi-base propellants, nitrocellulose-containing gunpowder, pharmaceuticals, and perfume oils (content, 0.1%) (Drzyzga, 1999, cited in European Commission, 2008; NCBI, 2021). Depending on its current or pending registration status, diphenylamine can be also used as a scald-suppression agent on fruits in storage in certain geographical regions, including the Americas (Johnson et al., 1997; Muñoz-Quezada et al., 2014), but has not been approved for this or similar uses since 2012 in the EU (European Commission, 2012; Dias et al., 2020).

(c) Former uses

In the EU, diphenylamine was used until 2003 as a colouring agent in low-taxed fuels and heating oils to distinguish them from other fuels (European Commission, 2008). This use was voided in 2001 for gas oils and kerosene (Commission Decision 2001/574/EC; European Commission, 2001). In the past, diphenylamine was also reportedly used in veterinary medicine as an additive in anti-screw worm mixtures and as an active ingredient in biocidal products (Drzyzga, 1999, cited in European Commission, 2008). However, more recent information indicates that diphenylamine is no longer used in veterinary products in the EU and United Kingdom (European Commission, 2008). Also in the EU, commercial use as a stabilizer for carbon tetrachloride is now no longer of importance because the production and use of carbon tetrachloride have been strongly regulated since 1994 (Council Regulation (EC) 3093/94) (European Commission, 2008).

1.3 Detection and quantification

1.3.1 Air

Diphenylamine in the air can be collected on a fibreglass filter (OSHA, 1989). The filter is then extracted with methyl alcohol, and diphenylamine is detected by high-performance liquid chromatography with an ultraviolet detector.

Various solid sorbents (Amberlite XAD-2, Amberlite XAD-4, Supelpak 2, Florisil, and the sorbent bound with octadecyl silica, C-18) have been shown to efficiently retain diphenylamine from the air under different sampling conditions (Gagoulia et al., 2011). Diphenylamine was recovered using low volumes of ethyl acetate or acetone and detected with gas chromatography.

Solid-phase microextraction (SPME) has been used for analyses of diphenylamine in storage environments (Song et al., 2014). Samples were taken from the air in various rooms using an SPME fibre and a portable pump with a flow rate of 1 L/minute for 30 minutes. Detection and identification of diphenylamine were performed using gas chromatography with mass spectroscopy (GC-MS).

1.3.2 Water

Diphenylamine can be extracted from water using methylene chloride, with > 90% recovery by continuous extraction techniques (US EPA, 2000). Detection analysis is performed by gas chromatography-atomic fluorescence.

Seventeen components of three diphenylamine derivatives can be analysed by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) and gas chromatography-tandem mass spectrometry, and quantified by flame ionization detection (Zhang et al., 2020).

1.3.3 Soil, sediment, and consumer products

Several methods and techniques are used to evaluate levels of diphenylamine.

(a) Chromatography with nitrogen phosphorous

Diphenylamine is extracted with acetone, and the extraction is followed by liquid-liquid partitioning. Subsequent detection can be performed by gas chromatography with nitrogen-phosphorous detection (GC-NPD) (Garrido et al., 1998).

(b) Gas chromatography-mass spectrometry

Residues are extracted with acetonitrile and transferred to acetone. Then GC-MS is applied in the selective-ion monitoring mode (Yu et al., 1997). Residues can also be extracted with dichloromethane. The GC-MS is applied on the residue dissolved in acetone (Robatscher et al., 2012).

(c) Liquid chromatography with electrochemical detection

Residues are extracted with dichloromethane, dissolved in methanol, filtered, and then injected into the chromatograph (Olek, 1988).

(d) Ultraviolet and visible spectrophotometric methods

The diphenylamine residue is dissolved in methanol, filtered, then injected into the chromatograph, using gradient reversed-phase liquid chromatography with ultraviolet-visible absorption and atmospheric pressure chemical ionization detection (LC-UV-vis-APCI-MS) (Rudell et al., 2005).

(e) Fluorimetric methods

To extract the diphenylamine residue, a mobile phase consisting of methanol/water and fluorescence detection is used, followed by reversed-phase high-performance liquid chromatographic (RP-HPLC) method (Saad et al., 2004).

Another study evaluated the potential of combining normal, synchronous, and derived fluorimetry with multivariate methods for the quantitative analysis of diphenylamine in fruit samples to validate a rapid, specific, and sensitive method to determine diphenylamine in food products (Farokhcheh & Alizadeh, 2013).

In whole milk (FAO, 2004), diphenylamine is extracted with acetonitrile divided with hexane to remove fat. The extract is evaporated, re-dissolved in hexane, and analysed by GC with mass-selective detection (GC-MSD). The method for animal tissues is similar, except that after evaporation, the residue is re-dissolved in a small volume of acetonitrile, diluted with water, and partitioned in hexane. The hexane solution is then analysed by GC-MSD (FAO, 2004).

1.3.4 Human biomarkers

Diphenylamine is transformed into hydroxylated metabolites and is rapidly excreted; therefore, it does not bioaccumulate (Alexander et al., 1965; European Commission, 2008).

There are no validated biomarkers of diphenylamine exposure in humans. [The Working Group noted the metabolites 4-hydroxydiphenylamine

and 4,4′-dihydroxydiphenylamine, described in Section 4.1, as possible targets for biomarker development.]

1.4 Occurrence and exposure

1.4.1 Occurrence in the environment, food, and consumer products

Table 1.1 presents a summary of the studies that evaluated the occurrence of diphenylamine in the environment, and in food commodities and consumer products.

Diphenylamine released as a result of use of weapons and ammunition in military bases causes contamination of soil and water (Drzyzga, 2003). A series of studies found high concentrations of diphenylamine and its nitrate derivatives in groundwater at these military bases.

In a study conducted in the province of Jaén, Spain (Robles-Molina et al., 2014), 83 surface-water samples were collected over 20 months in 3 rivers, 5 reservoirs, and 11 wetlands in order to monitor a group of 373 organic pollutants, including diphenylamine, belonging to different compound categories. Diphenylamine was found in 72.7% of the river samples (average concentration, 148.9 ng/L; C_{max}, 220.4 ng/L), in 20% of the studied wetlands (average, 178.9 ng/L; C_{max}, 195.5 ng/L), and in all reservoirs studied.

Diphenylamine is one of the most prevalent compounds found in air and sediment samples from the USA (Chicago) and in electronic-waste ("e-waste") and residential-dust samples from the USA and Canada (Wu et al., 2020).

The same study (Wu et al., 2020) evaluated phenolic and amino antioxidants and ultraviolet filters. The concentrations of 47 such compounds and their transformation products were measured in 20 samples of atmospheric particles collected in the USA (Chicago), 21 e-waste dust samples from Canada, 32 samples of residential dust from Canada and the USA, and 10 sediment samples collected from the Chicago Sanitary and Ship Canal, USA. Diphenylamine was one of the most prevalent compounds of those measured. Total concentrations of diphenylamine were significantly higher in the e-waste dust than in the Canadian residential dust. In addition, diphenylamine was the predominant amino antioxidant found in the US residential dust, but comprised only 4.4% of amino antioxidants in Canadian residential dust, suggesting regional variations in diphenylamine use. The sediment samples showed relatively high levels of other substances measured.

In China, Liu et al. (2019) evaluated the presence of two types of secondary aromatic amines in dust samples from rubber surfaces of outdoor playgrounds and from residential homes, and found diphenylamine in all the playground dust samples at concentrations of 2.33–32.6 ng/g, with a geometric mean of 8.02 ng/g. In indoor dust from residential homes, diphenylamine concentrations ranged from 8.71 to 129 ng/g, with a geometric mean of 25.5 ng/g.

The US EPA (1998) has assessed the dietary risk posed by diphenylamine. The anticipated residue concentration (ARC) for the overall population of the USA represents 2.27% of the reference dose (RfD). Non-nursing infants aged < 1 year had an ARC of 20.8% of the RfD, which was considered an acceptable dietary exposure risk.

Robatscher et al. (2012) evaluated the potential of fruit storage facilities to contaminate apples that had not been treated with diphenylamine. Diphenylamine (in quantities up to 917 g) was found on the walls of a storage room and was associated with cross-contamination of the untreated apples stored within, even years after the last diphenylamine treatment. Of 689 apple samples, 481 samples contained diphenylamine at concentrations ranging from 0.41 to 2 mg/kg, which exceeds the current EU maximum residue limit (MRL) of 0.05 mg/kg (Reg. (EU) 2018/1515) (European Commission, 2018).

Table 1.1 Concentrations of diphenylamine in the environment, and in food and consumer products

Occurrence context	Monitoring method	Analytical technique	No. of samples tested (*n*)	Concentration of diphenylamine			Reference
				Detection frequency (%)	Median (IQR)	Other measure	
During 20 months (April 2009 to November 2010), 83 surface-water samples from 19 sampling sites were collected in the province of Jaén, Spain	Representative water samples from 3 rivers, 5 reservoirs, and 11 wetlands were collected in amber glass bottles with Teflon caps (1 L)	LC-TOFMS for the analysis of 340 compounds, and GC-MS/MS for the analysis of 63 organic contaminants (30 of these compounds were also analysed by LC-TOFMS)	Guadalquivir river and tributary rivers (Guadalimar and Jandulilla river), *n* = 11	72.7%	NR	Average, 148.9 ng/L (C_{max}, 220.4 ng/L)	Robles-Molina et al. (2014)
			10 wetlands, *n* = 11	20%	NR	Average, 178.9 ng/L (C_{max}, 195.5 ng/L)	
			Giribaile reservoirs, *n* = 9	66.7%		Average, 113.0 ng/L (C_{max}, 170.2 ng/L)	
			Quiebrajano reservoirs, *n* = 5	40%	NR	Average, 57.8 ng/L (C_{max}, 64.2 ng/L)	
			Rublar reservoirs, *n* = 10	30%	NR	Average, 128.7 ng/L (C_{max}, 136.6 ng/L)	
			La Fernandina reservoirs, *n* = 11	45.5%	NR	Average, 141.1 ng/L (C_{max}, 203.2 ng/L)	
			Guadalen reservoirs, *n* = 11	36.4%	NR	Average, 125.7 ng/L (C_{max}, 181.2 ng/L)	

Table 1.1 (continued)

Occurrence context	Monitoring method	Analytical technique	No. of samples tested (*n*)	Concentration of diphenylamine			Reference
				Detection frequency (%)	Median (IQR)	Other measure	
E-waste dismantling facility, Ontario, Canada, 2016; houses in Ontario, Canada, 2015; houses in Indiana, USA, in 2013; Chicago Sanitary and Ship Canal, USA, 2013; atmospheric particles in Chicago, USA, September 2018 to April 2019	E-waste dust samples were collected from the floor, work benches, and sorting bins	In each case, half of the sample extract was diluted with hexane and half with methanol. Half of the samples were then analysed by electron impact GC-MS; the other half were analysed by positive or negative ion LC-MS/MS	E-waste dust, *n* = 21	100%	199 mg/g (81.8–439 mg/g)	NR	Wu et al. (2020)
	Residential floor dust samples were collected using a small vacuum cleaner fitted with a precleaned polyester sock inserted at the end of the hose attachment		Residential floor (Ontario), *n* = 20	25%	5.73 ng/g (< LOD to 10.6 ng/g)	NR	
			Residential floor (Indiana), *n* = 20	100%	13.4 ng/g (5.70–53.6 ng/g)	NR	
	Superficial sediment samples were collected from Chicago Sanitary and Ship Canal		Superficial sediment samples, *n* = 10	80%	7.70 ng/g (< LOD to 505 ng/g)	NR	
	Atmospheric particles were collected on quartz fibre filters using a high-volume air sampler (815 m^3 of air was sampled for 24 h every 12 days)		Samples of atmospheric particles, *n* = 20	85%	0.85 pg/m^3 (< LOD to 3.08 pg/m^3)	NR	

Table 1.1 (continued)

Occurrence context	Monitoring method	Analytical technique	No. of samples tested (*n*)	Concentration of diphenylamine			Reference
				Detection frequency (%)	Median (IQR)	Other measure	
Dust samples collected from outdoor rubber playgrounds and residential houses in March 2016, in Beijing, China	Using a wool paint brush, each dust sample was swept onto aluminium foil from the rubber ground, sealed in polyethylene zip bag	UHPLC interfaced with an API 5500 triple-quadrupole mass spectrometer	Dust from outdoor rubber playgrounds, *n* = 30	100%	NR	Geometric mean, 8.02 ng/g (range, 2.33–32.6 ng/g)	Liu et al. (2019)
	In the living room, 0.5 g of indoor dust was collected from the surfaces of upholstery, electronic fans, furniture, and windowsills (sampling procedure similar to above)		Dust from indoor residential houses, *n* = 30	100%	NR	Geometric mean, 25.5 ng/g (range, 8.71–129 ng/g)	

Table 1.1 (continued)

Occurrence context	Monitoring method	Analytical technique	No. of samples tested (*n*)	Concentration of diphenylamine			Reference
				Detection frequency (%)	Median (IQR)	Other measure	
DPA presence in fruit storage facilities	DPA residues in commercially stored apples	GC analysis was performed on an Agilent 6890 Series GC system equipped with an HP 5973 mass selective detector	Apple samples, *n* = 689	85% (587 samples with some level of DPA)	NR	106 samples containing residues at 0.01–0.40 mg/kg 481 samples containing residues at 0.41–2.00 mg/kg 102 samples were < LOD Untreated apples stored for several months in eight different storage rooms that had been used previously for DPA treatment, 0.01–0.07 mg/kg	Robatscher et al. (2012)
	Activated carbon was removed from CO_2 scrubbers		Activated carbon, 2 g	0	NR	< LOD	

Table 1.1 (continued)

Occurrence context	Monitoring method	Analytical technique	No. of samples tested (*n*)	Concentration of diphenylamine			Reference
				Detection frequency (%)	Median (IQR)	Other measure	
DPA presence in fruit storage facilities (cont.)	DPA extraction from storage cell wall paint		12 storage rooms (a sample of each of approximately 2 cm × 2 cm cell wall paint)	75% (8 samples with some level of DPA; 4 rooms < LOD)	NR	DPA amounts exceeding 1000 mg/m^2 in wall paint from storage rooms that had been nebulized with DPA for 3 years Walls of storage rooms in which drenched apples had been stored were contaminated with DPA at 150–300 mg/m^2 Storage room that had never been used for storage of DPA-treated apples yielded DPA residues of 21.0 mg/m^2	Robatscher et al. (2012 (cont.)
	Silica cartridges were installed on the air outlet of a pump and placed into a contaminated storage cell		Three consecutive silica cartridges	NR	NR	DPA measured in the air of storage rooms ranged from 0.9 to 7.3 µg/m^3 and showed strong temperature dependence, with the highest values measured at 20 °C and the lowest at 1 °C	
Grey partridge (*Perdix perdrix*) eggs, collected on 12 intensively cultivated areas of farmland in France, 2010–2011	Eggs from hatched, destroyed, and deserted clutches of radio-tagged grey partridge females; intact failed eggs were opened in the laboratory to examine their contents, including developing embryos	GC-MS/MS and LC-MS/MS screening and measuring about 500 compounds	139 eggs of 52 clutches	NA	NA	Fate: hatching, dead embryo, stage 11 days, 0.01 mg/kg Fate: hatching, infertile, < 0.01 mg/kg Fate: failure, dead embryo, stage 20 days, 0.019 mg/kg	Bro et al. (2016)

Table 1.1 (continued)

Occurrence context	Monitoring method	Analytical technique	No. of samples tested (*n*)	Concentration of diphenylamine			Reference
				Detection frequency (%)	Median (IQR)	Other measure	
Baby food from local markets, Spain, 2012	Baby food samples were purchased from different local markets	LC-MS and LC-MS/MS mode experiments, obtaining a reduction of these effects when working in LC-MS/MS	Fruit-based baby food, *n* = 25	NA	NA	< LOD (full scan, 5.0 μg/kg; LC-MS/MS, 3.0 μg/kg)	Gilbert-López et al. (2012)
Meals of urban and rural schools, Maule Region, Chile, 2010–2011	Presence of pesticide residues (including DPA) in apples	GC-MS	190 school children; 14 schools considered, DPA residues found in 9	Summer, 72% of children consumed fruit treated with DPA Autumn, 50% of children consumed fruit treated with DPA	NR	Summer (mg/kg apple): School (S): S2 = 0.26; S3 = 0.23; S4 = 0.77; S5 = 0.02; S6 = 0.01; S7 = 0.65; S8 = 3.89; S9 = 1.11; S10 = 2.01 Autumn (mg/kg apple): School: S1 = 0.12; S3 = 0.10; S4 = 0.53; S5 = 0.01; S9 = 0.02; S12 = 0.68; S14 = 0.45	Muñoz-Quezada et al. (2014)
Meals prepared and supplied by company cafeterias and by schools, hospitals, and rest homes; samples collected February–December 2005, Italy	Presence of pesticide residues (including DPA) in meals	MS	50 complete meals	[12%]	[1.726 μg]	Quantity of DPA in fruit: Range: 0.0484–132.5 μg per fruit (*n* = 6)	Lorenzin (2007)

DPA, diphenylamine; e-waste, electronic waste; GC, gas chromatography; GC-MS, gas chromatography with mass spectrometry; GC-MS/MS, gas chromatography triple – quadrupole mass spectrometry; HPLC, high-performance liquid chromatography; IQR, interquartile range; LC-MS, liquid chromatography with mass spectrometry; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LC-TOFMS, liquid chromatography electrospray time-of flight mass spectrometry; LOD, limit of detection; MS, mass spectrometry; NA, not applicable; NR, not reported; UHPLC, ultrahigh-performance liquid chromatography.

In a study by Bro et al. (2016) in France, analysis was carried out on a total of 139 eggs from 52 grey partridge clutches collected from 12 intensively cultivated areas of farmland. A total of 15 different compounds, including diphenylamine, were detected in 24 clutches. Diphenylamine concentrations ranged between < 0.01 and 0.019 mg/kg.

In a study by Gilbert-López et al. (2012) in Spain, liquid chromatography-electrospray ionization-ion trap tandem mass spectrometry was used to quantify multiple residues of 10 fungicides, including diphenylamine, in fruit-based baby foods. The limit of detection was 3.0 µg/kg for diphenylamine. None of the analysed samples exceeded the EU standard for infant feeding (EU No. 578/2012) (European Commission, 2018).

In a study by Muñoz-Quezada et al. (2014) in Talca Province, Chile, pesticide concentrations were measured in samples collected from school meals in 14 urban and rural schools in summer and autumn. Families were surveyed about their children's vegetable consumption in school and at home, the use of pesticides in the home, and other sociodemographic variables. Vegetables contained the highest pesticide concentration at both time points, both in urban and rural schools. In summer, diphenylamine residues were found in samples from nine schools. During the 4 days before sample collection in the summer, 72% (n = 136) of the schoolchildren had consumed fruits (apples) that had been treated with diphenylamine, and 65% had consumed fruit (oranges and apples) that had been treated with more than one type of pesticide. In autumn, 70.3% (n = 128) of the children consumed a vegetable or a fruit that had been treated with diphenylamine. The risk of consuming vegetables containing diphenylamine was 2.9 times higher in urban schoolchildren than in rural schoolchildren.

The Italian Ready-Meal Residue Project, promoted by the Pesticides Working Group of Italian environmental agencies (Lorenzin, 2007), evaluated the number of pesticides in pre-prepared meals (first course, side dish, fruit, bread, and wine). In 2005, 50 complete meals were analysed. The results showed residues of pesticides in 39 meals, with an average number of 2.4 pesticides, and a maximum of 10, in each meal. Diphenylamine was one of the most common pesticides found in fruit (6 meals).

1.4.2 Occupational exposure

The most relevant routes of occupational exposure to diphenylamine are respiratory (inhalation) and dermal (skin contact) (European Commission, 2008).

In a study by Gagoulia et al. (2011), a simple method was developed to determine and monitor diphenylamine in the indoor air of two apple-storage plants from September 2006 to March 2007 in Greece. Diphenylamine was detected in indoor air at concentrations ranging between 1.6 and 580 µg/m^3 (Table 1.2). When evaluating the presence of diphenylamine in the air during a typical working day after diphenylamine application in both apple-storage plants, the highest concentrations of diphenylamine residues (483.5 µg/m^3 and 580 µg/m^3) were recorded in the afternoon. Lower concentrations of diphenylamine (3.7–16.8 µg/m^3) were detected in the air from other areas of the building, such as office areas and the sorting line, probably because of the greater distance between these areas and the diphenylamine application area. Indoor air concentrations of diphenylamine 3–4 months after diphenylamine application ranged from 1.6 to 6.9 µg/m^3; these levels were attributed to the desorption of diphenylamine from the building walls.

In a review of occupational exposure by inhalation (European Commission, 2008), data were considered from a study carried out in two rubber-antioxidant factories in 1989–1999 (Table 1.2). In one of these factories, during the bagging of diphenylamine flakes, values of up to 161.2 mg/m^3 were reported, with a measurement

Table 1.2 Occupational exposure to diphenylamine in workplace air

Exposure context	Monitoring method	Analytical technique	No. of samples tested	Concentration of diphenylamine		Reference
				Median	Other measure	
Workers' exposure in two apple-storage buildings located in two different agricultural areas in Greece, 2006–2007	Air sampling and analytical methodology were applied in the field to measure DPA levels in the air	GC-NPD analysis GC-MS analysis	2 apple storage plants; 33 air samples	NR	Range, 1.6–580 μg/m^3	Gagoulia et al. (2011)
Workers' exposure in two rubber-antioxidant factories, 1989–1999	Air monitoring (measurement duration, ≤ 420 minutes)	NR	122	0.92 mg/m^3	Range, 0.1–162 mg/m^3 90th percentile, 0.3 and 1.05 mg/m^3 95th percentile, 1.65 mg/m^3	European Commission (2008)
Workers' exposure in rubber-manufacturing industry, 1990s	Air samples taken over a 3 h period in two stable positions near the mixing and personal air samples were taken over 2 h period during a normal work day from five workers involved in different operations (mixing, weighing, calendering, compounding and extruding)	GC-MS	7	NR	DPA detected in the stationary air samples collected near the mixing and calendering areas	Fracasso et al. (1999)

DPA, diphenylamine; GC-MS, gas chromatography with mass spectrometry; GC-NPD, gas chromatography-nitrogen phosphorus detector; NR, not reported.

duration of up to 420 minutes, and a 95th percentile for the collective measurement of 1.65 mg/m^3. Concentrations during the "bagging of diphenylamine-chips" activity reached 0.4 mg/m^3 (duration, 60 minutes), with a 90th percentile of 0.3 mg/m^3. Since some exposure information was missing, the ECHA risk analysis used the Estimation and Assessment of Substance Exposure (EASE) model (August 1997) to evaluate the effects of various production parameters and diphenylamine physical states (liquids or flakes). Exposure levels were found to be similar to the measured levels across a variety of modelled parameters tested and for both physical states (European Commission, 2008).

Owing to the lack of data for dermal exposure, estimations of skin exposure were also performed using the EASE model. In this case, the input parameters used in the EASE model were non-dispersive use, direct and intermittent handling, an exposed area of 210 cm^2, and the use of suitable gloves with a protection efficiency of 90%. These input parameters led to exposure levels of 2.1–21 mg/person per day, which was considered to represent the reasonable worst case. Using the same model, the dermal exposure assessment was also carried out for a worker who did not wear personal protective equipment and was exposed to diphenylamine-containing lubricants. The estimated exposure levels (42–126 mg/person per day) were calculated for a 1% diphenylamine formulation over an exposed skin area of 840 cm^2. For this occupation, exposure by inhalation was considered negligible unless diphenylamine was in aerosol form (European Commission, 2008).

Mixers, loaders, and applicators of pesticides may also be exposed to diphenylamine during and after regular use in agricultural and other settings. The pesticide handlers may be exposed to diphenylamine used as a drench on apples after harvest (US EPA, 1998). A study developed in a rubber manufacturing industry located in Italy (Fracasso et al., 1999) detected diphenylamine through GC-MS analysis of airborne extracts on the basis of similarity of the mass spectra index to that in the Wiley library system. Diphenylamine was detected in ambient air samples taken over a 3-hour period in two stable positions near the mixing Banbury mixer and calendering areas, probably produced by degradation processes facilitated by the high temperatures (100–200 °C) to which the raw materials (e.g. antioxidants) are subjected in these workplaces (Table 1.2).

1.4.3 Exposure of the general population

According to the European Commission (2008), the route of exposure for consumers is oral intake by eating fruits and vegetable foods that have been preserved with diphenylamine, but dermal exposure from lubricants in consumer products is also possible.

In the Total Diet Study by the Food and Drug Administration, conducted between 1986 and 1991 (Gunderson, 1995), the average daily intake of diphenylamine was determined for eight age groups as follows: 6–11 months, 0.0034 µg/kg body weight (bw) per day; 2 years, 0.0410 µg/kg bw per day; girls aged 14–16 years, 0.0073 µg/kg bw per day; boys aged 14–16 years, 0.0099 µg/kg bw per day; women aged 25–30 years, 0.0074 µg/kg bw per day; men aged 25–30 years, 0.0051 µg/kg bw per day; women aged 60–65 years, 0.0079 µg/kg bw per day; and men aged 60–65 years, 0.0065 µg/kg bw per day.

1.5 Regulations and guidelines

1.5.1 Exposure limits and guidelines

(a) Occupational exposure limits

In the USA, the Occupational Safety and Health Administration (OSHA) and the National Institute for Occupational Safety and Health (NIOSH) both recommend an 8-hour time-weighted average (TWA) limit of 10 mg/m^3 to be applied only in construction and

Table 1.3 Occupational exposure limits for diphenylamine in various countries

Country	8-hour TWA (mg/m³)	Short-term (15 minutes) (mg/m³)	Reference
Australia	10		IFA (2021)
Austria	5	10	IFA (2021)
Belgium	10		IFA (2021)
Canada – province of Ontario	10		IFA (2021)
Canada – province of Quebec	10		IFA (2021)
China	10		IFA (2021)
Denmark	5	10	IFA (2021)
Finland	5	10	IFA (2021)
France	10		IFA (2021)
Germany	5	10	IFA (2021)
Ireland	10	20	IFA (2021)
New Zealand	10		IFA (2021)
Italy	10		European Commission (2008)
Netherlands	0.7		European Commission (2008)
Norway	5		IFA (2021)
Poland	8		IFA (2021)
Republic of Korea	10		IFA (2021)
Romania	4	6	IFA (2021)
Singapore	10		IFA (2021)
Spain	10		IFA (2021)
Sweden	4	12	IFA (2021)
Switzerland	10		IFA (2021)
United Kingdom	10	20	IFA (2021)
USA – NIOSH	10		IFA (2021)

NIOSH, National Institute for Occupational Safety and Health; TWA, time-weighted average.

maritime settings. The American Conference of Governmental Industrial Hygienists (ACGIH) and the Division of Occupational Safety and Health of California (Cal/OSHA) recommend the same value for the 8-hour TWA, but do not restrict to specific types of occupational settings (OSHA, 2020). The same exposure limit is used by Safe Work Australia (2019) (Table 1.3).

Several European countries have national occupational limits for diphenylamine (also summarized in Table 1.3) (European Commission, 2008).

(b) Environmental exposure limits

In the USA (United States Government, 2014), the tolerances for residues of diphenylamine are as follows: apple wet pomace, 30.0 mg/kg; apple from pre-harvest or post-harvest use, including use of impregnated wraps, 10.0 mg/kg; cattle fat, cattle meat, cattle meat by-products, except liver, goat fat and meat and goat meat by-products, except liver, horse fat and meat and horse meat by-products, except liver, milk, sheep fat and meat, and sheep meat by-products, except liver, 0.01 mg/kg; cattle, goat, horse, and sheep liver, 0.1 mg/kg.

According to the Codex Alimentarius (FAO, 2021), the MRLs that exist for diphenylamine in food are: apple, 10 mg/kg; apple juice, 0.5 mg/kg; cattle kidney and meat, 0.01 mg/kg; cattle liver, 0.05 mg/kg; milk and milk fats, 0.01 mg/kg; and pear, 5 mg/kg. The MRLs for apple and for processed foods accommodate post-harvest treatment of the commodity. Most non-European

countries define MRLs in food on the basis of these Codex Alimentarius indications (FAO, 2021). At the EU level, the value of 0.05 mg/kg is used for all commodities (Reg. (EU) 2018/1515) (European Commission, 2018).

The state drinking-water guideline in the USA is 175 µg/L (US EPA, 1993).

According to the harmonized classification and labelling framework implemented in the EU (Classification, Labelling and Packaging (CLP) Regulation, 1272/2008/EC), diphenylamine has the following classification: acute toxicity category 3; specific target organ toxicity-repeated exposure category 2; aquatic acute 1; aquatic chronic 1. Employers are obliged under the CLP Regulation to minimize worker exposure to diphenylamine and must arrange for medical surveillance of exposed workers (Council Directive 98/24/EC; European Commission, 1998).

1.5.2 Reference values for biological monitoring of exposure

No reference values related to diphenylamine biological monitoring were available to the Working Group.

2. Cancer in Humans

No epidemiological studies were available that directly investigated the relationship between exposure to diphenylamine and cancer risk. Although there was a case–control study on occupational exposures (workers employed in gunpowder production mentioning use of diphenylamine) and bladder cancer risk (Nizamova, 1991), the study was considered by the Working Group to be uninformative and was excluded here since there was no information on the risk of cancer in relation to diphenylamine exposure specifically.

3. Cancer in Experimental Animals

See Table 3.1.

3.1 Mouse

3.1.1 Oral administration (feed)

In a well-conducted study of chronic toxicity and carcinogenicity that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female Crj:BDF_1 [B6D2F_1/Crlj] mice (age, 6 weeks) were given feed containing diphenylamine (purity, 100.5%) at a concentration of 0, 250, 1000, or 4000 ppm for the control group and the groups at the lowest, intermediate, and highest dose, respectively, for 104 weeks (JBRC, 2011a, b). The survival rate of males at the highest dose was significantly lower than that of the controls, probably due to urinary retention. The highest dose level was considered to exceed the maximum tolerated dose. The survival rate of females at the highest dose was significantly higher than that of controls. At study termination, survival was: 31/50, 29/50, 29/50, and 16/50 in males, and 23/50, 25/50, 25/50, and 35/50 in females, for the control group and the groups at the lowest, intermediate, and highest dose, respectively. The body weights at the highest dose were significantly decreased in males and females compared with their respective controls. All mice underwent complete necropsy. All organs and tissues were sampled for histopathology in all the animals.

In male mice, there was a significant positive trend ($P < 0.05$, Peto test) in the incidence of haemangioma in the liver, haemangioma or haemangiosarcoma (combined) in the liver, and haemangiomas in all organs. The incidence of haemangioma or haemangiosarcoma (combined) was significantly increased ($P < 0.01$, Fisher exact test) both in the spleen and in all organs (spleen, liver, subcutis, bone marrow, and heart) combined in the group at the intermediate dose: for the spleen – control, 1/50 (2%);

Table 3.1 Studies of carcinogenicity with diphenylamine in experimental animals

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, Crj:BDF_1 [$B6D2F_1$/Crlj] (M) 6 wk 104 wk JBRC (2011a, b)	Oral administration (feed) Diphenylamine, 100.5% Feed 0, 250, 1000, 4000 ppm (w/w), 1×/day 50, 50, 50, 50 31, 29, 29, 16	*Liver* Haemangioma 2/50, 2/50, 5/50, 3/50 Haemangiosarcoma 0/50, 1/50, 2/50, 1/50 Haemangioma or haemangiosarcoma (combined) 2/50, 3/50, 7/50, 4/50 *Spleen* Haemangioma 1/50, 0/50, 6/50, 2/50 Haemangiosarcoma 0/50, 0/50, 3/50, 1/50 Haemangioma or haemangiosarcoma (combined) 1/50 (2%), 0/50, 9/50 (18%)*, 3/50 (6%) *All organs (spleen, liver, subcutis, bone marrow, and heart)* Haemangioma 3/50 (6%), 2/50 (4%), 10/50 (20%)*, 6/50 (12%) Haemangiosarcoma 0/50, 1/50, 4/50, 1/50 Haemangioma or haemangiosarcoma (combined) 3/50 (6%), 3/50 (6%), 14/50 (28%)*, 6/50 (12%)	 $P < 0.05$, Peto trend test NS $P < 0.05$ by Peto trend test NS NS *$P < 0.01$, Fisher exact test $P < 0.05$, Peto trend test; *$P < 0.05$, Fisher exact test NS *$P < 0.01$, Fisher exact test	Principal strengths: multiple doses used; duration of exposure and observation was adequate; well-conducted GLP study; adequate number of mice per group Historical controls: haemangioma or haemangiosarcoma (combined) of the spleen: 107/2244 (4.8%); range, 0–14%; haemangioma in all organs: 145/2245 (6.5%); range, 0–18%; haemangioma or haemangiosarcoma (combined) in all organs: 279/2245 (12.4%); range, 0–22%; liver haemangioma: 70/2245 (3.1%); range, 0–14%

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	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, Crj:BDF_1 [$B6D2F_1$/Crlj] (F) 6 wk 104 wk JBRC (2011a, b)	Oral administration (feed) Diphenylamine, 100.5% Feed 0, 250, 1000, 4000 ppm (w/w), 1×/day 50, 50, 50, 50 23, 25, 25, 35	*Uterus*: histiocytic sarcoma 8/50 (16%), 7/50 (14%), 17/50 (34%)*, 12/50 (24%)	*$P < 0.05$, Fisher exact test	Principal strengths: multiple doses used; the duration of exposure and observation was adequate; well-conducted GLP study; adequate number of mice per group Historical controls: histiocytic sarcoma of the uterus, 464/2245 (20.7%); range, 0–34%
Full carcinogenicity Mouse, NMRI (M) 8 wk 126 wk Holmberg et al. (1983)	Oral administration (gavage) Diphenylamine, ≥ 99% Soybean oil 0, 300 mg/kg bw 1×/wk for 18 mo (78 wk) 30, 125 NR	Total tumours (all types) 22.2%, 22.9%	NS	Principal limitations: only one sex used; only one dose used; unusual dosing regimen Other comments: after 26 wk, 28 animals were killed in the diphenylamine-treated group, and 7 animals in the vehicle control group; after 52 wk, 24 animals were killed in the diphenylamine-treated group, and 7 animals in the vehicle control group In both groups, the most common tumour types were lymphoma and alveolar adenoma: diphenylamine-treated group, lymphoma (8.3%) and alveolar adenoma (16.5%); vehicle control group, lymphoma (11.1%) and alveolar adenoma (11.1%)

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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (M) 6 wk 104 wk JBRC (2011c, d)	Oral administration (feed) Diphenylamine, 100.5% Feed 0, 250, 1000, 4000 ppm (w/w), 1×/day 50, 50, 50, 50 37, 40, 43, 41	*Spleen* Haemangiosarcoma 0/50, 0/50, 0/50, 3/50 (6%) Haemangioma or haemangiosarcoma (combined) 0/50, 1/50 (2%), 0/50, 3/50 (6%) *Subcutis* Fibroma 2/50, 11/50*, 3/50, 2/50 Fibrosarcoma 0/50, 2/50, 0/50, 1/50 Fibroma or fibrosarcoma (combined) 2/50, 13/50*, 3/50, 3/50 Haemangiosarcoma 0/50, 0/50, 0/50, 1/50 *All organs* Haemangioma 0/50, 1/50, 0/50, 1/50 Haemangiosarcoma 0/50, 0/50, 0/50, 4/50 (8%) Haemangioma or haemangiosarcoma (combined) 0/50, 1/50 (2%), 0/50, 5/50 (10%)* *Testis*: interstitial cell tumour 37/50, 40/50, 46/50*, 46/50*	 $P < 0.01$, Peto trend test and Cochran–Armitage test $P < 0.05$, Peto trend test and Cochran–Armitage test *$P < 0.01$, Fisher exact test NS *$P < 0.01$, Fisher exact test NS NS $P < 0.01$, Peto trend test and Cochran–Armitage test *$P < 0.05$, Fisher exact test $P < 0.05$ by Peto trend test and Cochran–Armitage test; *$P = 0.05$, Fisher exact test	Principal strengths: multiple doses used; the duration of exposure and observation was adequate; well-conducted GLP study; adequate number of rats per group Historical controls: haemangiosarcoma in the spleen, 7/2748 (0.3%); range, 0–4%; haemangiosarcoma in all organs, 8/2748 (0.3%); range, 0–4%; haemangioma or haemangiosarcoma (combined) in all organs, 19/2748 (0.7%); range, 0–4%

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	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/DuCrlCrlj (F) 6 wk 104 wk JBRC (2011c, d)	Oral administration (feed) Diphenylamine, 100.5% Feed 0, 250, 1000, 4000 ppm (w/w), 1×/day 50, 50, 50, 50 40, 43, 45, 43	*Uterus* Adenocarcinoma 1/50 (2%), 0/50, 0/50, 4/50 (8%) Adenoma or adenocarcinoma (combined) 1/50 (2%), 1/50 (2%), 0/50, 4/50 (8%) *Spleen*: mononuclear cell leukaemia 3/50 (6%), 2/50 (4%), 0/50, 5/50 (10%)	$P < 0.01$, Peto trend test and Cochran–Armitage test $P < 0.05$, Peto trend test and Cochran–Armitage test $P < 0.05$ by Peto trend test	Principal strengths: multiple doses used; the duration of exposure and observation was adequate; well-conducted GLP study; adequate number of rats per group Historical controls: adenocarcinoma of the uterus, 15/2544 (0.6%); range, 0–4%; adenoma or adenocarcinoma of the uterus, 22/2544 (0.9%); range, 0–4%; mononuclear cell leukaemia of the spleen, 314/2547 (12.3%); range, 2–26%

bw, body weight; F, female; GLP, Good Laboratory Practice; M, male; mo, month; NR, not reported; NS, not significant; ppm, parts per million; w/w, weight per weight; wk, week.

lowest dose, 0/50; intermediate dose, 9/50 (18%); and highest dose, 3/50 (6%); and for all organs combined – control, 3/50 (6%); lowest dose, 3/50 (6%); intermediate dose, 14/50 (28%); and highest dose, 6/50 (12%). The incidence of haemangioma or haemangiosarcoma (combined) at the intermediate dose both in the spleen and in all organs combined exceeded the upper bound of the range observed in historical controls from this laboratory – spleen, 107/2244 (4.8%); range, 0–14%; and all organs combined, 279/2245 (12.4%); range, 0–22%. The incidence of haemangioma in all organs combined was significantly increased at the intermediate dose: control, 3/50 (6%); lowest dose, 2/50 (4%); intermediate dose, 10/50 (20%); and highest dose, 6/50 (12%); $P < 0.05$, Fisher exact test. The incidence of haemangioma in all organs combined at the intermediate dose exceeded the upper bound of the range observed in historical controls – 145/2245 (6.5%); range, 0–18%.

In female mice, there was a significant increase in the incidence of histiocytic sarcoma of the uterus at the intermediate dose: control, 8/50 (16%); lowest dose, 7/50 (14%); intermediate dose, 17/50 (34%); and highest dose, 12/50 (24%); $P < 0.05$, Fisher exact test. The incidence of histiocytic sarcoma of the uterus at the intermediate dose was at the upper bound of the range observed in historical controls from this laboratory – 464/2245 (20.7%); range, 10–34%.

In all treated groups of male and female mice, diphenylamine caused methaemoglobinaemia, anaemia, increased haematopoiesis of the bone marrow, splenic enlargement, haematopoiesis, and hemosiderosis (JBRC, 2011a, b). [The Working Group noted that this was a well-conducted study that complied with GLP, the number of animals per group was adequate, the study used both sexes and multiple dose groups, and the duration of exposure and observation was adequate.]

3.1.2 Oral administration (gavage)

A group of 125 male NMRI mice (age, 8 weeks) was treated with diphenylamine (purity, ≥ 99%) at a dose of 300 mg/kg bw in soybean oil by gavage once per week for 18 months (78 weeks). A control group of 30 male NMRI mice was given the vehicle only (soybean oil, 10 mL per kg bw) using the same protocol (Holmberg et al., 1983). Groups of mice were killed at 26 weeks (7 controls and 28 diphenylamine-treated mice) and 52 weeks (7 controls and 24 diphenylamine-treated mice). The remaining mice were observed up to experimental week 126. Diphenylamine decreased the mean body weight but not the survival of the treated animals compared with vehicle controls. Histopathological examination was performed on main organs and tissues.

There were no changes in the frequency of any type of tumour in treated animals compared with vehicle controls. [The Working Group noted that only one sex and dose were used, and that the dosing regimen was unusual.]

3.2 Rat

3.2.1 Oral administration (feed)

In a well-conducted chronic toxicity and carcinogenicity study that complied with GLP, groups of 50 male and 50 female F344/DuCrlCrlj rats (age, 6 weeks) were given feed containing diphenylamine (purity, 100.5%) at a dose of 0, 250, 1000, or 4000 ppm for the control group and the groups at the lowest, intermediate, and highest dose, respectively, for 104 weeks (JBRC, 2011c, d). Survival analysis showed no differences between the treated groups and their respective control groups. At study termination, survival was: 37/50, 40/50, 43/50, and 41/50 in males, and 40/50, 43/50, 45/50, and 43/50 in females, for the control group and the groups at the lowest, intermediate, and highest dose, respectively. At termination of treatment, the body weights of males

at the highest dose and females at the intermediate and highest dose were significantly lower than those of their respective controls. Food consumption was decreased in males at the highest dose for most of the duration of the study. Food consumption was also decreased in females at the intermediate and highest dose for most (weeks 0–78) of the duration of the study. All rats underwent complete necropsy. All organs and tissues were sampled for histopathology in all the animals.

In male rats, there was a significant positive trend in the incidence of haemangiosarcoma in the spleen ($P < 0.01$, Peto and Cochran–Armitage tests), of haemangiosarcoma in all organs (spleen and subcutis) combined ($P < 0.01$, Peto and Cochran–Armitage tests), and of haemangioma or haemangiosarcoma (combined) in the spleen ($P < 0.05$, Peto and Cochran–Armitage tests). The incidence of haemangioma or haemangiosarcoma (combined) in all organs combined was significantly increased at the highest dose: control, 0/50; lowest dose, 1/50 (2%); intermediate dose, 0/50; and highest dose, 5/50 (10%); $P < 0.05$, Fisher exact test. The incidence of haemangiosarcoma in the spleen (6%), haemangiosarcoma in all organs combined (8%), and of haemangioma or haemangiosarcoma (combined) in all organs combined (10%), all at the highest dose, exceeded the overall incidence and upper bound of the range for these tumours observed in historical controls from this laboratory – incidence, 7/2748 (0.3%), 8/2748 (0.3%), and 19/2748 (0.7%), respectively; all ranges: 0–4%. The incidence of subcutis fibroma was 2/50, 11/50, 3/50, and 2/50; the incidence of subcutis fibrosarcoma was 0/50, 2/50, 0/50, and 1/50; and the incidence of fibroma or fibrosarcoma (combined) of the subcutis was 2/50, 13/50, 3/50, and 3/50 in the control groups and in the groups at the lowest, intermediate, and highest dose, respectively. The incidence of fibroma of the subcutis and of fibroma or fibrosarcoma (combined) of the subcutis was significantly increased at the lowest dose compared with controls ($P < 0.01$, Fisher exact test). There was a significant positive trend in the incidence of interstitial cell tumour of the testis – control, 37/50; lowest dose, 40/50; intermediate dose, 46/50; highest dose, 46/50; $P < 0.05$, Peto and Cochran–Armitage tests – with a significant increase ($P < 0.05$, Fisher exact test) at the intermediate and highest dose.

In female rats, there was a significant positive trend in the incidence of adenocarcinoma of the uterus – control, 1/50 (2%); lowest dose, 0/50; intermediate dose, 0/50; and highest dose, 4/50 (8%); $P < 0.01$, Peto and Cochran–Armitage tests – and of adenoma or adenocarcinoma (combined) of the uterus –control, 1/50 (2%); lowest dose, 1/50 (2%); intermediate dose, 0/50; and highest dose, 4/50 (8%); $P < 0.05$, Peto and Cochran–Armitage tests. The incidence of adenocarcinoma of the uterus at the highest dose and adenoma or adenocarcinoma (combined) of the uterus at the highest dose exceeded the upper bound of the range observed in historical controls from this laboratory – incidence of adenocarcinoma of the uterus, 15/2544 (0.6%); range, 0–4%; and incidence of adenoma or adenocarcinoma (combined) of the uterus, 22/2544 (0.9%); range, 0–4%). A significant positive trend in the incidence of mononuclear cell leukaemia of the spleen ($P < 0.05$, Peto test) was also observed. The incidence of mononuclear cell leukaemia of the spleen in all dose groups – control, 3/50 (6%); lowest dose, 2/50 (4%), intermediate dose, 0/50; and highest dose, 5/50 (10%) – did not exceed the upper bound of the range (2–26%) observed in historical controls from this laboratory.

In treated males (at the intermediate and highest dose) and females (at all doses), diphenylamine caused methaemoglobinaemia. Anaemia occurred in males (at the highest dose) and females (at the intermediate and highest dose). Splenic enlargement, increased haematopoiesis, and haemosiderosis were observed in the spleen of treated male rats. Splenic enlargement, capsular hyperplasia, angiectasis, and fibrosis were

observed in the spleen of treated female rats (JBRC, 2011c, d). [The Working Group noted that this was a well-conducted GLP study that used an adequate number of animals per group, males and females, and multiple dose groups, and with the duration of exposure and observation was adequate.]

In another study, groups of 20 male and 20 female weanling Slonaker-Addis strain rats were given feed containing diphenylamine (purity, ≥ 99.9%) at a concentration of 0 (control), 0.001%, 0.01%, 0.1%, 0.5%, or 1.0% for 2 years (Thomas et al., 1967a). All rats surviving for at least 640 days (including those that survived until study termination at 734 days) were given a complete postmortem examination. The incidence of tumours of any type was not affected by diphenylamine treatment. [The Working Group noted that this study was inadequate for the evaluation of the carcinogenicity of diphenylamine in experimental animals due to the small number of animals and lack of details regarding the postmortem examination.]

3.2.2 Oral administration (gavage)

Twenty female Sprague-Dawley rats (age, 50–55 days) were given a single dose of diphenylamine [purity unspecified] of 300 mg per rat (in sesame oil) by gavage. Complete necropsy was performed 6 months after diphenylamine administration. A group of 89 female Sprague-Dawley rats were given sesame oil only and served as controls. No increased incidence of tumours of any type was reported (Griswold et al., 1966). [The Working Group noted that this study was inadequate for the evaluation of the carcinogenicity of diphenylamine in experimental animals due to the limited duration of observation, small number of animals, and the administration of a single dose.]

3.3 Dog

Oral administration (feed)

Four groups of two male and two female beagle dogs (age, 8 months) were given feed containing diphenylamine (purity, ≥ 99.9%) at a concentration of 0, 0.01%, 0.1%, or 1.0% for the control group and the groups at the lowest, intermediate, and highest dose, respectively, for 2 years. No neoplasms were reported in any treatment group (Thomas et al., 1967b). [The Working Group noted that this study was inadequate for the evaluation of the carcinogenicity of diphenylamine in experimental animals due to the small number of animals, lack of details regarding the postmortem evaluation, and limited duration of observation.]

3.4 Evidence synthesis for cancer in experimental animals

The carcinogenicity of diphenylamine has been assessed in one well-conducted GLP study in male and female Crj:BDF$_1$ mice (JBRC, 2011a, b) and in one well-conducted GLP study in male and female F344/DuCrlCrlj rats (JBRC, 2011c, d) treated by oral administration (in the feed); in two additional studies in male and female Slonaker-Addis strain rats (Thomas et al., 1967a) and male and female beagle dogs (Thomas et al., 1967b) treated by oral administration (in the feed); in one study in female Sprague-Dawley rats treated by oral administration (gavage) (Griswold et al., 1966), and in one study in male NMRI mice treated by oral administration (gavage) (Holmberg et al., 1983).

In the well-conducted GLP study in male and female Crj:BDF$_1$ mice treated by oral administration (JBRC, 2011a, b), there was a significant positive trend in the incidence of haemangioma in the liver, haemangioma or haemangiosarcoma (combined) in the liver, and haemangioma in all organs combined in male mice. The incidence

of haemangioma or haemangiosarcoma (combined) was significantly increased both in the spleen and in all organs combined in male mice at the intermediate dose. The incidence of haemangioma in all organs combined was significantly increased in male mice at the intermediate dose. In female mice at the intermediate dose, oral administration of diphenylamine caused a significant increase in the incidence of histiocytic sarcoma of the uterus (JBRC, 2011a, b).

In a well-conducted GLP study in male and female F344/DuCrlCrlj rats treated by oral administration (JBRC, 2011c, d), there was a significant positive trend in the incidence of haemangiosarcoma in the spleen, haemangiosarcoma in all organs combined, and haemangioma or haemangiosarcoma (combined) in the spleen of male rats. The incidence of haemangioma or haemangiosarcoma (combined) in all organs combined was significantly increased in male rats at the highest dose. The incidence of fibroma and of fibroma or fibrosarcoma (combined) of the subcutis was significantly increased in male rats at the lowest dose. There was a significant positive trend in the incidence of interstitial cell tumours of the testis, with a significant increase in the incidence in male rats at the intermediate dose and highest dose. In female rats, there was a significant positive trend in the incidence of adenocarcinoma of the uterus and of adenoma or adenocarcinoma (combined) of the uterus. A significant positive trend in the incidence of mononuclear cell leukaemia of the spleen was also observed in female rats (JBRC, 2011c, d).

There was no significant increase in the incidence of tumours in the study in male NMRI mice treated by oral administration (Holmberg et al., 1983).

Both studies in male and female weanling Slonaker-Addis strain rats (Thomas et al., 1967a) and in male and female beagle dogs (Thomas et al., 1967b) treated by oral administration, and the one study in female Sprague-Dawley rats treated by oral administration (Griswold et al., 1966), were judged to be inadequate for the evaluation of the carcinogenicity of diphenylamine in experimental animals.

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

(a) Exposed humans

Only one study on the absorption, distribution, metabolism, and excretion of diphenylamine in humans was available. Diphenylamine was found to be metabolized to 4-hydroxydiphenylamine and 4,4′-dihydroxydiphenylamine after analysis of the urine of two human subjects for 24 hours after administration of a single oral dose of 100 mg of diphenylamine (Alexander et al., 1965). As well as the two identified metabolites, diphenylamine was also shown to be excreted in its unmetabolized form into the urine. No 2-hydroxydiphenylamine was found in the urine. The findings in the urine samples collected 24 hours after oral dosing suggested that diphenylamine is absorbed in humans via the gastrointestinal tract; however, the extent and rate of absorption is unclear. Data on other routes of absorption in humans were not available.

Piechocki et al. (2018) reported the accidental exposure of a 23-year-old patient to diphenylamine in the workplace, resulting in methaemoglobinaemia. [The Working Group noted that this study was not informative because the patient was co-exposed to 1,4-diaminobenzene and the precise amount and duration of exposure were not reported.]

(b) Human cells in vitro

Most metabolites of diphenylamine undergo conjugation. Fig 4.1 illustrates the proposed metabolic pathways for diphenylamine. Green et al. (1998) reported on the direct N-glucuronidation of diphenylamine by human UDP-glucuronosyltransferase 1A3 (UGT1A3) transiently expressed in human embryonic kidney cells (HEK293). However, the rate of glucuronide formation by UGT1A3 was low compared with that by UGT1A4 (Green et al., 1998). [The Working Group noted that this suggests that this enzyme is not a major contributor to the metabolic clearance of diphenylamine in vivo.] Similarly, the metabolism of diphenylamine was shown to be catalysed by human UGT1A8 transfected-HEK293 cells with low glucuronidation rates (Cheng et al., 1998).

As a part of the Toxicity Forecaster/Toxicity Testing in the 21st Century (ToxCast/Tox21) analysis (Section 4.2.4), the intrinsic hepatic clearance rate in vitro for diphenylamine was measured to be 64.57 µL/minute per 10^6 hepatocytes from a human donor pool. The in vitro and computationally derived estimates of pharmacokinetic parameters therefore included half-life and volume of distribution values of 7.35 hours and 0.62 L/kg, respectively (US EPA, 2021).

4.1.2 Experimental systems

(a) Absorption and distribution

Diphenylamine was found to be well absorbed in male and female Sprague-Dawley rats; 68–89% of an oral dose of [^{14}C]-labelled diphenylamine of 5 mg/kg bw was recovered in the urine after 168 hours (WHO, 1998). Adequate absorption was observed across experimental systems. About 85–91% of the daily dose was recovered in the urine of two lactating Toggenburg goats given [^{14}C]-labelled diphenylamine at 50 mg/kg bw per day by oral administration for 7 days. In goats, diphenylamine was reported to distribute both as parent and as metabolites to the liver, kidney, leg muscle, loin muscle, back fat, omental fat, and milk (WHO, 1998). No appreciable tissue accumulation of diphenylamine was noted in male and female rats tested over a wide dose range (5 and 750 mg/kg bw) on the basis of percentage of radiolabelled dose in the carcass and tissues (WHO, 1998).

(b) Metabolism

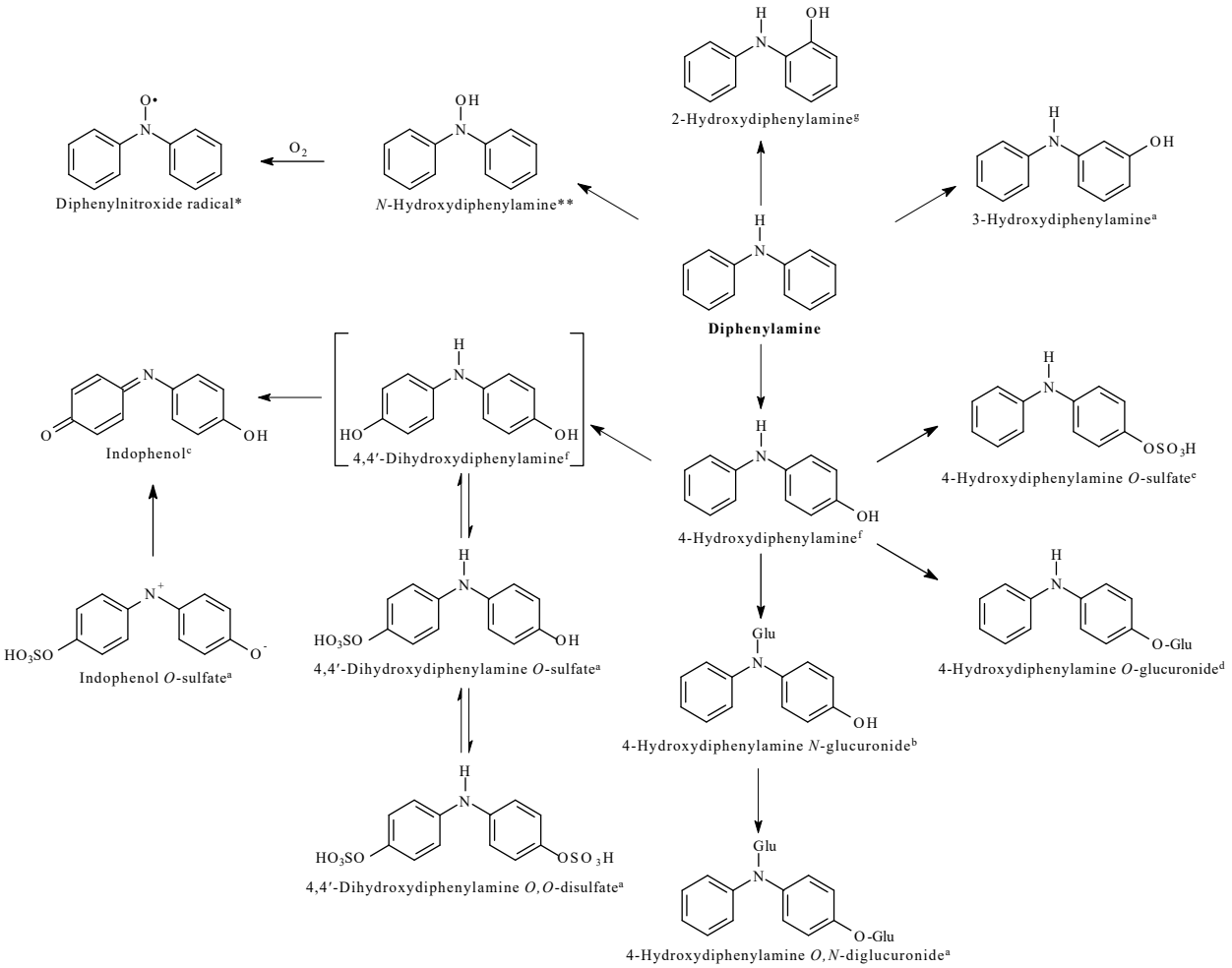
See Fig. 4.1.

Diphenylamine undergoes rapid and extensive metabolism by hydroxylation followed by conjugation (Alexander et al., 1964, 1965; WHO, 1998). A total of 12 metabolites of diphenylamine were identified in rats given oral doses at 5 or 750 mg/kg bw (WHO, 1998), with less than 3% of the administered dose remaining as parent compound in the urine and faeces. Metabolites of diphenylamine in rats include 4-hydroxydiphenylamine, 3-hydroxydiphenylamine, 2-hydroxy-diphenylamine, 4-hydroxydiphenylamine *O*-sulfate, 4-hydroxydiphenylamine *O*-glucuronide, 4-hydroxydiphenylamine *N*-glucuronide, 4-hydroxydiphenylamine *O,N*-diglucuronide, 4,4′-dihydroxydiphenylamine, 4,4′-dihydroxydiphenylamine *O*-sulfate, 4,4′-dihydroxydiphenylamine *O,O*-disulfate, indophenol, and indophenol *O*-sulfate.

Diphenylamine was also shown to be metabolized to 4-hydroxydiphenylamine and 4,4′-dihydroxydiphenylamine in goats, hens, and dogs (DeEds, 1963; WHO, 1998). [The Working Group noted that DeEds (1963) did not provide adequate experimental evidence for their findings in dogs.] 2-Hydroxydiphenylamine was identified as a minor metabolite of diphenylamine in rabbits (Alexander et al., 1964, 1965). 2-Hydroxydiphenylamine was also reported to be a metabolite in rats (WHO, 1998); however, it was not detected in rat urine by Alexander et al. (1965).

Conjugates of 4-hydroxydiphenylamine were identified as the major metabolites of diphenyl-

Fig. 4.1 Metabolic pathways for diphenylamine



Glu, glucuronate.
[a] Rat.
[b] Rat and dog.
[c] Rat and goat.
[d] Rat, rabbit, goat, and hen.
[e] Rat, rabbit, dog, goat, and hen.
[f] Rat, rabbit, dog, goat, hen and human.
[g] Rat [contradictory findings], rabbit, and hen.
* Microsomal systems in hogs and mice.
** Hydroxylamine derivative as hypothesized metabolic intermediate (Appel et al., 1987; Valvis et al., 1990).
Adapted from WHO (1998).

amine in the urine of rats injected intraperitoneally with 5 mg of diphenylamine (Alexander et al., 1964). In a rabbit, 5 g of diphenylamine was orally administered as a suspension and as a divided dose of 1 g over a period of 9 days. *O*-Sulfate and *O*-glucuronide were detected as the primary conjugates of 4-hydroxydiphenylamine in rabbit urine (Alexander et al., 1965).

O-Sulfate and *N*-glucuronide conjugates of 4-hydroxydiphenylamine and 4,4′-dihydroxydiphenylamine were also detected as the products of metabolism in the urine and faeces of albino rats and beagle dogs in a 2-year feeding study (DeEds, 1963). [The Working Group noted that DeEds (1963) did not provide adequate experimental evidence for their findings.]

Furthermore, N-hydroxylation of diphenylamine was hypothesized as a potential metabolic pathway in rats, rabbits, and cats (Alexander et al., 1964, 1965). [The Working Group noted that it is difficult to detect *N*-hydroxydiphenylamine due to its chemical instability.] Under acidic conditions of urine hydrolysis in vitro, *N*-hydroxydiphenylamine was shown to rearrange to diphenylamine and 4-hydroxydiphenylamine. After a single intraperitoneal injection of 5 mg of *N*-hydroxydiphenylamine in male white rats [the Working Group noted that the strain was not provided], neither *N*-hydroxydiphenylamine nor diphenylamine were detected in the hydrolysed urine. Instead, 4-hydroxydiphenylamine and 4,4′-dihydroxydiphenylamine were detected, possibly due to the chemical rearrangement of *N*-hydroxydiphenylamine in vivo (Alexander et al., 1964, 1965).

Additional evidence for the formation of *N*-hydroxydiphenylamine in vivo is indirect and associated with methaemoglobin formation in rats, mice, and cats after diphenylamine exposure (Alexander et al., 1965; Nomura, 1977). The kinetics of methaemoglobin formation were studied in male ddY mice for 96 hours after intraperitoneal injection with a single dose of diphenylamine at 103 mg/kg bw. Methaemoglobin concentrations in the blood peaked rapidly about 30 minutes after administration and decreased to levels that were similar to those of controls after 90 minutes (Nomura, 1977). No significant formation of methaemoglobin was detected 48 hours after three consecutive days of intraperitoneal injections in male ddY mice (Nomura, 1977). Similarly, methaemoglobin in rat blood was shown to reach peak concentrations 30–35 hours after oral administration (gavage) of diphenylamine at half the median lethal dose (½ LD_{50}) (Volodchenko, 1975). [The Working Group noted that, overall, the N-hydroxylation of diphenylamine in vivo is probable and supported by the evidence of formation of methaemoglobin; however, it has not been chemically detected or conclusively determined (Alexander et al., 1964, 1965; Volodchenko, 1975; Nomura, 1977; Appel et al., 1987; Semak & Pikulev, 1993).]

Acellular assays using hog liver microsomes also showed that diphenylamine is a good substrate for mixed function amine oxidase and can undergo bio-oxidation in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen to yield its respective nitroxide free radical, diphenylnitroxide (Valvis et al., 1990). Bio-oxidation was rate-limited by substrate inhibition at higher diphenylamine concentrations although the yield over time was noted to be high (Valvis et al., 1990). Additional acellular assays using mouse microsomes provided more evidence for production of diphenylnitroxide radicals after incubation with diphenylhydroxylamine, a potential metabolite of diphenylamine (Appel et al., 1987). More recently, diphenylamino radical formation was detected after oxidation of diphenylamine in an acellular system (Son & Choi, 2021).

(c) Excretion

Urine is a major route of excretion for diphenylamine in rats, rabbits, dogs, and goats, with bile and faeces contributing to a lesser extent

(DeEds 1963; Alexander et al., 1965; WHO, 1998). Diphenylamine was shown to be excreted primarily as its metabolite 4-hydroxydiphenylamine, its conjugates, and 4,4′-dihydroxydiphenylamine in rat and rabbit urine (DeEds, 1963; Alexander et al., 1964, 1965), in rat bile (Alexander et al., 1964, 1965), and in rat and dog faeces (DeEds, 1963), but not as metabolites in the urine and faeces of goat and hen (WHO, 1998). [The Working Group noted that WHO (1998) contained limited experimental and analytical details.] In rabbits, 2-hydroxydiphenylamine and unchanged diphenylamine were also detected in the urine (Alexander et al., 1965). A 4-day feeding study in a Holstein dairy cow given diphenylamine at 5 ppm showed excretion of 1.4% of the administered dose in the faeces, but no diphenylamine was detected in the urine or milk (Gutenmann & Lisk, 1975). [The Working Group noted that the analytical method used (gas chromatography) could not have detected the metabolites of diphenylamine, which are the primary forms in which diphenylamine is eliminated in urine across species.]

Excretion of diphenylamine is rapid. Urine, faeces, and milk collected cumulatively in goats orally dosed with radiolabelled diphenylamine at 50 mg/kg bw per day for 7 days showed that the administered dose was largely excreted within 24 hours after each dose (WHO, 1998). In Sprague-Dawley rats, up to 72% of the administered dose was reported to be excreted in the urine within 24 hours (WHO, 1998). In male white rats injected with radiolabelled diphenylamine intraperitoneally or intravenously (with bile duct cannulation) at a dose of 5 mg/kg bw, there was 75% recovery of the radiolabel in the urine after 48 hours and 25% in the bile after 6 hours, respectively (Alexander et al., 1965).

4.2 Evidence relevant to key characteristics of carcinogens

4.2.1 Is genotoxic

(a) Humans

(i) Exposed humans

No genotoxicity studies in exposed humans were available to the Working Group. However, Fracasso et al. (1999) detected diphenylamine along with five other chemicals in stationary workplace air samples collected over a 3-hour period and in personal air samples collected over a 2-hour period during a typical work day from five workers employed in different rubber-processing operations. The mutagenic activity of the air samples was determined by a plate incorporation assay using *Salmonella typhimurium* strains TA98NR, TA98, YG1021, and TA100 (Table 4.1).

The results showed direct and indirect frameshift mutagenicity induced by both the ambient and personal air samples. No mutation was induced in the *S. typhimurium* TA100 strain, except for the air sample from one worker. The high levels of mutagenic activity in the ambient and personal air samples compared with negative controls indicate the presence of substances with high genotoxic potency (Fracasso et al., 1999). [The Working Group noted that the air samples contained a mixture of chemicals including diphenylamine; however, it was not possible to conclusively establish a causative link between genotoxicity and exposure to diphenylamine only. Furthermore, the precise concentration of diphenylamine in the air samples and the duration of exposure were not reported.]

(ii) Human cells in vitro

See Table 4.2.

In the study by Ardito et al. (1996), diphenylamine significantly increased the frequency of sister-chromatid exchange in cultured human peripheral blood lymphocytes treated with a non-cytotoxic concentration of 6 μg/mL (but not

Table 4.1 Genetic and related effects of diphenylamine in exposed humans

Test system (species, strain)	End-point	Description of exposed and controls	Results[a]	Comments	Reference
Salmonella typhimurium, TA98NR, TA98, and YG1021	Reverse mutation	Personal air samples collected over a 2 h period during a typical work day from five workers employed in different rubber-processing operations. Control air samples from factories offices included.	(+)	The air samples contained a mixture of chemicals including diphenylamine; however, it was not possible to conclusively establish a causative link between genotoxicity and exposure to diphenylamine only.	Fracasso et al. (1999)
TA100	Reverse mutation	Personal air samples collected over a 2 h period during a typical work day from five workers employed in different rubber-processing operations. Control air samples included.	(–)	The air samples contained a mixture of chemicals including diphenylamine; however, it was not possible to conclusively establish a causative link between genotoxicity and exposure to diphenylamine only.	Fracasso et al. (1999)

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

Table 4.2 Genetic and related effects of diphenylamine in human cells in vitro

End-point	Tissue, cell type	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Micronucleus formation	Peripheral blood lymphocytes	+	NT	1.25 µg/mL	Purity, NR; 48 h exposure; statistically significant at this concentration versus the negative and solvent controls.	Santovito et al. (2012)
Sister-chromatid exchange	Peripheral blood lymphocytes	(+)	NT	3.5×10^{-5} M (6 µg/mL), 48 h exposure	Chemical source and purity, NR; increase is small and within 1 SD of control; method inconsistent with OECD test guideline to support clear negatives; S9 from phenobarbital/benzoflavone-induced rat liver.	Ardito et al. (1996)
		(–)	(–)	3.5×10^{-5} M (6 µg/mL), 4 h exposure		

HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; OECD, Organisation for Economic Cooperation and Development; S9, 9000 × *g* supernatant; SD, standard deviation.

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

0.6 µg/mL) for 48 hours. However, no difference was observed when lymphocytes were exposed for 4 hours at 6 µg/mL with and without metabolic activation (Ardito et al., 1996). [The Working Group noted that dose-dependent trends could not be established for diphenylamine owing to cytotoxicity after 48 hours of exposure at higher concentrations (60 µg/mL). The Working Group also noted cautious interpretation of the positive result since the increase, although statistically significant, was small and within only one standard deviation of the control. The number of well-spread metaphases scored in the cultures for each concentration, particularly in the 4-hour treatment group, were 10 times less than that suggested to be required to support a clear negative result (OECD test guideline, TG473; OECD, 2016). Furthermore, the Working Group noted that chemical source and purity were not reported.]

Another study investigated the potential of diphenylamine to induce chromosomal damage within a dose range comparable to that used in the two lowest treatment groups (6 and 0.6 µg/mL) in Ardito et al. (1996) with a similar exposure duration of 48 hours (Santovito et al., 2012). In human peripheral blood lymphocytes, diphenylamine significantly increased the frequency of micronucleus formation at concentrations of 1.25, 2.5, 5, and 10 µg/mL, but not at 0.625 µg/mL, compared with the negative and solvent (1% dimethyl sulfoxide, DMSO) controls. Moreover, diphenylamine was shown to induce an increase in the frequency of micronucleus formation with statistical significance at all treatment concentrations except 1.25 µg/mL when compared with 0.625 µg/mL. None of the tested concentrations were cytotoxic (Santovito et al., 2012).

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3.

Diphenylamine was reported to give negative results for the induction of micronuclei in the bone marrow of ICR mice exposed at concentrations of 250–1000 mg/kg bw (males) and 375–1500 mg/kg bw (females) (WHO, 1998; European Commission, 2008).

Diphenylamine at concentrations of 1450–2900 µmol/kg bw (plus sodium nitrite) was also shown to lack mutagenic activity in a host-mediated mouse assay when injected intraperitoneally together with *S. typhimurium* TA1950 as a genetic indicator organism (Braun et al., 1977).

(ii) Non-human mammalian cells in vitro

See Table 4.4.

Negative results for DNA single-strand breaks were reported for diphenylamine in Chinese hamster V79 cells (Appel et al., 1987). [The Working Group noted that the doses tested were not indicated.] Diphenylhydroxylamine [*N*-hydroxydiphenylamine], a proposed metabolite of diphenylamine (Section 4.1.2), was shown to cause DNA breaks in Chinese hamster V79 cells (Appel et al., 1987). [The Working Group noted that this was possibly due to its auto-oxidation to the diphenylnitroxide radical.]

Diphenylamine produced negative results for unscheduled DNA synthesis when tested at the highest non-cytotoxic concentration of 100 µM [mol/L] without metabolic activation in cultured rat hepatocytes (Probst et al., 1981). [The Working Group noted the challenges associated with detecting low levels of DNA repair using the autoradiographic method, and the potential ability of a chemical to inhibit DNA repair enzymes, resulting in a negative DNA-repair response.]

Furthermore, diphenylamine was found to be non-mutagenic in the L5178Y mouse lymphoma thymidine kinase ($Tk^{+/-}$) assay in the presence of metabolic activation (Amacher et al., 1980) after 3 hours of treatment at concentrations of up to 6.75×10^{-5} M. Cytotoxicity was observed at higher concentrations (9×10^{-5} M to 21.36×10^{-5} M). In another study reported in

Table 4.3 Genetic and related effects of diphenylamine in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LEC or HIC)	Route, duration, dosing regimen	Comments	Reference
Micronucleus formation	Mouse, ICR (M, F)	Bone marrow	(–)	1000 mg/kg bw (M) 1500 mg/kg bw (F)	Oral administration (gavage); 24, 48, and 72 h	Purity, 99.9%	WHO (1998); European Commission (2008)
Mutation (host-mediated assay)	Mouse, NMRI (M)	*S. typhimurium* TA1950 from peritoneal cavity	–	1450–2900 µmol/kg bw (+ sodium nitrite)	Oral administration (gavage) and intraperitoneal injection of bacteria	"Pure" (but % not given)	Braun et al. (1977)
Oxidative DNA damage (8-OHdG)	Rat, Wistar (M)	Liver	+	0.09 mg/kg bw per day	Oral administration (gavage); 10 days	Purity, 99.9%	Lodovici et al. (1997)

bw, body weight; F, female; HIC, highest ineffective concentration; LEC, lowest effective concentration; M, male; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.
[a] +, positive; –, negative; (–), negative in a study of limited quality.

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

End-point	Species, cell type	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA single-strand breaks, alkaline elution	Chinese hamster, V79 lung cells	(–)	NT	NR	Chemical source and purity, NR; scant information on analytical methods; dose for diphenylamine not reported, inferred from results for *N*-nitrosodiphenylamine (diphenylamine metabolite).	Appel et al. (1987)
Unscheduled DNA synthesis	F344 rat, hepatocytes	–	NT	100 nmol/mL [100 µM; ~17 µg/mL]	Low levels of DNA repair potentially not detected by autoradiography.	Probst et al. (1981)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/*Tk*$^{+/-}$ lymphoma cells	NT	–	6.75×10^{-5} M [67.5 µM; ~11.5 µg/mL]	Dose as high as 21.36×10^{-5} M tested; however, cytotoxicity occurred at 9.00×10^{-5} M; S9 from Aroclor-1254-induced male (Sprague Dawley) rat liver.	Amacher et al. (1980)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/*Tk*$^{+/-}$ lymphoma cells	(+)	(+)	5–80 µg/mL	Weakly positive; dose range cytotoxic and mutation frequency did not increase with dose; effect with or without metabolic activation, NR; purity, ≥ 93%.	WHO (1998)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; S9, 9000 × *g* supernatant.
[a] –, negative; (+) or (–), positive or negative in a study of limited quality.

WHO (1998), diphenylamine was found to give weak positive results in the L5178Y ($Tk^{+/-}$) assay over a concentration range of 5–80 µg/mL. [The Working Group noted that the exposure duration and whether this effect was observed with or without metabolic activation were not specified. The report also noted that the dose range was cytotoxic, and the mutation frequency did not increase with dose.]

(iii) Non-mammalian experimental systems

See Table 4.5.

Wakabayashi et al. (1982) found that diphenylamine at 1.0 µmol/plate induced mutations in *S. typhimurium* TA98 but only in the presence of the co-mutagen norharman and with metabolic activation. No mutagenic activity was reported for diphenylamine (without norharman) in the presence or absence of metabolic activation in *S. typhimurium* TA98 or TA100. Epler et al. (1978) also reported that diphenylamine with metabolic activation was not mutagenic in *S. typhimurium* TA100. [The Working Group noted that the dose was not clearly reported in this study.] Similarly, diphenylamine at 100 µg/plate was not found to be mutagenic in *S. typhimurium* TA1538, with or without metabolic activation (Ferretti et al., 1977). [The Working Group noted that positive and negative controls were not included in this study.]

Diphenylamine (dissolved in ethanol) was not mutagenic at a concentration of 3 µmol/plate when spot-tested in *S. typhimurium* TA98, TA100, TA1535, and TA1537, with or without metabolic activation (Florin et al., 1980). [The Working Group noted that, although diphenylamine was reported to be non-mutagenic, there were challenges interpreting the results, and that diphenylamine precipitated at this concentration.] However, Zeiger et al. (1988) tested diphenylamine in similar *S. typhimurium* strains (TA97, TA98, TA100, and TA1535) over a range of concentrations with and without metabolic activation and conclusively determined it to be non-mutagenic. Another study also reported a lack of a mutagenic response with diphenylamine in a modified Ames test in gradient plates and at concentrations ranging from approximately 0.1 to 1000 µg/mL (McMahon et al., 1979; Probst et al., 1981). The bacterial strains tested were *S. typhimurium* (G46, C3076, D3052, TA1535, TA1537, TA1538, TA100, and TA98) and *Escherichia coli* (WP2 and WP2 *uvrA*$^-$) with and without metabolic activation (McMahon et al., 1979; Probst et al., 1981). [The Working Group noted that it was not clear whether negative and positive controls were tested or whether cytotoxicity occurred concurrently in this study. The chemical source but not the purity was reported.] McGregor et al. (1980) also reported a negative mutagenic response with diphenylamine in bacterial and yeast systems. The test systems included *S. typhimurium* (TA1535, TA1537, TA1538, TA98, and TA100), *E. coli* (W3110/polA$^+$ and p3478/polA$^-$), and *Saccharomyces cerevisiae* (D5), with and without metabolic activation (McGregor et al., 1980). [The Working Group noted that no sufficient information on the experimental specifications or dose of the diphenylamine tested were provided.] Moreover, diphenylamine tested negative for mutagenicity in another short-term assay, the SOS chromotest, conducted in *E. coli* PQ37, with and without metabolic activation (von der Hude et al., 1988). Kubo et al. (2002) reported a negative mutagenic response for diphenylamine (1 mM) in *S. typhimurium* strains TA98 and TA100, with and without metabolic activation. Diphenylamine at concentration ranges of 6.67–333 µg/plate and 10–667 µg/plate did not induce mutations in *S. typhimurium* strains TA98 and TA100, TA1535, TA1537, and TA1538 (WHO, 1998). [The Working Group noted that it was not clear whether this effect was with or without metabolic activation.]

Comet assays conducted in haemocytes of adult fatmucket mussels (*Lampsilis siliquoidea)* showed greater percentage of tail DNA when exposed to diphenylamine for 28 days;

Table 4.5 Genetic and related effects of diphenylamine 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			
Lampsilis siliquoidea	DNA damage (comet assay), haemocytes	(+)	NT	0.3 µg/g dw of sediment	Purity, > 95%; no dose-dependent effect; statistical significance attained only at 0.3 but not 2.6, 4.6, 6.6, or 11.6 µg/g dw.	Prosser et al. (2017)
Saccharomyces cerevisiae, D5	Mitotic recombination	(–)	(–)	NR	Purity, NR; dose, NR; S9 from Aroclor-1254-induced male rat liver.	McGregor et al. (1980)
Salmonella typhimurium, TA98 and TA100	Reverse mutation	–	–	1.0 µmol/ plate (without norharman)	Purity, NR; S9 from male rat liver.	Wakabayashi et al. (1982)
TA100	Reverse mutation	NT	(–)	NR	S9 from Aroclor-1254-induced male rat liver.	Epler et al. (1978)
TA1535, TA1537, TA1538, TA98, and TA100	Reverse mutation	(–)	(–)	NR	Purity, NR; liver S9.	McGregor et al. (1980)
TA98, TA100, TA1535, and TA1537	Reverse mutation	(–)	(–)	3 µmol/plate	Precipitation of diphenylamine; S9 from Aroclor-1254-induced male (Sprague-Dawley) rat liver.	Florin et al. (1980)
G46, C3076, D3052, TA1535, TA1537, TA1538, TA100, and TA98	Reverse mutation	(–)	(–)	0.1 µg/mL to 1000 µg/mL	Purity, NR; controls, NR; cytotoxicity, NR; S9 from Aroclor-1254-induced male (Fischer) rat liver.	McMahon et al. (1979); Probst et al. (1981)
TA97, TA98, TA100, and TA1535	Reverse mutation	–	–	333 µg/plate	Dose inferred from secondary reference; S9 from Aroclor-1254-induced rat or hamster liver.	Zeiger et al. (1988)
TA98 and TA100	Reverse mutation	–	–	1 mM	Purity, NR; rat liver S9.	Kubo et al. (2002)
TA98, TA100, TA1535, TA1537, and TA1538	Reverse mutation	(–)	(–)	667 µg/plate	Purity, 99.9%.	WHO (1998)
TA1538	Reverse mutation	(–)	(–)	100 µg/plate	Single dose; purity, NR; no replicates; no positive or negative controls.	Ferretti et al. (1977)
Escherichia coli, W3110/ polA$^+$ and p3478/polA$^-$	Reverse mutation	(–)	(–)	NR	Purity, NR; dose, NR.	McGregor et al. (1980)

Table 4.5 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
PQ37, SOS chromotest	DNA damage	(–)	(–)	Limit of solubility or 100 mM	Purity, NR; concentration tested not clear.	von der Hude et al. (1988)
WP2 and WP2 *uvrA*$^-$	Reverse mutation	(–)	(–)	0.1 µg/mL to 1000 µg/mL	Purity, NR; controls, NR; cytotoxicity evaluations, NR.	McMahon et al. (1979); Probst et al. (1981)

dw, dry weight; HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; NR, not reported; S9, 9000 × *g* supernatant.

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

however, statistical significance was reported only at the lowest evaluated concentration of 0.3 µg/g dry weight (dw) of sediment (Prosser et al., 2017). No dose–response related effects were observed across the remaining doses of 2.6, 4.6, 6.6, and 11.6 µg/g dw of sediment measured at the start of the experiment. [The Working Group noted that these concentrations translate to 39.56 µg/L in the overlying water for the highest dose group. Water (containing diphenylamine) was replenished in the tanks after 14 days. The Working Group also noted that the diphenylamine concentrations in the water decreased after exposure initiation within each 14-day period.]

4.2.2 Induces oxidative stress

(a) Humans

(i) Exposed humans

No studies were available to the Working Group.

(ii) Human cells in vitro

Diphenylamine at concentrations of 10^{-4} and 10^{-5} M significantly induced increased superoxide anion production by phagocytosing human blood-derived polymorphonuclear leukocytes (Vandenbroucke-Grauls et al., 1984). Furthermore, diphenylamine (at concentrations greater than 0.05 mM) was shown to enhance lipid peroxidation via an intermediate nitrogen-based radical by increasing lipid hydroperoxide formation and oxygen consumption in erythrocytes obtained from healthy donors, therefore contributing to peroxidative stress (Sugihara et al., 1993).

(b) Experimental systems

(i) Non-human mammals in vivo

Diphenylamine was shown to induce oxidative stress in male Wistar rats exposed at a dose of 0.09–1.4 mg/kg bw per day for 10 days by gavage, as determined by the presence of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in liver DNA, a biomarker of oxidative DNA damage (Lodovici et al., 1997) (see Table 4.3).

Regional glutathione concentrations in the kidney cortex were found to be reduced 1 hour after a single oral dose of diphenylamine of 200, 400, or 600 mg/kg bw, and 4 hours after 400 and 600 mg/kg bw in male Syrian hamsters. However, no significant changes in glutathione levels were observed in the renal outer medulla or papilla at any dose tested (Lenz, 1996). [The Working Group noted that measurements of glutathione concentration in the renal papilla might not be reflective of oxidative stress at the capillary endothelium (i.e. decreased renal papillary glutathione levels may not correlate with renal papillary necrosis).]

Diphenylamine induced microsomal and cytosolic glutathione *S*-transferase (GST) activities by 2- and 1.3-fold, respectively, compared with controls, in male albino rats given a single oral dose at one third of the LD_{50} (Semak & Pikulev, 1993). [The Working Group noted that the strain was not reported, and the dose was unclear.]

Oral administration of diphenylamine at a dose of 400, 600, or 800 mg/kg bw per day in peanut oil for 9 days induced renal papillotoxicity in male Syrian hamsters. Exposure to diphenylamine in DMSO, which is a potent scavenger of oxygen-free radicals, inhibited this effect. Pre-treatment of hamsters with DMSO significantly reduced the renal toxicity at day 3 (Lenz & Carlton, 1991).

(ii) Non-human mammalian experimental systems

As discussed in Section 4.1.2, the formation of the diphenylnitroxide free radical has been reported in mammalian microsomal systems treated with diphenylamine in vitro (Appel et al., 1987; Valvis et al., 1990). The rate of oxygen consumption during diphenylamine bio-oxidation was found to be nonlinear and exhibited substrate

inhibition kinetics at diphenylamine concentrations greater than approximately 250 nmol/mL (Valvis et al., 1990).

4.2.3 Evidence relevant to other key characteristics

(a) Humans

Regarding immunosuppression, in studies conducted in vitro with natural killer (NK) cells enriched from human lymphocytes (effector) and a human myeloid leukaemia cell line (target), diphenylamine reduced NK cell activity in a dose-dependent manner with almost no activity observed at a concentration of 1 mM. Diphenylamine was shown to noncompetitively inhibit the kinetics of NK-mediated target cell lysis. However, it did not affect the effector–target cell binding at 1 mM but instead considerably reduced the level and activity of intracellular lysosomal enzymes (Verhoef & Sharma, 1983).

(b) Experimental systems

Regarding immortalization, pre-treatment of normal rat kidney cells with diphenylamine at concentrations of 2.5–20 µg/mL without metabolic activation did not increase the frequency of viral transformation by murine sarcoma virus. With metabolic activation, an increase of 2.5-fold in the frequency of transformation by murine sarcoma virus was induced by diphenylamine; however, this was not found to be statistically significant when compared with controls (Wilson & Khoobyarian, 1982). [The Working Group noted that the mechanisms of chemical carcinogenesis for this assay system were not clearly defined.]

Regarding alterations in cell proliferation, cell death, or nutrient supply, in male and female Fischer 344 rats exposed to diphenylamine at a dose of 1000 mg/kg bw per day for 28 days, necrosis and degeneration of the kidney tubules and erosion of the forestomach were induced (Yoshida et al., 1989). These changes were associated with increased blood leukocyte counts, bone marrow hyperplasia, and forestomach hyperplasia. [The Working Group considered that the increase in leukocytes and bone marrow hyperplasia, which increased the leukocyte counts, were a secondary response to tissue necrosis and degeneration in the kidney and forestomach erosion, and the forestomach hyperplasia was an indicator of mucosal repair in the forestomach.] Exposure of male Sprague-Dawley rats to diphenylamine at 1% in the feed also induced hyperplasia of the tubular cells in the collecting ducts of the kidney at 5 weeks (Evan & Gardner, 1976; Evan et al., 1978). Gershbein (1975) reported that diphenylamine accelerated the rate of liver regeneration in partially hepatectomized male rats treated via the diet (0.5%) for 10 continuous days, when compared with controls. [The Working Group noted that the rat strain was not reported.]

4.2.4 High-throughput in vitro toxicity screening data evaluation

The analysis of the in vitro bioactivity of the agents reviewed in *IARC Monographs* Volume 130 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). Diphenylamine was one of thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes of the US EPA and the United States National Institutes of Health. Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2021). A supplementary table (Annex 2, Supplementary material for Section 4, Mechanistic Evidence, web only; available from: https://publications.iarc.fr/611) provides a summary of the findings (including the assay name, the corresponding key characteristic, the resulting "hit calls" both positive and negative, and any reported caution flags) for diphenyl-

amine. The results were generated with the software "kc-hits" (key characteristics of carcinogens – high-throughput screening discovery tool) (available from: https://gitlab.com/i1650/kc-hits) using the US EPA ToxCast and Tox21 assay data and the curated mapping of key characteristics to assays available at the time of the evaluations performed for the present monograph. Findings and interpretations from these high-throughput assays for diphenylamine are discussed below.

After mapping against the key characteristics of carcinogens, the ToxCast/Tox21 database contained 294 assays in which diphenylamine was tested. Of these, diphenylamine was found to be active and without caution flags in 14 assays relevant to the key characteristics of carcinogens. [The Working Group noted that the cytotoxic limit for diphenylamine is 8.97 μM.]

Diphenylamine was active in six assays mapped to key characteristic 8 (KC8), "modulates receptor-mediated effects". These assays included: activation of the estrogen response element with a half-maximal activity concentration (AC_{50}) of 52.3 μM; the peroxisome proliferator-activated response element (AC_{50}, 17.7 μM); the pregnane X receptor (PXR) response element (AC_{50}, 35.2 μM); and the human estrogen receptor α (AC_{50}, 47.3 μM). The PXR assay was conducted in the HepG2 cell line; all other assays were conducted in a metabolically enhanced HG19 variant of the HepG2 cell line. Diphenylamine was active in one assay with metabolically competent HepaRG cells that measured changes in the expression of the transcription factors for CYP2B6 ($AC_{50,}$ 23.8 μM). The chemical was also active in one assay with a human adrenal gland cell line, H295R (AC_{50}, 26.3 μM).

In addition, diphenylamine was active in eight assays mapped to KC10, "alters cell proliferation, cell death, or nutrient supply"; however, these assays reported a loss of cell viability.

5. Summary of Data Reported

5.1 Exposure characterization

Diphenylamine is a High Production Volume chemical that is predominantly used in lubricants and greases, hydraulic fluids, metal working fluids, dyes and textile treatment products, including leather and fur. It is also used as an intermediate in the manufacture of other substances, including antioxidants in the rubber and elastomer industries. In addition, it is applied in agriculture to prevent scalding on apples and pears. Use of diphenylamine in agriculture is prohibited in the European Union; however, it is frequently applied to post-harvest fruit in agricultural markets in the USA.

The most relevant occupational exposure routes are respiratory and dermal. The main source of occupational exposure to diphenylamine is during its production and further processing. Pesticide mixers, loaders, and applicators can be exposed to diphenylamine during and after regular use in agriculture and other settings.

Environmental exposure to diphenylamine occurs through the air, in sediment around military bases, sewage, residential dust, electronic waste dust, fruit storage facilities, eggs, water, and fruit (both for infants and other ages). The main route of exposure for the general population is oral intake of diphenylamine, primarily through ingestion of fruit and vegetables. The second is dermal exposure through the use of lubricants.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

Treatment with diphenylamine caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in two species.

Diphenylamine was administered by oral administration (in the feed) in one study in male and female Crj:BDF_1 mice. In males, diphenylamine caused an increase in the incidence of haemangioma or haemangiosarcoma (combined) of the liver, of the spleen, and of all organs combined. In females, diphenylamine caused an increase in the incidence of histiocytic sarcoma of the uterus.

Diphenylamine was administered by oral administration (in the feed) in one study in male and female F344/DuCrj rats. In males, diphenylamine caused an increase in the incidence of haemangiosarcoma and haemangioma or haemangiosarcoma (combined) of the spleen and of all organs combined, and of fibroma or fibrosarcoma (combined) of the subcutis. In females, diphenylamine caused an increase in the incidence of adenocarcinoma and adenoma or adenocarcinoma (combined) of the uterus and mononuclear cell leukaemia of the spleen.

5.4 Mechanistic evidence

In two human subjects administered a single oral dose, diphenylamine was absorbed and excreted in the urine as parent compound and/or metabolites. Studies in rats, rabbits, goats, cows, dogs, and laying hens treated with diphenylamine by oral administration showed absorption, tissue distribution without appreciable accumulation, metabolism, and rapid excretion primarily in the urine. In in vitro microsomal systems exposed to diphenylamine, the formation of the diphenylnitroxide free radical has been reported.

Overall, the mechanistic evidence for diphenylamine regarding the key characteristics of carcinogens ("is genotoxic", "induces oxidative stress", "is immunosuppressive", and "alters cell proliferation, cell death, or nutrient supply") is suggestive but incoherent across different experimental systems. There were no studies in humans with exposure specifically attributable to diphenylamine only.

The mechanistic evidence that diphenylamine is genotoxic is suggestive but incoherent across different experimental systems. Diphenylamine gave positive results for micronucleus formation in human peripheral blood lymphocytes in vitro in one study but negative results in the bone marrow of mice in another study. In a few studies, diphenylamine with and without metabolic activation gave negative results for mutagenicity in non-human mammalian systems in vitro and in non-mammalian experimental systems including multiple strains of bacteria. The mechanistic evidence that diphenylamine causes oxidative stress is suggestive based on two studies with positive results in human cells in vitro, four studies with positive results in rodents, and one positive result in vitro using mammalian microsomes.

The mechanistic evidence is also suggestive for the key characteristics "is immunosuppressive" and "alters cell proliferation, cell death, or nutrient supply" based on a few studies. Regarding immunosuppression, diphenylamine reduced human natural killer cell activity in a dose-dependent manner in vitro in one study. Regarding alterations in cell proliferation, cell death, or nutrient supply, diphenylamine induced hyperplasia in several tissues of rats in two studies, and one study reported that diphenylamine accelerated rat liver regeneration.

Diphenylamine 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 diphenylamine.

6.2 Cancer in experimental animals

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

6.3 Mechanistic evidence

There is *limited mechanistic evidence*.

6.4 Overall evaluation

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

6.5 Rationale

The Group 2B evaluation for diphenylamine is based on *sufficient evidence* for cancer in experimental animals. This *sufficient evidence* in experimental animals is based on an increased incidence of either malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in two species. The evidence regarding cancer in humans is *inadequate* because no studies were available. The mechanistic evidence was *limited* as the findings regarding key characteristics of carcinogens across experimental systems, including in some studies using human cells in vitro, were suggestive, but incoherent.

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N-METHYLOLACRYLAMIDE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 924-42-5

Chem. Abstr. Serv. name: *N*-(hydroxymethyl) acrylamide

EC/List No.: 213-103-2

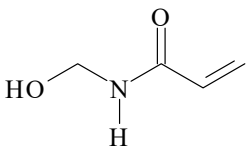
IUPAC systematic name: *N*-(hydroxymethyl) prop-2-enamide

Synonyms: *N*-methylolacrylamide, *N*-methanol-acrylamide, monomethylolacrylamide, *N*-(hydroxymethyl)-2-propenamide, NMA, N-MAM, and other depositor-supplied synonyms and acronyms (NCBI, 2021).

1.1.2 Structural and molecular information

Relative molecular mass: 101.10 (NCBI, 2021)

Chemical structure:



Molecular formula: $C_4H_7NO_2$

1.1.3 Chemical and physical properties

Description: white crystals as a solid, colourless or slightly yellow in aqueous solutions, with a formaldehyde-like odour (ECHA, 2017; NCBI, 2021)

Boiling point: 277 °C (NCBI, 2021)

Melting point: 74.5 °C (NCBI, 2021)

Density: 1.07 g/cm^3 at 25 °C (IFA, 2021)

Vapour pressure: 26.7–40.0 hPa at 25 °C (ECHA, 2021)

Solubility: soluble in water (IFA, 2021); soluble in polar solvents (alcohols) and not soluble in nonpolar solvents (hydrocarbon, chloroform) (ECHA, 2017)

Flash point: > 93 °C (ECHA, 2017)

Stability: sensitive to light, polymerization is possible (IFA, 2021)

Reactivity: tends to polymerize spontaneously and exothermically above 50 °C in the absence of stabilizers (ECHA, 2017; IFA, 2021).

Octanol/water partition coefficient (P): log K_{ow}, –1.81 (NCBI, 2021), log K_{ow}, –1.81 at 20 °C and pH 7 for a 48% aqueous solution (ECHA, 2021).

1.1.4 Impurities

Relevant impurities of toxicological significance are acrylamide (≤ 10%) and formaldehyde (≤ 2%), which are both residues from the production process (ECHA, 2017). Additional impurities can be polymers of *N*-methylolacrylamide (1–2%). The substance is essentially marketed as an aqueous solution only, to which additives such as mequinol (4-methoxyphenol; ≤ 30 ppm), oxygen, or cupric ions are added that function as stabilizers to prevent polymerization. The concentration of the solutions ranges between 40% and 85% (w/w).

1.2 Production and use

1.2.1 Production process

N-Methylolacrylamide is produced in an alkaline environment by hydroxymethylation of acrylamide with formaldehyde in the presence of copper(I) chloride, which acts as a polymerization inhibitor (Feuer & Lynch, 1953; Ashford, 1994; NCBI, 2021).

1.2.2 Production volume

N-Methylolacrylamide is listed as a High Production Volume chemical by the Organization for Economic Co-operation and Development (OECD) (OECD, 2004, 2009). In 2016, the United States Environmental Protection Agency (US EPA) estimated an aggregated production volume of 1 000 000–10 000 000 lb [~450–4500 tonnes] in the USA (NCBI, 2021). The substance is registered under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation, and 1000–10 000 tonnes per annum are manufactured in and/or imported to the European Economic Area (ECHA, 2021). [The Working Group noted that information on production volumes outside of the abovementioned areas was not available.]

1.2.3 Uses

N-Methylolacrylamide is used as an intermediate for the production of *N*,*N*′-methylenebisacrylamide (Lundberg, 1946; Feuer & Lynch, 1953) and, together with *N*,*N*′-methylenebisacrylamide, for the manufacture of a variety of polymers with acrylic and vinylic monomers, such as acrylonitrile, acrylamide, and substituted acrylamides (NCBI, 2021). Free-radical copolymerization using *N*-methylolacrylamide specifically provides stability to the polymer network via cross-linking (Kamogawa, 1967). Polymers based on *N*-methylolacrylamide are ultimately used in a multitude of industries as adhesives, inks and paints, antistatic compounds, thermoplastic and chromatographic resins, coatings, rubbers, plastics, paper, and textile finishes (NCBI, 2021). Specific examples are food contact plastics (US FDA, 2021), certain laminar flooring sealants and wood glues (DeLima Associates, 2021), viscosity adjustors of paints and colourants, adhesives and binders for papermaking, and finishing and dispersing agents for crease-resistant and antiwrinkle fabrics (ECHA, 2021). *N*-Methylolacrylamide-based polymers are also used as coatings for controlled delivery systems of drugs (Siemoneit et al., 2006; Singh et al., 2006; Yang et al., 2011) and fertilizers (Xie et al., 2012; Louzri & Bennour, 2018), energy storage electrolytes (Silvaraj et al., 2021), as grouting agents to reduce water leakages (Weideborg et al., 2001) and in the production of activity-specific clothing with high moisture absorption and release capabilities (Chaudhuri & Wu, 2020). Specific niche applications involve the use of *N*-methylolacrylamide-based polymers in test kits for medical diagnostics (Albers et al., 2010; Reddy et al., 2012; Sullivan et al., 2021), bioimaging (Mahapatra et al., 2020), food analyses (Hakkoymaz & Mazi, 2020; Zhang et al., 2020), and in experimental dental primers (Fukushima et al., 2001).

1.3 Detection and quantification

1.3.1. Environmental samples

No analytical methods were available that specifically described the quantitation of *N*-methylolacrylamide in air, water, and soil samples, or in consumer products, with the exception of the detection of *N*-methylolacrylamide in drainage water by high-performance liquid chromatography-ultraviolet (HPLC-UV), with a limit of detection of 5 µg/L [no additional analytical details were provided] (Weideborg et al., 2001). An analytical method based on gas chromatography (GC) and tandem mass spectrometry (MS/MS) has been reported for the analysis of the sum of acrylamide and *N*-methylolacrylamide in aqueous samples including drinking-water, brewed coffee, and water extracts of snuff (Pérez & Osterman-Golkar, 2003). L-Valine has been used as a nucleophilic trapping agent during sample preparation for both acrylamide and *N*-methylolacrylamide. The two reaction products are then converted to a single pentafluorophenylthiohydantoin derivative using pentafluorophenyl isothiocyanate during further sample preparation. The limit of detection for the sum of acrylamide and *N*-methylolacrylamide was estimated to be ~0.003 µg/L.

1.3.2. Biological specimens

No specific biomarkers for *N*-methylolacrylamide could be traced in the literature; however, there were multiple methods for the determination of haemoglobin adducts of acrylamide based on GC or HPLC and MS/MS analyses (Bergmark et al., 1993; Vesper et al., 2006; Schettgen et al., 2010; von Stedingk et al., 2010; Yang et al., 2018). These methods can also be used to analyse haemoglobin adducts of *N*-methylolacrylamide (Hagmar et al., 2001). All methods are based on a modified Edman degradation using pentafluorophenyl isothiocyanate which, similarly to the analyses of aqueous samples described above (see Section 1.3.1), converts the haemoglobin adducts of acrylamide and/or *N*-methylolacrylamide at the N-terminal valine of haemoglobin to a single pentafluorophenylthiohydantoin derivative. The methods have been used for several decades and there are only minor variations in the limits of detection, which are in a narrow range around 0.01 µg/L blood. As previously mentioned, the methods per se cannot distinguish between exposures to acrylamide or *N*-methylolacrylamide unless the exact source of exposure is known, e.g. grouting agents containing monomers of *N*-methylolacrylamide (Hagmar et al., 2001).

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

N-Methylolacrylamide is not known to occur naturally. It may enter the environment via its use in polyacrylamide polymers and other applications described in Section 1.2.3. The US EPA Toxic Release Inventory reported that, between 2009 and 2019, 2.9–146 tonnes of *N*-methylolacrylamide (3–13 tonnes for most years except for 2016 to 2018, when it was 129–146 tonnes) were released annually on-site (air emission, 5%; surface water discharge, < 0.1%; and land release, 95%) by 26–33 facilities (US EPA, 2021). Facilities emitting or sending *N*-methylolacrylamide off-site included hazardous-waste treatment and disposal (2009–2019, 411 tonnes), manufacture of all plastic material and resin (56 tonnes), manufacture of other basic organic chemicals (0.6 tonnes), manufacture of all other miscellaneous chemical products and preparations (4 tonnes), manufacture of synthetic rubber (0.4 tonnes), and manufacture of paint and coatings (0.2 tonnes).

On the basis of similar chemical characteristics to those of acrylamide, *N*-methylolacrylamide would be expected to have a low bioconcentration potential and be readily biodegradable

(Weideborg et al., 2001). Transient environmental contamination has been reported after use in grouts in tunnels in Sweden and Norway, where *N*-methylolacrylamide-based products were used as a replacement for the more hazardous acrylamide-based grouts (Hagmar et al., 2001; Weideborg et al., 2001). A grouting agent containing about 37% *N*-methylolacrylamide and about 1–5% acrylamide in a quantity of 1500 tonnes was used to prevent water leaks in a tunnel in Sweden over a period of 2 months in 1997 (Hagmar et al., 2001). Incomplete polymerization caused leakage of the grouting agents into a brook, leading to contamination of groundwater and wells in the area, with highest concentrations in the brook water (at the end of the 2-month period) of 92 mg/L for acrylamide and 342 mg/L for *N*-methylolacrylamide (Godin et al., 2002). Weideborg et al. (2001) described a similar situation in a tunnel in Norway, where the same grouting agent was used between 1995 and 1997. Both acrylamide and *N*-methylolacrylamide were monitored in drainage water during the entire period. The highest measured concentrations were 9.7 mg/L and 16.6 mg/L, respectively, and decreased to non-detectable when the grouting activities ceased (Weideborg et al., 2001). [The Working Group noted that the water contamination with acrylamide observed by the authors was much higher than expected from the grout formula (water levels for acrylamide were similar to those for *N*-methylolacrylamide, despite the latter being ~10 times more concentrated than acrylamide in the original product). They attributed this observation to the chemical instability of *N*-methylolacrylamide, which tends to rapidly degrade to acrylamide.]

1.4.2 Occupational exposure

Several reports indicated that occupational exposure to *N*-methylolacrylamide occurred in construction workers in Sweden and Norway, where *N*-methylolacrylamide-based grouting agents were used in waterproofing tunnels. In Sweden, Hagmar et al. (2001) described airborne exposure conditions of workers who pumped the grouting agent under pressure into drill holes. The study reported two measurements of 0.27 and 0.34 mg/m^3, which were the sum of acrylamide and *N*-methylolacrylamide concentrations, taken by the construction company's health service (Hagmar et al., 2001). The authors further indicated that *N*-methylolacrylamide represented 50% of the sum based on two additional samples [levels not reported] taken on the day operations were stopped after the leakage was discovered, and that none of the workers wore adequate protective equipment. [No information was provided about sampling and analysis of these air samples.] The study also reported quantification of haemoglobin adducts in 228 workers (210 exposed, and 18 unexposed) who provided blood samples approximately 1 month after the end of the 2-month grouting operation. Although it was impossible to differentiate between adducts originating from acrylamide or *N*-methylolacrylamide, adduct levels were higher in exposed workers than in unexposed referents. In a similar occupational setting in Norway, Kjuus et al. (2004) measured haemoglobin adducts in 23 exposed workers and 8 unexposed referents. Distinct from the previous study, workers had been engaged in grouting work for an average of 19 months (rather than 2 months), and blood samples were taken 2–5 months after the cessation of work (instead of 1 month). Mean adduct levels were also higher in the exposed workers than in the unexposed referents (Kjuus et al., 2004). In both studies, the higher level of adducts in exposed workers was mainly attributed to dermal exposure (Hagmar et al., 2001; Kjuus et al., 2004). [The Working Group noted that, despite the inability to differentiate adducts from *N*-methylolacrylamide and those from acrylamide, the combined evidence of the studies in Sweden and Norway demonstrated possible internal exposure to *N*-methylolacrylamide

from both the dermal, and, probably to a lesser extent, airborne routes.]

Some information was available to the Working Group regarding the extent of the use of *N*-methylolacrylamide-based grouts, for Europe from a commissioned United Kingdom risk assessment document prepared in 2000 after the incidents in Sweden and Norway (Risk & Policy Analysts Limited, 2000), and for the USA from the United States Federal Register about the proposal for a ban on these products in 1991 by the US EPA (Office of the Federal Register, 1991), followed in 2002 by the withdrawal of the proposed ban (Office of the Federal Register, 2002). According to the United Kingdom document, acrylamide and *N*-methylolacrylamide-based grouts were not produced in Europe as of 2000, and their use was rare. They were, however, in much wider use before the incidents in Sweden and Norway. According to the US EPA proposal for a ban, acrylamide-based grouts represented approximately half of the total chemical grout usage in the USA in 1989, with ~300 tonnes consumed (of which ~10% was *N*-methylolacrylamide-based), mostly used for sewer operations. The US EPA withdrew the proposal for a ban in 2002, proposing that affordable and appropriate protective equipment had become available such that a ban of the products was no longer warranted. In a case report of toxicity in two acrylamide-grout workers, it was reported in 2017 that acrylamide grouts were widely used in the Republic of Korea (Kim et al., 2017). [To the Working Group, this would suggest that occupational exposure via *N*-methylolacrylamide-based grouts may also have occurred recently in this country.]

Exposure to *N*-methylolacrylamide in sealant was also described for four workers working in a window-manufacturing company that used an interlayer product similar to the grouting agent described in the Swedish tunnel studies mentioned above (Paulsson et al., 2006).

No direct information on exposure to *N*-methylolacrylamide for other uses or types of workplaces was available to the Working Group. The National Institute for Occupational Safety and Health (NIOSH) National Occupational Exposure Survey estimated that 20 665 workers (13 852 of whom were women) were exposed to *N*-methylolacrylamide in 1981–1983 (NIOSH, 1983, 1988a, b, 1990). These included mainly workers in the textile-mill product industry (major occupation as "mixing and blending machine operators") and in the apparel and other textile-product industries (major occupation as "textile sewing machine operators"). In 1982, 101 510 000 workers were employed in the USA (Silvestri et al., 1983); thus 0.02% of the working population in the USA in 1982 was potentially occupationally exposed to *N*-methylolacrylamide. [The Working Group noted that it is unclear how representative these estimates are of current exposure prevalence.] During 2003–2018, 98% of *N*-methylolacrylamide used in the Nordic countries (i.e. Denmark, Finland, Norway, and Sweden) was used in the manufacture of chemicals and chemical products (SPIN, 2021). [The Working Group noted that these numbers for Europe are consistent with values for toxic releases for 2009–2019 reported by the US EPA (see Section 1.4.1), primarily in plastics material and resin manufacturing (after excluding landfills).]

[In terms of co-exposures, at least in the grouting sector, the Working Group noted that acrylamide is omnipresent alongside *N*-methylolacrylamide, both as a component of grouting agents as well as a product of *N*-methylolacrylamide degradation. No other specific co-exposures were noted, but it is expected that the various uses described in Section 1.2.3 also imply potential exposure to multiple chemicals (e.g. in paints or adhesives).]

1.4.3 Exposure of the general population

No empirical measurement data were available to the Working Group regarding exposure of the general population. In a recent report on polymers containing *N*-methylolacrylamide in Australia, the National Industrial Chemicals Notification and Assessment Scheme indicated that products manufactured using these polymers can contain low levels of *N*-methylolacrylamide as an impurity, and that consumers might be exposed dermally or through inhalation when in contact with coated surfaces, although it was concluded that this type of exposure would be too low to pose a health risk (National Industrial Chemicals Notification and Assessment Scheme, 2019).

1.5 Regulations and guidelines

There are no reported occupational standard or guidelines for *N*-methylolacrylamide. According to the harmonized classification and labelling framework implemented in the European Union, Classification, Labelling and Packaging (CLP) Regulation (1272/2008/EC), *N*-methylolacrylamide has the following classification: mutagen 1B; carcinogen 1B; specific target organ toxicity-repeated exposure category 1. Employers are obligated under the CLP Regulation to minimize worker exposure to *N*-methylolacrylamide and must arrange for medical surveillance of exposed workers (Council Directive 98/24/EC; European Council, 1998). *N*-Methylolacrylamide is also regulated under the United States Food and Drug Administration, listed as a minor monomer in the production of acrylic plastics in contact with food. Its use is restricted in plastic items for repeated food contact and the main polymer material cannot include more than 5% in weight of polymer units derived by copolymerization with *N*-methylolacrylamide (US FDA, 2020). Acrylamide residues in polyacrylamide polymers are also regulated under the European regulation on cosmetic products. The final cosmetic product maximum residual acrylamide content (including *N*-methylolacrylamide) is 0.1 mg/kg and 0.5 mg/kg for body-leave-on and other products, respectively (European Council, 2009). *N*-Methylolacrylamide is also regulated under the European regulation on plastic materials and articles intended to come into contact with food, with permitted use as a monomer. *N*-Methylolacrylamide should not be released to foods in quantities exceeding 0.01 mg/kg of food (European Commission, 2011). Pregnant workers and workers who have recently given birth or are breastfeeding may not be exposed; young persons (age < 18 years) may not be exposed at the workplace (European Council, 1992, 1994). In 2021, the European Commission developed a Regulatory Management Option Analysis (RMOA) aiming to assess the regulatory needs for *N*-methylolacrylamide. This assessment, performed by the Swedish Chemicals Agency, concluded that *N*-methylolacrylamide fulfils the criteria for inclusion in the Candidate List of Substances of Very High Concern (SVHC) according to Article 57 (a) and (b) of REACH (ECHA, 2021). The RMOA report also states that this inclusion will raise awareness of the substance, represents an incentive for substitution, and may furthermore prevent regrettable substitution of acrylamide with *N*-methylolacrylamide, as acrylamide is already included in the Candidate list of SVHC.

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1.

Table 3.1 Studies of carcinogenicity in experimental animals exposed to *N*-methylolacrylamide

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 8 wk 105 wk NTP (1989)	Oral administration (gavage) *N*-Methylolacrylamide, ~98% Deionized water 0, 25, 50 mg/kg bw 5 days/wk for 103 wk 50, 50, 50 30, 20, 21	*Harderian gland* Adenoma 1/48, 14/49*, 29/50*	$P < 0.001$, Cochran–Armitage trend test, life-table trend test, logistic-regression trend test *$P < 0.001$, Fisher exact test, life-table test, logistic regression test	Principal strengths: well-conducted study that complied with GLP; covered most of the life span; used multiple doses; used males and females; adequate number of mice per group Historical controls: hepatocellular carcinoma, gavage studies, 56/347 (16.1 ± 8.03%), range, 4–28%; hepatocellular carcinoma, all routes, 379/2032 (18.7 ± 6.50%), range, 8–30%; hepatocellular adenoma or carcinoma (combined), gavage studies, 106/347 (30.5 ± 5.83%), range, 20–36%; hepatocellular adenoma or carcinoma (combined), all routes, 609/2032 (30.0 ± 7.59%), range, 16–58% No historical control data for Harderian gland carcinoma
		Carcinoma 1/48, 0/49, 2/50	NS	
		Adenoma or carcinoma (combined) 2/48, 14/49*, 30/50*	$P < 0.001$, Cochran–Armitage trend test, life-table trend test, logistic-regression trend test *$P < 0.001$, Fisher exact test, life-table test, logistic regression test	
		Liver Hepatocellular adenoma 8/50, 4/50, 19/50*	$P = 0.005$, Cochran–Armitage trend test; $P < 0.001$, life-table trend test; $P = 0.002$, logistic-regression trend test *$P = 0.012$, Fisher exact test; $P = 0.004$, logistic regression test	
		Hepatocellular carcinoma 6/50 (12%), 13/50 (26%)*, 12/50 (24%)**	$P = 0.027$, life-table trend test *$P = 0.023$, logistic regression test; $P = 0.012$, life-table test **$P = 0.031$, life-table test	
		Hepatocellular adenoma or carcinoma (combined) 12/50, 17/50, 26/50*	$P = 0.003$, Cochran–Armitage trend test; $P < 0.001$, life-table trend test, logistic regression trend test *$P < 0.001$, life-table test; $P = 0.001$, logistic regression test; $P = 0.004$, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 8 wk 105 wk NTP (1989) (cont.)		*Lung* Bronchioloalveolar adenoma 3/49, 6/50, 11/50* Bronchioloalveolar carcinoma 2/49, 4/50, 10/50* Bronchioloalveolar adenoma or carcinoma (combined) 5/49, 10/50, 18/50*	*P* = 0.005, life-table trend test; *P* = 0.010, logistic-regression trend test **P* = 0.006, life-table test; *P* = 0.015, logistic regression test; *P* = 0.022, Fisher exact test *P* = 0.003, life-table trend test; *P* = 0.005, logistic-regression trend test **P* = 0.006, life-table test; *P* = 0.011, logistic regression test; *P* = 0.015, Fisher exact test *P* = 0.001, Cochran–Armitage trend test; *P* < 0.001, life-table trend test, logistic-regression trend test **P* < 0.001, life-table test; *P* = 0.001, logistic regression test; *P* = 0.002, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 8 wk 105 wk NTP (1989)	Oral administration (gavage) *N*-Methylolacrylamide, ~98% Deionized water 0, 25, 50 mg/kg bw 5 days/wk for 103 wk 50, 50, 50 41, 35, 33	*Harderian gland* Adenoma 5/47, 8/45, 20/48*	*P* < 0.001, Cochran–Armitage trend test, life-table trend test, logistic-regression trend test **P* < 0.001, life-table test, logistic regression test, Fisher exact test	Principal strengths: well-conducted study that complied with GLP; covered most of the life span; used multiple doses; used males and females; adequate number of mice per group Historical controls: Harderian gland carcinoma, gavage studies, 3/350 (0.9 ± 1.57%), range, 0–4%; Harderian gland carcinoma, all routes, 7/2040 (0.3 ± 0.88%), range, 0–4%; bronchioloalveolar carcinoma, gavage studies, 8/349 (2.3 ± 1.80%), range, 0–6%; bronchioloalveolar carcinoma, all routes, 45/2026 (2.2 ± 1.78%), range, 0–6%; bronchioloalveolar adenoma or carcinoma (combined), gavage studies, 33/349 (9.5 ± 3.66%), range, 4–14.3%; bronchioloalveolar adenoma or carcinoma (combined): all routes, 145/2026 (7.2 ± 4.21%), range, 0–16%
		Carcinoma 0/47, 3/45 (6.7%), 2/48 (4.2%)	NS	
		Adenoma or carcinoma (combined) 5/47, 11/45*, 22/48**	*P* < 0.001, Cochran–Armitage trend test, life-table trend test, logistic-regression trend test **P* = 0.031, logistic regression test ***P* < 0.001, life-table test, logistic regression test, Fisher exact test	
		Liver Hepatocellular adenoma 3/50, 4/50, 17/49*	*P* < 0.001, Cochran–Armitage trend test, life-table trend test, logistic-regression trend test **P* < 0.001, life-table test, logistic regression test, Fisher exact test	
		Hepatocellular carcinoma 3/50, 3/50, 2/49	NS	
		Hepatocellular adenoma or carcinoma (combined) 6/50, 7/50, 17/49*	*P* = 0.004, Cochran–Armitage trend test; *P* = 0.001, life-table trend test; *P* = 0.002, logistic-regression trend test **P* = 0.002, life-table test; *P* = 0.003, logistic regression test; *P* = 0.007, Fisher exact test	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 8 wk 105 wk NTP (1989) (cont.)		*Lung* Bronchioloalveolar adenoma 4/50, 4/50, 7/49 Bronchioloalveolar carcinoma 2/50, 5/50, 7/49*	 NS *P* = 0.034, life-table trend test **P* = 0.045, life-table test	
		Bronchioloalveolar adenoma or carcinoma (combined) 6/50, 8/50, 13/49*	*P* = 0.019, life-table trend test; *P* = 0.042, logistic-regression trend test; *P* = 0.041, Cochran–Armitage trend test **P* = 0.025, life-table test	
		Ovary: benign granulosa cell tumours 0/50, 5/45*, 5/47**	*P* = 0.017, life-table trend test; *P* = 0.017, logistic-regression trend test; *P* = 0.031 Cochran–Armitage trend test ** *P* = 0.015, life-table test; *P* = 0.015, logistic regression test; *P* = 0.021, Fisher exact test ** *P* = 0.016, life-table test; *P* = 0.016, logistic regression test; *P* = 0.024, Fisher exact test	
Full carcinogenicity Mouse, C57BL/6 (M) 15–18 wk 30 wk Tennant et al. (1995)	Oral administration (gavage) *N*-Methylolacrylamide, assumed to be ~98% purity Corn oil 0, 50 mg/kg bw 5×/wk for 24 wk 5, 5 5, 5	*Liver*: tumours 0/5, 0/5	 NA	Principal limitations: small number of mice per group; an unspecified number of mice were killed at interim; inadequate duration

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, C57BL/6 (F) 15–18 wk 30 wk Tennant et al. (1995)	Oral administration (gavage) *N*-Methylolacrylamide, assumed to be ~98% purity Corn oil 0, 50 mg/kg bw 5×/wk for 24 wk 5, 5 5, 4	*Liver*: tumours 0/5, 0/5	NA	Principal limitations: small number of mice per group; an unspecified number of mice were killed at interim; inadequate duration
Full carcinogenicity Mouse, C57BL/6 *p53*$^{+/-}$ (M) 15–18 wk 30 wk Tennant et al. (1995)	Oral administration (gavage) *N*-Methylolacrylamide, assumed to be ~98% purity Corn oil 0, 25, 50 mg/kg bw 5×/wk for 24 wk 7, 7, 10 7, 6, 8	*Liver*: tumours 0/7, 0/7, 0/10	NA	Principal limitations: small number of mice per group; an unspecified number of mice were killed at interim
Full carcinogenicity Mouse, C57BL/6 *p53*$^{+/-}$ (F) 15–18 wk 30 wk Tennant et al. (1995)	Oral administration (gavage) *N*-Methylolacrylamide, assumed to be ~98% purity Corn oil 0, 25, 50 mg/kg bw 5×/wk for 24 wk 7, 7, 10 7, 6, 8	*Liver*: tumours 0/7, 0/7, 0/10	NA	Principal limitations: small number of mice per group; an unspecified number of mice were killed at interim

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, $CB6F_1$ (M) 7 wk 26 wk Tsuji et al. (2015)	Oral administration (drinking-water) *N*-Methylolacrylamide, NR Water 0, 135 mg/kg bw, ad libitum 5, 5 5, 5	*Lung* Adenoma 0/5, 0/5 Adenocarcinoma 0/5, 0/5	 NA NA	Principal limitations: only one sex used; only one dose used; small number of mice per group; inadequate duration of experiment
Full carcinogenicity Mouse (transgenic, Tg), $CB6F_1$ *rasH2* (M) 7 wk 26 wk Tsuji et al. (2015)	Oral administration (drinking-water) *N*-Methylolacrylamide, NR Water 0, 135 mg/kg bw, ad libitum 5, 5 5, 4	*Lung* Adenoma 0/5, 5/5* Adenocarcinoma 0/5, 3/5	 *[$P = 0.0040$, one-tailed Fisher exact test] [NS]	Principal limitations: only one sex used; only one dose used; small number of mice per group; lack of historical control data
Full carcinogenicity Rat, F344/N (M) 7 wk 105 wk NTP (1989)	Oral administration (gavage) *N*-Methylolacrylamide, ~98% Deionized water 0, 6, 12 mg/kg bw 5 days/wk for 103 wk 50, 50, 50 28, 22, 27	No significant increase in tumour incidence in treated animals		Principal strengths: well-conducted study that complied with GLP; covers most of the life span; used multiple doses; used males and females; adequate number of rats per group

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (F) 7 wk 105 wk NTP (1989)	Oral administration (gavage) *N*-Methylolacrylamide, ~98% Deionized water 0, 6, 12 mg/kg bw 5 days/wk for 103 wk 50, 50, 50 35, 22, 33	No significant increase in tumour incidence in treated animals		Principal strengths: well-conducted study that complied with GLP; covers most of the life span; used multiple doses; used males and females; adequate number of rats per group

bw, body weight; F, female; GLP, Good Laboratory Practice; M, male; NA, not applicable; NR, not reported; NS, not significant; wk, week.

3.1 Mouse

3.1.1 Oral administration (gavage)

In a well-conducted study that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female B6C3F$_1$ mice (age, 8 weeks) were given *N*-methylolacrylamide (purity, approximately 98%) at a dose of 0, 25, or 50 mg/kg body weight (bw) for the control group and the groups at the lower and higher dose, respectively, in deionized water, by gavage, 5 days per week for 105 weeks (NTP, 1989). Surviving animals were killed at age 113 weeks. At study termination, survival was 30/50, 20/50, and 21/50 in males and 41/50, 35/50, and 33/50 in females, for the control group and the groups at the lower and higher dose, respectively. The mean body weights of all groups of treated mice were significantly increased, being up to 13% (males) and 25% (females) higher than those of controls. *N*-Methylolacrylamide treatment had no significant effect on survival. All mice underwent complete necropsy. Histopathological evaluation was performed on main tissues and organs.

In male mice, there was a significant positive trend in the incidence of Harderian gland adenoma ($P < 0.001$; Cochran–Armitage trend test, logistic-regression trend test and life-table trend test). The incidence of Harderian gland adenoma was significantly increased ($P < 0.001$; Fisher exact test, logistic regression test and life-table test) in all exposed groups. No significant changes were reported for the incidence of Harderian gland carcinoma [and no data on historical controls were reported]. [The Working Group noted that the reported increased incidence of Harderian gland adenoma or carcinoma (combined) in males may not have been related to treatment, in view of the lack of a significant positive trend in the incidence of Harderian gland carcinoma, lack of a significant increase in the incidence of Harderian gland carcinoma at the lower and higher dose, and lack of data on Harderian gland carcinoma in historical controls, making the contribution of the numerical increase at the higher dose negligible.] There was a significant positive trend in the incidence of hepatocellular adenoma ($P = 0.005$, Cochran–Armitage trend test; $P < 0.001$, life-table trend test; $P = 0.002$, logistic-regression trend test) with the incidence being significantly increased in males at the higher dose ($P = 0.004$, logistic regression test; $P = 0.012$, Fisher exact test). There was a significant positive trend in the incidence of hepatocellular carcinoma ($P = 0.027$, life-table trend test). The incidence of hepatocellular carcinoma in males – controls, 6/50 (12%); lower dose, 13/50 (26%); and higher dose, 12/50 (24%) – was significantly increased at the lower dose ($P = 0.023$, logistic regression test; $P = 0.012$, life-table test) and higher dose ($P = 0.031$, life-table test), but did not exceed the upper bound of the range observed in historical controls in this laboratory – gavage, 56/347 (mean ± standard deviation, 16.1 ± 8.03%); range, 4–28% – and all routes, 379/2032 (18.7 ± 6.50%); range, 8–30%). There was a significant positive trend in the incidence of hepatocellular adenoma or carcinoma (combined) – control, 12/50 (24%); lower dose, 17/50 (34%); and higher dose, 26/50 (52%); $P = 0.003$, Cochran–Armitage trend test; $P < 0.001$, life-table trend test and logistic-regression trend test – and a significant increase in the incidence at the highest dose ($P = 0.001$, logistic regression test; $P = 0.004$, Fisher exact test) that exceeded the upper bound of the range observed in historical controls in this laboratory – gavage, 106/347 (mean ± standard deviation, 30.5 ± 5.83%); range, 20–36% – and all routes, 609/2032 (30.0 ± 7.59%); range, 16–58%. There were significant positive trends in the incidence of bronchioloalveolar adenoma ($P = 0.010$, logistic-regression trend test; $P = 0.005$, life-table trend test) and bronchioloalveolar carcinoma ($P = 0.005$, logistic-regression trend test; $P = 0.003$, life-table trend test). The incidence of both bronchioloalveolar adenoma and of

bronchioloalveolar carcinoma was significantly increased at the higher dose ($P = 0.022$, Fisher exact test; $P = 0.006$, life-table test; $P = 0.015$, logistic regression test; and $P = 0.015$, Fisher exact test; $P = 0.006$, life-table test; $P = 0.011$, logistic regression test, respectively) compared with controls. There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) ($P = 0.001$, Cochran–Armitage trend test; $P < 0.001$, logistic-regression trend test and life-table trend test), with the incidence being significantly increased at the higher dose ($P = 0.002$, Fisher exact test; $P < 0.001$, life-table test; $P = 0.001$, logistic regression test).

In female mice, there was a significant positive trend in the incidence of Harderian gland adenoma ($P < 0.001$; Cochran–Armitage trend test, logistic-regression trend test, and life-table trend test), with the incidence being significantly increased at the higher dose ($P < 0.001$, Fisher exact test, life-table test, and logistic regression test). Although there was no significant positive trend in the incidence of Harderian gland carcinoma – controls, 0/47; lower dose, 3/45 (6.7%); and higher dose 2/48 (4.2%) – the incidence exceeded the upper bound of the range observed in historical controls in this laboratory – gavage, 3/350 (mean ± standard deviation, 0.9 ± 1.57%); range, 0–4% – and all routes, 7/2040 (0.3 ± 0.88%); range, 0–4%. A significant positive trend in the incidence of Harderian gland adenoma or carcinoma (combined) was observed ($P < 0.001$, Cochran–Armitage trend test, logistic-regression trend test, and life-table trend test), with a significant increase in incidence at both the lower and higher doses ($P = 0.031$, logistic regression test; and $P < 0.001$, Fisher exact test, logistic regression test and life-table test, respectively). There was a significant positive trend in the incidence of hepatocellular adenoma ($P < 0.001$, Cochran–Armitage trend test, life-table trend test, and logistic-regression trend test), with the incidence being significantly increased at the higher dose ($P < 0.001$, Fisher exact test, logistic regression test, and life-table test). The incidence of hepatocellular carcinoma was: controls, 3/50; lower dose, 3/50; and higher dose, 2/49. [A significant positive trend in the incidence of hepatocellular adenoma or carcinoma (combined) and a significant increase in the incidence at the higher dose were reported, but the Working Group concluded that this was attributable to the increased incidence of hepatocellular adenoma alone.] There was a significant positive trend in the incidence of bronchioloalveolar carcinoma ($P = 0.034$, life-table trend test) with the incidence being significantly increased at the higher dose ($P = 0.045$, life-table test). There was a significant positive trend in the incidence of bronchioloalveolar adenoma or carcinoma (combined) – controls, 6/50 (12%); lower dose, 8/50 (16%); and higher dose, 13/49 (26%); $P = 0.019$, life-table trend test; $P = 0.041$, Cochran–Armitage trend test; $P = 0.042$, logistic-regression trend test – and a significant increase in the incidence at the higher dose ($P = 0.025$, life-table test) that exceeded the upper bound of the range observed in historical controls in this laboratory – gavage, 33/349 (mean ± standard deviation, 9.5 ± 3.66%); range, 4–14.3% – and all routes, 145/2026 (7.2 ± 4.21%); range, 0–16.0%. There was a significant positive trend in the incidence of benign granulosa cell tumours of the ovary ($P = 0.031$, Cochran–Armitage trend test; $P = 0.017$, life-table trend test and logistic-regression trend test) with the incidence being significantly increased at both the lower and higher doses ($P = 0.021$, Fisher exact test; $P = 0.015$, logistic regression test and life-table test; and $P = 0.024$, Fisher exact test; $P = 0.016$, logistic regression test and life-table test, respectively).

Regarding non-neoplastic lesions, chronic inflammation and alveolar epithelium hyperplasia were observed at an increased incidence in the lungs of treated male and female mice. These two lesions generally occurred together and appeared to be part of the same lesion (NTP, 1989). [The

Working Group noted that this was a well-conducted GLP study, males and females were used, the duration of exposure and observation was adequate, and an adequate number of animals per group and multiple doses were used.]

A concurrent study (Tennant et al., 1995) in male and female C57BL/6 mice homozygous (wildtype) or hemizygous for *Tp53* (C57BL/6 *p53*$^{+/-}$) was performed using the same chemical doses as the above study by the NTP (1989). Treatment began in a staggered fashion at age 15–18 weeks. Male mice were housed singly, and female mice were housed in groups. The authors stated that the wildtype sibling groups and hemizygous *Tp53* mouse control group contained 10 mice each [15 mice were probably used for the hemizygous *Tp53* mouse control group], and the hemizygous *Tp53* treatment groups contained 15 (lower dose) or 20 (higher dose) mice. *N*-Methylolacrylamide [purity assumed to be approximately 98%] was administered daily, by gavage, five times per week, for 24 weeks, at a dose of 0, 25 mg/kg bw (lower dose, hemizygous *Tp53* treatment groups only), or 50 mg/kg bw (higher dose) in corn oil. Mice were held an additional 6 weeks. An unspecified number of animals were killed at interim. All mice underwent gross necropsy and microscopic examination of gross lesions and of the liver.

There was no significant effect of *N*-methylolacrylamide treatment on survival. *N*-Methylolacrylamide treatment decreased body-weight gain in all groups of treated male mice (highest decrease in wildtype siblings at the higher dose). No liver tumours were observed in any groups of mice (Tennant et al., 1995). [The Working Group noted the small number of animals per group and the inadequate duration of the study. Therefore, the Working Group judged the study in wildtype mice inadequate for the evaluation of the carcinogenicity of *N*-methylolacrylamide in experimental animals.]

3.1.2 Oral administration (drinking-water)

A group of eight $CB6F_1$ *rasH2* transgenic mice (Tg) and a group of eight non-Tg mice (age, 7 weeks) were given drinking-water containing *N*-methylolacrylamide [purity not reported] at a dose of 135 mg/kg bw (1000 ppm) ad libitum for up to 26 weeks. One control group of eight Tg mice and one control group of eight non-Tg mice received drinking-water alone, ad libitum. After 4 weeks of treatment, three mice from each group were killed, and the study was continued until experimental week 26. Full histopathological examination was performed on major tissues and organs (Tsuji et al., 2015). None of the non-Tg mice died during the experimental period; one mouse from the *N*-methylolacrylamide-treated group of Tg mice died on the day of necropsy (at experimental week 26). No significant difference in average body weight was observed in the groups of Tg or non-Tg mice treated with *N*-methylolacrylamide compared with their respective controls.

In the *N*-methylolacrylamide-treated group of Tg mice, there was a significant increase in the incidence of adenoma of the lung [$P = 0.0040$, Fisher exact test]. In both groups of non-Tg mice, no lung tumours were observed. [The Working Group noted that the study had several limitations: the small number of animals per group, the lack of data on historical controls for Tg mice, the administration of a single dose, the use of only one sex and, the short duration of the study for non-Tg mice. Despite the small numbers of animals analysed, the incidence of adenoma in the lung of Tg mice was 0/5 in the untreated group and 5/5 in the *N*-methylolacrylamide-treated group, representing a significant increase in the treated group of $P < 0.005$ by Fisher exact test, a highly significant *P* value. The Working Group considered that this increase was treatment-related.]

3.2 Rat

Oral administration (gavage)

In a well-conducted study that complied with GLP, groups of 50 male and 50 female F344/N rats (age, 7 weeks) were given *N*-methylolacrylamide (purity, approximately 98%) at a dose of 0, 6, or 12 mg/kg bw in deionized water, for the control group and the groups at the lower and higher dose, respectively, by gavage, on 5 days per week for 105 weeks (NTP, 1989). Surviving animals were killed at age 112 weeks. The mean body weights of males at the higher dose were 6–7% lower than those of controls. The mean body weights of females at the higher dose were 5–6% lower than those of controls. *N*-Methylolacrylamide treatment has no significant effect on survival. At study termination, survival was 28/50, 22/50, and 27/50 for males; and 35/50, 22/50 and 33/50 for females, for the control group and the groups at the lower and higher dose, respectively. All rats underwent complete necropsy. Histopathological evaluation was performed on the main tissues and organs.

No increased incidence of any neoplasm was attributable to the administration of *N*-methylolacrylamide in male and female rats (NTP, 1989). [The Working Group noted that this was a well-conducted GLP study, both sexes were used, the duration of exposure and observation was adequate, and an adequate number of animals per group and multiple doses were used.]

3.3 Evidence synthesis for cancer in experimental animals

The carcinogenicity of *N*-methylolacrylamide has been assessed in one well-conducted GLP study in male and female B6C3F_1 mice (NTP, 1989) and one well-conducted study in male and female F344/N rats (NTP, 1989) treated by oral administration (gavage), in a concurrent oral administration (gavage) study in male and female C57BL/6 mice homozygous (wildtype) or hemizygous for *Tp53* (C57BL/6 *p53*$^{+/-}$) (Tennant et al., 1995), and in an oral administration study (drinking-water) in male CB6F_1 *rasH2* transgenic mice (Tg) and non-Tg mice (Tsuji et al., 2015).

In the well-conducted GLP study in male and female B6C3F_1 mice treated by oral administration (gavage) (NTP, 1989), there was a significant positive trend in the incidence of Harderian gland adenoma in male mice with the incidence being significantly increased in all treated groups. In male mice, there was a significant positive trend in the incidence of hepatocellular adenoma, of hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined) with a significant increase in the incidence of hepatocellular adenoma at the higher dose, of hepatocellular carcinoma at all doses, and of hepatocellular adenoma or carcinoma (combined) at the higher dose. In male mice, there was a significant positive trend in the incidence of bronchioloalveolar adenoma, of bronchioloalveolar carcinoma, and of bronchioloalveolar adenoma or carcinoma (combined) with a significant increase in the incidence at the highest dose. In female mice, there was a significant positive trend in the incidence of Harderian gland adenoma and of Harderian gland adenoma or carcinoma (combined), with the incidence being significantly increased for Harderian gland adenoma in females at the highest dose, and for Harderian gland adenoma or carcinoma (combined) in all treated groups of females. There was a significant positive trend in the incidence of hepatocellular adenoma in female mice, with the incidence being significantly increased at the higher dose. There was a significant positive trend in the incidence of bronchioloalveolar carcinoma and of bronchioloalveolar adenoma or carcinoma (combined) in female mice, with the incidence being significantly increased at the highest dose. The incidence of benign granulosa cell tumours of the ovary was significantly increased in both treated

groups, with a significant positive trend (NTP, 1989).

In the study in male $CB6F_1$ *rasH2* transgenic mice (Tg) and non-Tg mice given drinking-water containing *N*-methylolacrylamide, there was a significant increase in the incidence of adenoma of the lung in the group of treated Tg mice. No lung tumours were observed in treated and control non-Tg mice (Tsuji et al., 2015).

In the study by Tennant et al. (1995) in male and female C57BL/6 mice homozygous (wildtype) or hemizygous for *Tp53* (C57BL/6 $p53^{+/-}$) treated by gavage, no liver tumours were observed in any groups of hemizygous mice; the study in homozygous mice was considered inadequate for the evaluation of the carcinogenicity of *N*-methylolacrylamide in experimental animals.

In the well-conducted GLP study in male and female F344/N rats treated by gavage (NTP, 1989), no increased incidence of any neoplasm was attributable to the administration of *N*-methylolacrylamide in male or female rats.

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

N-Methylolacrylamide was widely distributed in the blood and tissues of male rats given a single intravenous injection of 140 mg/kg bw (Hashimoto & Aldridge, 1970) and of male mice given a single dose by intraperitoneal injection (150 mg/kg bw) or by oral administration (in the drinking-water) (150 or 1.5 mg/kg bw) (Witt et al., 2003). In the study by Witt et al. (2003, there was no difference in tissue distribution between intraperitoneal and oral administration in mice, and for both administration routes the tissue/blood concentration ratios were < 1. In the same study, comparison of the excretion profiles after oral or intraperitoneal administration indicated lower absorption via the oral route as the percent of the administered dose recovered in urine was higher after intraperitoneal injection.

In rats given a single intravenous injection of 140 mg/kg bw, the blood concentration of *N*-methylolacrylamide decreased rapidly, with a half-life of 1.55 hours and a first-order rate of elimination of 0.45 per hour from the blood with distribution in total body water (Edwards, 1975). *N*-Methylolacrylamide was also excreted in the urine and faeces of mice; the percentage of the administered dose recovered in the urine was higher after intraperitoneal injection than after oral administration, whereas the percentage of the administered dose recovered in the faeces was higher after oral administration. For both intraperitoneal injection and oral administration, about 10% of the administered dose was exhaled as radiolabelled carbon dioxide ($^{14}CO_2$) after either intraperitoneal injection or oral administration of *N*-methylolacrylamide [^{14}C]-labelled at the hydroxymethyl group (Witt et al., 2003).

A rapid decrease in liver gluthatione levels was observed after a single intravenous injection of *N*-methylolacrylamide, which was suggestive of conjugation with glutathione as a metabolic pathway (Edwards, 1975). This was further confirmed by the identification of gluthatione conjugates in the bile of exposed rats (Edwards, 1975) and by demonstration of the reaction of *N*-methylolacrylamide with glutathione in vitro (Hashimoto & Aldridge, 1970; Edwards, 1975).

After a single intraperitoneal injection or oral administration of *N*-methylolacrylamide in mice, about 10% of the administered dose was excreted unchanged and 10% as *N*-acetyl-*S*-(3-hydroxymethyl-amino-3-oxopropyl)cysteine (a metabolite derived from reaction with gluta-

thione) in the urine during the first 24 hours of administration (Witt et al., 2003). No evidence was found for the conversion of *N*-methylolacrylamide to acrylamide in vivo (Edwards, 1975). In contrast, *N*-(2-carbamoyl-2-hydroxyethyl)valine (GAVal) adducts, which are derived from glycidamide (the reactive metabolite of acrylamide), were observed in rats given *N*-methylolacrylamide as a single dose at 71 mg/kg bw by gavage (Fennell et al., 2003). [The Working Group noted that this suggests oxidation of *N*-methylolacrylamide, either directly or indirectly after conversion to acrylamide. The Working Group also noted that the purity of the *N*-methylolacryamide used in the study was reported to be 99%. In addition, analytical assessment of purity was performed and there was no indication that either acrylamide or formaldehyde were present. The Working Group considered the identification of glycidamide-specific adducts as an important finding, since glycidamide is genotoxic. However, the Working Group also noted that the possible metabolism of *N*-methylolacrylamide directly or indirectly to glycidamide is not supported by the results of other studies.]

4.2 Evidence relevant to key characteristics of carcinogens

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

(a) Humans

(i) Exposed humans

See Table 4.1.

No studies on DNA adducts were available to the Working Group.

Haemoglobin adducts were reported in the blood of tunnel workers (Hagmar et al., 2001; Kjuus et al., 2004) and glass workers (Paulsson et al., 2006) exposed to grout or sealant containing acrylamide and *N*-methylolacrylamide. [The Working Group noted that it was not possible to attribute these effects only to *N*-methylolacrylamide in these studies.]

(ii) Human cells in vitro

No data were available to the Working Group.

(b) Experimental systems

Haemoglobin adducts were reported in rats given *N*-methylolacrylamide either at 142 mg/kg bw as a single intraperitoneal injection (Paulsson et al., 2002) or at 71 mg/kg bw by gavage (Fennell et al., 2003). In mice, haemoglobin adduct levels were increased after a single intraperitoneal injection of *N*-methylolacrylamide at a dose of 35, 71, and 142 mg/kg bw (Paulsson et al., 2002).

4.2.2 Is genotoxic

(a) Humans

(i) Exposed humans

See Table 4.2.

Chromosomal alterations in blood lymphocytes of tunnel workers exposed to grout containing *N*-methylolacrylamide and acrylamide were assessed in one study. The findings were negative for chromosomal aberrations and breaks, and positive for chromatid gaps (but without an exposure–response relationship) (Kjuus et al., 2005). [The Working Group noted that it was not possible to attribute the effects only to *N*-methylolacrylamide in this study.]

(ii) Human cells in vitro

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3.

N-Methylolacrylamide induced dominant lethal (germ cell) mutations in male mice after 13 weeks of oral administration via the drinking-water (Chapin et al., 1995; Witt et al., 2003). No induction of dominant lethal mutations in

Table 4.1 Haemoglobin adducts in humans exposed to *N*-methylolacrylamide

End-point	Tissue or cell type	Description of exposed and controls	Results[a]	Comments	Reference
Hb adducts	Blood	210 tunnel workers (3 F/207 M; age, 20–62 years) exposed to grout containing *N*-methylolacrylamide and acrylamide; and 18 controls (7 F/11 M, all non-smokers)	(+)	Increased Hb adduct level was observed for 74 of the exposed workers. Causative effect of *N*-methylolacrylamide alone could not be demonstrated as there was co-exposure to other substances including acrylamide.	Hagmar et al. (2001)
Hb adducts	Blood	23 tunnel workers exposed to grout containing *N*-methylolacrylamide and acrylamide (12 smokers and 11 non-smokers); and 3 controls (2 smokers and 1 non-smoker). The blood samples were collected 60–143 days (mean, 84 days) after the end of working with the grout	(+)	Increased Hb adduct level was observed for two of the exposed workers. Causative effect of *N*-methylolacrylamide alone could not be demonstrated as there was co-exposure to other substances including acrylamide.	Kjuus et al. (2004)
Hb adducts	Blood	A case study of 4 glass workers exposed to sealant containing *N*-methylolacrylamide and acrylamide; and 1 control	(+)	Increased Hb adduct level was observed for one of the exposed workers. Causative effect of *N*-methylolacrylamide alone could not be demonstrated as there was co-exposure to other substances including acrylamide.	Paulsson et al. (2006)

M, male; F, female; Hb, haemoglobin.

[a] (+), positive result in a study of limited quality.

Table 4.2 Genetic and related effects of *N*-methylolacrylamide in exposed humans

End-point	Tissue or cell type	Description of exposed and controls	Results[a]	Comments	Reference
Chromosomal aberrations	Blood lymphocytes	25 tunnel workers exposed to grout containing *N*-methylolacrylamide (26–31%), acrylamide (2.5–5.4%), methylene-bis-acrylamide (0.02–0.03%), methylic diesters (12–17%), formaldehyde (0.9%), and water, were compared with 25 age- and sex-matched tunnel workers who had not been exposed to the grout; both smokers and non-smokers were included; exposure was assessed by questionnaire	(–)	There was co-exposure to other substances including acrylamide.	Kjuus et al. (2005)
Chromosomal gaps and breaks, chromatid breaks	Blood lymphocytes		(–)	There was co-exposure to other substances including acrylamide.	Kjuus et al. (2005)
Chromatid gaps	Blood lymphocytes		(+)	Significant increase; however, there was no exposure–response relationship. Causative effect of *N*-methylolacrylamide alone could not be demonstrated as there was co-exposure to other substances including acrylamide.	Kjuus et al. (2005)

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

Table 4.3 Genetic and related effects of *N*-methylolacrylamide in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Dominant lethal test (mutation)	Mouse, CD-1 Swiss (M)	Ovary/uterus after mating	+	180 ppm [56.8 mg/kg bw]	Oral via drinking-water, 13 wk	Significant increase in total post-implantation losses and early fetal resorptions; dose calculated using water consumption from the post-mating week; no positive control; purity, 97–99%.	Chapin et al. (1995)
Dominant lethal test (mutation)	Mouse, CD-1 Swiss (M)	Ovary/uterus after mating	+	360 ppm [112.5 mg/kg bw]	Oral via drinking-water, 13 wk	Significant increase in total post-implantation losses, early fetal resorptions, and decrease in live fetuses; dose calculated using water consumption from the post-mating week; no positive control; purity, 97–99%.	Chapin et al. (1995)
Dominant lethal test (mutation)	Mouse, $B6C3F_1$ (M)	Ovary/uterus after mating	–	150 mg/kg bw	Intraperitoneal injection, ×1	No positive control; purity, ~98%.	Witt et al. (2003)
Dominant lethal test (mutation)	Mouse, $B6C3F_1$ (M)	Ovary/uterus after mating	–	50 mg/kg bw	Intraperitoneal injection, ×5	No positive control; purity, ~98%.	Witt et al. (2003)
Dominant lethal test (mutation)	Mouse, $B6C3F_1$ (M)	Ovary/uterus after mating	+	180 ppm (37 mg/kg bw)	Oral via drinking-water, 13 wk	Significant increase in total post-implantation losses and early fetal resorptions; three sets of mating occurred; females were killed and the uterine contents were assessed 2 wk post-mating; dose is estimated using data on water consumption from Chapin et al. (1995); no positive control; purity, ~98%.	Witt et al. (2003)
Micronucleus formation	Mouse, $B6C3F_1$ (M)	Bone marrow PCE	–	150 mg/kg bw	Intraperitoneal injection, ×2	Purity, ~98%.	NTP (1989)
Micronucleus formation	Mouse, CBA (M)	Peripheral blood PCE	+	142 mg/kg bw	Intraperitoneal injection, ×1	Dose-dependent increase; flow cytometric measurements were applied; purity, ~48% in water.	Paulsson et al. (2002)
Micronucleus formation	Rat, Sprague-Dawley (M)	Bone marrow PCE	(–)	142 mg/kg bw	Intraperitoneal injection, ×1	Flow cytometric measurements were applied; purity, ~48% in water.	Paulsson et al. (2002)

Table 4.3 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Micronucleus formation	Mouse, $B6C3F_1$ (M)	Bone marrow PCE	–	150 mg/kg bw	Intraperitoneal injection, ×2 (vehicle, corn oil)	Purity, ~98%.	Witt et al. (2003)
Micronucleus formation	Mouse, $B6C3F_1$ (M)	Bone marrow PCE	–	112 mg/kg bw	Intraperitoneal injection, ×2 (vehicle, PBS)	Purity, ~98%.	Witt et al. (2003)
Micronucleus formation	Mouse, $B6C3F_1$ (M)	Bone marrow PCE	–	150 mg/kg bw	Gavage, ×2 (vehicle, PBS)	Purity, ~98%.	Witt et al. (2003)
Micronucleus formation	Mouse, $B6C3F_1$ (M)	Bone marrow and peripheral blood PCE	–	720 ppm (168 mg/kg bw)	Gavage, daily for 31 days (vehicle, water)	Purity, ~98%.	Witt et al. (2003)
Micronucleus formation	Mouse, $B6C3F_1$ (M)	Bone marrow and peripheral blood PCE	–	720 ppm (~120–125 mg/kg bw)	Oral via drinking-water, 13 wk	Purity, ~98%.	Witt et al. (2003)

bw, body weight; HID, highest ineffective dose; LED, lowest effective dose; M, male; PBS, phosphate-buffered saline; PCE, polychromatic erythrocytes; ppm, parts per million; wk, week.
[a] +, positive; –, negative; (–), negative in a study of limited quality.

male mice was seen after a single intraperitoneal injection or five repeated intraperitoneal injections (Witt et al., 2003). [The Working Group noted that there was no positive control for the dominant lethal test reported in Witt et al. (2003). However, the Working Group considered that the positive result in mice treated by oral administration is sufficient to demonstrate the proficiency of the laboratory in the conduct of the test.]

A significant increase in the frequency of micronucleated erythrocytes was observed in peripheral blood of male mice given a single intraperitoneal injection of *N*-methylolacrylamide (Paulsson et al., 2002). The same route of administration and dose did not induce micronucleus formation in the bone marrow erythrocytes of male rats (Paulsson et al., 2002). Several other studies also reported negative findings regarding the induction of micronucleus formation in bone marrow and peripheral blood cells of male mice exposed to *N*-methylolacrylamide, after either intraperitoneal injection (NTP, 1989; Witt et al., 2003) or oral administration (Witt et al., 2003). [The Working Group noted that several of the studies on micronucleus formation in vivo did not report evidence of bone marrow exposure to *N*-methylolacrylamide; however, the Working Group considered that the toxicokinetic data reported in Witt et al. (2003) showing detection of radiolabelled *N*-methylolacrylamide in blood/plasma and several tissues were sufficient to conclude that this substance is systemically available after both intraperitoneal injection and oral administration. The Working Group also noted that the blood/tissue ratio was < 1, which would indicate that *N*-methylolacrylamide and/or its metabolites are only taken up to some extent by tissues other than blood.]

(ii) Non-human mammalian cells in vitro

See Table 4.4.

An increased frequency of mutation was seen in L5178Y $Tk^{+/-}$ mouse lymphoma cell cultures after incubation with *N*-methylolacrylamide in the presence and absence of metabolic activation (Kirkland & Fowler, 2010).

In one study, *N*-methylolacrylamide with and without metabolic activation induced chromosomal aberrations in Chinese hamster ovary (CHO) cells (NTP, 1989). In another study with CHO cells, induction of chromosomal aberrations was seen only in the absence, but not in the presence, of metabolic activation (Kirkland & Fowler, 2010).

The frequency of sister-chromatid exchanges was increased in CHO cells treated with *N*-methylolacrylamide without metabolic activation. In the presence of metabolic activation, the frequency of sister-chromatid exchange was weakly increased (NTP, 1989).

(iii) Non-mammalian experimental systems

See Table 4.5.

N-Methylolacrylamide did not induce mutation in any of several strains of *Salmonella typhimurium* in the presence or absence of metabolic activation (Hashimoto & Tanii, 1985; Zeiger et al., 1988; NTP, 1989). [The Working Group noted that the mutagenic effect of *N*-methylolacrylamide has not been tested in the strains *Escherichia coli* WP2 (pKM101), *E. coli* WP2 *uvrA* (pKM101), or *S. typhimurium* TA102, which are able to detect cross-linking agents and oxidizing mutagens.]

4.2.3 Other key characteristics of carcinogens

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Regarding oxidative stress, *N*-methylolacrylamide showed reactivity towards glutathione in vitro (Hashimoto & Aldridge, 1970; Edwards, 1975), and a decrease in liver glutathione levels was observed in rats given a single intravenous injection of *N*-methylolacrylamide (Edwards, 1975).

Table 4.4 Genetic and related effects of *N*-methylolacrylamide in non-human mammalian cells in vitro

End-point	Species, cell type	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y *Tk*$^{+/-}$ lymphoma cells	+	+	303.3 µg/mL –S9, 404.4 µg/mL +S9	Exposure, 3 h; S9 from Aroclor-1254-induced rat liver; purity, NR.	Kirkland & Fowler (2010)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y *Tk*$^{+/-}$ lymphoma cells	+	NT	202.2 µg/mL	Exposure, 24 h; purity, NR.	Kirkland & Fowler (2010)
Chromosomal aberrations	Chinese hamster, ovary (CHO) cells	+	+	250 µg/mL –S9, 2500 µg/mL +S9	No information on whether the increase is statistically significant (no *P* values given); S9 from Aroclor-1254-induced rat liver; purity, ~98%.	NTP (1989)
Chromosomal aberrations	Chinese hamster, ovary (CHO) cells	+	NT	375 µg/mL	No information on whether the increase is statistically significant (no *P* values given); purity, ~98%.	NTP (1989)
Chromosomal aberrations	Chinese hamster, ovary (CHO) cells	+	–	202.2 µg/mL	Exposure, 3 h; S9 from Aroclor-1254-induced rat liver; purity, NR.	Kirkland & Fowler (2010)
Chromosomal aberrations	Chinese hamster, ovary (CHO) cells	+	NT	202.2 µg/mL	Exposure, 20 h; purity, NR.	Kirkland & Fowler (2010)
Sister-chromatid exchange	Chinese hamster, ovary (CHO) cells	+	NT	250 µg/mL	No information on whether the increase is statistically significant (no *P* values given); the highest tested dose is given; purity, ~98%.	NTP (1989)
Sister-chromatid exchange	Chinese hamster, ovary (CHO) cells	NT	+	1700 µg/mL	Weakly positive; no information on whether the increase is statistically significant; S9 from Aroclor-1254-induced rat liver; purity, ~98%.	NTP (1989)

LEC, lowest effective concentration; HIC, highest ineffective concentration; NR, not reported; NT, not tested; Tk, thymidine kinase; S9, 9000 × *g* supernatant.

[a] +, positive; –, negative.

Table 4.5 Genetic and related effects of *N*-methylolacrylamide in non-mammalian experimental systems

Species, strain	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium, TA97, TA98, TA100, and TA1535	Reverse mutation	–	–	10 000 µg/plate	Some cytotoxicity was observed; S9 from Aroclor-1254-induced male Syrian hamster liver or male Sprague-Dawley rat liver; purity, ~98%.	NTP (1989) Zeiger et al. (1988)
Salmonella typhimurium, TA98, TA100, TA1535, TA1537, and TA1538	Reverse mutation	–	–	5000 µg/plate	S9 from Aroclor-1254-induced rat liver; purity, > 95%.	Hashimoto & Tanii (1985)

LEC, lowest effective concentration; HIC, highest ineffective concentration; S9, 9000 × *g* supernatant.

[a] –, negative.

Regarding chronic inflammation, in a 13 week study in F344/N rats treated by gavage, *N*-methylolacrylamide caused inflammation and/or haemorrhage of the urinary bladder mucosa at doses of 25 mg/kg bw or greater (NTP, 1989; also reported in Bucher et al., 1990). [The Working Group noted that the mucosal lesions of the urinary bladder were reportedly associated with urinary retention, which was secondary to defects in neural control of the bladder in this 13 week study, and not a direct induction of chronic inflammation by *N*-methylolacrylamide in the bladder.] In $B6C3F_1$ mice, 2-year exposure to *N*-methylolacrylamide administered by gavage caused chronic inflammation of the lung (NTP, 1989). [The Working Group noted that chronic inflammation of the lung was probably attributable to infection with Sendai virus in this 2-year study and was not a direct induction of chronic inflammation by *N*-methylolacrylamide in the lung.] RasH2 transgenic and non-transgenic mice received drinking-water containing *N*-methylolacrylamide at a dose of 135 mg/kg bw per day for 4 weeks. Gene ontology enrichment analysis of non-neoplastic regions of the lungs showed that inflammation-related genes were differentially expressed in both transgenic and non-transgenic mice compared with untreated mice (Tsuji et al., 2015).

Regarding alterations in cell proliferation, cell death, or nutrient supply, hyperplasia and dysplasia of the tracheal mucosa and bronchiolar epithelial hyperplasia of the lung in male and female F344/N rats, and bronchial epithelial hyperplasia of the lung in male and female $B6C3F_1$ mice, were observed after exposure to *N*-methylolacrylamide by gavage for 16 days (NTP, 1989). In $B6C3F_1$ mice, 2-year exposure to *N*-methylolacrylamide by gavage caused alveolar epithelial hyperplasia of the lung (NTP, 1989). [The Working Group noted that alveolar epithelial hyperplasia of the lung observed in this 2-year study was probably attributable to infection with Sendai virus and was not direct induction of cell proliferation by *N*-methylolacrylamide in the lung.]

4.2.4 High-throughput in vitro toxicity screening data evaluation

N-Methylolacrylamide has not been tested in the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA (Thomas et al., 2018).

4.3 Other relevant evidence

Hepatocellular necrosis was observed in rats and mice exposed to *N*-methylolacrylamide by gavage for 16 days and 13 weeks, respectively (NTP, 1989).

N-Methylolacrylamide has been found to be neurotoxic in rats and mice (NTP, 1989; Bucher et al., 1990).

5. Summary of Data Reported

5.1 Exposure characterization

N-Methylolacrylamide is a High Production Volume chemical that is used as an intermediate in the manufacture of some chemicals and acrylamide-based polymers that ultimately appear in a variety of adhesives, sealants, inks, resins, paints, plastics, paper, and textile finishes.

While industrial uses of *N*-methylolacrylamide permit its release into the environment, it is not expected to bioaccumulate and should be readily biodegradable. The only documented environmental contamination involved transient presence in drainage and groundwater after grouting operations in nearby tunnels in Norway and Sweden.

The most substantial human exposures to *N*-methylolacrylamide probably occur in occupational settings, particularly during industrial

processes using *N*-methylolacrylamide (e.g. in textile treatment resins and polymers), although very little empirical information on occupational exposure was available to the Working Group. Documented worker-exposure events involved the use of a grout containing *N*-methylolacrylamide in the construction of one tunnel in Norway and one in Sweden. Airborne exposure was in the milligram per cubic metre range, but most workers also had dermal contact. Additionally, a case study reported the use of *N*-methylolacrylamide as a sealant in the window-manufacturing industry and as hazardous waste generated in the two aforementioned tunnels. Both situations involved co-exposure to acrylamide contained within the *N*-methylolacrylamide products used.

While the general population might be exposed to *N*-methylolacrylamide through uses of products containing its associated polymers, no quantitative information was available to the Working Group.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

Treatment with *N*-methylolacrylamide caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in both sexes of a single species in a well-conducted study that complied with Good Laboratory Practice (GLP).

N-Methylolacrylamide was administered by oral administration (gavage) in one well-conducted GLP study in male and female B6C3F$_1$ mice. In males, *N*-methylolacrylamide caused an increase in the incidence of hepatocellular carcinoma, hepatocellular adenoma or carcinoma (combined), bronchioloalveolar carcinoma, and bronchioloalveolar adenoma or carcinoma (combined). In females, *N*-methylolacrylamide caused an increase in the incidence of bronchioloalveolar carcinoma, bronchioloalveolar adenoma or carcinoma (combined), and Harderian gland adenoma or carcinoma (combined).

5.4 Mechanistic evidence

No data on absorption, distribution, metabolism, or excretion in humans were available. One study in mice showed that *N*-methylolacrylamide is widely distributed after oral administration and intraperitoneal injection. There is also evidence that conjugation with glutathione is a metabolic pathway. In mice, parent *N*-methylolacrylamide and/or metabolites are excreted in the urine and faeces and exhaled as carbon dioxide.

Overall, the mechanistic evidence that *N*-methylolacrylamide exhibits key characteristics of carcinogens ("is electrophilic or metabolically activated", "is genotoxic", "induces oxidative stress", "induces chronic inflammation", and "alters cell proliferation, cell death, or nutrient supply") is suggestive but incoherent across experimental systems. There were no studies in humans with exposure specifically attributable to *N*-methylolacrylamide and no studies using human cells in vitro.

There is suggestive evidence that *N*-methylolacrylamide is electrophilic. In two studies, haemoglobin adducts were detected in rodents after intraperitoneal injection. There is suggestive but incoherent evidence that *N*-methylolacrylamide is genotoxic in different experimental systems. In two studies, *N*-methylolacrylamide gave positive results in the dominant lethal test in mice when administered orally, but not by intraperitoneal injection. In rodents, results were positive for micronucleus formation in only one of seven tests. *N*-Methylolacrylamide induced mutations at the *Tk* locus in L5178Y mouse lymphoma cells with

and without metabolic activation in one study. *N*-Methylolacrylamide with and without metabolic activation gave positive results in Chinese hamster ovary (CHO) cells for chromosomal aberrations in two studies and for sister-chromatid exchange in one study. *N*-Methylolacrylamide gave negative results for mutation in bacteria both with and without metabolic activation.

Regarding the key characteristics "induces oxidative stress", "induces chronic inflammation", and "alters cell proliferation, cell death, or nutrient supply", there is suggestive mechanistic evidence. *N*-Methylolacrylamide showed reactivity to glutathione in vitro in two studies and caused decreased liver glutathione levels in rats in another study, indicative of increased oxidative stress. Oral administration of *N*-methylolacrylamide caused chronic inflammation in the lung of mice in one study and increased the expression of inflammation-related genes in the lung of mice 4 weeks after exposure in another. Acute and chronic exposure to *N*-methylolacrylamide by gavage induced epithelial hyperplasia in the respiratory tract of rodents.

N-Methylolacrylamide has not been tested in 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 *N*-methylolacrylamide.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of *N*-methylolacrylamide.

6.3 Mechanistic evidence

There is *limited mechanistic evidence.*

6.4 Overall evaluation

N-Methylolacrylamide is *possibly carcinogenic to humans (Group 2B).*

6.5 Rationale

The Group 2B evaluation for *N*-methylolacrylamide is based on *sufficient evidence* for cancer in experimental animals. This *sufficient evidence* in experimental animals is based on an increased incidence of either malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in both sexes of a single species in a well-conducted study that complied with Good Laboratory Practice. The evidence regarding cancer in humans is *inadequate* because no studies were available. The mechanistic evidence was *limited* as the findings regarding key characteristics of carcinogens across experimental systems were suggestive, but incoherent.

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ISOPHORONE

1. Exposure Characterization

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 78-59-1

EC/List No.: 201-126-0

Chem. Abstr. Serv. name: isophorone

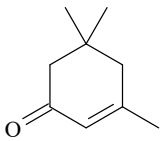
IUPAC systematic name: 3,5,5-trimethyl-cyclohex-2-en-1-one

Synonyms: 3,5,5-trimethylcyclohex-2-enone; 3,5,5-trimethyl-2-cyclohexene-1-one; 1,1,3-trimethyl-3-cyclohexene-5-none; 3,5,5-trimethyl-2-cyclohexen-1-one; isoacetophorone; isooctopherone; α-isophorone; 3,5,5-trimethyl-2-cyclohexenone; and other depositor-supplied synonyms and acronyms (NCBI, 2021).

1.1.2 Structural and molecular information

Relative molecular mass: 138.21 (NCBI, 2021)

Chemical structure:



Molecular formula: $C_9H_{14}O$

1.1.3 Chemical and physical properties

Description: colourless liquid with a peppermint-like odour (IFA, 2021a) or camphor-like odour (NCBI, 2021)

Odour threshold: odour may be noticeable at concentrations of 2–5 ppm (NCBI, 2021)

Boiling point: 215 °C (NCBI, 2021)

Melting point: –8.1 °C (NCBI, 2021)

Density: 0.92 g/cm³ at 20 °C (IFA, 2021a)

Relative vapour density: 4.77 (air = 1) (IFA, 2021a)

Vapour pressure: 0.59 hPa at 25 °C (IFA, 2021a)

Auto-ignition temperature: 460–470 °C at 1013 hPa (ECHA, 2021a)

Lower explosion limit: 0.87 vol.% (50 g/m³) (IFA, 2021a)

Upper explosion limit: 3.8 vol.% (220 g/m³) (IFA, 2021a)

Solubility: sparingly soluble (12 g/L at 20 °C) in water (IFA, 2021a); soluble in ether, acetone, and alcohol; high solvent power for vinyl resins, and cellulose esters (NCBI, 2021)

Flash point: 84 °C (NCBI, 2021)

Stability and reactivity: the combustible substance can react dangerously with air, and the formation of peroxides is possible (IFA, 2021a); exposure to sunlight in aqueous

solutions can result in the formation of photodimers by 2+2 photocycloaddition (Gonçalves et al., 1998)

Octanol/water partition coefficient (P): log K_{ow} = 1.70 (NCBI, 2021)

Conversion factor: 1 ppm is equivalent to 5.74 mg/m^3 at 1013 mbar [101.3 kPa] and 20 °C (IFA, 2021a)

Dynamic viscosity: 2.62 cP at 20 °C [2.62 × 10^{-5} hPa.s] (NCBI, 2021).

1.1.4 Impurities

Impurities include up to 4% isomeric β-isophorone (3,5,5-trimethyl-3-cyclohexen-1-one) and traces (< 1%) of 1,3,5-trimethylbenzene, mesityl oxide (2-methyl-2 pentene-4-one), phorone (2,6-dimethyl-2, 5-heptadiene-4-one), and isoxylitones (NCBI, 2021).

1.2 Production and use

1.2.1 Production process

Isophorone is produced by the aldol condensation of acetone at high temperature (200 °C) and pressure (3.6 MPa) in the presence of aqueous potassium hydroxide. Alternatively, isophorone can be manufactured using calcium oxide, hydroxide, or carbide, or mixtures thereof, at atmospheric pressure and high temperature (350 °C) (NCBI, 2021).

1.2.2 Production volume

Isophorone is listed as a High Production Volume chemical by the Organisation for Economic Co-operation and Development (OECD) (OECD, 2004, 2009). Worldwide production capacity has been estimated at 50 000 tons [45 400 tonnes] in 1990 (NCBI, 2021). In 2016, the United States Environmental Protection Agency (US EPA) estimated an aggregated production volume of 10 000 000–50 000 000 pounds [~4536–22 680 tonnes] in the USA (US EPA, 2016). Isophorone is registered under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation, and more than 100 tonnes per year are manufactured in and/or imported to the European Economic Area (ECHA, 2021a).

1.2.3 Uses

Isophorone is a widely used solvent and chemical intermediate used at industrial sites and in the manufacture of lacquers and vinyl/acetate-based polymers, inks and paints, nitrocellulose finishes, and washing and cleaning products (US EPA, 2000; ECHA, 2021a; NCBI, 2021). Isophorone is used in the manufacture of agrochemicals and is a constituent of certain pesticides. For example, in the USA, isophorone is exempt from the requirement of a tolerance when used as an inert ingredient in pesticide formulations applied to beet, ginseng, rice, spinach, sugar beet, and Swiss chard (Federal Register, 2006). Isophorone is also used as an intermediate for the manufacture of other chemicals, such as 3,5-xylenol, 3,3,5-trimethylcyclohexanol, and trimethylcyclohexanone (US EPA, 2000).

1.3 Detection and quantification

The methodology used to measure isophorone in environmental samples is broadly synonymous with the approach used for other organic solvents, i.e. typically involving solid- or liquid-phase extraction followed by chromatographic analysis. The volatility of isophorone permits determination by headspace analysis, whereby sorbents can be used to extract volatilized isophorone from the headspace above samples, or the headspace can be sampled and analysed inline. Selected representative methods for the analysis of isophorone in different sample matrices are summarized in Table 1.1.

Table 1.1 Representative methods for the detection and quantification of isophorone in various matrices

Sample matrix (method number)	Sample preparation	Analytical technique	LOD (unless otherwise specified)	Reference
Air				
Indoor/workplace air (NIOSH Method 2556)	Solid-phase extraction (XAD-4 resin collection matrix)	GC-FID	1 µg per sample; working range, 0.24–33.2 µg/m^3 for a typical 25 L sample	NIOSH (2003), based on Levin & Carleborg (1987)
Headspace over electrical equipment	Sample components heated in a headspace chamber for inline analysis	GC-MS	NR	Paz et al. (2012)
Water				
Drinking-water (US EPA Method 525.3)	Solid-phase extraction (styrene divinylbenzene or divinylbenzene *N*-vinylpyrrolidone copolymer sorbents)	GC-MS	0.004–0.014 µg/L	US EPA (2012)
Industrial wastewater (US EPA Method 609)	Solvent extraction (methylene chloride)	GC-FID or GC-ECD	5.7 µg/L	US EPA (1984)
Lake water	Solid-phase microextraction (polydimethylsiloxane-coated fibre)	GC-FID	15 µg/L	Horng & Huang (1994)
Seawater	HSPME (activated carbon fibre)	GC	1.4–3.2 ng/L [0.0014–0.0032 µg/L]	Ma et al. (2009)
Soil or sediment				
Lake sediment	Solvent extraction (diethyl ether), centrifugation, and fractionation by gel permeation column chromatography	GC-MS	NR	McFall et al. (1985)
Lake sediment	Freeze-drying, homogenization, Soxhlet extraction with acetone and *n*-hexane, cleaning with alumina/silica adsorption chromatography	GC-MS	NR	Wang et al. (2002)
Soil, experimentally treated	HSPME (divinylbenzene/carboxen/polydimethylsiloxane fibre)	GC-Q-TOF-MS	NR	Brown et al. (2021)
Soil, spiked samples	Shaken and centrifuged with water, methanol or dichloromethane and extracted by florisil column	GC-MS	NR	Singh et al. (1998)
Food				
Oysters and clams, environmental samples	Solvent extraction (diethyl ether), homogenization, centrifugation, and fractionation by gel permeation column chromatography	GC-MS	NR	McFall et al. (1985)
32 supermarket-bought food samples, including soft and alcoholic drinks, condiments, grains, vegetables, spices, seafood, dairy and meat products	Solid and semi-solid samples were homogenized, suspended in distilled water, and saturated with sodium chloride, magnetically stirred at 60 °C and underwent HSPME (polydimethylsiloxane/divinylbenzene fibre)	GC-MS	0.5 pg/mL [0.0005 µg/L]	Kataoka et al. (2007)

Table 1.1 (continued)

Sample matrix (method number)	Sample preparation	Analytical technique	LOD (unless otherwise specified)	Reference
Fish, multiple species, environmental samples	Homogenized samples underwent Soxhlet extraction with acetone and hexane, concentration, and cleaning with gel permeation chromatography	GC-MS	0.02 mg/kg	Camanzo et al. (1987)
Honey	Storage at –18 °C; sample vials were maintained at 60 °C in a water bath during HSPME (divinylbenzene/carboxen/polydimethylsiloxane fibre)	GC-MS	NR	Alissandrakis et al. (2007)
Honey	Magnetically stirred and maintained at 70 °C during HSPME (divinylbenzene/carboxen/polydimethylsiloxane fibre)	GC-MS	NR	El-Sayed et al. (2018)
Honey	Magnetically stirred and maintained at 60 °C during HSPME (polyacrylate fibre)	GC-MS	NR	Anand et al. (2019)
Saffron	Steam distillation to extract essential oil, received in ethyl acetate	GC-MS	NR	Liu et al. (2018)
Saffron	Vials heated to 50 °C in water bath and headspace directly sampled into PTR-TOF-MS instrument	PTR-TOF-MS	NR	Masi et al. (2016)
Jiashi muskmelon juice	Heated to 40 °C and magnetically stirred for HSPME (polydimethylsiloxane/divinylbenzene/carboxen fibre)	GC-MS	NR	Pang et al. (2012)
Other consumer products				
Clofibrate preparations	Dissolution of 0.5 mL of capsule content in 0.5 mL of chloroform and injection into GC-MS	GC-MS	NR	Johansson & Ryhage (1976)
Inflatable aquatic toys and swimming learning devices	Solvent extraction from plastic matrix with dichloromethane, filtration, and volatile fraction isolation with SAFE	GC-MS	NR	Wiedmer et al. (2017), Wiedmer & Buettner (2018)
Human biospecimens				
Urine	HSPME (divinylbenzene/carboxen/polydimethylsiloxane fibre)	GC-TOF-MS	NR	Hanai et al. (2012)
Plasma (unspecified origin), spiked	Centrifugation with acetonitrile and extraction by florisil column	GC-MS	NR	Singh et al. (1998)

FID, flame ionization detector; GC-ECD, gas chromatography with electron capture detection; GC-FID, gas chromatography with flame ionization detection; GC-MS, gas chromatography with mass spectrometry; GC-TOF, gas chromatography time-of-flight; GC-Q-TOF-MS, gas chromatography quadrupole time-of-flight mass spectrometry; HSPME, headspace solid-phase microextraction; LOD, limit of detection; NIOSH, National Institute for Occupational Safety and Health; NR, not reported; PTR-TOF-MS, proton-transfer-reaction time-of-flight mass spectrometry; SAFE, solvent-assisted flavour evaporation; US EPA, United States Environmental Protection Agency.

1.3.1 Air

Established methods exist for the measurement of isophorone in indoor workplace air, with protocols dated as early as 1955 (Kacy & Cope, 1955). Modern methods commonly involve the use of sampling tubes containing a solid adsorbent matrix through which a volume of air is passed and can be used in conjunction with personal air samplers. Early approaches to this method employed adsorption on a charcoal matrix (White et al., 1970), e.g. National Institute for Occupational Safety and Health (NIOSH) Method 2508 (NIOSH, 2003). Owing to the decomposition of isophorone desorbed on charcoal, polymer-based collection matrices are now favoured (Brown & Purnell, 1979; Levin & Carleborg, 1987). These developments formed the basis of (superseding) NIOSH Method 2556, which uses a XAD-4 resin mesh as the adsorptive collection matrix with desorption using diethyl ether and analysis by gas chromatography (GC) with flame ionization detection (FID). This method has a limit of detection (LOD) of 1 µg per sample and has achieved average recoveries of isophorone of 94.1%, in the range of 55–831 µg (Levin & Carleborg, 1987; NIOSH, 2003). One study made inline measurements of emissions of isophorone and other volatile organic compounds (VOCs) from headspace chambers in which electronic components were heated to 75–200 °C. Analysis was performed using gas chromatography-mass spectrometry (GC-MS) (Paz et al., 2012). [The Working Group noted that this study was conducted in the context of detecting the onset of equipment overheating and electrical fires in on-board instruments found in aircraft, submarines, and other vessels. While determining human exposure was not the primary rationale for this study, such electrical emissions may still be a relevant potential source of isophorone.]

Methods for the determination of isophorone in workplace air have also been developed in China. These methods are based on the earlier principle of adsorption to a charcoal matrix but report good recoveries (> 90%) if the analysis is carried out within 10 days of sampling (Kang et al., 2006; Chen & He, 2009).

1.3.2 Water

Several methods have been used to measure isophorone in various aqueous samples, including drinking-water (US EPA, 2012), natural waters (Sheldon & Hites, 1978; Horng & Huang, 1994; Ma et al., 2009), industrial effluents (US EPA, 1984), and landfill leachate (Ghassemi et al., 1984). Solid-phase or solvent extraction techniques are capable of separating isophorone from an aqueous matrix before chromatographic analysis. The US EPA has developed several approved methods for the determination of isophorone in water. US EPA Method 525.3 (US EPA, 2012) is the most recent issue of a series of methods used to detect isophorone and more than 100 other organic chemicals in drinking-water. In brief, 1 L of sample is passed through a solid-phase extraction (SPE) device. Analytes are eluted from the SPE device with organic solvents. The eluent is then dried by passing through an anhydrous sodium sulfate column, concentrated by evaporation with nitrogen gas, and made up to a volume of 1 mL with ethyl acetate and internal standard solutions. Samples are then analysed by GC-MS, with reported LODs of 0.004–0.014 µg/L. US EPA Method 609 (US EPA, 1984) covers the determination of isophorone and selected nitroaromatics in industrial wastewater using solvent extraction with methylene chloride [dichloromethane]. Extracts are dried, concentrated to a volume of 10 mL in hexane and can be analysed by GC with FID, or GC with electron capture detection (ECD), although the former is recommended since the LOD is lower (LOD of 5.7 µg/L with GC-FID compared with 15.7 µg/L with GC-ECD). The abovementioned analytes were also analysed using an SPE approach in

lake water (Horng & Huang, 1994). Low concentrations of isophorone (LODs, 1.4–3.2 ng/L) have also been measured in sea water by a method using GC coupled with headspace solid-phase microextraction (HSPME) with activated carbon fibre (Ma et al., 2009).

1.3.3 Soil and sediment

No methods were identified for the measurement of isophorone in natural soils.

Isophorone has been measured in lake sediments using both solvent (diethyl ether) (McFall et al., 1985) and Soxhlet (Wang et al., 2002) extraction methods followed by chromatographic clean-up of extracts and analysis by GC-MS. Two studies have reported isophorone measurements made in soils subjected to various aerobic and anaerobic conditions in vitro (Brown et al., 2021) and spiked with known quantities of isophorone (Singh et al., 1998). These studies used HSPME followed by gas chromatography quadrupole time-of-flight mass spectrometry (GC-Q-TOF-MS), and florisil column extraction followed by GC-MS, respectively. [The Working Group noted that although the soils analysed in these two studies were subjected to experimental conditions, the sample preparation, extraction, and analysis steps are probably applicable to environmentally sampled or natural soils.]

1.3.4 Food and consumer products

Methods have been described for the determination of isophorone in various edible samples, including fish and shellfish sampled in their natural habitat (McFall et al., 1985; Camanzo et al., 1987) and shop-bought food items (Kataoka et al., 2007). Isophorone was measured in samples of oyster and clam using solvent extraction with diethyl ether, homogenization, and centrifugation, followed by fractionation by gel-permeation chromatography and analysis by GC-MS (McFall et al., 1985). A similar approach, using Soxhlet extraction with GC-MS, was used to analyse isophorone in several fish species (Camanzo et al., 1987). Kataoka et al. (2007) collected samples of 32 food items and determined isophorone content using HSPME and GC-MS, with a reported LOD of 0.5 pg/mL [0.0005 µg/L] and recoveries of > 84% [the Working Group noted the versatility of this method across the wide variety of food items analysed].

Additionally, several reports have described methods for the chemical profiling of a variety of natural products cultivated for human consumption (see Section 1.4.1). These methods have assessed the VOC composition of different samples of which isophorone is a major constituent. The rationales for undertaking such analyses include the VOC profiling of honeys for identification and for description of antifungal, medicinal and aromatic properties (Alissandrakis et al., 2007; El-Sayed et al., 2018; Anand et al., 2019); the VOC profiling of plant extracts (e.g. *Clerodendrum infortunatum* L.; Gera et al., 2020) to determine taxonomy and aromatherapeutic and pharmaceutical applications; the analysis of saffron for the purposes of geographical and commercial discrimination (Masi et al., 2016; Liu et al., 2018); the analysis of the intermediate distillate of the Chinese traditional medicine, Xingnaojing injection, to optimize its extraction process (Fang et al., 2017); and the analysis of Jiashi muskmelon juice for odour profiling – a major determinant of consumer acceptance (Pang et al., 2012). [The Working Group noted that, while the rationales for the abovementioned analyses were not related to human exposure, the methods used would be applicable to determinations of isophorone made in this context.] Selected methods for measure of isophorone in these items are summarized in Table 1.1 and mostly involved HSPME techniques or direct headspace analysis by GC-MS.

Other consumer products for which methods of isophorone determination have been reported

in the context of human exposure include clofibrate (a pharmaceutical used to control high blood levels of cholesterol and triglyceride), which was analysed directly by GC-MS after dissolution in chloroform (Johansson & Ryhage, 1976). Children's inflatable aquatic toys and learning devices (armbands, beach balls and bathing rings) were also analysed by GC-MS after solvent extraction with dichloromethane and filtration (Wiedmer et al., 2017; Wiedmer & Buettner, 2018). [The Working Group noted that the two studies quantifying isophorone in aquatic toys used a combination of sensory analyses (i.e. a panel of trained assessors) and instrumental analyses to identify the principal chemical signatures of odours, a technique termed "gas chromatography-olfactometry". The instrumental aspects of this approach are relevant to exposure assessment more broadly.]

1.3.5 Biological specimens

Although isophorone is mostly excreted via the urine in animal systems, data on excretion in humans are sparse (see Section 4.1 of the present monograph). There are no standardized protocols for the measurement of biomarkers of isophorone exposure in humans, and only one study reporting the determination of isophorone in human urine was available. In this study with a case–control design (Hanai et al., 2012), isophorone and other urinary VOCs were investigated as potential non-invasive diagnostic markers of lung cancer. Urine samples were frozen at −80 °C until use and, after thawing, underwent centrifugation and filtration. In an approach similar to that used by Ma et al. (2009) for sea water samples, isophorone and other VOCs were extracted from urine samples by HSPME using divinylbenzene/carboxen/polydimethylsiloxane fibres. Fibres with adsorbed compounds were analysed by GC-TOF-MS [limits of detection were not reported]. [The Working Group noted that although there was a lack of studies reporting methods for isophorone determination in human urine, methods used for other aqueous samples and in animals (e.g. hexane extraction of rabbit urine followed by GC analysis; Dutertre Catella et al., 1978) may be used for the analysis of human urine.]

Finally, an experimental study reported isophorone measurements on spiked plasma samples that underwent florisil column extraction and analysis by GC-MS (Singh et al., 1998). [The Working Group noted that the authors did not specify the origin (i.e. human or animal) of the plasma used in this experiment, and that samples were spiked with isophorone. Nevertheless, the study provided an example of isophorone determination in a plasma matrix, which may be relevant to human biomonitoring.]

1.4 Occurrence and exposure

1.4.1 Occurrence in the environment, food, and consumer products

The wide use of isophorone as a chemical intermediate and solvent for lacquers, inks, vinyl resins, herbicides, copolymers, coatings, and other products in a variety of industrial settings permits its entry into the environment from urban centres and industrial sites via atmospheric emissions due to volatilization; and via water and soil contamination due to waste disposal, industrial effluents and runoff. While isophorone is rapidly removed from the air by photochemical breakdown, and to a lesser extent washout, it may persist in natural waters and soil for longer periods. In water, volatilization and sorption to sediments and particulates are not expected to be significant removal mechanisms of isophorone and, in soils, microbial degradation is expected to occur (ATSDR, 2018). Isophorone is also present in food items, and in products whose manufacture involves its application, including food packaging (Sasaki et al., 2005; Skjevrak et al., 2005) and children's aquatic

toys (Wiedmer et al., 2017; Wiedmer & Buettner, 2018). While the dominant sources of isophorone in the environment appear to be anthropogenic in nature, it has been found to occur naturally, including in several botanical specimens, such as cranberries (NCBI, 2021) and saffron, and in honey (see Section 1.3.4), and in the defensive froth or secretions of grasshoppers (Eisner et al., 1971). [The Working Group noted that the precise origin of isophorone in these natural specimens is a subject of inquiry.] Concentrations of isophorone reported in different environmental media, including food and other consumer products, are summarized in Table 1.2 and described throughout the following sections. [The Working Group noted that many of the measurements reviewed throughout Section 1.4.1 were made for method development and validation purposes and do not necessarily reflect the actual distribution of isophorone in the environment.]

(a) Environmental occurrence

There is a notable scarcity of ambient air measurements of isophorone in the literature, despite its volatility and known sources of atmospheric emissions. The US EPA publishes national estimates of isophorone emissions via its National Emissions Inventory (NEI) on the basis of data provided by state, local, and tribal air agencies, and supplemented by data collected by the US EPA. Estimated isophorone emissions by sector in the USA in 2017 are presented in Table 1.3. This suggests that the five sectors with the highest emissions of isophorone are coal-powered electricity generation, waste disposal, industrial surface coating and solvent use, industrial processes not elsewhere classified, and chemical manufacturing, which contribute 38%, 29%, 19%, 6.5%, and 3.4%, respectively, of total emissions (US EPA, 2017). Atmospheric emissions of isophorone may be produced by coal combustion; isophorone was measured at a concentration of 490 ppb [0.49 mg/kg] in coal fly ash from a power station in the USA (Harrison et al., 1985). Overheating electrical components have also been shown to be a source of atmospheric emission of isophorone. Under experimental conditions, a resistor heated at a constant temperature of 200 °C for 5 hours emitted isophorone at 128 ng/g of component per hour (Paz et al., 2012). [The Working Group noted that these data indicated that isophorone exposure may occur as a result of electrical and other fires involving the combustion of isophorone-containing materials. The former is of relevance to those involved in the burning of electronic waste, an activity that is particularly prevalent in low- and middle-income countries such as those in West Africa and from where occurrence and exposure data for isophorone appear to be absent.]

Data on the occurrence of isophorone in surface waters (excluding effluents from industrial sites) are sparse, with the few measurements available from the USA not detecting the compound or detecting trace amounts (< 2 µg/L) (Sheldon & Hites, 1978; US EPA, 1982; Hall Jr et al., 1987). Lake sediments in both the USA and China have been found to contain isophorone. Mean concentrations at three sites at Lake Pontchartrain, Louisiana, USA, between May and June 1980, were between 0.9 and 12 ng/g [0.0009–0.012 mg/kg] (McFall et al., 1985). Much higher concentrations (1.01–17.21 mg/kg) were measured in five sediment samples collected from Donghu Lake, Wuhan, China, in November 2000 (Wang et al., 2002). A small number of studies (US EPA, 1974, 1975; Keith et al., 1976; Feng et al., 2020) have measured isophorone in drinking-water. A range of 1.5–2.9 µg/L was reported for an unknown number of samples collected in New Orleans, USA in 1974 (US EPA, 1974). Isophorone was detected in 3 out of 11 samples collected in Philadelphia, USA, between 1975 and 1977 (Suffet et al., 1980), but the concentrations were not reported. A study conducted in China comparing methods for the removal of isophorone from reservoir-sourced drinking-water

Table 1.2 Occurrence of isophorone in environmental samples, food, consumer products, and biological specimens

Sample type	Location and collection date	No. of samples	Mean (range)	Analytical method	Comments	Reference
Atmospheric emissions						
Coal fly ash	The Four Corners coal-fired power station, New Mexico, USA, December 1979	1	490 ppb [0.49 mg/kg]	GC-MS	Collected from an electrostatic precipitator	Harrison et al. (1985)
Natural waters and sediments						
River water	Delaware River, USA, August 1977 and March 1978	Not well reported, but interpreted as 16	"trace", i.e. < 0.01 ppb [0.01 µg/L]	GC-MS		Sheldon & Hites (1978)
River water	Potomac River, Quantico, Virginia, USA, Spring 1986	1	< 2 µg/L	GC-MS		Hall Jr et al. (1987)
River water	Olentangy River	1	ND, < 5 µg/L	GC-FID		US EPA (1982)
Lake sediment	Lake Pontchartrain, Louisiana, USA, May–June 1980	10 in total, collected from 3 sites	3 sites: 0.9 ng/g (mean of 8 samples); 12 ng/g (1 sample); 10 ng/g (1 sample)	GC-MS	Lake Pontchartrain is a brackish estuary in the Gulf of Mexico	McFall et al. (1985)
Lake sediment	Donghu Lake, Wuhan, China, November 2000	5	7.9 mg/kg (1.01–17.21 mg/kg)	GC-MS		Wang et al. (2002)

Table 1.2 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Analytical method	Comments	Reference
Industrial effluents						
Sewer pump sample receiving wastes from phenolic resins, vinyl acetate, and polyvinyl chloride process areas	USA	1	40.5 µg/L	GC-FID		US EPA (1982)
Brine sample from holding tank receiving washings from ships delivering various commodities	USA	1	< 50 µg/L			
Secondary sewage effluent	Columbus, Ohio, USA	1	120 µg/L			
Final effluent from UNOX treatment system receiving wastes from plants producing plasticizers, butyl rubber, and olefins	USA	1	< 5 µg/L			
Final effluent from organic chemical plants producing nitrobenzene, *ortho*-dichlorobenzene, *ortho*-nitrophenol, aniline, and oil additives	USA	1	< 20 µg/L			
Timber products	USA	2 (positive samples)	83 µg/L (55–111 µg/L)	GC-MS		US EPA (1983), cited in ATSDR (2018)
Petroleum refining		1 (positive sample)	1380 µg/L			
Paint and ink		5 (positive samples)	185 µg/L (24–946 µg/L)			
Pulp and paper		1 (positive sample)	753 µg/L			
Auto and other laundries		2 (positive samples)	43 µg/L (43–44 µg/L)			
Pharmaceuticals		1 (positive sample)	237 µg/L			
Foundries		1 (positive sample)	136 µg/L			
Transportation equipment		2 (positive samples)	173 µg/L (28–318 µg/L)			
Publicly owned treatment works		15 (positive samples)	11.5 µg/L (4.2–114 µg/L)			
Drinking-water						
Drinking-water	Philadelphia, USA, 1975–1977	12	NR	GC-MS	Detected in 17% of samples	Keith et al. (1976), cited in ATSDR (2018)

Table 1.2 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Analytical method	Comments	Reference
Drinking-water	New Orleans, USA, 1974	NR	(1.5–9.5 µg/L)	GC-MS		US EPA (1974) cited in ATSDR (2018)
Drinking-water	Cincinnati, USA	NR	≤ 0.02 µg/L	NR		US EPA (1975) cited in ATSDR (2018)
Raw drinking-water (pre-traditional treatment)	Yellow River, eastern China, October–November 2018	5	0.338 ng/L (0.132–0.521 ng/L) [0.338×10^{-3} µg/L ($0.132–0.521 \times 10^{-3}$ µg/L)]	GC-MS	Reservoir-sourced water from two plants using alternative treatment processes was compared	Feng et al. (2020)
Finished drinking-water (traditional treatment)		5	0.2 ng/L (0.07–0.49 ng/L) [0.2×10^{-3} µg/L ($0.07–0.49 \times 10^{-3}$ µg/L)]			
Raw drinking-water (pre-advanced oxidation treatment)		5	1.92 ng/L (0.41–5.18 ng/L) [0.129×10^{-2} µg/L ($0.041–0.518 \times 10^{-2}$ µg/L)]			
Finished drinking-water (advanced oxidation treatment)		5	0.33 ng/L (0.17–0.60 ng/L) [0.33×10^{-3} µg/L ($0.17–0.60 \times 10^{-3}$ µg/L)]			
Swimming-pool water						
Chlorinated water from public, private, outdoor and indoor pools, sports pools, hot tubs, water slides, paddling pools, and recreational pools	Poland	50	Quantified in 89% of samples (LOQ, 0.75 µg/L); for those in which quantified, 0.8 µg/L (range, 0.75–1.0 µg/L)	GC-MS		Lempart et al. (2020)
Dietary occurrence						
Oysters	Lake Pontchartrain, Louisiana, USA, May–June 1980	8	38 ng/g	GC-MS	Environmental samples	McFall et al. (1985)
Clams		2	ND			
Fish, including carp, bass, catfish, pumpkinseed, bowfin, and pike	Lake Michigan tributaries, USA, 1983	28 composite samples from a total of 140 fish caught	[0.76 mg/kg] (< LOD to 3.61 mg/kg) [760 ng/g (< LOD to 3610 ng/g)]	GC-MS	Environmental samples; concentrations reported in wet weight; LOD not reported, but 0.02 ng/g used in Working Group calculation of mean for < LOD values reported as "0.*" in the article	Camanzo et al. (1987)

Table 1.2 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Analytical method	Comments	Reference
"Green tea A"	Japan (supermarket-bought products)	3 per product	129 ± 1 pg/g (standard deviation)	GC-MS	Concentrations in liquid samples are expressed as pg/mL, semi-solid and solid as pg/g	Kataoka et al. (2007)
"Green tea B"			647 ± 5 pg/g			
Liquor			21 ± 1 pg/mL			
Sake			340 ± 19 pg/mL			
Soy sauce (weak)			88 ± 4 pg/mL			
Soy sauce (strong)			3306 ± 107 pg/mL			
Tomato juice			994 ± 43 pg/mL			
Tomato juice			262 ± 14 pg/mL			
Milk			200 ± 9 pg/mL			
Honey			4142 ± 141 pg/mL			
Maple syrup			1252 ± 37 pg/g			
Sugar			1568 ± 22 pg/g			
Soybean flour			13 258 ± 312 pg/g			
Miso			3322 ± 74 pg/g			
Wheat flour			1674 ± 25 pg/g			
Rice			2868 ± 67 pg/g			
Cod roe			260 ± 4 pg/g			
Sea urchin			2868 ± 67 pg/g			
Scallop			238 ± 11 pg/g			
Fresh fish			150 ± 8 pg/g			
Chicken			184 ± 14 pg/g			
Pork			220 ± 10 pg/g			
Worcester sauce, potato, carrot, green pepper, mustard, wasabi, white pepper, dried bonito, egg yolk, beef			ND (LOD, 0.5 pg/mL)			
Thyme honey	Greece	28	46 ng/kg (0–1114 ng/kg) [0.046 ng/g (0–1.1 ng/g)]	GC-MS		Alissandrakis et al. (2007)

Table 1.2 (continued)

Sample type	Location and collection date	No. of samples	Mean (range)	Analytical method	Comments	Reference
Grains and their products	Japan, 2002–2003	17	1.55 ng/g (0.8–2.8 ng/g)	GC-MS		Sasaki et al. (2005)
Beans and their products		30	3.0 ng/g (< 0.1–8.9 ng/g)			
Vegetables and their products		26	0.2 ng/g (< 0.1–1.7 ng/g)			
Fish		10	0.3 ng/g (< 0.1–1.8 ng/g)			
Meat		5	< 0.1 ng/g (< 0.1 to < 0.1 ng/g)			
Milk and butter		2	< 0.1 ng/g (< 0.1 to < 0.1 ng/g)			
Sake		3	0.1 ng/g (< 0.1 to < 0.2 ng/g)			
Food containers		8	12.6 ng/g (4–19 ng/g)		Containers for rice, soy sauce, miso, and beans, made from either polyethylene terephthalate, polyethylene, or polypropylene	
Water exposed to polyolefin bottles at ambient temperature for 72 h	Norway	3	≤ 4 µg/L	GC-MS		Skjevrak et al. (2005)
Consumer product occurrence						
Inflatable pool toys: armbands, bathing rings, and beach balls	Online suppliers located in Germany	20	Detected in 8/20 samples; for those in which detected, [1.95 g/kg] (< 0.16–5.25 g/kg) [1080 mg/kg (< 160–5250 mg/kg)]	GC-MS	0.08 mg/kg was assigned to values reported as < 0.16 mg/kg (LOQ) in the Working Group calculations of mean	Wiedmer & Buettner (2018)
Biological specimens						
Urine	Pennsylvania, USA	20	130 nM (39–1412 nM) [18 µg/L (5.4–195 µg/L)]	GC-TOF-MS	Control participants in a lung cancer case–control study	Hanai et al. (2012)

GC-FID, gas chromatography with flame-ionization detection; GC-MS, gas chromatography with mass spectrometry; GC-TOF-MS, gas chromatography time-of-flight with mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; ND, not detected; NR, not reported; ppb, parts per billion.

Table 1.3 Estimated isophorone emissions in the USA in 2017 by emission sector[a]

Sector	Estimated emissions		Contribution to total estimated emissions (%)[b]
	(pounds)	(tonnes)	
Fuel combustion – electric generation – coal	117 802	[53.4]	38%
Waste disposal	89 208	[40.5]	29%
Solvent use – industrial surface coating and solvent use	60 097	[27.3]	19%
Industrial processes – not elsewhere classified	20 475	[9.3]	6.5%
Industrial processes – chemical manufacturing	10 550	[4.8]	3.4%
Industrial processes – ferrous metals	6256	[2.8]	2%
Fuel combustion – industrial boilers, ices – coal	3307	[1.5]	1%
Solvent use – consumer and commercial solvent use	1659	[0.75]	< 1%
Solvent use – graphic arts	1136	[0.52]	< 1%
Fuel combustion – commercial/institutional – coal	749	[0.34]	< 1%
Other fuel combustion	960	[0.44]	< 1%
Other industrial processes	236	[0.11]	< 1%
Bulk gasoline terminals	166	[0.08]	< 1%
Solvent use – degreasing	0.19	[0.09×10^{-3}]	< 1%

[a] The 10 highest emitting sectors are displayed with the 15 remaining sectors collapsed.
[b] Calculated by the Working Group using National Emissions Inventory Data (US EPA, 2017).

reported concentrations that were all below 5.18 ng/L [0.005 µg/L] (Feng et al., 2020).

The highest environmental concentrations of isophorone have been measured in industrial effluents (Table 1.2): concentrations were 120 µg/L in sewage effluent, 185 µg/L in paint and ink effluent, 237 µg/L in pharmaceutical effluent, 753 µg/L in pulp and paper effluent, and as high as 1380 µg/L in effluent from petroleum refining (US EPA, 1982, 1983). Isophorone has also been found in the turf crumb rubber of synthetic sports pitches, which is made from recycled tyres (Perkins et al., 2019).

(b) Dietary exposure

Although represented by only a few studies and individual samples, a substantial variety of food items have been analysed for isophorone content. As mentioned in Section 1.3.4 of the present monograph, some of these studies were conducted for purposes other than human exposure assessment, such as determining the VOC profile of honey, a large fraction of which includes isophorone. Mean concentrations ranged from 0.046 ng/g (Alissandrakis et al., 2007) to 4.12 ng/g (Kataoka et al., 2007). In environmental samples, isophorone was found at a concentration of 38 ng/g in an oyster collected from Lake Pontchartrain, Louisiana, USA, in 1980, but was not detected in two samples from clam (McFall et al., 1985). Two studies conducted in Japan (Sasaki et al., 2005; Kataoka et al., 2007) measured isophorone in a large variety of supermarket-bought food items. These results are summarized in Table 1.2; the majority of samples contained isophorone at less than 1 ng/g, but relatively higher concentrations were found in polished rice (2.8 ng/g), miso (8.9 ng/g), spinach (1.7 ng/g), sole (1.8 ng/g) (Sasaki et al., 2005); and rice and sea urchin (both, 2.9 ng/g), miso (3.3 ng/g), honey (4.1 ng/g), strong soy sauce (3.3 ng/g), soy sauce (5.2 ng/g), fermented soybeans (5.4 ng/g), and soybean flour (13.3 ng/g) (Kataoka et al., 2007). The highest isophorone concentrations in food reported by any study were found in samples of various fish species

collected in 1983 from Lake Michigan tributaries, USA, where there were known influxes of industrial effluent (Camanzo et al., 1987). [A mean concentration of 0.76 mg/kg (range, < LOD to 3.61 mg/kg) [760 ng/g (range, < LOD to 3610 ng/g)] was calculated.] [The Working Group noted that, although these samples were sourced directly from the environment, they pointed to potentially high human exposures from food sourced from polluted areas.]

The origin of isophorone in many food items was not clear; it may be naturally occurring or a result of contamination, including with herbicides and pesticides, some of which include isophorone as a major constituent, e.g. 10–20% in one herbicide (Arysta LifeScience, 2013) and as high as 60% in another (Bayer Crop Science, 2010), both of which are used on beetroot, rice, beans, spinach, and sugar beet (Federal Register, 2006). Isophorone has been detected in food packaging at concentrations many times higher than in the food items themselves. The average isophorone concentration measured in containers made from polyethylene terephthalate, polyethylene, and polypropylene for soy sauce, polished rice, miso, and beans was 12.6 ng/g (range, 4–19 ng/g). The corresponding isophorone concentrations measured in the foods in these containers ranged from 1 to 3.5 ng/g, and estimated migration levels (determined by filling containers with dichloromethane and leaving them at 25 °C for 1 hour) ranged from < 50 to 150 pg/cm^2 [0.05–0.15 ng/cm^2] (Sasaki et al., 2005). In another study (Skjevrak et al., 2005), water exposed to polyolefin bottles at ambient temperature for 72 hours was found to contain an isophorone concentration up to 4 µg/L.

(c) Consumer products

Notably high concentrations of isophorone (160–5250 mg/kg) have been measured in 40% of tested inflatable swimming-pool toys and learning devices, including armbands, bathing rings, and beach balls (Wiedmer & Buettner, 2018). [Direct exposure to these products is most likely to occur among children.] Isophorone has also been shown to migrate from these products and other swimwear (e.g. goggles, earplugs, flip-flops, and swimming caps) into swimming-pool water. In a survey of water from 50 public, private, outdoor and indoor pools, sports pools, hot tubs, water slides, paddling pools, and recreational pools in Poland, isophorone was quantified in 89% of samples, with a mean concentration of 0.8 µg/L (0.75–1.0 µg/L) among those samples in which isophorone was quantified (Lempart et al., 2020). In another study (Danish Ministry of the Environment, 2007), isophorone was detected in a school bag, pencil case, and eraser.

Like other VOCs, isophorone is a component of tobacco smoke (Yang et al., 2006).

1.4.2 Occupational exposure

Few estimates are available of the number of workers exposed to isophorone worldwide. NIOSH estimated in 1978 that about 1.5 million workers were potentially exposed to isophorone in the USA (NIOSH, 1978a). However, the NIOSH National Occupational Exposure Survey estimated that just 47 097 workers (10 353 of whom were women) were exposed to isophorone in 1981–1983, with the highest numbers seen among operators of printing machines, painting and paint spraying machines, textile machines, and miscellaneous machines, as well as hand packers and packagers, assemblers, non-construction labourers, unspecified mechanics and repairers. The industries with the highest representation among exposed workers were rubber and miscellaneous plastics products, printing and publishing, fabricated metal, chemicals and allied products, and miscellaneous manufacturing industries (NIOSH, 1990). [The Working Group noted that it is unclear how representative these estimates are of current exposure prevalence.]

No studies on occupational exposure of workers exposed to isophorone during its manufacture were available to the Working Group. Isophorone has been measured in air in several occupational settings where isophorone is used as an ink in screen-printing and other types of printing and coating, and in plastics manufacture. Although isophorone is a constituent of some herbicides, e.g. Betanal (Bayer Crop Science, 2010) and Satunil (Arysta LifeScience, 2013), no information was available on exposures during manufacture or use of these herbicides. [The Working Group noted that the available data were sparse and were collected in only a few countries.]

In an epidemiological study by Rodrigues et al. (2020), the authors reported isophorone exposures in three facilities, located in East Fishkill in New York, Burlington in Vermont, and San José in California, USA, in which the following operations were carried out: semiconductor manufacture, masking, and module manufacture; and the manufacture of printers, hard disk drives, tape drives, and Winchester disks. [The Working Group noted that, although isophorone was present in at least one of these facilities, no information was provided as to in which operation the isophorone occurred.]

An Institut national de recherche et de sécurité (INRS) database called Solvex (INRS, 2021) is derived from a French occupational exposure database (COLCHIC) of measurements taken by French prevention authorities for risk assessment purposes since 1987 (Mater et al., 2016). Solvex provides summary statistics for personal measurements by industry, occupation, or task. The industry categories that showed sufficient data on isophorone exposure (more than 50 samples) that could be used to calculate statistics were printing, and reproduction of documents (100 results; mean, 0.99 mg/m^3; range, 0.15–12 mg/m^3), metallurgy (83 results; mean, 0.65 mg/m^3; range, 0.05–21 mg/m^3), manufacture of metal products (62 results; mean, 0.65 mg/m^3; range, 0.05–10 mg/m^3), and manufacture of electrical equipment (57 results; mean, 0.62 mg/m^3; range, 0.04–3 mg/m^3). Of these measurements, 88% had been taken before 2001.

Compliance measurements are also available from the United States Occupational Safety and Health Administration (OSHA) (OSHA, 2021) and discussed in Lavoué et al. (2013). Between 1984 and 2020, 755 personal isophorone measurements varying in duration between 11 and 880 minutes were collected. Of the 8% of measurements that were made after 2000, isophorone was not detected in ~30%. The isophorone measurements made between 1984 and 2020 ranged from < 0.015 ppm [< 0.09 mg/m^3] (the smallest reported detected value) to 40 ppm [230 mg/m^3] (interquartile interval, < 0.015–0.6 ppm [< 0.09–3.4 mg/m^3]). The three most visited industries were: commercial printing, not elsewhere classified (n = 239; median < 0.015 ppm [< 0.09 mg/m^3]; 90th percentile, 2.3 ppm [13 mg/m^3]); plastics products, not elsewhere classified (n = 150; median < 0.015 ppm; 90th percentile, 1.1 ppm [6.3 mg/m^3]); and blank books and loose-leaf binders (n = 41; median, < 0.015 ppm [< 0.09 mg/m^3]; 90th percentile, 1.9 ppm [11 mg/m^3]).

Table 1.4 summarizes the results of the identified literature on occupational exposure measurements in specific workplaces.

A NIOSH health hazard evaluation was conducted during two visits in 1977 to a metals coating company near Chicago, Illinois, USA (NIOSH, 1978b). Personal breathing zone (PBZ) and area-sample air measurements were collected in three coating lines and two reclaimed-solvent areas using charcoal sampling tubes and analysed using GC. Isophorone was below the LOD in several air measurements collected in one reclaimed-solvent area and 1.0 ppm [5.74 mg/m^3] in the second area. Isophorone and several other solvents were measured for workers carrying out different tasks on three coating lines during the first visit and on one coating line during the

Table 1.4 Occupational exposure to isophorone measured in workplace air

Occupational group, job type, location, and date	Monitoring method	Analytical method (LOD)	No. of samples	Mean (range)	Median (IQR)	Comments	Reference
Vinyl coating process in metals finishing company, Illinois, USA, 1977	Indoor PBZ air measurements, September 1977	GC of organic vapour charcoal sampling tubes (0.01 mg per tube)	15	NR (< 0.05–1.5 ppm) [< 1.60–8.61 mg/m^3]	ND (ND to 0.75 ppm) [ND to 4.30 mg/m^3]	7 samples, ND; tended to be due to short (< 3 h) sampling times	NIOSH (1978b)
	Indoor PBZ air measurements, November 1977		4	1.00 ppm (0.64–1.31 ppm) [5.74 mg/m^3 (3.67–7.52 mg/m^3)]	1.02 ppm (0.64–1.31 ppm) [5.85 mg/m^3 (3.67–7.52 mg/m^3)]		
Screen printer in specialty screen-printing operation, Pennsylvania, USA, 1978	Short-term and full-shift PBZ air measurements	GC of organic vapour charcoal sampling tubes (0.03 mg per tube)	7	NR (< 0.07–25.7 ppm) [< 0.40–148 mg/m^3]	ND (ND to 0.30 ppm) [ND to 1.72 mg/m^3]	All samples collected from same screen-printer; highest concentration was from short-term cleaning operation	NIOSH (1979)
Screen printing process using gloss vinyl inks in specialty decal company, Georgia, USA, 1982	Full-shift PBZ or area air measurements collected at flow rate of 50 or 100 cm^3/min, and 200 cm^3/min for short-term measurement	GC of organic vapour charcoal sampling tubes (0.01 mg per tube)	8	NR ([< 0.16]–3.4 ppm) [< 0.89–19.5 mg/m^3]	[1.3 ppm (ND to 2.15 ppm)] [7.5 mg/m^3 (ND to 14.4 mg/m^3)]	Flow rate of 100 cm^3/min gave highest concentrations	NIOSH (1983)
Plastic-product manufacturers, China, NR	Indoor area samples from 10 facilities	GBZ/T (method name) 160.55–2007 (NR)	10	[0.267 mg/m^3] (0.0065–2.1 mg/m^3)	0.65 × 10^{-2} mg/m^3 [0.65–0.725 × 10^{-2} mg/m^3]	Very little information given about methods; unclear whether 0.0065 mg/m^3 represents a measured value or LOD	Cai et al. (2019)

Table 1.4 (continued)

Occupational group, job type, location, and date	Monitoring method	Analytical method (LOD)	No. of samples	Mean (range)	Median (IQR)	Comments	Reference
Screen-printing process using high-isophorone inks and solvents, USA, 1982	Short-term (50–90 min) PBZ measurements, printing press	GC of organic vapour charcoal sampling tubes (NR)	18	23 ppm (SD, 5.4) [132 mg/m^3 (SD, 31)]	NR	Exposure calculated on TWA basis, but time frame unclear	Samimi (1982)
	Automatic dryer		19	9.5 ppm (SD, 3.3) [55 mg/m^3 (SD, 19)]	NR		
	Manual drying		15	15 ppm (SD, 4.1) [86 mg/m^3 (SD, 24)]	NR		
	Paint mixing		12	17.8 ppm (SD, 5.5) [102 mg/m^3 (SD, 32)]	NR		
	Screen wash		14	8.3 ppm (SD, 5.6) [48 mg/m^3 (SD, 32)]	NR		
Printing and reproduction of documents, France, 1987–2021	Personal samples, median duration, 127 min (range, 61–276 min)	Variable; INRS standard methods	100	0.99 mg/m^3 (range, 0.15–12 mg/m^3)	0.60 mg/m^3		INRS (2021)
Metallurgy, France, 1987–2021	Personal samples, median duration, 242 min (range, 70–450 min)		83	0.65 mg/m^3 (range, 0.05–21 mg/m^3)	0.25 mg/m^3		
Manufacture of metal products, France, 1987–2021	Personal samples, median duration, 230 min (range, 156–371 min)		62	0.69 mg/m^3 (range, 0.05–10 mg/m^3)	0.25 mg/m^3		
Manufacture of electrical equipment, France, 1987–2021	Personal samples, median duration, 118 min (range, 65–214 min)		57	0.62 mg/m^3 (range, 0.04–3 mg/m^3)	0.4 mg/m^3		

Table 1.4 (continued)

Occupational group, job type, location, and date	Monitoring method	Analytical method (LOD)	No. of samples	Mean (range)	Median (IQR)	Comments	Reference
Commercial printing, USA, 1984–2020	Personal OSHA compliance measurements varying from 11 to 360 min, from 20 companies	Variable; OSHA standard methods; smallest reported detected value, 0.015 ppm	239		[< 0.015 ppm (< 0.015–0.80 ppm); 90th percentile, 2.3 ppm; converted to < 0.086 mg/m^3 (< 0.086–4.59 mg/m^3); 90th percentile, 13.2 mg/m^3]	Calculated by Working Group using only measurements with duration > 10 min	OSHA (2021)
Plastics products, USA, 1984–2020	Personal OSHA compliance measurements varying from 13 to 389 min, from 14 companies		150		[< 0.015 ppm (< 0.015–0.61 ppm); 90th percentile, 1.1 ppm; converted to < 0.086 mg/m^3 (< 0.086–3.50 mg/m^3; 90th percentile, 6.3 mg/m^3]	Calculated by Working Group using only measurements with duration > 10 min	
Blank books and loose-leaf binders, USA, 1984–2020	Personal OSHA compliance measurements varying from 26 to 153 min, from 3 companies		41		[< 0.015 ppm (< 0.015–1.1 ppm); 90th percentile, 1.9 ppm; converted to < 0.086 mg/m^3 (< 0.086–6.3 mg/m^3; 90th percentile, 10.9 mg/m^3]	Calculated by Working Group using only measurements with duration > 10 min	

GC, gas chromatography; INRS, Institut national de recherche et de sécurité; IQR, interquartile range; LOD, limit of detection; min, minute; ND, not detectable; NR, not reported; OSHA, Occupational Safety and Health Administration; PBZ, personal breathing zone; ppm, parts per million; SD, standard deviation; TWA, time-weighted average.

second visit. [Ventilation was present, but its effectiveness was unclear.] Most of the general area samples in the coating lines contained non-detectable concentrations of isophorone. A PBZ sample for the finish coater collected over 4 hours contained non-detectable concentrations of isophorone. Time-weighted average (TWA) concentrations for exposure to isophorone were estimated at 1.5 ppm [8.61 mg/m^3] for the prime coater, and 0.75 [4.30 mg/m^3] and 0.97 ppm [5.57 mg/m^3] for the finish coater, in short-term samples collected over 1–1.5 hours. PBZ samples collected over 5.5–6 hours showed non-detectable concentrations of isophorone for the prime coat operator and, in three out of four samples, for the finish coat operator (the fourth sample contained isophorone at a concentration of 0.74 ppm [4.25 mg/m^3]). Short-term samples collected over a period of less than 35 minutes for the unwind operator, rewind operator, and finish coat operator all contained non-detectable concentrations of isophorone in the PBZ samples. The coating line for a different product was evaluated on the second visit and showed a mean isophorone concentration of 1.0 ppm [5.74 mg/m^3] in PBZ samples across the various tasks. [The Working Group noted that sampling times were generally longer during the second visit, which may have improved the ability to detect isophorone in the PBZ samples.]

NIOSH measured occupational exposures to isophorone and other solvents in several screen-printing operations in the USA between 1978 and 1984. [The Working Group noted that these investigations were generally triggered by workers' reports of nausea, headache, or eye and nose irritation.] Measurements were made in these studies using PBZ or area air sampling onto charcoal tubes, which were then analysed using GC.

Several PBZ samples were collected in 1977 at a small specialty screen-printing operation in Pittsburgh, Pennsylvania, USA (NIOSH, 1979). Of seven samples, all except two had non-detectable concentrations of isophorone (0.30 ppm [1.72 mg/m^3] during screen printing in an unventilated area and 25.7 ppm [148 mg/m^3] in a very short-term sample while cleaning the screens [ventilation effectiveness was unclear]; the LOD was 0.3 mg per sample.) [The Working Group noted this was more than five times the short-term ceiling limit value in effect at that time, 5 ppm [28.7 mg/m^3].]

In 1980, NIOSH (1981) measured isophorone exposures in a company in Ridgefield, New Jersey, USA, employing 54 workers to screen-print, cut, laminate, and sew decals. Isophorone was a component of the printing ink and was also used directly in spray bottles as a "reducer" and on the printing screens as an anti-static coating. [The Working Group noted that employees using these sprays reported acute respiratory and neurological symptoms.] Isophorone was detected in the PBZ air of only the screen printers: their full-shift TWA (8 hours) concentrations were 0.7 and 14 ppm [4.0 and 80.4 mg/m^3]. It was noted that the ventilation was poorly designed.

In a small specialty printing company in Augusta, Georgia, USA, PBZ and area air samples were collected in 1982 for two screen-printing workers using gloss vinyl inks containing 35–40% isophorone (NIOSH, 1983). Ventilation was considered poorly designed. The median air concentration across eight PBZ and area samples was 1.3 ppm [7.5 mg/m^3], with the highest concentrations noted near the drying racks (area sample, 2.5 ppm [14 mg/m^3]) and while printing decals (PBZ sample, 3.4 ppm [20 mg/m^3]).

Lastly, NIOSH (1984) investigated exposures to isophorone and other solvents at a silk-screen printing vinyl-wallcovering manufacturer in Chicago, USA, in 1984. Isophorone (a solvent in the "retarder") was not detected during screen printing operations at this facility. [The Working Group noted that the full-shift air sampling rates were lower than those used in previous NIOSH studies, which could have affected the detection limit, which was not given for isophorone.]

A study focusing on isophorone exposures among screen-printing workers was carried out in a 34 000 ft² [3160 m²] facility in the USA [location unspecified] (Samimi, 1982). Isophorone was a main constituent of the inks and ink thinners, ranging from 10% to 75% among the various products, which were used to screen-print plastic, paper, or metal sheets at this mostly unventilated workplace. The product was dried in a ventilated dryer or hung to dry in a [presumably unventilated] room. It was noted that isophorone exposures were highest for workers involved in press operations, drying operations, ink formulation, and screen cleaning. The authors collected 78 short-term (50–90 minutes) PBZ air samples using charcoal tubes among workers expected to have highest solvent exposures. [The Working Group noted that the sampling and analytical methods used in this study were similar to those used in the above series of NIOSH studies.] TWA PBZ exposure concentrations were generally highest among printing press operators (mean, 23 ppm [132 mg/m³] and workers involved in paint mixing (mean, 17.8 ppm [102 mg/m³]), but mean concentrations for all workers were above 8 ppm [46 mg/m³]. It was also noted that PBZ concentrations were higher than corresponding area samples. [The Working Group noted that this is a typical finding for many solvents, aerosols, and particulates studied in occupational settings.]

[The Working Group noted that it was unclear how representative of current exposure conditions these 40-year-old studies of screen-printing workers are. However, it is notable that a recent publication identified isophorone as "the most widely used screen-printing ink solvent (comprising 75% of the total solvent)" (Kiurski et al., 2016).]

A small study was carried out of indoor air in a paper and cardboard printing company in Slovakia in 2015 (Vilcekova & Meciarova, 2016). Quantitative measurements were made for total VOCs in a location with a floor area of 144.3 m². Although no quantitative exposure levels were measured for individual VOCs, qualitative analysis was done for isophorone and 20 other VOCs by sampling with a zNose 4300 electronic nose in four cycles ["cycle" was not defined] (Meciarova et al., 2014). Total VOC concentrations fluctuated throughout the day and were typically above 40 mg/m³, with the highest levels (spiking above 120 mg/m³) seen in the latest part of the 8-hour day. Isophorone was one of the three most commonly occurring individual VOCs, appearing in all four cycles.

Cai et al. (2019) measured isophorone and several other ketones and aldehydes in workplace air of the production workspaces of 10 large-scale plastic-product manufacturers in China. Air concentrations for seven of the plants were reported as 0.0065 mg/m³ [the Working Group interpreted this to be the LOD], and the highest concentration measured at a plant was 2.1 mg/m³. [The Working Group noted that few details were provided about the sampling and analytical methods used or about the facilities themselves in this study. All measured values were noted by the authors to be well below occupational exposure limits in China.]

In addition to the printing and coating operations described above, isophorone has occasionally been reported in office settings. A recent study by Davis et al. (2019) found isophorone to be emitted in 8% of 3D printers tested. A NIOSH investigation (NIOSH, 2014) of a large government office complex in the USA in 2011 noted that grab samples had been historically collected for isophorone, but no information was provided on whether isophorone was detected.

1.4.3 Exposure of the general population

Only one study reporting quantitative measurements of isophorone in the general population was available. A case–control study (Hanai et al., 2012; Table 1.2) measured isophorone in the headspace of urine samples from

20 lung cancer cases and 20 healthy controls for the purpose of identifying novel diagnostic markers of lung cancer. A mean concentration of 130 nM [18 µg/L] was reported among control participants, and concentrations ranged from 39 to 1412 nM [5.4–195 µg/L]. It has been reported that the most likely routes of isophorone exposure in the general population are the inhalation of contaminated air and the ingestion of contaminated drinking-water (ATSDR, 2018), as well as direct contact with lacquers, paints, inks, and adhesives (US EPA, 2000), and exposure as a bystander during professional spraying and agrochemical use (ECHA, 2022). [The Working Group noted that while exposure to chemicals and products containing high concentrations of isophorone is probably higher in occupational settings, exposure to such products also occurs among hobbyists in the general population. However, there are substantial gaps in the published data on environmental exposures. The relative importance of exposure to other sources of isophorone described in this section (food, food packaging, and inflatables) remains to be quantified. It was further noted that, in many populations, isophorone exposure via drinking-water may be higher among people drinking bottled water due to migration from plastic bottles.]

1.5 Regulations and guidelines

1.5.1 Exposure limits and guidelines

(a) Occupational exposure limits

The American Conference of Governmental Industrial Hygienists (ACGIH, 2001) recommended a 15-minute ceiling limit of 28 mg/m^3 for isophorone. There is no occupational exposure limit for the European Union, although occupational exposure limits have been defined by some European Union countries. This is the case for Germany, which has a MAK value (maximum workplace concentration) of 11 mg/m^3 (IFA, 2021b). European Union Council Directives state that pregnant or breast-feeding workers and persons under age 18 years may not be occupationally exposed to isophorone (Council directive 92/85/EEC; Council Directive 94/33/EC; European Council, 1992, 1994). Table 1.5 summarizes the current occupational exposure limits for isophorone in some countries with an 8-hour time-weighted average (TWA), or 15-minute short-term or ceiling limits (IFA, 2021b). In the USA, NIOSH has established an "immediately dangerous to life and health" (IDLH) limit for isophorone of 200 ppm on the basis of acute inhalation toxicity data in humans (NIOSH, 1994).

(b) Environmental exposure limits

In the USA, there were insufficient data for derivation of minimal risk levels (MRL) for inhalation exposure. For oral exposure of intermediate and long-term duration, MRLs of 3 mg/kg per day and 0.2 mg/kg per day, respectively, were derived – the latter based on lesions of the liver, kidney, and gastrointestinal tract in mice (ATSDR, 2018). The US EPA recommended ambient water quality criteria (AWQC) for human health are 34 µg/L for consumption of water and aquatic organisms and 1800 µg/L for consumption of aquatic organisms only (US EPA, 2015). Low reliability guideline trigger values of 120 µg/L for freshwater and 130 µg/L for marine water were derived in Australia and New Zealand (Australian and New Zealand Environment and Conservation Council, 2000).

Isophorone is a hazardous substance. According to the harmonized classification and labelling implemented in the European Union (Classification, Labelling and Packaging Regulation), isophorone is classified as a suspected human carcinogen on the basis of limited evidence of carcinogenicity in human studies and limited evidence of carcinogenicity in animal studies, i.e. carcinogen, category 2; acute toxicity, category 4; and is subject to several substance control regulations on general product

Table 1.5 Occupational exposure limits for isophorone in various countries

Country	8-hour TWA		Short-term (15 min)		Ceiling		Reference
	ppm	mg/m³	ppm	mg/m³	ppm	mg/m³	
Argentina	5	25	5	25			NICNAS (2013)
Australia					5	28	IFA (2021b)
Austria	2	11	2	11			IFA (2021b)
Belgium					5	28	IFA (2021b)
Canada – province of Ontario					5		IFA (2021b)
Canada – province of Quebec					5	28	IFA (2021b)
Chile					5	28	NICNAS (2013)
China						30	IFA (2021b)
Denmark	5	25			5	25	IFA (2021b)
Finland	1	5.7					IFA (2021b)
France					5	25	IFA (2021b)
Germany – AGS[a]	2	11	4	22			IFA (2021b)
Germany – DFG[a]	2	11	4	22			IFA (2021b)
Greece	5	25	5	25			NICNAS (2013)
ILO and WHO	2[c]	11[c]	5	29			ILO & WHO (2021)
Ireland[b]			5	25			IFA (2021b)
New Zealand					5	28	IFA (2021b)
Norway					5	25	IFA (2021b)
Poland		5				10	IFA (2021b)
Republic of Korea					5	25	IFA (2021b)
Romania	4.42	25	8	50			IFA (2021b)
Singapore			5	28			IFA (2021b)
South Africa			5	25			NICNAS (2013)
Spain			5	29			IFA (2021b)
Sweden			5	30			IFA (2021b)
Switzerland	2	11	4	22			IFA (2021b)
United Kingdom			5	29			IFA (2021b)
USA – ACGIH					5	28	NIOSH (1994)
USA – Cal/OSHA	4	23					OSHA (2020)
USA – NIOSH	4	23					IFA (2021b)
USA – OSHA	25	140					IFA (2021b)

ACGIH, American Conference of Governmental Industrial Hygienists; AGS, Ausschuss für Gefahrstoffe (German Committee on Hazardous Substances); Cal/OSHA, California Division of Occupational Safety and Health; DFG, Deutsche Forschungsgemeinschaft; ILO, International Labour Organization; JSOH, Japan Society for Occupational Health; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; ppm, parts per million; TWA, time-weighted average.

[a] Inhalable fraction and vapour.

[b] 15 min reference period.

[c] MAK, the maximum concentration in the workplace air which generally does not have known adverse effects on the health of the employee nor cause unreasonable annoyance (e.g. by a nauseous odour) during 8 h daily, assuming an average work week of 40 h.

safety, including medical devices and a ban from use in any cosmetic products and food contact materials in the European Union (ECHA, 2021b).

1.5.2 Reference values for biological monitoring of exposure

No reference values related to isophorone biological monitoring were available.

1.6 Quality of exposure assessment in key epidemiological studies of cancer

There was one case–control study on brain and other central nervous system (CNS) cancers available for review by the Working Group. The study was nested within a cohort of workers at three facilities manufacturing semi-conductor and electronic storage devices in the USA (Rodrigues et al., 2020). Further details on the exposure assessment for this study were also provided in Rodrigues et al. (2019).

Details on the selected domains of the exposure assessment review for these studies are summarized in Table S1.6 (Annex 1, Supplementary material for isophorone, Section 1, Exposure Characterization, available from: https://publications.iarc.fr/611).

1.6.1 Exposure assessment methods

The exposure assessment for the study by Rodrigues et al. (2020) was completed by conducting site visits at the three facilities and compiling more than 700 000 documents with site-specific job, task, and process information, and an industrial hygiene database with more than 10 000 samples across 31 chemicals and dusts of interest (including isophorone). Exposure was assigned by group exposure matrices and time period ("manufacturing era" to record periods where processes remained relatively stable) using division, department, and job title coupled with the sampling data, which were used to assign a mean exposure.

1.6.2 Critical review of exposure assessment methods

The exposure assessment for the study by Rodrigues et al. (2020) was well described for the cohort overall, and it was a key strength that such detailed and extensive site-specific information and a large database of industrial hygiene measurements were available to assign mean exposure by work group and over manufacturing eras. In the earlier work, the authors identified a confidence for each exposure estimate (Rodrigues et al., 2019). [The Working Group noted that although confidence estimates were apparently available, they were not used in the later analysis (Rodrigues et al., 2020).] The number of samples used for isophorone in particular was not reported (although availability of at least 20 samples across all three facilities was a criterion for inclusion) and so the strength of the exposure assessment for isophorone exposure with respect to hygiene data was difficult to evaluate. In addition, all 126 836 employees were categorized into 1 of 10 exposure groups per manufacturing era (three eras per facility), so there was likely to be substantial heterogeneity within each group. Job histories were only available for the parts of workers' careers that were spent at these facilities; this may not be a weakness if workers typically spent most or all of their career in these facilities, but if they spent significant time periods employed in other jobs, there could be an issue with exposure misclassification. An additional weakness was that industrial hygiene measurements were not randomly collected, such that their generalizability across the combination of chemical, facility, exposure group, and era was unclear.

2. Cancer in Humans

2.1 Cohort study

See Table 2.1.

Rodrigues et al. (2020) conducted a case–control study nested in a cohort of workers at three facilities manufacturing semiconductor and electronic storage devices located in East Fishkill in New York, Burlington in Vermont, and San José in California, USA. The study evaluated associations between CNS cancer and exposure to 31 agents of interest, including isophorone.

Deceased cases with a malignant CNS neoplasm were identified via National Death Index records or from death certificates. Incident cases of CNS cancer and date of diagnosis were identified through record linkage with the NY and CA state cancer registries. Ten controls per case were selected using incidence density sampling, matched on year of birth, facility, sex, and race. Ten primary exposure groups (PEGs) were created on the basis of type of production, tasks performed, and work environment with potential for exposure to chemical and physical agents. Mean concentrations were estimated for each chemical agent in each PEG using industrial hygiene data from the three facilities. Changes in the work environment over time were accounted for by use of manufacturing eras (i.e. PEG-exposure matrix by era for each chemical) when exposures associated with work processes remained relatively stable. Cumulative exposures to each chemical were estimated using work history variables, including department and job title assigned to one of the 10 PEGs and work start and end date in each facility and division, and mean concentrations for each exposure matrix cell (based on chemical, PEG, and era). Odds ratios were estimated for the risk of CNS cancer (incidence and mortality combined) using conditional logistic regression models, controlling for the matching variables and stratifying by facility. Exposure categories were tertiles of mg/m^3-years and the reference category was employees with no exposure to the particular chemical. The 120 CNS cancer cases and 1028 controls who had worked only at one facility were mostly male (88%) and White (93%).

Among the 1137 workers (119 cases and 1018 controls) in the analyses with cumulative exposure tertile, 728 (64%) were ever exposed to isophorone, including 239 in the highest tertile (> 5.09 mg/m^3-years) and 78 of the 119 included cases. Odds ratios were non-statistically significantly elevated in the highest tertile of cumulative exposure for isophorone at two of the three facilities. At one of the facilities (San José, CA), odds ratios were elevated in all three tertiles of exposure, although none were statistically significant. None of the tests for trend for isophorone (including at the San José facility) were statistically significant. [A strength of the study was the quantitative exposure assessment with detailed work history information and historical industrial hygiene data, although as the authors pointed out, none of the 31 chemicals identified as carcinogens or possible carcinogens is specifically a known cause of CNS cancer in humans. Isophorone results were presented in a table but not described by the authors in the text, because only chemicals with largely elevated odds ratios were discussed. The authors cited the small number of exposed cases and controls as a limitation but did not conduct analyses with three facilities combined controlling for this matching variable. Only stratified analyses by facility controlling for other three matching variables were conducted to "evaluate the internal consistency of results".]

2.2 Evidence synthesis for cancer in humans

One epidemiological study (Rodrigues et al., 2020) has been conducted on the carcinogenicity of isophorone. The study was a case–control

Table 2.1 Cohort studies on exposure to isophorone and cancer

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Rodrigues et al. (2020) East Fishkill (NY), Burlington (VT) and San José (CA), USA Deceased cases, 1965–1999; incident cases, 1976–1999 Nested case–control	Cases: 120 deceased cases of malignant neoplasms of the CNS were identified via National Death Index records or from death certificates. Incident cases of CNS cancer were identified through record linkage with the NY and CA state cancer registries. Population/eligibility characteristics: cases and controls nested in the cohort of 126 836 employees included in the 1965–1999 mortality study at all three facilities and 1976–1999 study of cancer incidence among 89 054 workers at NY and CA facilities Controls: 1028; 10 controls per case (alive at the time of the case's index date) were selected using incidence-density sampling from the cohort, matched on year of birth, facility, sex, and race Exposure assessment method: expert judgement; detailed and site-specific information obtained from site visits combined with hygiene measurement data to create work group-exposure matrices specific to manufacturing era	CNS, incidence	Cumulative exposure tertile, East Fishkill (NY) facility (OR): 0 > 0 to < 1.69 mg/m³-years 1.70–5.09 mg/m³-years > 5.09 mg/m³-years Trend-test *P* value, 0.73	 25 14 8 6	 1 1.33 (0.65–2.72) 0.70 (0.31–1.62) 1.05 (0.41–2.73)	Year of birth, sex, race	*Exposure assessment critique*: occupational histories are specific just to company jobs (information on other jobs held was not provided) but the impact of this is difficult to discern. There were no measurements specific to the worker, but industrial hygiene data for the specific locations was used to quantify exposure. A key strength was the detailed information on individual jobs. The main limitation is the potential for exposure misclassification, as the industrial hygiene samples were not randomly collected; instead, they were collected based on a combination of chemical, facility, exposure group, and era. It was unclear if this is particularly a problem for isophorone vs the other chemicals assessed. Regression models of isophorone and cumulative exposure tertile excluded 1 case and 10 controls, presumably for missing exposure information. *Other comments*: conducted sensitivity analysis with 5 yr exposure lag. *Strengths*: quantitative exposure assessment with detailed work history information and historical industrial hygiene data.
		CNS, incidence	Cumulative exposure tertile, Burlington (VT) facility (OR): 0 > 0 to < 1.69 mg/m³-years 1.70–5.09 mg/m³-years > 5.09 mg/m³-years Trend-test *P* value, 0.52	 8 2 4 3	 1 0.75 (0.15–3.71) 1.18 (0.32–4.39) 1.27 (0.27–6.06)	Year of birth, sex, race	

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site (histopathology), incidence or mortality	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Rodrigues et al. (2020) East Fishkill (NY), Burlington (VT) and San José (CA), USA Deceased cases, 1965–1999; incident cases, 1976–1999 Nested case–control (cont.)		CNS, incidence	Cumulative exposure tertile, San José (CA) facility (OR):			Year of birth, sex, race	*Limitations*: small number of exposed cases (including highly exposed cases in analysis by facility) and controls; stratified analyses by facility only and no analyses with all three facilities together; potential co-exposures to other occupational agents was also of concern.
			0	8	1		
			> 0 to < 1.69 mg/m³-years	13	1.45 (0.56–3.71)		
			1.70–5.09 mg/m³-years	13	1.60 (0.61–4.18)		
			> 5.09 mg/m³-years	15	1.18 (0.46–2.99)		
			Trend-test *P* value, 0.33				

CA, California; CI, confidence interval; CNS, central nervous system; NY, New York; OR, odds ratio; vs, versus; VT, Vermont.

study on exposure to 31 occupational agents and fatal and incident CNS cancers nested in a cohort of employees at three facilities. Cumulative occupational exposure to isophorone was estimated based on work history variables and estimates of mean concentrations from industrial hygiene data for 10 PEGs. Approximately 64% of the 1137 participants included (cases and controls) were ever exposed to isophorone (78 of 199 included cases). There were some weak positive associations observed in some categories of cumulative exposure in analysis stratified by three facilities, although none was significant and there was no trend. Although detailed and quantitative exposure estimation was performed, limitations included small numbers of exposed cases, including highly exposed cases in analysis by facility. Potential co-exposure to other occupational agents was also of concern.

3. Cancer in Experimental Animals

See Table 3.1.

3.1 Mouse

Oral administration (gavage)

In a well-conducted study that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female B6C3F$_1$ mice (age, 6–8 weeks) were given isophorone (purity, ≥ 94%) at a dose of 0, 250, or 500 mg/kg body weight (bw) in corn oil, for the control group and the groups at the lower and higher dose, respectively, by gavage, 5 days per week, for 103 weeks (NTP, 1986). At study termination, survival was 16/50, 16/50, and 19/50 in males, and 26/50, 35/50, and 34/50 in females, for the control group and the groups at the lower and higher dose, respectively. In females, there was a significant positive trend in survival in females, and the survival rates at both doses were significantly higher than that of the controls. In males, the survival rates of the treated groups were similar to that of the control group. A significant decrease in body-weight gain was observed in females at the higher dose (about 5% lower at the end of the exposure period compared with controls). No significant difference in body weight was observed in females at the lower dose or in males at either dose. All mice (except one missing male mouse in the control group) underwent complete necropsy, and histopathological examination was performed on all gross lesions, and main tissues and organs.

In male mice, there was a significant positive trend in the incidence of hepatocellular adenoma or carcinoma (combined) (P = 0.027, incidental tumour trend test; P = 0.025, Cochran–Armitage trend test), with incidence being significantly increased at the higher dose: control, 18/48 (37%); lower dose, 18/50 (36%); and higher dose, 29/50 (58%); P = 0.033, Fisher exact test. The incidence of hepatocellular adenoma was 6/48, 7/50, and 13/50, and the incidence of hepatocellular carcinoma was 14/48 (29%), 13/50 (26%), and 22/50 (44%), for the control group and the groups at the lower and higher dose, respectively. The incidence of hepatocellular carcinoma and of hepatocellular adenoma or carcinoma (combined) in males at the higher dose exceeded the upper bound of the ranges observed in historical controls in this laboratory – hepatocellular carcinoma, 218/1034 (mean ± standard deviation, 21.1 ± 7.6%); range, 8.3–36%; and hepatocellular adenoma or carcinoma (combined), 335/1034 (32.4 ± 9.4%); range, 14–50%. There was a significant positive trend in the incidence of fibrosarcoma of the subcutis (P = 0.044, life-table trend test; P = 0.019, incidental tumour trend test; P = 0.023, Cochran–Armitage trend test), with the increase in incidence being significant at the higher dose: control, 3/48; lower dose, 4/50; higher dose, 10/50; P = 0.042, Fisher exact test; P = 0.009, incidental tumour test. The incidence of fibroma of the subcutis was 0/48, 2/50, and 3/50, for the control group and the groups

Table 3.1 Studies of carcinogenicity in experimental animals exposed to isophorone

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6–8 wk 103 wk NTP (1986)	Oral administration (gavage) Isophorone, ≥ 94% Corn oil 0, 250, 500 mg/kg bw 1×/day, 5 days/wk, 103 wk 50, 50, 50 16, 16, 19	*Liver* Hepatocellular adenoma 6/48, 7/50, 13/50 Hepatocellular carcinoma 14/48, 13/50, 22/50 (44%) Hepatocellular adenoma or carcinoma (combined) 18/48, 18/50, 29/50 (58%)* *Subcutis* Fibroma 0/48, 2/50, 3/50 Fibrosarcoma 3/48, 4/50, 10/50 (20%)*	NS NS *P* = 0.027, incidental tumour trend test; *P* = 0.025, Cochran–Armitage trend test **P* = 0.033, Fisher exact test NS *P* = 0.044, life-table trend test; *P* = 0.019, incidental tumour trend test; *P* = 0.023, Cochran–Armitage trend test **P* = 0.042, Fisher exact test; *P* = 0.009, incidental tumour test	Principal strengths: well-conducted study that complied with GLP; used multiple doses; used males and females; adequate number of animals per group; adequate duration of exposure and observation Principal limitations: one control animal missing Other comments: survival rates of treated animals were similar to those of controls Historical controls: hepatocellular carcinoma, 218/1034 (21.1% ± 7.6%; range, 8.3–36%); hepatocellular adenoma or carcinoma (combined), 335/1034 (32.4% ± 9.4%; range, 14–50%); integumentary system (skin and subcutis: fibroma, fibrosarcoma, neurofibrosarcoma or sarcoma, combined), 70/1040 (6.7% ± 6.5%; range, 0–22%); subcutis fibrosarcoma, 28/1040 (2.7% ± 4.0%; range, 0–16.7%); lymphoma or leukaemia (combined) of the lymphohaematopoietic system, 132/1040 (12.7% ± 5.9%; range, 2.1–27.1%); and lymphoma of the lymphohaematopoietic system, 126/1040 (12.1% ± 5.1%; range, 2.1–22.0%)

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6–8 wk 103 wk NTP (1986) (cont.)		*Integumentary system (skin and subcutis)*: fibroma, fibrosarcoma, neurofibrosarcoma, or sarcoma (combined) 6/48, 8/50, 14/50 (28%)*	*P* = 0.034, incidental tumour trend test; *P* = 0.033, Cochran–Armitage trend test **P* = 0.048, Fisher exact test; *P* = 0.050, incidental tumour test	
		Lymphohaematopoietic system: malignant lymphoma 7/48, 18/50 (36%)*, 5/50	**P* = 0.013, Fisher exact test	
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6–8 wk 103 wk NTP (1986)	Oral administration (gavage) Isophorone, ≥ 94% Corn oil 0, 250, 500 mg/kg bw 1×/day, 5 days/wk, 103 wk 50, 50, 50 26, 35, 34	No significant increase in tumour incidence in treated animals		Principal strengths: well-conducted study that complied with GLP; used multiple doses; used males and females; adequate number of animals per group; adequate duration of exposure and observation Other comments: significant positive trend in survival

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Tumour incidence	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 6–7 wk 103 wk NTP (1986)	Oral administration (gavage) Isophorone, ≥ 94% Corn oil 0, 250, 500 mg/kg bw 1×/day, 5 days/wk, 103 wk 50, 50, 50 33, 33, 14	*Kidney* Tubular cell adenoma 0/50, 0/50, 2/50 Tubular cell adenocarcinoma 0/50, 3/50, 1/50 Tubular cell adenoma or tubular cell adenocarcinoma (combined) 0/50, 3/50 (6%), 3/50 (6%)* *Preputial gland*: carcinoma 0/50, 0/50, 5/50 (10%)*	 NS NS $P = 0.014$, life-table trend test; $P = 0.034$, incidental tumour trend test *$P = 0.025$, life-table test $P = 0.002$, life-table trend test; $P = 0.019$, incidental tumour trend test; $P = 0.006$, Cochran–Armitage trend test *$P = 0.028$, Fisher exact test; $P = 0.012$, life-table test	Principal strengths: well-conducted study that complied with GLP; used multiple doses; used males and females; adequate number of animals per group; adequate duration of exposure and observation Principal limitations: accidental kill by gavage errors was 4/50, 5/50, and 6/50 Other comments: there was a significant trend for decreased survival, and the survival rate at 500 mg/kg bw was decreased compared with controls Historical controls: tubular cell adenoma or tubular cell adenocarcinoma (combined) of the kidney, 4/1091 (0.4%); preputial gland tumours, 38/1094 (3.5%; range, 0–14%); and preputial gland carcinoma, 19/1094 (1.7%)
Full carcinogenicity Rat, F344/N (F) 6–7 wk 103 wk NTP (1986)	Oral administration (gavage) Isophorone, ≥ 94% Corn oil 0, 250, 500 mg/kg bw 1×/day, 5 days/wk, 103 wk 50, 50, 50 30, 23, 20	No significant increase in tumour incidence in treated animals		Principal strengths: well-conducted study that complied with GLP; used multiple doses; used males and females; adequate number of animals per group; adequate duration of exposure and observation Principal limitations: accidental kill by gavage errors was 1/50, 6/50, and 14/50 Other comments: survival rates were similar to those of controls

bw, body weight; F, female; GLP, Good Laboratory Practice; M, male; NS, not significant; wk, week.

at the lower and higher dose, respectively. In the subcutis, one sarcoma was observed at the higher dose, and one neurofibrosarcoma was observed in controls. In the skin, the incidence of fibroma was 2/48, 1/50, and 0/50, for the control group and the groups at the lower and higher dose, respectively; and one neurofibrosarcoma was observed at the lower dose. There was a significant positive trend ($P = 0.034$, incidental tumour trend test; $P = 0.033$, Cochran–Armitage trend test) in the incidence of mesenchymal tumours (fibroma, fibrosarcoma, neurofibrosarcoma or sarcoma, combined) of the integumentary system (skin and subcutis, combined) with the incidence being significantly increased at the higher dose: control, 6/48 (12%); lower dose, 8/50 (16%); and higher dose, 14/50 (28%); $P = 0.048$, Fisher exact test; $P = 0.050$, incidental tumour test. In addition, the incidence of mesenchymal tumours (fibroma, fibrosarcoma, neurofibrosarcoma, or sarcoma, combined) of the integumentary system in male mice at the higher dose exceeded the upper bound of the range observed in historical controls in this laboratory – 70/1040 (6.7 ± 6.5%); range, 0–22%. There was a significant increase in the incidence of malignant lymphoma of the lymphohaematopoietic system at the lower dose: control, 7/48 (15%); lower dose, 18/50 (36%); higher dose, 5/50 (10%); $P = 0.013$, Fisher exact test. The incidence of malignant lymphoma of the lymphohaematopoietic system at the lower dose exceeded the upper bound of the range observed in historical controls in this laboratory – 126/1040 (12.1 ± 5.1%); range, 2.1–22.0%.

In female mice, there was no significant increase in tumour incidence in any of the treated groups compared with controls (NTP, 1986).

[The Working Group noted this was a well-conducted GLP study that used multiple doses, an adequate number of animals per group, an adequate duration of exposure and observation, and males and females.]

3.2 Rat

3.2.1 Oral administration (gavage)

In a well-conducted GLP study, groups of 50 male and 50 female F344/N rats (age, 6–7 weeks) were given isophorone (purity, ≥ 94%) at a dose of 0, 250, or 500 mg/kg bw in corn oil, for the control group and the groups at the lower and higher dose, respectively, by gavage, 5 days per week, for 103 weeks (NTP, 1986). There was a significant negative trend in survival in males; survival at study termination was: 33/50, 33/50, and 14/50 in males, and 30/50, 23/50, and 20/50 in females, for the control group and the groups at the lower and higher dose, respectively. The survival rate was significantly decreased in males at the higher dose. Gavage errors accounted for all of the 36 accidental deaths of male and female rats. Deaths related to gavage error increased with dose in females. A decrease in body weight was observed in males and females at the higher dose (7% lower at the end of the exposure period). All rats underwent complete necropsy, and histopathological examination was performed on all gross lesions, and main tissues and organs.

In male rats, there was a significant positive trend in the incidence of tubular cell adenoma or tubular cell adenocarcinoma (combined) of the kidney ($P = 0.014$, life-table trend test; $P = 0.034$, incidental tumour trend test), with incidence being significantly increased at the higher dose: control 0/50, lower dose, 3/50 (6%); higher dose, 3/50 (6%); $P = 0.025$, life-table test. The incidence of tubular cell adenoma or carcinoma (combined) of the kidney in both treated groups (3/50, 6%) was higher by 15-fold than the incidence reported for historical controls in this laboratory (4/1091, 0.4%). [The range of incidence of kidney tubular cell tumours in the historical control groups was not reported.] The incidence of tubular cell adenoma of the kidney was 0/50, 0/50, and 2/50, and the incidence of tubular cell adenocarcinoma of the kidney was 0/50, 3/50,

and 1/50, for the control group and the groups at the lower and higher dose, respectively. There was a significant positive trend in the incidence of carcinoma of the preputial gland (P = 0.002, life-table trend test; P = 0.019, incidental tumour trend test; P = 0.006, Cochran–Armitage trend test), with the incidence being significantly increased at the higher dose – control, 0/50; lower dose, 0/50; and higher dose, 5/50 (10%); P = 0.028 Fisher exact test; P = 0.012, life-table test. The incidence of preputial cell carcinoma in historical controls was 19/1094 (1.7%). [The range of incidence of preputial gland carcinoma in historical control groups was not reported.]

Regarding non-neoplastic lesions in male rats, the incidence of tubular cell hyperplasia of the kidney was 0/50, 1/50, and 4/50, for the control group and the groups at the lower and higher dose, respectively.

In female rats, there was no significant increase in tumour incidence in any of the treated groups compared with controls (NTP, 1986).

[The Working Group noted that this was a well-conducted GLP study that used multiple doses, an adequate number of animals per group, an adequate duration of exposure and observation, and males and females. The Working Group also noted the high number of accidental gavage-related deaths in males and females in this study.]

3.2.2 Inhalation

Two groups of 10 male and 10 female Wistar rats [age not reported; body weight, approximately 140 g at 2 weeks before exposure] were exposed by inhalation (whole-body exposure) to isophorone at a concentration of 0 (control) or 250 ppm [1413 mg/m^3] for 6 hours per day, 5 days per week, for 18 months. In treated rats, slight conjunctivitis and irritation of the nasal mucosa with a bloody discharge were observed. Frequent haemorrhages were found with oedema in the alveoli of the lungs, and microvacuolization was found in the liver. Tumour incidence was not reported (Dutertre-Catella, 1976). [The Working Group noted that this study was inadequate for the evaluation of the carcinogenicity of isophorone in experimental animals due to incomplete reporting, and lack of details regarding the study design, postmortem evaluation, and tumour incidence.]

3.3 Evidence synthesis for cancer in experimental animals

The carcinogenicity of isophorone has been assessed in one well-conducted GLP study in male and female B6C3F$_1$ mice (NTP, 1986) and in one well-conducted GLP study in F344/N rats (NTP, 1986) treated by oral administration (gavage), and in one inhalation (whole-body exposure) study in male and female Wistar rats (Dutertre-Catella, 1976).

In the GLP study in male and female B6C3F$_1$ mice treated by gavage, there was a significant positive trend in the incidence of hepatocellular adenoma or carcinoma (combined) in male mice, with the incidence being significantly increased at the higher dose. There was a significant positive trend in the incidence of fibrosarcoma of the subcutis in male mice, with the incidence being significantly increased at the higher dose. There was a significant positive trend in the incidence of mesenchymal tumours (fibroma, fibrosarcoma, neurofibrosarcoma or sarcoma, combined) of the integumentary system (skin and subcutis, combined) in male mice, with the incidence being significantly increased at the higher dose. A significantly increased incidence of malignant lymphoma of the lymphohaematopoietic system in male mice was also reported at the lower dose. In female mice, there was no significant increase in tumour incidence in any of the treated groups compared with controls (NTP, 1986).

In the GLP study in male and female F344/N rats treated by gavage, there was a significant

positive trend in the incidence of tubular cell adenoma or tubular cell adenocarcinoma (combined) of the kidney in male rats, with the incidence being significantly increased at the higher dose. There was a significant positive trend in the incidence of carcinoma of the preputial gland, with the incidence being significantly increased at the higher dose. In female rats, there was no significant increase in tumour incidence in any of the treated groups compared with controls (NTP, 1986).

The one inhalation study in Wistar rats was judged to be inadequate for the evaluation of the carcinogenicity of isophorone in experimental animals (Dutertre-Catella, 1976).

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

Only one study was performed to study dermal absorption of isophorone in human skin. Low permeability (< 20%) was demonstrated, and no potential to damage the skin was observed after dermal application of isophorone (25 mg/mL) for 60 minutes (Fasano & McDougal, 2008). Urinary isophorone was detected at higher concentrations in 20 lung cancer patients than in 20 healthy control volunteers (Hanai et al., 2012). [The Working Group noted that there was pharmacokinetic disposition, but the significance of this study was not clear.]

4.1.2 Experimental systems

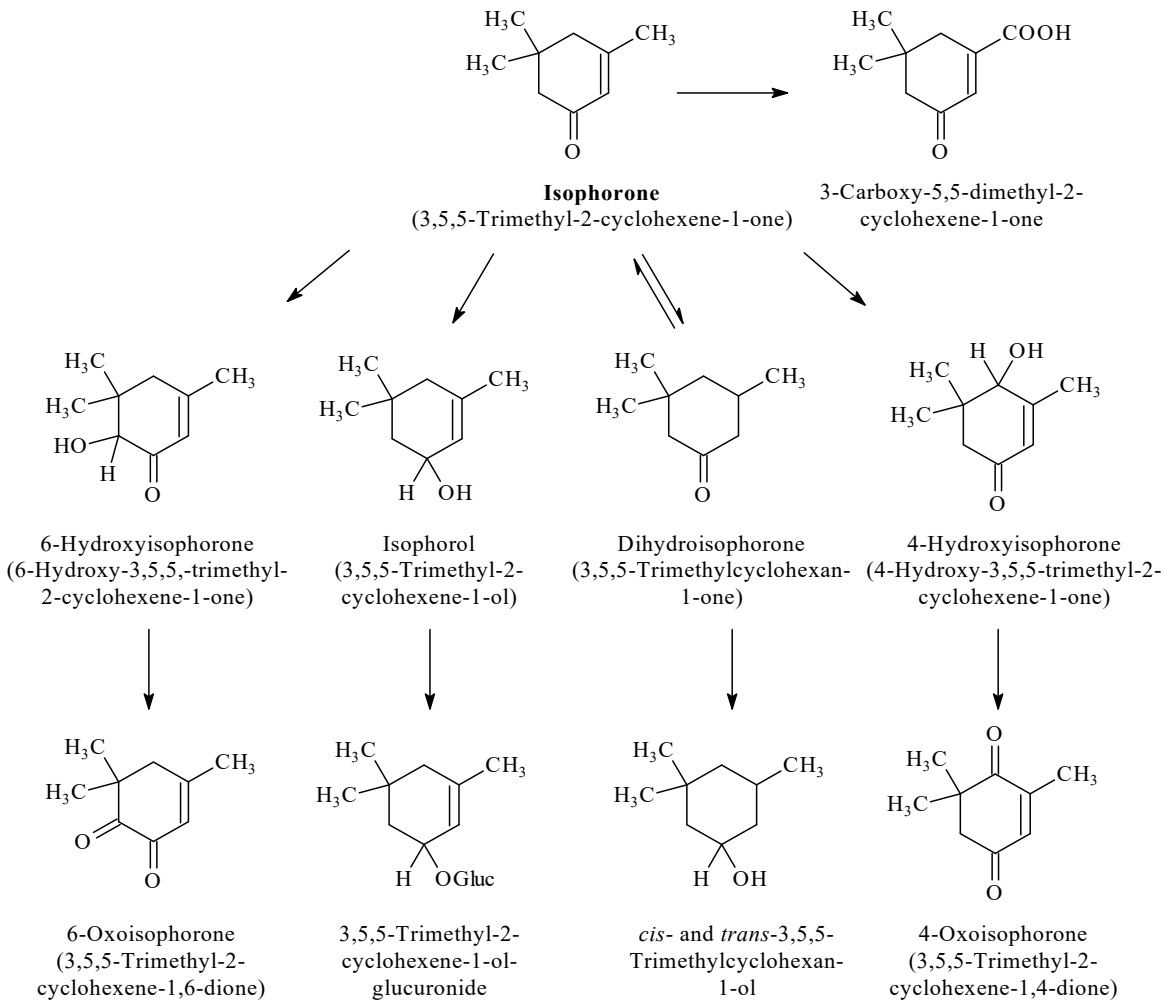
(a) Absorption and distribution

Studies in mice, rats, and rabbits show that isophorone is absorbed after inhalation, and after oral and dermal exposure (Dutertre-Catella et al., 1970, 1978; Dutertre-Catella, 1976). In six rats and one rabbit treated with isophorone (4 g/kg bw) by gavage, isophorone was detected 1 hour after treatment in the stomach, pancreas, and adrenal glands of both species, at concentrations ranging from 0.3 to 6.3 µg/g tissue wet weight. In six rats treated with isophorone (400 ppm, 2 mg/m^3) by inhalation for 4 hours, isophorone was detected in the kidneys, adrenal glands, liver, pancreas, and brain; levels in the kidneys were higher in males than in females. The presence of isophorone in all these organs decreased rapidly 1 hour after the end of the inhalation exposure (Dutertre-Catella, 1976). In another experiment in six rats treated by gavage, isophorone (1 g/kg bw) could not be detected in any organ 48 hours after treatment. In two rabbits treated with isophorone (1 g/kg bw) by gavage, most of the administered dose was detected in the blood at 10 minutes to 1 hour after treatment, with concentrations decreasing rapidly thereafter (to 50% of the administered dose at 3 hours and trace amounts at 21 hours) (Dutertre-Catella, 1976).

(b) Metabolism and excretion

Metabolites of isophorone have been identified in the urine of animals exposed to isophorone by oral administration (Dutertre-Catella et al., 1970, 1978; Dutertre-Catella, 1976). In rabbits and rats treated with isophorone (1 g/kg bw) by gavage, the following metabolites were identified in the urine: 3,5,5-trimethyl-2-cyclohexene-1-ol (isophorol) and its glucuronic conjugate; 3-carboxy-5,5-dimethyl-2-cyclohexene-1-one; 3,5,5-trimethylcyclohexan-1-one (dihydroisophorone); and *cis*- and *trans*-3,5,5-trimethylcyclohexan-1-ol (Dutertre-Catella, 1976; Dutertre-Catella et al., 1978). After isophorone ingestion, more dihydroisophorone and less isophorol was found in rat urine than in rabbit urine. Truhaut et al. (1973) identified isophorone in the urine of rats and rabbits given 3,5,5-trimethylcyclohexan-1-one (dihydroisophorone) (1 g/kg bw) by gavage (Truhaut et al.,

Fig. 4.1 Metabolic scheme for isophorone



Gluc, glucuronide.
Created by the Working Group.

1973). Dutertre-Catella (1976) and Dutertre-Catella et al. (1978) proposed that the metabolism of isophorone involves methyl oxidation to 3-carboxy-5,5-dimethyl-2-cyclohexene-l-one, reduction of the ketone group to isophorol, reduction of the ring double bond to dihydroisophorone, and dismutation of dihydroisophorone to *cis*- and *trans*-3,5,5-trimethylcyclohexan-1-ol (Dutertre-Catella, 1976; Dutertre-Catella et al., 1978). The metabolic pathways of isophorone are presented in Fig. 4.1. [The Working Group noted that the enzymes implicated in the metabolism of isophorone in rodents have not been identified.]

Studies in rats and rabbits suggest that urine is the predominant route of elimination of isophorone (Dutertre-Catella et al., 1970, 1978; Truhaut et al., 1973; Dutertre-Catella, 1976). After oral administration of isophorone, rats and rabbits excreted unchanged isophorone and metabolites in the urine, and unchanged isophorone in the expired air. Forty-eight hours after ingestion of dihydroisophorone (a metabolite of isophorone) by rats or rabbits, an estimated 50–70% of the

administered dose was present as glucuronic conjugates in the urine (Dutertre-Catella, 1976; Dutertre-Catella et al., 1978). [The Working Group noted that the rate and extent of excretion were not reported.]

4.2 Evidence relevant to key characteristics of carcinogens

4.2.1 Is genotoxic

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.1.

Intraperitoneal injection of isophorone did not result in micronucleus formation in bone marrow cells (polychromatic erythrocytes) of male and female CD-1 mice (O'Donoghue et al., 1988).

(ii) Non-human mammalian cells in vitro

See Table 4.2.

Tests for unscheduled DNA synthesis in rat primary hepatocytes treated with isophorone were reported to give weakly positive results in two studies (Selden et al., 1994) [the Working Group noted the use of a non-standard protocol in one of the studies], although the same test system gave a negative result in a third study (O'Donoghue et al., 1988). Gene mutation studies in mouse L5178Y/*Tk*$^{+/-}$ lymphoma cells treated with isophorone (at concentrations ranging from 400 to 1500 µg/mL) gave one positive result without metabolic activation at non-cytotoxic concentrations (McGregor et al., 1988), three additional positive results without metabolic activation (NTP, 1986; Tennant et al., 1987; Honma et al., 1999b) [but the Working Group noted concerns over cytotoxicity and/or study design], and three studies gave negative or equivocal results with and without metabolic activation (O'Donoghue et al., 1988; Sofuni et al., 1996; Honma et al., 1999a). Tests for chromosome aberrations were reported to give positive results with and without metabolic activation in one study in Chinese hamster lung fibroblast cells (Matsuoka et al., 1996) [the Working Group noted that a non-standard protocol was used and no indication of cytotoxicity was provided], but negative results were reported in three additional studies in Chinese hamster ovary cells (with isophorone at concentrations up to 1600 µg/mL) with and without metabolic activation (NTP, 1986; Tennant et al., 1987; Gulati et al., 1989). In Chinese hamster ovary cells, tests for sister-chromatid exchange were reported to give positive results without metabolic activation in two studies (Tennant et al., 1987; Gulati et al., 1989) [the Working Group noted that no indication of cytotoxicity was provided], but results were negative with or without metabolic activation (with isophorone at concentrations of up to 1000 µg/mL) in a third study (NTP, 1986).

(iii) Non-mammalian experimental systems

See Table 4.3.

Isophorone did not induce micronucleus formation in a hen's egg test (Greywe et al., 2012). Isophorone exposure by feeding or injection gave negative results in the sex-linked recessive lethal test in *Drosophila melanogaster* (Foureman et al., 1994). Isophorone did not induce mutation in *Salmonella typhimurium* in the presence or absence of metabolic activation (Mortelmans et al., 1986; NTP, 1986; Tennant et al., 1987; Kubo et al., 2002).

4.2.2 Evidence relevant to other key characteristics

(a) Humans

No data were available to the Working Group.

Table 4.1 Genetic and related effects of isophorone 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, CD-1 (M, F)	Bone marrow, polychromatic erythrocytes	–	0.54 mL/kg [500 mg/kg bw]	Intraperitoneal injection, mice killed after 12, 24, and 48 h	Only one dose tested	O'Donoghue et al. (1988)
DNA binding	Mouse, $B6C3F_1$ (M, F)	Liver, kidney	–	500 mg/kg bw	Gavage, mice killed after 24 h	Non-standard assay, only one dose tested	Thier et al. (1990)
DNA binding	Rat, F344 (M, F)	Liver, kidney	–	500 mg/kg bw	Gavage, rats killed after 24 h	Non-standard assay, only one dose tested	Thier et al. (1990)

bw, body weight; F, female; HID, highest ineffective dose; LED, lowest effective dose; M, male.

[a] –, negative

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

End-point	Species, cell type	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Unscheduled DNA synthesis	Rat, primary hepatocytes	–	NT	0.2 µL/mL [185 µg/mL]	GLP study (6 concentrations tested, biological triplicates, positive control).	O'Donoghue et al. (1988)
Unscheduled DNA synthesis	Rat, primary hepatocytes	(+)	NT	5.75 mM [795 µg/mL]	Non-standard protocol (5 concentrations tested, biological triplicates).	Selden et al. (1994)
Unscheduled DNA synthesis	Rat, primary hepatocytes	(+)	NT	5 mM [691 µg/mL]	Standard protocol (only one concentration tested, no biological triplicates).	Selden et al. (1994)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/ *Tk*$^{+/-}$ lymphoma cells	(+)	NT	1200 µg/mL	Positive only at concentration with cytotoxicity > 80%; positive controls not included.	NTP (1986)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/ *Tk*$^{+/-}$ lymphoma cells	(+)	NT	400 µg/mL	No indication of cytotoxicity; number of experiments, NR.	Tennant et al. (1987)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/ *Tk*$^{+/-}$ lymphoma cells	+	NT	800 µg/mL	Well-conducted study (5 concentrations tested, biological triplicates, positive control).	McGregor et al. (1988)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/ *Tk*$^{+/-}$ lymphoma cells	–	–	1300 µg/mL	Only one experiment; positive controls included.	O'Donoghue et al. (1988)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/ *Tk*$^{+/-}$ lymphoma cells	+/–	–	1500 µg/mL	Probably negative.	Sofuni et al. (1996)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/ *Tk*$^{+/-}$ lymphoma cells	–	+/–	1500 µg/mL	Probably negative, positive (+S9) in only one laboratory; the mutation frequency with isophorone was < 2 times the spontaneous mutation frequency.	Honma et al. (1999a)
Gene mutation, *Tk*$^{+/-}$	Mouse, L5178Y/ *Tk*$^{+/-}$ lymphoma cells	(+)	NT	1500 µg/mL	Non-standard protocol with long-term treatment; only one experiment.	Honma et al. (1999b)
Chromosome aberrations	Chinese hamster, ovary cells	–	–	1000 µg/mL		NTP (1986)
Chromosome aberrations	Chinese hamster, ovary cells	–	–	1600 µg/mL	No indication of cytotoxicity or the number of experiments performed.	Tennant et al. (1987)
Chromosome aberrations	Chinese hamster, ovary cells	–	–	1600 µg/mL		Gulati et al. (1989)

Table 4.2 (continued)

End-point	Species, cell type	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Chromosome aberrations	Chinese hamster, lung cells	(+)	(+)	1250 µg/mL –S9 1500 µg/mL +S9	Non-standard protocol; no indication of cytotoxicity.	Matsuoka et al. (1996)
Sister-chromatid exchange	Chinese hamster, ovary cells	–	–	1000 µg/mL		NTP (1986)
Sister-chromatid exchange	Chinese hamster, ovary cells	(+)	NT	500 µg/mL	No indication of cytotoxicity; number of experiments, NR.	Tennant et al. (1987)
Sister chromatid exchange	Chinese hamster, ovary cells	(+)	–	500 µg/mL –S9 1600 µg/mL +S9	Positive in only one experiment (of two); no indication of cytotoxicity.	Gulati et al. (1989)

GLP, Good Laboratory Practice; HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; S9, 9000 × *g* supernatant (liver); Tk, thymidine kinase.

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

Table 4.3 Genetic and related effects of isophorone 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			
Hen's egg	Micronucleus formation (blood erythrocytes)	–	NT	10 mg/65 g egg	Single and repeated treatments	Greywe et al. (2012)
Drosophila melanogaster	Sex-linked recessive lethal mutations	–	NT	2000 µg/mL (feeding) or 12 500 µg/mL (injection)		Foureman et al. (1994)
Salmonella typhimurium	Mutation	–	–	10 000 µg/plate	No indication of the strain tested	Tennant et al. (1987)
Salmonella typhimurium TA98 and TA100	Mutation	–	–	1 mM [138.21 µg/mL]		Kubo et al. (2002)
Salmonella typhimurium TA98, TA100, TA1537, and TA1538	Mutation	–	–	10 000 µg/plate		Mortelmans et al. (1986)
Salmonella typhimurium TA98, TA100, TA1535, and TA1537	Mutation	–	–	10 000 µg/plate		NTP (1986)

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

[a] –, negative.

(b) Experimental systems

Regarding electrophilicity, there was no covalent binding of isophorone or its metabolites to DNA in the liver or kidney of Fischer 344 rats and B6C3F$_1$ mice 24 hours after administration of isophorone by gavage (500 mg/kg/bw) (Thier et al., 1990; see Table 4.1).

Regarding oxidative stress, a single dose of isophorone (500 mg/kg/bw) administered by intraperitoneal injection to male Sprague-Dawley rats caused significant depletion of hepatic, testicular, and epididymal glutathione (Gandy et al., 1990). Using a bacterial (*Escherichia coli)* reporter assay expressing 14 different stress response genes, isophorone (0.6–36 g/L) significantly induced the expression of catalase-peroxidase (*KatG*) (Nobels et al., 2011).

Regarding immunosuppression, leukopenia without any change in differential or erythrocyte counts was observed in male Sprague-Dawley rats exposed to isophorone (67 or 90 ppm) for 4 hours (Brondeau et al., 1990).

Regarding immortalization, a transformation study was performed in BALB/c-3T3 mouse cells (Matthews et al., 1993). In one experiment, isophorone at up to 1.34 mM did not induce cell transformation. In two additional experiments, cell transformation was observed at 0.5 and 2.67 mM. [The Working Group noted that inconsistencies between the three experiments confounded interpretation of the study.]

Regarding alterations in cell proliferation, cell death, or nutrient supply, in studies in B6C3F$_1$ mice and F344/N rats exposed to isophorone (250 and 500 mg/kg bw per day) by gavage for 103 weeks (see Section 3), there was a slight increase in the incidence of renal tubular cell hyperplasia of a single tubule in the kidney [which is a rare lesion] and a significant increase [P = 0.028] in the incidence of epithelial hyperplasia of the renal pelvis in both treated groups of male rats compared with controls (Bucher et al., 1986; NTP, 1986). [The Working Group noted that there was no dose-dependent effect on the incidence of renal pelvis hyperplasia. The Working Group also noted that there were no effects on renal histopathology in 16-day and 13-week studies of isophorone administered at higher dose levels (Bucher et al., 1986; NTP, 1986).]

4.2.3 High-throughput in vitro toxicity screening data evaluation

The analysis of the in vitro bioactivity of the agents reviewed in *IARC Monographs* Volume 130 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). Isophorone was one of thousands of chemicals tested across the large assay battery of the Tox21 and ToxCast research programmes of the US EPA and the United States National Institutes of Health. Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is publicly available (US EPA, 2021). A supplementary table (Annex 2, Supplementary material for Section 4, Mechanistic Evidence, web only; available from: https://publications.iarc.fr/611) provides a summary of the findings including the assay name, the corresponding key characteristic, the resulting "hit calls" both positive and negative, and any reported caution flags for isophorone. The results were generated with the software "kc-hits" (key characteristics of carcinogens – high-throughput screening discovery tool) (available from: https://gitlab.com/i1650/kc-hits) using the US EPA ToxCast and Tox21 assay data and the curated mapping of key characteristics to assays available at the time of the evaluations performed for the present monograph. Findings and interpretations from these high-throughput assays for isophorone are discussed below.

After mapping against the key characteristics of carcinogens, the ToxCast/Tox21 database contained 291 assays in which isophorone was tested. Of these, it was found to be active and without caution flags in seven assays relevant to the key characteristics of carcinogens. [The Working Group noted that the cytotoxic limit for isophorone is 14.8 μM.]

Isophorone was active in five assays mapped to key characteristic 8 (KC8), "modulates receptor-mediated effects". It was active in one assay related to G protein-coupled receptor (GPCR) binding activity in human platelets with a half-maximal activity concentration (AC_{50}) of 22.7 μM. In HepG2 cells, isophorone activated the nuclear receptor subfamily 1, group I, member 2 (NR1I2) (AC_{50}, 48.7 μM); the nuclear receptor subfamily 1, group H, member 3 (NR1H3) (AC_{50}, 36 μM); the nuclear receptor subfamily 1, group H, member 2 (NR1H2) (AC_{50}, 63.3 μM); and the retinoid X receptor, β (RXRB) (AC_{50}, 51.4 μM).

In addition, isophorone was active in two assays mapped to KC10, "alters cell proliferation, cell death, or nutrient supply", in HEK293 cells. The assay measurements were performed 32 and 40 hours after exposure, with AC_{50}s of 40.93 and 36.74 μM, respectively.

4.3 Other relevant evidence

Several studies reported effects related to α_{2u}-globulin in the kidney of male rats. An increase in α_{2u}-globulin was reported in the kidneys of male Sprague-Dawley rats treated with isophorone (150 mg/kg bw per day) by gavage for 14 days (Saito et al., 1992). In addition, isophorone (207 mg/kg bw per day) administered by gavage for seven consecutive days caused an increase in urinary and renal α_{2u}-globulin and hyaline droplets in the renal proximal convoluted tubule epithelial cells of male Sprague-Dawley rats (Saito et al., 1996). In a 2-year study in male and female F334/N rats treated with isophorone by gavage (250 and 500 mg/kg bw per day), the incidence of nephropathy in treated and control male rats was similar, with greater severity in males at the lower dose, and an increase in the incidence of nephropathy in female rats compared with controls. In male rats only, there was also an increase in the incidence of mineralization of the renal tubule epithelial cells (most often found in the medullary collecting ducts and occurring coincidentally with lesions of chronic nephropathy); a significant increase in the incidence of epithelial hyperplasia of the renal pelvis; and an increase in the incidence of other non-neoplastic lesions (including a low incidence of hyperplasia that was described as confined to one tubule) and renal tumours (NTP, 1986). [The Working Group noted that α_{2u}-globulin has not been determined to be relevant to carcinogenesis in other organs besides the kidney.] Male NCI-Black-Reiter (NBR) rats, which do not synthesize α_{2u}-globulin, did not exhibit hyaline droplet formation and nephrotoxicity (necrosis, exfoliation, and regeneration of renal tubule epithelial cells) after exposure to isophorone (1000 mg/kg bw per day) by gavage for 4 days (Dietrich & Swenberg, 1991). [The Working Group noted that major limitations of this study were that a positive control (i.e. a strain of male rat that produces α_{2u}-globulin) was not included, and longer time-points were not evaluated.]

Lehman-McKeeman et al. (1990) reported that isophorone bound to α_{2u}-globulin extracted from the urine of male Sprague-Dawley rats and reduced its lysosomal degradation in vitro. Furthermore, Borghoff et al. (1991) determined that isophorone competed with 2,2,4-trimethylpentane for binding to α_{2u}-globulin in protein extracts isolated from the kidney of male Fischer 344 rats. [The Working Group noted that it has been suggested that the binding of isophorone to α_{2u}-globulin is reversible on the basis of modelling predictions (Borghoff et al., 1991) but this has not been conclusively demonstrated experimentally.]

IARC has established seven criteria that need to be fully met to conclude that an agent induces

tumours of the kidney by an α_{2u}-globulin-associated response (IARC, 1999). For isophorone, only one of the seven criteria was met, that is, identification of the accumulating protein as α_{2u}-globulin. The remaining six criteria were not met, specifically: (i) lack of genotoxic activity of the agent and/or metabolite (see Section 4.2.1) [the Working Group noted that there is mixed evidence for genotoxicity of isophorone in non-human mammalian experimental systems]; (ii) male rat specificity for nephropathy and renal tumorigenicity [the Working Group noted that there is no evidence for α_{2u}-globulin-dependent renal hyperplasia or tumorigenicity (using the NBR rat strain), and isophorone induced tumours at other sites in which α_{2u}-globulin has not been demonstrated to be relevant to carcinogenesis, i.e. the preputial gland in male rats and the liver, subcutis, and lymphohaematopoietic system in male mice (see Section 3)]; (iii) induction of the characteristic sequence of histopathological changes associated with α_{2u}-globulin accumulation [the Working Group noted that Saito et al. (1992, 1996) only showed increased α_{2u}-globulin accumulation and hyaline droplets, whereas NTP (1986) reported nephrotoxic effects (including increased incidence of tubular cell hyperplasia confined to a single tubule, and tumours) but no α_{2u}-globulin accumulation and hyaline droplets, and no increased injury to kidney proximal tubule epithelial cells that would be characteristic of α_{2u}-globulin nephropathy in 16-day, 13-week, or 2-year studies]; (iv) reversible binding of the chemical or metabolite to α_{2u}-globulin [the Working Group noted that reversible binding of isophorone has been predicted but not determined experimentally]; (v) induction of sustained increases in cell proliferation in the renal cortex; and (vi) similarities in dose–response relationships for the tumour outcome and for histopathological end-points associated with α_{2u}-globulin nephropathy. [The Working Group noted that the NTP (1986) study did not report α_{2u}-globulin accumulation and hyaline droplets so these end-points could not be correlated to kidney tumours in male rats.]

While such data can be informative in interpreting the relevance to humans of kidney tumours observed in rodents, the findings did not fulfil all criteria required in order to conclude that the induction of renal tumours by isophorone operates via a mechanism associated with α_{2u}-globulin in male rats (IARC, 1999).

5. Summary of Data Reported

5.1 Exposure characterization

Isophorone is a High Production Volume chemical that is widely used as a solvent and also as a chemical intermediate in the manufacture of a variety of products, including polymers and their precursors, lacquers, inks, paints, nitrocellulose finishes, and cleaning products. It is also used in the production of agrochemicals and is a constituent of certain pesticides. The widescale industrial use of isophorone permits its release into the environment, primarily through atmospheric release at urban and industrial centres, but also via industrial effluents. The most substantial human exposures to isophorone probably occur in occupational settings, particularly as airborne exposure during industrial processes using isophorone or products containing isophorone. In particular, printing and screen printing; coating, painting and spray painting; machine operating; plastics production; packaging; and cleaning may be notable sources of occupational exposure, although few recent quantitative data were available to the Working Group. Isophorone has been detected and quantified in a variety of environmental samples and products, notably drinking-water, pesticides, food items and food packaging, inflatable pool toys, and other polymer-based products. No quantitative data on exposure to isophorone in the general population were available to the Working Group.

5.2 Cancer in humans

The available evidence on cancer in humans consisted of a single study investigating the association between cancers of the central nervous system and exposure to isophorone. The study observed some weakly elevated risk estimates in some categories of cumulative exposure in analyses conducted by facility, but none of the elevations were statistically significant. No trend with increasing exposure category was present. The study was only weakly informative due to small numbers of exposed cases, in particular, small numbers of highly exposed cases, and the potential for co-exposures to other agents used in the workplace. The study did not permit a conclusion to be drawn about the presence or absence of a causal association between exposure to isophorone and cancer risk.

5.3 Cancer in experimental animals

Treatment with isophorone caused an increase in the incidence of either malignant neoplasms or an appropriate combination of benign and malignant neoplasms in two species.

Isophorone was administered by oral administration (gavage) in one study in $B6C3F_1$ mice. In males, isophorone caused an increase in the incidence of fibrosarcoma of the subcutis, mesenchymal tumours (fibroma, fibrosarcoma, neurofibrosarcoma, or sarcoma, combined) of the integumentary system (skin and subcutis, combined), hepatocellular adenoma or carcinoma (combined), and malignant lymphoma of the lymphohaematopoietic system.

Isophorone was administered by oral administration (gavage) in one study in F344/N rats. In males, isophorone caused an increase in the incidence of carcinoma of the preputial gland, and tubular cell adenoma or tubular cell adenocarcinoma (combined) of the kidney.

5.4 Mechanistic evidence

Only one study on the absorption, distribution, metabolism, and excretion of isophorone in humans was available; this study demonstrated poor dermal absorption. Studies in rodents and rabbits demonstrated that isophorone is rapidly absorbed via multiple routes and is excreted as parent compound and/or metabolites in the urine.

Overall, the mechanistic evidence that isophorone exhibits the key characteristics of carcinogens ("is genotoxic", "induces oxidative stress", "is immunosuppressive", and "alters cell proliferation, cell death, or nutrient supply") is suggestive but inconsistent across experimental systems. No studies relevant to the key characteristics were available in exposed humans or human cells in vitro. The mechanistic evidence on whether isophorone is genotoxic was inconsistent across non-human mammalian and non-mammalian experimental systems. One study on micronucleus formation in mice exposed to isophorone by intraperitoneal injection gave negative results. In one study in mammalian cells, there was a positive response without metabolic activation, but for several other studies in mammalian cells, results were negative or equivocal with and without metabolic activation. Isophorone with and without metabolic activation was not mutagenic in non-mammalian experimental systems (including bacteria) in multiple studies. Regarding the key characteristics "induces oxidative stress", "is immunosuppressive", and "alters cell proliferation, cell death, or nutrient supply", there was a paucity of available data. One study in rats provided evidence that isophorone induces oxidative stress, whereas in another study in rats isophorone caused leukopenia. Rare instances of renal tubular cell hyperplasia of a single tubule in the kidney and a significant increase in the incidence of epithelial hyperplasia of the renal pelvis were reported in male rats exposed to isophorone by gavage in a 2-year study. Regarding whether

isophorone exhibits the key characteristic "is electrophilic or metabolically activated", negative results were reported in one study on DNA binding in the liver and kidney of rodents treated with isophorone by gavage. Isophorone was largely inactive at non-cytotoxic concentrations 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 isophorone.

6.2 Cancer in experimental animals

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

6.3 Mechanistic evidence

There is *limited mechanistic evidence*.

6.4 Overall evaluation

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

6.5 Rationale

The Group 2B evaluation for isophorone is based on *sufficient evidence* for cancer in experimental animals. This *sufficient evidence* in experimental animals is based on an increased incidence of either malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in two species. The evidence for cancer in humans was *inadequate*. There was only one study on cancers of the central nervous system, which was not sufficiently informative to permit a conclusion to be drawn about the presence or absence of a causal association due to small numbers of exposed cases, in particular, small numbers of highly exposed cases, in an analysis by facility. Potential co-exposure to other agents used in the workplace was also of concern. The mechanistic evidence was *limited* because the findings regarding key characteristics of carcinogens across experimental systems were suggestive, but inconsistent.

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LIST OF ABBREVIATIONS

AC_{50}	half-maximal activity concentration
ACGIH	American Conference of Governmental Industrial Hygienists
ADP	adenosine diphosphate
AML	acute myeloid leukaemia
ARC	anticipated residue concentration
ATP	Danish Supplementary Pension Fund Register (Arbejdsmarkedets Tillægspension)
ATSDR	Agency for Toxic Substances and Disease Registry
BAT	biological tolerance value (Biologischer Arbeitsstoff-Toleranz-Wert)
BEI	biological exposure indices
bw	body weight
CAS	Chemical Abstracts Service
cDNA	complementary DNA
CHO	Chinese hamster ovary
CLL	chronic lymphocytic leukaemia
CYP	cytochrome P450
DFG	Deutsche Forschungsgemeinschaft (The German Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area of the German Research Foundation)
DGUV	German Statutory Accident Insurance (Deutsche Gesetzliche Unfallversicherung)
2,3-DHBA	2,3-dihydroxybenzoic acid
DMSO	dimethyl sulfoxide
DSE	Danish five-digit branch/industry code (Danmarks Statistisk Erhvervsgrupperingskode)
EASE	Estimation and Assessment of Substance Exposure
ECD	electron capture detection
e-waste	electronic waste
FID	flame ionization detection
FINJEM	Finnish job-exposure matrix
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
GC-MS/MS	gas chromatography-tandem mass spectrometry
GC-Q-TOF-MS	gas chromatography quadrupole time of flight mass spectrometry
GLP	Good Laboratory Practice
GPT	glutamic--pyruvic transaminase
GST	glutathione *S*-transferase
HHE	Health Hazard Evaluation Program

HSPME	headspace solid-phase microextraction
IDLH	immediately dangerous to life and health
ISIC	International Standard Industrial Classification of all Economic Activities
IWS	Industrywide Studies
IUPAC	International Union of Pure and Applied Chemistry
KC	key characteristic
LMICs	low- and middle-income countries
LOD	limit of detection
MRL	minimal risk level
MTD	maximum tolerated dose
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NCI	National Cancer Institute
ND	not detected
NHEXAS	National Human Exposure Assessment Survey
NHL	non-Hodgkin lymphoma
NIOSH	National Institute for Occupational Health and Safety
NK	natural killer
NOCCA	Nordic Occupational Cancer Study
NOES	National Occupational Exposure Survey
NR	not reported
NS	not significant
NTP	National Toxicology Program
NWQMC	National Water Quality Monitoring Council
8-OHdG	8-hydroxy-2-deoxyguanosine
OSHA	Occupational Safety and Health Administration
PBZ	personal breathing zone
PEG	primary exposure group
ppm	parts per million
RCC	renal cell carcinoma
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
REL	recommended exposure limit
RMOA	Regulatory Management Option Analysis
SIR	standardized incidence ratio
SPE	solid-phase extraction
SPME	solid-phase microextraction
TBARS	thiobarbituric acid-reactive substances
TLV	threshold limit value
Tox21	Toxicology in the 21st Century
ToxCast	Toxicity Forecaster
TRI	Toxic Release Inventory
TWA	time-weighted average
US EPA	United States Environmental Protection Agency
US FDA	United States Food and Drug Administration
VOC	volatile organic compound
v/v	volume per volume

ANNEX 1. SUPPLEMENTARY MATERIAL FOR 1,1,1-TRICHLOROETHANE, SECTION 1, EXPOSURE CHARACTERIZATION

These supplementary web-only tables are available from: https://publications.iarc.fr/611.

These tables were produced in draft form by the Working Group and were subsequently fact-checked but not edited.

Please report any errors to imo@iarc.who.int.

ANNEX 2. SUPPLEMENTARY MATERIAL FOR SECTION 4, MECHANISTIC EVIDENCE

These supplementary web-only tables (available from: https://publications.iarc.fr/611) contain summaries of the findings (including the assay name, the corresponding key characteristic, the resulting "hit calls" both positive and negative, and any reported caution flags) for those chemicals evaluated in the present volume that have been tested in high-throughput screening assays performed by the United States Environmental Protection Agency (US EPA) and the United States National Institutes of Health. The results were generated by the Working Group using the software "kc-hits" (key characteristics of carcinogens – high-throughput screening discovery tool) available from https://gitlab.com/i1650/kc-hits.git, with the US EPA Toxicity Forecaster (ToxCast) assay data and the curated mapping of key characteristics to assays available at the time of the evaluations performed for *IARC Monographs* Volume 130. Data were available for 1,1,1-trichloroethane, 1,2-diphenylhydrazine, diphenylamine, and isophorone, but not *N*-methylolacrylamide.

Please report any errors to imo@iarc.who.int.

ANNEX 3. SUPPLEMENTARY MATERIAL FOR ISOPHORONE, SECTION 1, EXPOSURE CHARACTERIZATION

This supplementary web-only table is available from: https://publications.iarc.fr/611.

This table was produced in draft form by the Working Group and was subsequently fact-checked but not edited.

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Table S1.6 Exposure assessment review and critique for epidemiological studies on cancer and exposure to isophorone

SUMMARY OF FINAL EVALUATIONS

Summary of final evaluations for Volume 130

Agent	Evidence stream			Overall evaluation
	Cancer in humans	Cancer in experimental animals	Mechanistic evidence	
1,1,1-Trichloroethane	*Limited*	*Sufficient*	*Limited*	Group 2A
1,2-Diphenylhydrazine	*Inadequate*	*Sufficient*	*Limited*	Group 2B
Diphenylamine	*Inadequate*	*Sufficient*	*Limited*	Group 2B
N-Methylolacrylamide	*Inadequate*	*Sufficient*	*Limited*	Group 2B
Isophorone	*Inadequate*	*Sufficient*	*Limited*	Group 2B

IARC MONOGRAPHS

This volume of the *IARC Monographs* provides evaluations of the carcinogenicity of five industrial chemicals: 1,1,1-trichloroethane, 1,2-diphenylhydrazine, diphenylamine, *N*-methylolacrylamide, and isophorone.

1,1,1-Trichloroethane was used extensively until the 1990s as a solvent, metal degreaser, chemical intermediate. Since the Montreal Protocol on Substances that Deplete the Ozone Layer, production and use have dwindled, and it is now mostly used as a chemical feedstock in closed systems and for "essential uses". Poorly documented non-essential uses might occur in low-income and middle-income countries.

1,2-Diphenylhydrazine was primarily used as an intermediate in the manufacture of benzidine dyes, which has ceased in the USA and European Union, although production might occur elsewhere. Additional uses include as an intermediate in drug manufacture.

Diphenylamine, *N*-methylolacrylamide, and isophorone are High Production Volume chemicals and intermediates used for a wide range of industrial applications. The use of diphenylamine in agrochemicals to prevent fruit scalding is prohibited in the European Union, but ongoing in the USA and elsewhere. Isophorone has been detected in numerous polymer-based products from food packaging to aquatic inflatables, and in food items, possibly because of agrochemical contamination or migration from packaging.

For all agents, data were sparse regarding exposure levels (apart from 1,1,1-trichloroethane, for which data were available mainly on exposures pre-dating the adoption of the Montreal Protocol), but indicated that exposures are higher in occupational situations than in the general population.

An *IARC Monographs* Working Group reviewed evidence from cancer studies in humans (available for 1,1,1-trichloroethane), cancer bioassays in experimental animals, and mechanistic studies to assess the carcinogenic hazard to humans of exposure to these agents and concluded that:

- 1,1,1-Trichloroethane is *probably carcinogenic to humans (Group 2A)*
- 1,2-Diphenylhydrazine, diphenylamine, *N*-methylolacrylamide, and isophorone are *possibly carcinogenic to humans (Group 2B)*.

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