IARC MONOGRAPHS

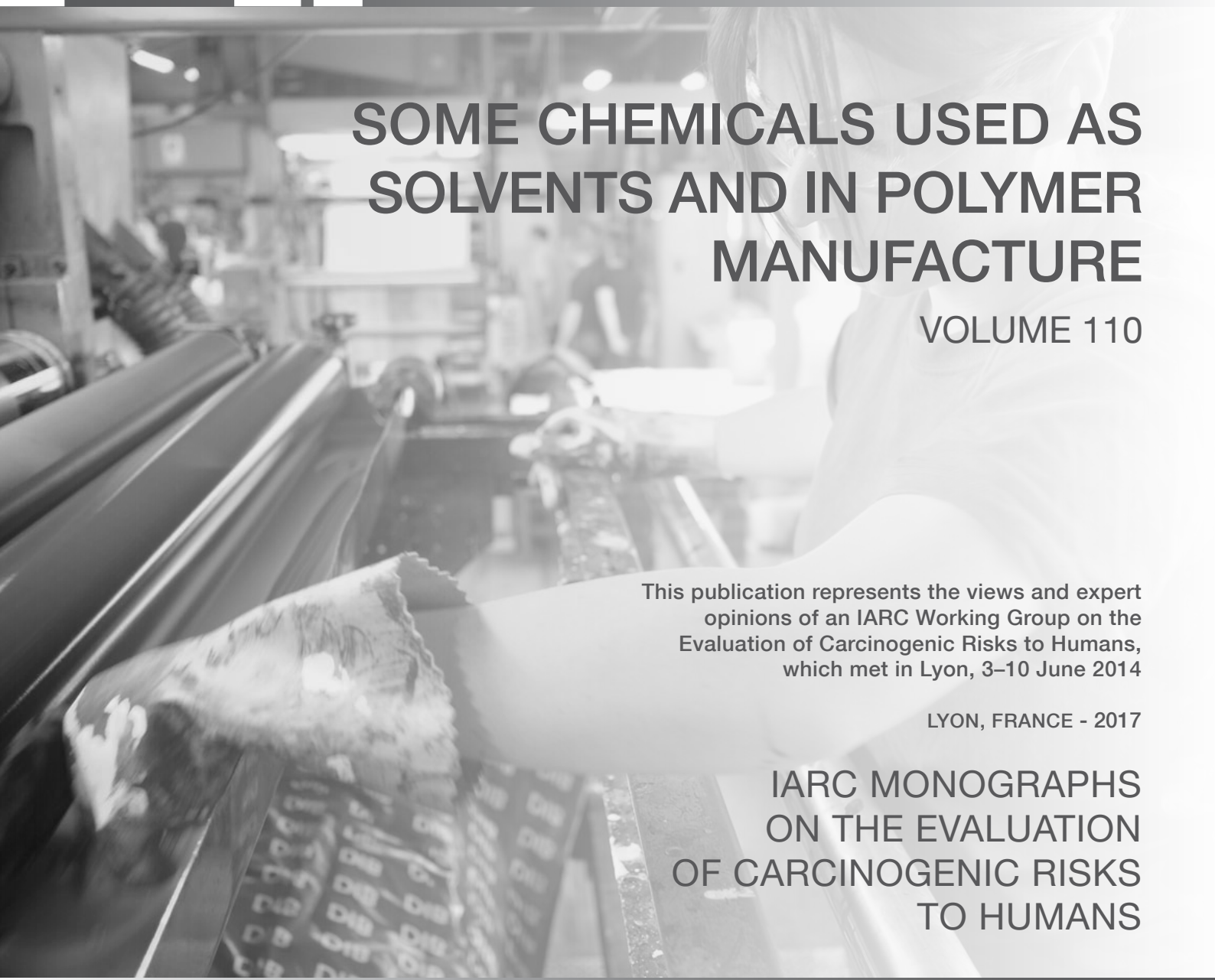
SOME CHEMICALS USED AS SOLVENTS AND IN POLYMER MANUFACTURE

VOLUME 110

IARC MONOGRAPHS
ON THE EVALUATION
OF CARCINOGENIC RISKS
TO HUMANS

International Agency for Research on Cancer
World Health Organization

IARC MONOGRAPHS



SOME CHEMICALS USED AS SOLVENTS AND IN POLYMER MANUFACTURE

VOLUME 110

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 3–10 June 2014

LYON, FRANCE - 2017

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

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IARC MONOGRAPHS

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

This programme has been supported since 1982 by Cooperative Agreement U01 CA33193 with the United States National Cancer Institute, Department of Health and Human Services. Additional support has been provided since 1986 by the European Commission Directorate-General for Employment, Social Affairs, and Inclusion, initially by the Unit of Health, Safety and Hygiene at Work, and since 2014 by the European Union Programme for Employment and Social Innovation "EaSI" (2014–2020) (for further information please consult: http://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.

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

On-line publication, December 2016

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

Corrigenda to the IARC Monographs are published online at http://monographs.iarc.fr/ENG/Publications/corrigenda.php
To report an error, please contact: editimo@iarc.fr

Co-funded by the European Union

IARC Library Cataloguing in Publication Data

Perfluorooctanoic acid, tetrafluoroethylene, dichloromethane, 1,2-dichloropropane, and 1,3-propane sultone / IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2014.

(IARC monographs on the evaluation of carcinogenic risks to humans ; volume 110)

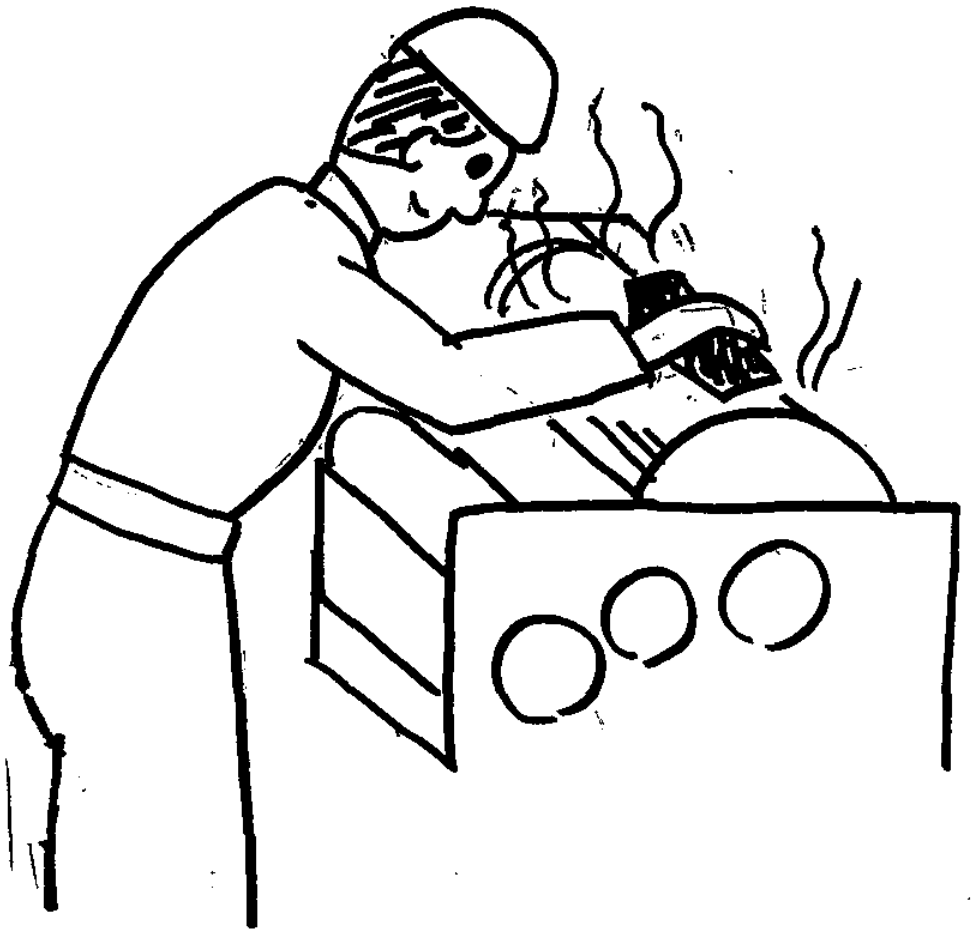
1. Neoplasms – etiology 2. Carcinogens 3. Caprylates – adverse effects 4. Fluorocarbons – adverse effects
5. Methylene Chloride – adverse effects 6. Propane – adverse effects 7. Thiophenes – adverse effects 8. Risk Factors
9. Occupational Exposure – adverse effects

I. International Agency for Research on Cancer II. Series

ISBN 978-92-832-0176-2 (NLM Classification: W1)
ISSN 1017-1606

PRINTED IN FRANCE

Cover image: The photo on the cover shows the cleaning and emptying of a printing machine in a printing workshop © Guillaume J. Plisson for the Institut national de recherche et de sécurité pour la prévention des accidents du travail et des maladies professionnelles (INRS)



This sketch illustrates an example of occupational exposure to 1,2-dichloropropane, a solvent that is used for cleaning offset printing presses. Unprotected workers who clean the printing machines by hand are exposed to high levels of 1,2-dichloropropane vapour at close proximity, particularly in small, poorly ventilated rooms. This type of specific and intense exposure to 1,2-dichloropropane was observed in epidemiological studies on which the Working Group based its evaluation.

The sketch was drawn by Ms Tomoko Terashima and kindly provided by Mr Kenichi Kamae, Ministry of Health, Labour and Welfare, Japan.

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NOTE TO THE READER

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

Inclusion of an agent in the *Monographs* does not imply that it is a carcinogen, only that the published data have been examined. Equally, the fact that an agent has not yet been evaluated in a *Monograph* does not mean that it is not carcinogenic. Similarly, identification of cancer sites with *sufficient evidence* or *limited evidence* in humans should not be viewed as precluding the possibility that an agent may cause cancer at other sites.

The evaluations of carcinogenic risk are made by international working groups of independent scientists and are qualitative in nature. No recommendation is given for regulation or legislation.

Anyone who is aware of published data that may alter the evaluation of the carcinogenic risk of an agent to humans is encouraged to make this information available to the Section of IARC Monographs, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France, in order that the agent may be considered for re-evaluation by a future Working Group.

Although every effort is made to prepare the *Monographs* as accurately as possible, mistakes may occur. Readers are requested to communicate any errors to the Section of IARC Monographs, so that corrections can be reported in future volumes.

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[2] Scott Bartell has received research funding from the C8 class action settlement agreement between DuPont and plaintiffs. Funds were administered by the Garden City Group (Melville, New York) that reports to the court. The research was independent of either party to the lawsuit.

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[6] John Cherrie has received significant research funding from the Association of Plastic Manufacturers Europe (APME, till 2012), the European Chemical Industry Council (CEFIC), the European Solvents Industry Group (ESIG, till 2012), CONCAWE and International Paint.

[7] 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.

[8] Josje Arts is senior toxicologist of Akzo Nobel, the Netherlands. Her participation is sponsored by the three organizations for which she is an observer, the REACH Chlorsolv Consortium, the European Chlorinated Solvent Association (ECSA), and the Halogenated Solvents Industry Alliance (HSIA).

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PREAMBLE

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

A. GENERAL PRINCIPLES AND PROCEDURES

1. Background

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

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

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

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

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

2. Objective and scope

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

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

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

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

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

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

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

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

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

3. Selection of agents for review

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

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

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

4. Data for the *Monographs*

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

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

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

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

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

5. Meeting participants

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

(a) The Working Group

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

(b) Invited Specialists

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

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

(c) Representatives of national and international health agencies

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

(d) Observers with relevant scientific credentials

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

(e) The IARC Secretariat

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

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

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

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

6. Working procedures

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

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

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

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

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

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

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

B. SCIENTIFIC REVIEW AND EVALUATION

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

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

Exposure data

Studies of cancer in humans

Studies of cancer in experimental animals

Mechanistic and other relevant data

Summary

Evaluation and rationale

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

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

1. Exposure data

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

(a) General information on the agent

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

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

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

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

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

(b) Analysis and detection

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

(c) Production and use

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

which may give rise to different impurities, are described.

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

(d) Occurrence and exposure

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

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

(e) Regulations and guidelines

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

2. Studies of cancer in humans

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

(a) Types of study considered

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

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

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

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

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

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

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

(b) Quality of studies considered

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

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

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

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

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

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

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

(c) Meta-analyses and pooled analyses

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

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

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

(d) Temporal effects

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

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

(e) Use of biomarkers in epidemiological studies

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

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

(f) Criteria for causality

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

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

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

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

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

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

3. Studies of cancer in experimental animals

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

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

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

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

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

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

(a) Qualitative aspects

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

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

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

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

(b) Quantitative aspects

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

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

(c) Statistical analyses

Factors considered include the adequacy of the information given for each treatment group: (i) number of animals studied and number examined histologically, (ii) number of animals with a given tumour type and (iii) length of survival. The statistical methods used should be clearly stated and should be the generally accepted techniques refined for this purpose (Peto et al., 1980; Gart et al., 1986; Portier & Bailer, 1989; Bieler & Williams, 1993). The choice of the most appropriate statistical method requires consideration of whether or not there are differences in survival among the treatment groups; for example, reduced survival because of non-tumour-related mortality can preclude the occurrence of tumours later in life. When detailed information on survival is not available, comparisons of the proportions of tumour-bearing animals among the effective number of animals (alive at the time the first tumour was discovered) can be useful when significant differences in survival occur before tumours appear. The lethality of the tumour also requires consideration: for rapidly fatal tumours, the time of death provides an indication of the time of tumour onset and can be assessed using life-table methods; non-fatal or incidental tumours that do not affect survival can be assessed using methods such as the Mantel-Haenzel test for changes in tumour prevalence. Because tumour lethality is often difficult to determine, methods such as the Poly-K test that do not require such information can also be used. When results are available on the number and size of tumours seen in experimental animals (e.g. papillomas on mouse skin, liver tumours observed through nuclear magnetic resonance tomography), other more complicated statistical procedures may be needed (Sherman et al., 1994; Dunson et al., 2003).

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

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

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

4. Mechanistic and other relevant data

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

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

(a) Toxicokinetic data

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

(b) Data on mechanisms of carcinogenesis

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

(i) Changes in physiology

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

(ii) Functional changes at the cellular level

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

(iii) Changes at the molecular level

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

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

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

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

Positive results in tests using prokaryotes, lower eukaryotes, insects, plants and cultured mammalian cells suggest that genetic and related effects could occur in mammals. Results from such tests may also give information on the types of genetic effect produced and on the involvement of metabolic activation. Some end-points described are clearly genetic in nature (e.g. gene mutations), while others are associated with genetic effects (e.g. unscheduled DNA synthesis). In-vitro tests for tumour promotion, cell transformation and gap–junction intercellular communication may be sensitive to changes that are not necessarily the result of genetic alterations but that may have specific relevance to the process of carcinogenesis. Critical appraisals of these tests have been published (Montesano et al., 1986; McGregor et al., 1999).

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

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

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

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

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

(c) Other data relevant to mechanisms

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

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

(d) Susceptibility data

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

(e) Data on other adverse effects

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

5. Summary

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

(a) Exposure data

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

(b) Cancer in humans

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

(c) Cancer in experimental animals

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

(d) Mechanistic and other relevant data

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

6. Evaluation and rationale

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

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

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

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

(a) Carcinogenicity in humans

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

***Sufficient evidence of carcinogenicity*:**

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

***Limited evidence of carcinogenicity*:**

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

***Inadequate evidence of carcinogenicity*:**

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

***Evidence suggesting lack of carcinogenicity*:**

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

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

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

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

(b) Carcinogenicity in experimental animals

Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multistage process of carcinogenesis should be considered in evaluating the degree of evidence of carcinogenicity in experimental animals.

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

***Sufficient evidence of carcinogenicity*:**

The Working Group considers that a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well conducted study, ideally conducted under Good Laboratory Practices, can also provide *sufficient evidence*.

A single study in one species and sex might be considered to provide *sufficient evidence of carcinogenicity* when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.

***Limited evidence of carcinogenicity*:**

The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

***Inadequate evidence of carcinogenicity*:**

The studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect because of major qualitative or quantitative limitations, or no data on cancer in experimental animals are available.

***Evidence suggesting lack of carcinogenicity*:**

Adequate studies involving at least two species are available which show that, within the limits of the tests used, the agent is not carcinogenic. A conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the species, tumour sites, age at exposure, and conditions and levels of exposure studied.

(c) Mechanistic and other relevant data

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

The strength of the evidence that any carcinogenic effect observed is due to a particular mechanism is evaluated, using terms such as 'weak', 'moderate' or 'strong'. The Working Group then assesses whether that particular mechanism is likely to be operative in humans. The strongest indications that a particular mechanism operates in humans derive from data on humans or biological specimens obtained from exposed humans. The data may be considered to be especially relevant if they show that the agent in question has caused changes in exposed humans that are on the causal pathway to carcinogenesis. Such data may, however, never become available, because it is at least conceivable that certain compounds may be kept from human use solely on the basis of evidence of their toxicity and/or carcinogenicity in experimental systems.

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

For complex exposures, including occupational and industrial exposures, the chemical composition and the potential contribution of carcinogens known to be present are considered by the Working Group in its overall evaluation of human carcinogenicity. The Working Group also determines the extent to which the materials tested in experimental systems are related to those to which humans are exposed.

(d) Overall evaluation

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

An evaluation may be made for a group of agents that have been evaluated by the Working Group. In addition, when supporting data indicate that other related agents, for which there is no direct evidence of their capacity to induce cancer in humans or in animals, may also be carcinogenic, a statement describing the rationale for this conclusion is added to the evaluation narrative; an additional evaluation may be made for this broader group of agents if the strength of the evidence warrants it.

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

Group 1: The agent is carcinogenic to humans.

This category is used when there is *sufficient evidence of carcinogenicity* in humans. Exceptionally, an agent may be placed in this category when evidence of carcinogenicity in humans is less than *sufficient* but there is *sufficient evidence of carcinogenicity* in experimental animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity.

Group 2.

This category includes agents for which, at one extreme, the degree of evidence of carcinogenicity in humans is almost *sufficient*, as well as those for which, at the other extreme, there are no human data but for which there is evidence of carcinogenicity in experimental animals. Agents are assigned to either Group 2A (*probably carcinogenic to humans*) or Group 2B (*possibly carcinogenic to humans*) on the basis of epidemiological and experimental evidence of carcinogenicity and mechanistic and other relevant data. The terms *probably carcinogenic* and *possibly carcinogenic* have no quantitative significance and are used simply as descriptors of different levels of evidence of human carcinogenicity, with *probably carcinogenic* signifying a higher level of evidence than *possibly carcinogenic*.

Group 2A: The agent is probably carcinogenic to humans.

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

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

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

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

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

Exceptionally, agents for which the evidence of carcinogenicity is *inadequate* in humans but *sufficient* in experimental animals may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans.

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

An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that further research is needed,

especially when exposures are widespread or the cancer data are consistent with differing interpretations.

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

This category is used for agents for which there is *evidence suggesting lack of carcinogenicity* in humans and in experimental animals. In some instances, agents for which there is *inadequate evidence of carcinogenicity* in humans but *evidence suggesting lack of carcinogenicity* in experimental animals, consistently and strongly supported by a broad range of mechanistic and other relevant data, may be classified in this group.

(e) Rationale

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

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GENERAL REMARKS

This one-hundred-and-tenth volume of the *IARC Monographs* contains evaluations of the carcinogenic hazard to humans of perfluorooctanoic acid, tetrafluoroethylene, 1,2-dichloropropane, dichloromethane, and 1,3-propane sultone. Exposure measurements and biomonitoring studies have shown that workers and the general population are exposed to these agents. All except one of these agents, perfluorooctanoic acid, were evaluated previously in Volume 71 of the *IARC Monographs* (IARC, 1999), when the Working Group classified three (tetrafluoroethylene, dichloromethane, and 1,3-propane sultone) as *possibly carcinogenic to humans* (Group 2B) and one (1,2-dichloropropane) as *not classifiable as to its carcinogenicity to humans* (Group 3). Since the previous evaluations, new data have become available, and the Preamble to the *IARC Monographs* has been modified to permit more explicit consideration of mechanistic data. A summary of the findings of this volume appears in *The Lancet Oncology* (Benbrahim-Tallaa et al., 2014).

Perfluorooctanoic acid

Interference with steroidogenic enzymes is a putative mechanism that may result in testicular carcinogenesis. The evidence that perfluorooctanoic acid could cause cancer of the testis was considered credible by the Working Group and unlikely to be explained by bias and confounding, but this cancer is rare and the conclusion was based on small numbers of cases. Taking into account the data in humans and experimental animals, and the mechanistic data on perfluorooctanoic acid, a plausible hypothesis for perfluorooctanoic acid-related carcinogenesis in the testes involves perturbation of molecular pathways related to testosterone, estradiol, and estrogen receptor, including during development. However, the lack of strong data precludes the establishment of a causal relationship between perturbation of these pathways and increased risk of cancer, with respect to human testicular cancer in general, as well as perfluorooctanoic acid-induced cancers in particular. If established, causal relationships between sex-hormone perturbations and specific cancers in humans could have implications for identifying the causes of hormone-related cancer based on mechanistic data.

1,2-Dichloropropane and dichloromethane

Biomonitoring studies have shown that workers and the general population are exposed to 1,2-dichloropropane and dichloromethane. The Working Group noted that a reported cluster of cancers of the biliary tract in workers at small

printing plants in Japan was very unusual, given the rarity of the outcome, the young ages at diagnosis, the absence of other known risk factors among the cases, and the very high relative risk, as well as the specificity and the intensity of the exposures (Kumagai et al., 2013; Kumagai, 2014). The Working Group recalled a previously reported cluster of four cases of angiosarcoma in workers exposed to vinyl chloride in a single chemical plant in the USA in January 1974. That cluster was extremely unusual in that the incidence of angiosarcoma at that time in the USA was only about 25 cases per year. An IARC Working Group determined later that the association between angiosarcoma and exposure to vinyl chloride was causal, based on investigation of this cluster in the USA and others elsewhere, and an earlier study in experimental animals (IARC, 2008).

1,2-Dichloropropane and dichloromethane are used as chemical intermediates and in paint stripping, but their use in the cleaning of printing presses in Japan resulted in exposure to both agents at remarkably high concentrations. The use of 1,2-dichloropropane for printing-press cleaning was reported to be widespread in Japan in the mid-1990s after the decline in use of 1,1,1-trichloroethane (although no specific statistics were available). Offset printing machines at typical small and medium-sized printing firms located in urban areas in Japan tended to be installed in small rooms with poor ventilation. When printers wiped the machines with cleaning cloths imbibed with volatile agents, the agents evaporated fully into the room to create high concentrations in the air. The sensitive control of room temperature and moisture to ensure product quality prevented air circulation, which resulted in much higher concentration of the agents near the breathing zone of the worker. This unique work environment and usage had not been observed previously. No information was available to the Working Group on whether 1,2-dichloropropane was used similarly in the 1990s in countries other than Japan; to date, this specific exposure setting has not been reported elsewhere.

In the cluster of cancers of the biliary tract in Japan, the Working Group noted the rapid work of the Japanese investigators and the Japanese government to confirm the cluster, and to provide epidemiological data on the exposed cohort that enabled estimation of the relative risk for those exposed to 1,2-dichloropropane only; this evidence played an important role in the decision of the Working Group regarding the evidence for carcinogenicity of 1,2-dichloropropane in humans. The Working Group also noted that there is a need for further epidemiological studies of those exposed occupationally either to dichloromethane alone (without 1,2-dichloropropane), or 1,2-dichloropropane alone (without dichloromethane), at different levels, with a focus on detecting cancers of the biliary tract. There is also the question of whether the combination of 1,2-dichloropropane and dichloromethane is synergistic, such that the exposure to both is more potent than exposure to either one separately. Furthermore, the Working Group noted that past cohort studies of printers might be re-examined in more detail with regard to exposure to dichloromethane and 1,2-dichloropropane, and separating out cancers of the biliary tract from the combined category of liver and biliary tract. In addition, further experimental studies are needed to understand the mechanisms of co-exposure to 1,2-dichloropropane and dichloromethane.

1,3-Propane sultone

In making its evaluation, the Working Group took into consideration the data demonstrating that 1,3-propane sultone is a strong, direct-acting alkylating agent that reacts with DNA and protein. A comprehensive review of agents

with similar direct alkylating activity, including those previously evaluated for carcinogenicity by IARC, may be warranted to identify agents with similar carcinogenic potential.

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PERFLUOROOCTANOIC ACID

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 335-67-1

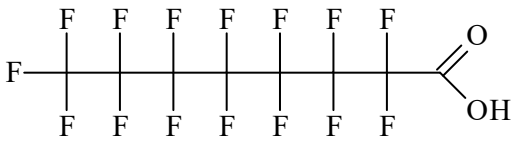
Chem. Abstr. Serv. Name: Perfluorooctanoic acid

IUPAC Name: 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid

Synonyms: PFOA; pentadecafluoro-1-octanoic acid; pentadecafluoro-n-octanoic acid; pentadecaflurooctanoic acid; perfluorocaprylic acid; perfluoroctanoic acid; perfluoroheptanecarboxylic acid; APFO; ammonium perfluorooctanoate

Isomers and Salts: There are 39 possible structural isomers of pentadecafluorooctanoic acid (1 with chain length 8, 5 with chain length 7, 13 with chain length 6, 16 with chain length 5, and 4 with chain length 4). These isomers can also exist as the ammonium, sodium, or potassium salt (Nielsen, 2012). Fig. 1.1 presents the few isomers and salts that have Chemical Abstracts Service (CAS) references.

1.1.2 Structural and molecular formulae, and relative molecular mass: straight-chain isomer



Molecular formula: $C_8HF_{15}O_2$
Relative molecular mass: 414

1.1.3 Chemical and physical properties of the pure substance: straight-chain isomer

From HSDB (2014), unless otherwise indicated

Description: White to off-white powder

Boiling point: 192.4 °C

Melting point: 54.3 °C

Density: 1.792 g/cm^3 at 20 °C

Solubility: 9.5 g/L in water at 25 °C

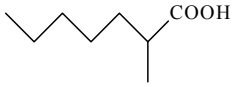
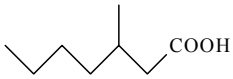
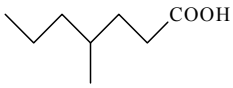
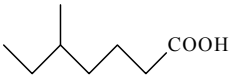
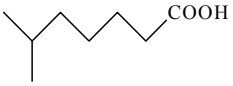
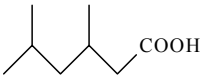
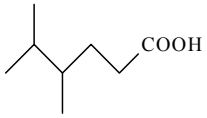
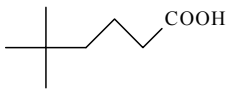
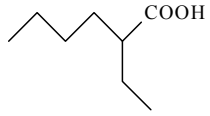
Vapour pressure: 0.0023 kPa at 20 °C (extrapolated); 0.127 kPa at 59.25 °C (measured) (ATSDR, 2009); 0.070 kPa at 25 °C

Stability: When heated to decomposition it emits toxic vapours of hydrogen fluoride

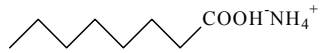
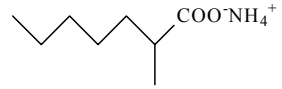
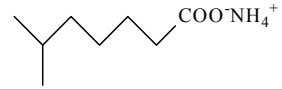
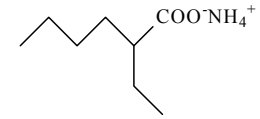
Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), 1 mg/m^3 = 16.9 ppm.

Fig. 1.1 Structures of isomers and salts of perfluorooctanoic acid (PFOA)

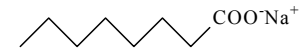
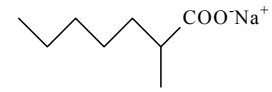
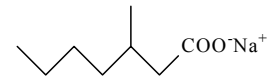
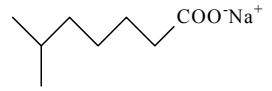
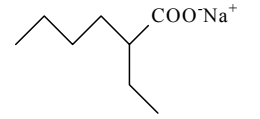
a. PFOA isomers

Carbon chain length and structure	CAS registry number
8 	335-67-1
7 	207678-51-1
7 	705240-04-6
7 	1144512-18-4
7 	909009-42-3
7 	15166-06-0
6 	1144512-35-5
6 	1192593-79-5
6 	1144512-36-6
6 	1144512-34-4
6 	35605-76-6

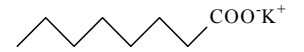
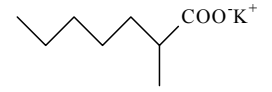
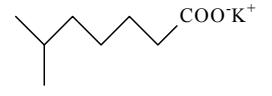
b. Ammonium salts of PFOA isomers

Carbon chain length and structure	CAS registry number
8 	3825-26-1
7 	207678-62-4
7 	19742-57-5
6 	13058-06-5

c. Sodium salts of PFOA isomers

Carbon chain length and structure	CAS registry number
8 	335-95-5
7 	207678-72-6
7 	646-84-4
7 	18017-22-6
6 	1195164-59-0

d. Potassium salts of PFOA isomers

Carbon chain length and structure	CAS registry number
8 	2395-00-8
7 	207678-65-7
7 	29457-73-6

Adapted from Nielsen (2012)
CAS, Chemical Abstracts Service

Table 1.1 Selected methods for the analysis of perfluorooctanoic acid (PFOA)

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Drinking-water	Adsorb on polystyrene divinylbenzene; elute methanol; reconstitute in water/methanol with ^{13}C-PFOA internal standard	HPLC-MS/MS	1.7 ng/L	EPA (2009a) Method 537-1
Indoor and outdoor air	Collect particle-bound PFOA on glass fibre filters; elute methanol	HPLC-TOF/MS	1 pg/m^3	Barber et al. (2007)
Human serum	Precipitate proteins with formic acid; solid phase extraction clean-up	HPLC-MS/MS	0.1 ng/mL	Kuklenyik et al. (2005)
Human milk	Precipitate proteins with formic acid; solid phase extraction clean-up	HPLC-MS/MS	0.2 ng/mL	Kuklenyik et al. (2004)
Animal tissue	Add homogenized tissue to buffered tetra-n-butylammonium hydrogensulfate solution; Extract with *tert*-butyl methyl ether	HPLC-TOF/MS	1.25 ng/g ww	Berger & Haukås (2005)
Soil	Rehydrate soil to ~50% moisture; extract with acetonitrile/water; sonicate and centrifuge; decant supernatant	HPLC-MS/MS	180 fg on column	Washington et al. (2008)
Foods and food packaging	Methanol extraction	HPLC-MS/MS	0.5 ng/g ww	Tittlemeier et al. (2007)

HPLC-MS/MS, high-performance liquid chromatography-mass spectrometry/mass spectrometry; MS, mass spectrometry; TOF, time-of-flight mass spectrometry; ww, wet weight

1.1.4 Technical products and impurities

See Fig. 1.1

Perfluorooctanoic acid (PFOA) produced by the electrochemical fluorination (ECF) method, before 2002, was reported to have a consistent isomer composition of 78% linear isomer (standard deviation, 1.2%) and 22% branched-chain isomer (standard deviation, 1.2%) in 18 production lots over a 20-year period, as determined by ^{19}F nuclear magnetic resonance. PFOA produced by the telomerization method (major use from 2002 to present) is typically an isomerically pure, linear product (Benskin et al., 2010).

PFOA produced by ECF was reported to contain the following impurities: perfluorohexanoate, 0.73%; perfluoroheptanoate, 3.7%; perfluorononanoate, 0.2%; perfluorodecanoate, 0.0005%; perfluoroundecanoate, 0.0008%; and perfluorododecanoate, 0.0008% (Benskin et al., 2010).

1.1.5 Analysis

Selected methods for the analysis of PFOA in various matrices are listed in Table 1.1. Methods for the trace analysis of PFOA in human serum and milk, in food and consumer products, as well as in environmental samples such as wildlife, water, solid matrices, and air have been reviewed (ATSDR, 2009; Jahnke & Berger, 2009).

1.2 Production and use

1.2.1 Production process

Perfluoroalkyls have been manufactured industrially by two methods: electrochemical fluorination (ECF) and telomerization. The two techniques can be distinguished based on the isomeric profile of their products. ECF (major use from the 1950s to 2002) results in a product containing both linear and branched isomers, while telomerization (major use from 2002 to

Table 1.2 Production volumes for perfluorooctanoic acid (PFOA) in the USA, 1986–2002

Substance produced	Production volume range (pounds)				
	1986	1990	1994	1998	2002
Perfluorooctanoic acid	10 000–500 000	Not reported	10 000–500 000	10 000–500 000	10 000–500 000
Ammonium perfluorooctanoate	10 000–500 000	10 000–500 000	10 000–500 000	10 000–500 000	500 000–1 000 000

From ATSDR (2009); reported under the United States Environmental Protection Agency Inventory Update Rule
Note: 10 000–500 000 pounds corresponds to approx. 4.5–227 tonnes; and 500 000–1 000 000 pounds corresponds to approx. 227–454 tonnes

present) typically yields an isomerically pure, linear product (ATSDR, 2009).

During the ECF process, an organic acyl backbone structure is dissolved in a solution of aqueous hydrogen fluoride. A direct electrical current is then passed through the solution, which replaces all of the hydrogens on the molecule with fluorines. Perfluoroacyl fluorides produced by ECF are hydrolysed to form the perfluorocarboxylic acid, which is then separated via distillation (ATSDR, 2009).

From 1947 until 2002, ECF was used worldwide to manufacture most (80–90% in 2000) PFOA, as the ammonium salt. The largest production sites were in the USA and Belgium, the next largest were in Italy, and small-scale producers were located in Japan. From about 1975 to the present, the remaining 10–20% of ammonium perfluorooctanoate was manufactured by direct oxidation of perfluorooctyl iodide at one site in Germany, and at least one site in Japan. In 1999, the global annual production of ammonium perfluorooctanoate was approximately 260 tonnes. By 2002, the principal worldwide manufacturer of ammonium perfluorooctanoate using ECF had discontinued external sales and ceased production, leaving only a few relatively small producers in Europe and in Asia (Prevedouros et al., 2006). Production volumes of PFOA, as both the acid and the ammonium salt, in the USA from 1986 to 2002 are shown in Table 1.2.

The telomerization process begins with the preparation of pentafluoroiodoethane from tetrafluoroethane. Tetrafluoroethane is then added to the product at a molar ratio that gives a product of desired chain length, and the final product is oxidized to form the carboxylic acid. The telomerization process produces linear perfluorocarboxylic acids with even numbers of carbon atoms (ATSDR, 2009).

New production capacity for ammonium perfluorooctanoate based on perfluorooctyl iodide commenced in the USA in late 2002. In 2006, the eight major manufacturers of PFOA in the USA joined the 2010/2015 PFOA Stewardship Program, a voluntary programme run by the United States Environmental Protection Agency (EPA) with the aim of reducing facility emissions and product content of PFOA, its precursors, and higher homologues by 95% by 2010, compared with the year 2000 (EPA, 2014). These manufacturers also agreed to the goal of totally eliminating these substances from emissions and product contents by 2015. Six of the eight manufacturers reported at least 95% reduction in emissions of PFOA by the end of 2010 in the USA. Substantial reductions in product content were also reported by these manufacturers for 2010 relative to 2000, both in the USA and in global operations. In a few cases, data were withheld by the manufacturers to protect business interests – particularly for non-USA operations and for precursors (EPA, 2014). Ammonium perfluorooctanoate is currently manufactured in Japan via oxidation of a mixture of linear fluorotelomer olefins (Prevedouros et al., 2006).

1.2.2 Uses

PFOA and it salts have been used as emulsifiers to solubilize fluoromonomers and to facilitate their aqueous polymerization in the production of fluoropolymers such as polytetrafluoroethylene and fluoroelastomers, used as non-stick coatings on cookware, membranes for clothing that are both waterproof and breathable, electrical-wire casing, fire- and chemical-resistant tubing, and plumber's thread-seal tape (ATSDR, 2009). Fluoropolymer manufacture is the single largest direct use of the ammonium salts of PFOA (Prevedouros et al., 2006).

PFOA has also been used in cosmetics, greases and lubricants, paints, polishes, adhesives, and fluorinated surfactants (HSDB, 2014). Widespread use of perfluorocarboxylates, including PFOA, and derivatives as additives in industrial and consumer products in 1966 included metal cleaners, electrolytic-plating baths, self-shine floor polishes, cement, fire-fighting formulations, varnishes, emulsion polymerization, lubricants, gasoline, and paper, leather, and textile treatments (Prevedouros et al., 2006). PFOA has found use as a grease and water-repellent coating in food packaging (Fromme et al., 2009).

Perfluorocarboxylates, including PFOA, were used as a component in aqueous fire-fighting foam from about 1965 to 1975. These formulations were used by the military (e.g. at aircraft bases and aboard ship) and in oil and gas production, refining industries, and airports worldwide (Prevedouros et al., 2006).

1.3 Occurrence and exposure

1.3.1 Environmental occurrence

The sources of emissions of PFOA to the environment are: (a) their manufacture, use and disposal; (b) their presence as impurities in substances that are emitted to the environment; and (c) precursor substances that degrade abiotically or biotically in the environment (Buck et al., 2011). One reference defined all chemicals with a C_7F_{15} or C_8F_{17} perfluorinated alkyl moiety and a direct bond to any chemical moiety other than a fluorine, chlorine, or bromine atom, as potential precursors of PFOA (Environment Canada, 2012). For example, 8:2 polyfluoroalkyl phosphates have been measured in human serum and can be metabolized to 8:2 fluorotelomer alcohol (8:2 FTOH) and/or PFOA in animal models (Lee & Mabury, 2011; Environment Canada, 2012). However, the extent to which the various precursors are metabolized in humans, and their relative contribution to serum concentrations of PFOA, are not well understood.

Under normal environmental conditions, PFOA is highly persistent, with photodegradation and hydrolysis half-lives of months to years, and insignificant biotic degradation (Environment Canada, 2012). It has low to moderate potential to accumulate in aquatic species, but does appear to accumulate in some terrestrial and marine mammals (Environment Canada, 2012).

(a) Natural occurrence

PFOA is not known to occur naturally.

(b) Air

Although PFOA is not routinely monitored in air, sporadic measurements have been reported. Fromme et al. (2009) reviewed the literature and reported site mean concentrations of PFOA in air ranging from 1.4 to 552 pg/m^3 from 11 rural and urban outdoor sampling sites in Japan, Canada, the United Kingdom, Norway, Ireland, and the USA; the highest measurements were from urban locations or adjacent to busy roads. PFOA and 8:2 FTOH have been found in remote Arctic areas far from known sources, suggesting long-range aerial transport. Concentrations of PFOA ranging from 0.012 to 0.147 ng/L were reported in polar ice caps in the High Arctic in 2006 (Environment Canada, 2012).

(c) Water

Samples from potable water supplies without known point sources of perfluorooctanoate contamination typically contain perfluorooctanoate at < 1 ng/L, or at levels below the detection limit (Fromme et al., 2009). However, higher concentrations in drinking-water have been reported for some locations. For example, Kim et al. (2011b) reported average concentration of perfluorooctanoate of 5.4 ng/L, and a maximum concentration of 33 ng/L, for 15 tap-water samples collected in 8 cities in the Republic of Korea. Surface water from Boulder basin of Lake Mead, the Hoover dam, and the lower Colorado River in the USA had average concentrations of perfluorooctanoate that were below the method reporting limit of 5 ng/L; however, samples affected by run-off from municipal wastewater treatment facilities had average concentrations of perfluorooctanoate ranging from 26 to 120 ng/L (Quiñones & Snyder, 2009).

Concentrations of perfluorooctanoate in water were measured in six public-water districts and for selected private wells in West Virginia, USA; these concentrations differed substantially by water district, varying by about three orders of magnitude (Fig. 1.2; Shin et al., 2011a). Perfluorooctanoate has also been measured at concentrations exceeding 1 ng/L in many of more than 8000 samples of surface water and groundwater collected in the region surrounding a large fluoropolymer-production facility in West Virginia, USA, probably due to direct emissions to the Ohio River, the air, and long-term transport through the vadose zone (DuPont, 2010). The highest off-site environmental concentrations of PFOA were predicted to occur about 1 mile [1.6 km] away from the production facility, and average concentrations in drinking-water ranged from < 0.05 to 10.1 μg/L in 2002–2004 (Paustenbach et al., 2007).

(d) Food

PFOA may be found in food due to contamination of plants and animals, and/or via transfer from food-packaging materials. Trudel et al. (2008) summarized several studies reporting measurements of PFOA in food in North America and Europe. Among the food categories, snacks and potatoes were reported to have the highest concentrations of PFOA (up to 3 ng/g wet weight), followed by packaged cereal products, meat, and North American fish/shellfish (up to 0.5, 1.0, and 2.0 ng/g, respectively). A list of measurements of PFOA concentrations in various foods is provided in Table 1.3.

(e) Dust

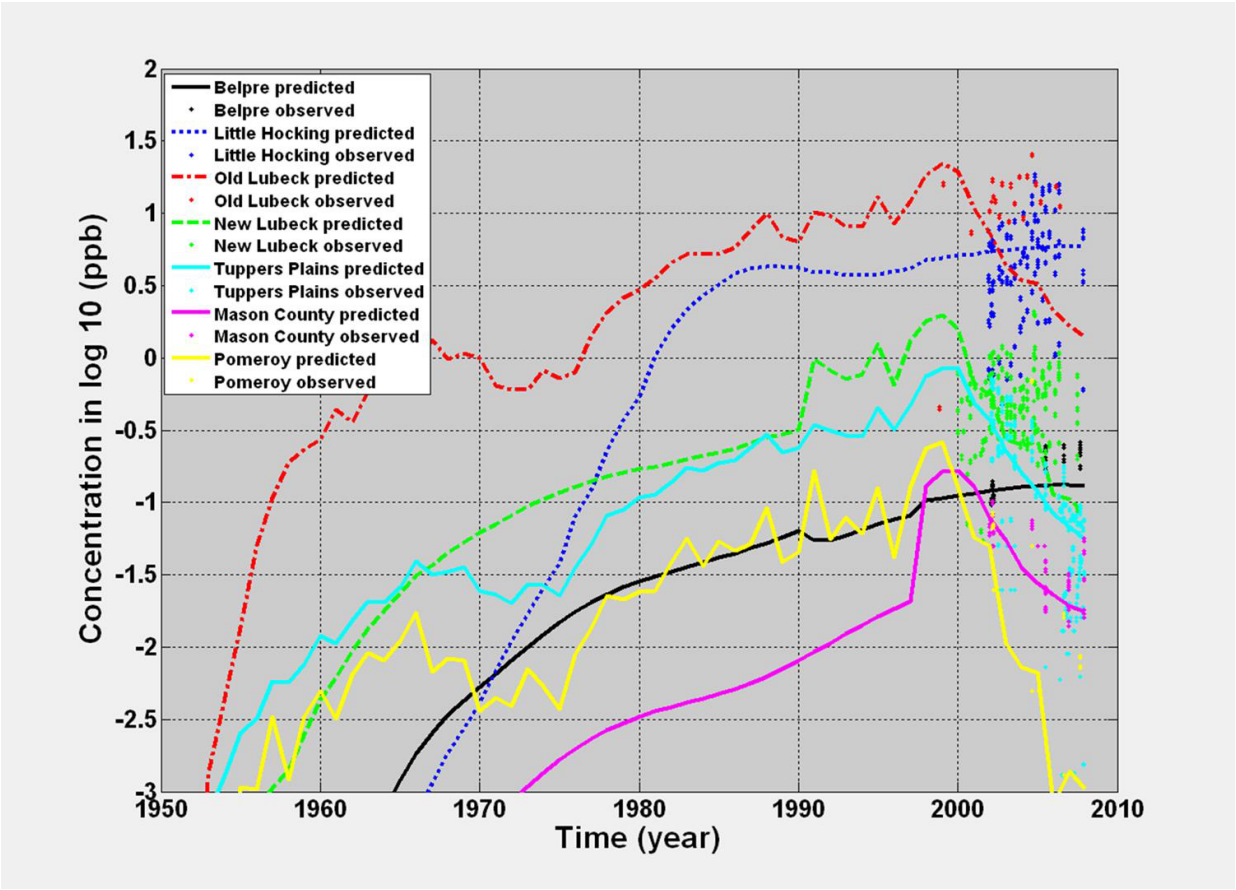
Trudel et al. (2008) and Fromme et al. (2009) reviewed the literature and estimated the concentrations of PFOA in dust in typical indoor environments as 100 ng/g and 19.72 ng/g, respectively.

Several studies suggested that the potential contribution of dust ingestion to exposure was higher than previously estimated. For example, one study in the USA reported a median concentration of PFOA in dust of 142 ng/g, and a 95th percentile of 1200 ng/g in dust collected at 102 homes and 10 day-care centres in Ohio and North Carolina, USA, in 2000–2001 (Strynar & Lindstrom, 2008). A study of 102 homes in Vancouver, Canada, reported median concentrations of 30 ng/g for PFOA in dust, 63 ng/g for 8:2 FTOH, and 1362 ng/g for the sum of polyfluoroalkyl phosphoric acid diesters containing at least one 8:2 polyfluoroalkyl group, suggesting that these potential precursors may contribute substantially to the body burden of PFOA if efficiently metabolized in the human body (De Silva et al., 2012).

1.3.2 Occupational exposure

In occupational settings, the primary routes of exposure are thought to be dermal and by inhalation (IFA, 2014). Studies of occupational

Fig. 1.2 Measured and modelled concentrations of perfluorooctanoic acid (PFOA) in water for the six public water districts in the C8 Health Project/C8 Science Panel studies, USA



For the Lubeck water district, different well locations were used before 1991 ("Old Lubeck") and after 1991 ("New Lubeck")
ppb, parts per billion
Reprinted with permission from Shin HM, Vieira VM, Ryan PB et al. Environmental fate and transport modelling for perfluorooctanoic acid emitted from the Washington Works Facility in West Virginia. Environmental Science and Technology, Volume 45, pages 1435–1442. Copyright (2011) American Chemical Society (Shin et al., 2011a)

exposure have typically described exposures to ammonium perfluorooctanoate, a salt of PFOA that is often produced in industry (Lundin et al., 2009; Woskie et al., 2012).

Woskie et al. (2012) summarized measurements of ammonium perfluorooctanoate in 2125 blood samples collected from workers in a fluoropolymer-production facility in West Virginia, USA, in 1972–2004; there was a peak in median serum concentrations in 2000 that exceeded 1000 µg/L in most highly exposed groups when PFOA was at the point of highest use. In 2000–2004, median serum concentration of perfluorooctanoate among these workers was 240 µg/L. Measured serum concentrations were paired with work histories to construct a model predicting serum concentration by job-exposure group from 1950 to 2004; in most years, the highest exposures were predicted for operators exposed to the fine powder or granular polytetrafluoroethylene chemical, for whom the predicted serum perfluorooctanoate concentration peaked in 1980, exceeding 6000 µg/L, and declined to about 2000 µg/L in 2004. Predicted

Table 1.3 Concentrations of perfluorooctanoic acid (PFOA) in food and drinking-water

Food category	Concentration (ng/g wet weight)	Year of sampling	Country or region	Reference
Meat products	< 0.4–2.6	2004	Canada	Tittlemeier et al. (2007)
Meat products (*n* = 8)	< 0.071	2006	Catalonia, Spain	Ericson et al. (2008)
Fish, marine	< 0.5	2004	Canada	Tittlemeier et al. (2007)
Fish, freshwater	< 0.5	2004	Canada	Tittlemeier et al. (2007)
Fish, freshwater	< 2	1998	Canada	Tittlemeier et al. (2007)
Trout (*n* = 47)	< 2–24	2006	Sauerland, Germany	Wilhelm et al. (2008)
Trout (*n* = 39)	< 2–5	2007	Sauerland, Germany	Wilhelm et al. (2008)
Other fish (*n* = 33)	< 2–8	2006	Sauerland, Germany	Wilhelm et al. (2008)
Other fish (*n* = 73)	< 2	2007	Sauerland, Germany	Wilhelm et al. (2008)
White fish (*n* = 2)	< 0.065	2006	Catalonia, Spain	Ericson et al. (2008)
Seafood (*n* = 2)	< 0.029	2006	Catalonia, Spain	Ericson et al. (2008)
Fish (muscle tissue)	< 0.2–5	2005	Germany	Gruber et al. (2007)
Fish (liver)	< 0.2–9	2005	Germany	Gruber et al. (2007)
Pizza	0.74	1998	Canada	Tittlemeier et al. (2007)
Microwave popcorn	3.6	1999	Canada	Tittlemeier et al. (2007)
Cereal products (*n* = 72)	ND–0.5	1999–2007	Europe and North America	Trudel et al. (2008)
Cereals (*n* = 6)	ND	1999–2007	Europe and North America	Trudel et al. (2008)
Cereals (*n* = 2)	< 0.080	2006	Catalonia, Spain	Ericson et al. (2008)
Dairy products (*n* = 6)	ND	1999–2007	Europe and North America	Trudel et al. (2008)
Dairy products (*n* = 2)	< 0.040	2006	Catalonia, Spain	Ericson et al. (2008)
Eggs (*n* = 86)	ND	1999–2007	Europe and North America	Trudel et al. (2008)
Eggs (*n* = 2)	< 0.055	2006	Catalonia, Spain	Ericson et al. (2008)
Fats and oils (*n* = 2)	ND	1999–2007	Europe and North America	Trudel et al. (2008)
Margarine	< 0.115	2006	Catalonia, Spain	Ericson et al. (2008)
Oil	< 0.247	2006	Catalonia, Spain	Ericson et al. (2008)
Fish and shellfish (*n* = 155)	ND–2	1999–2007	Europe and North America	Trudel et al. (2008)
Tinned fish	< 0.126	2006	Catalan, Spain	Ericson et al. (2008)
Blue fish	< 0.132	2006	Catalonia, Spain	Ericson et al. (2008)
Fruits (*n* = 76)	ND–0.3	1999–2007	Europe and North America	Trudel et al. (2008)
Fruits (*n* = 2)	< 0.036	2006	Catalonia, Spain	Ericson et al. (2008)
Meat (*n* = 262)	ND–1	1999–2007	Europe and North America	Trudel et al. (2008)
Milk (*n* = 82)	ND	1999–2007	Europe and North America	Trudel et al. (2008)
Whole milk (*n* = 2)	0.056	2006	Catalonia, Spain	Ericson et al. (2008)
Semi-skimmed milk	< 0.028	2006	Catalonia, Spain	Ericson et al. (2008)
Potatoes (*n* = 26)	0.4–2	1999–2007	Europe and North America	Trudel et al. (2008)
Potatoes	< 0.2–3	2006	Germany	Gruber et al. (2007)
Poultry (*n* = 78)	ND	1999–2007	Europe and North America	Trudel et al. (2008)
Snacks (*n* = 4)	0.9–3	1999–2007	Europe and North America	Trudel et al. (2008)
Sweets (*n* = 2)	ND	1999–2007	Europe and North America	Trudel et al. (2008)
Tap water (*n* = 102)	0.009–0.02	1999–2007	North America	Trudel et al. (2008)
Tap water (*n* = 28)	ND–0.2	1999–2007	Europe	Trudel et al. (2008)

Table 1.3 (continued)

Food category	Concentration (ng/g wet weight)	Year of sampling	Country or region	Reference
Vegetables (n = 77)	ND–0.3	1999–2007	Europe and North America	Trudel et al. (2008)
Vegetables (n = 2)	< 0.027	2006	Catalonia, Spain	Ericson et al. (2008)
Pulses (n = 2)	< 0.045	2006	Catalonia, Spain	Ericson et al. (2008)
Water-based drinks (n = 2)	ND	1999–2007	Europe and North America	Trudel et al. (2008)

ND, not detected

serum concentrations in operators exposed to fluorinated ethylene propylene/perfluoroalkoxy were < 1000 µg/L before 1975, increasing to about 2000 µg/L by 2004. Predicted serum concentrations for operators using the fine powder/granular polytetrafluoroethylene finish declined from about 1500–2000 µg/L in 1950–1980 to about 500–1000 µg/L in 1990–2004. Predicted serum concentrations for job-exposure groups with intermittent direct or plant background exposures were < 1600 µg/L in all years.

Another study of 506 fluoropolymer-production workers in Belgium, Minnesota, and Alabama, USA, reported a median serum concentration of perfluorooctanoate of 1100 µg/L in 2000 (Olsen & Zobel, 2007). Median serum concentrations of perfluorooctanoate were 650, 950, and 1510 µg/L among workers at the facilities in Belgium, Minnesota, and Alabama, respectively.

In both studies described above, serum perfluorooctanoate measurements exceeded 10 000 µg/L for some workers (Olsen & Zobel, 2007; Woskie et al., 2012). No measurements of PFOA, ammonium perfluorooctanoate, or precursors in workplace air, work surfaces, or skin were reported in these studies of occupational exposure. In a separate study, Kaiser et al. (2010) reported eight-hour time-weighted average (TWA) concentrations of PFOA in air ranging from 0.004–0.065 mg/m^3 near process sumps in an unidentified facility producing ammonium perfluorooctanoate and PFOA.

In China, 48 workers involved in the manufacture of footware had mean serum concentrations of PFOA of 6.93 µg/L (range, 0.17–117.7 µg/L) (Zhang et al., 2011).

As part of an international epidemiological study of workers in six plants manufacturing polytetrafluoroethylene in Germany, the Netherlands, Italy, the United Kingdom, New Jersey, and West Virginia, Sleeuwenhoek & Cherrie (2012) estimated exposure to ammonium perfluorooctanoate by inhalation and dermal routes using modelling. The exposure reconstructions were made using descriptive information about the workplace environment and work processes, including changes over time in local ventilation, use of respiratory protective equipment, working in a confined space, outdoor work, cleanliness and the level of involvement of the workers in the process (for example, operator or supervisor). There were very few measurements of exposure available from the plants (all unpublished) and so the exposure estimates were expressed on an arbitrary dimensionless scale. In each plant, the highest estimated exposures to ammonium perfluorooctanoate were considered to have occurred in the polymerization area, with an annual decline in exposure varying from 2.2% to 5.5%. At any point in time, the differences between plants in the average estimated exposure level for polymerization workers were up to about fivefold. Among workers in the six plants whose jobs involved exposure to both tetrafluoroethylene and ammonium perfluorooctanoate, the correlation between the two

exposure estimates was 0.72 (Sleeuwenhoek & Cherrie, 2012). There were some workers with no exposure to ammonium perfluorooctanoate and low-to-moderate exposure to tetrafluoroethylene, but no workers who were exposed to ammonium perfluorooctanoate without tetrafluoroethylene exposure (Sleeuwenhoek & Cherrie, 2012; Consonni et al., 2013).

1.3.3 Exposure in the general population

(a) Serum concentrations

Human exposure to PFOA has often been assessed using measured or predicted concentrations of perfluorooctanoate in serum or plasma (Eriksen et al., 2009; Fromme et al., 2009; Bonefeld-Jorgensen et al., 2011; Barry et al., 2013; Vieira et al., 2013a; Hardell et al., 2014). The pharmacokinetics of PFOA differ widely between species, with short half-lives and strong sex differences in rats, but a half-life of 2.3–3.5 years and no observed sex differences in humans (Olsen et al., 2007; Bartell et al., 2010). The Canadian Health Measures Survey reported that the median and geometric mean plasma concentrations of perfluorooctanoate among Canadians aged 20–79 years in 2007–2009 were both 2.5 µg/L, and the 95th percentile was 5.5 µg/L (Environment Canada, 2012).

The California Environmental Contaminant Biomonitoring Program reported median serum measurements of perfluorooctanoate of 2.49 µg/L for 1337 teachers and school administrators in 2011–2014, and 0.474 µg/L for 77 pregnant women in 2010–2011 (California Department of Public Health, 2014). Yeung et al. (2013) reported a median serum PFOA concentration of 2.34 µg/L among 25 Australian liver donors in 2007–2009, noting a substantial decline in serum PFOA compared with previous reports of pooled Australian samples in 2002–2003 [7.6 µg/L] (Kärrman et al., 2006) and 2006–2007 [6.4 µg/L] (Toms et al., 2009). In a study of 413 pregnant and nursing women in Sweden, serum PFOA concentrations declined by an average of 3.1% per year (95% CI, 1.8–4.4%) from 1996–2010 (Glynn et al., 2012).

Geometric mean serum concentrations of perfluorooctanoate in the USA population based on serum measurements from the National Health and Nutrition Examination Survey were 5.2 µg/L, 3.9 µg/L, 3.9 µg/L, and 4.1 µg/L in 1999–2000, 2003–2004, 2005–2006, and 2007–2008, respectively, with similar concentrations in different age groups, but slightly higher concentrations in males than females (Calafat et al., 2007a; Kato et al., 2011). The 95th percentile of serum perfluorooctanoate concentrations did not exceed 12 µg/L in any of those years (Kato et al., 2011). Pooled samples from 3802 Australian residents in 2002–2003 yielded a mean perfluorooctanoate serum concentration of 7.6 µg/L (Kärrman et al., 2006) – a value roughly consistent with the geometric mean in the USA, considering that these measurements were positively skewed. Smaller studies of general populations in Europe, Asia, and the USA for samples collected in 1989–2006 have produced similar findings, with reported average concentrations ranging from 1.6 to 11.6 µg/L (Fromme et al., 2009).

Several studies of serum measurements of perfluorooctanoate are available for stored samples collected before the 1990s. Olsen et al. (2005) reported a median serum concentration of perfluorooctanoate of 2.3 µg/L for 178 blood samples collected in Maryland, USA, in 1974, and Harada et al. (2004) reported a geometric mean serum concentration of perfluorooctanoate of 0.2 µg/L for 39 blood samples collected from females in Miyagi, Japan, in 1977. Haug et al. (2009) reported serum concentrations of perfluorooctanoate in samples from a biobank of hospital patients in Norway, pooling samples by year (n > 19 for most years) for the period 1977–2006. Perfluorooctanoate serum concentrations in this study rose from 0.58 µg/L in 1977

Table 1.4 Concentrations of perfluorooctanoic acid (PFOA) in human breast milk

Food category	Concentration (ng/L)	Year of sampling	Country or region	Reference
Breast milk (n = 19)	47–210 [100%]	2004	China	So et al. (2006)
Breast milk (n = 70)	< 200–460 [16%]	2006	Bavaria, Germany	Völkel et al. (2008)
Breast milk (n = 203)	80–610 [55%]	2007	North Rhine-Westphalia, Germany	Bernsmann & Fürst (2008)
Breast milk (n = 51)	< LOD–340 [44%]	2007	Japan	Nakata et al. (2007)
Breast milk (n = 31)	50–300	1999–2007	Europe and North America	Trudel et al. (2008)
Breast milk (n = 12)	< 209–492	2004	Sweden	Kärrman et al. (2007)

LOD, limit of detection

to 1.3 µg/L in 1980, 3.3 µg/L in 1990, and 4.5 µg/L in 2000, falling to 2.7 µg/L in 2006.

Mean concentrations of perfluorooctanoate were measured in 258 samples of blood, serum, or plasma collected from men between 2000 and 2004 in the USA (Michigan) (5.7 µg/L; < 3–14.7 µg/L), Colombia (6.2 µg/L; 3.9–12.2 µg/L), Brazil (< 20 µg/L), Belgium (5.0 µg/L; 1.1–13 µg/L), Italy (< 3 µg/L), Poland (20.5 µg/L; 11–40 µg/L), India (3.5 µg/L; < 3–3.5 µg/L), Malaysia (< 10 µg/L), suggesting the presence of specific sources of PFOA in this country (Kannan et al., 2004). Relatively higher concentrations of PFOA were reported in the Republic of Korea (35.5 µg/L; < 15–71.4 µg/L) (Kannan et al., 2004).

Overall, the published data suggested that serum PFOA concentrations in the general population increased over time until about 2000, and have remained constant or decreased since that time.

Higher serum concentrations of perfluorooctanoate have been reported in general populations near production facilities and other known exposure sources. For example, the geometric mean serum concentration of perfluorooctanoate in 2005–2006 among 69030 residents living near a production facility in West Virginia, USA, was 32.9 µg/L (standard deviation, 241 µg/L). Exposures in that community varied substantially across six water districts; the mean serum concentration of PFOA was about 16 µg/L in the two water districts with the lowest water concentrations of PFOA, and 228 µg/L in the water district with the highest concentrations (Frisbee et al., 2009). A study of 641 residents of Arnsberg, Germany, in 2006 reported mean serum concentrations of perfluorooctanoate of 24.6, 26.7, and 28.5 µg/L in children, mothers, and men, respectively, due to surface water contamination from upstream agricultural use of soil conditioner mingled with industrial waste (Hölzer et al., 2008).

(b) Breast milk

PFOA has been measured in breast milk; these data are presented in Table 1.4.

In North Rhine-Westphalia, Germany, more than half of the samples analysed in 2007 (n = 203) contained PFOA; concentrations up to 610 ng/L have been reported (Bernsmann & Fürst, 2008). In China, PFOA was measured in 100% of the breast milk samples analysed (n = 19) in 2004 (So et al., 2006).

(c) Exposure sources

As PFOA and its precursors are not routinely or systematically monitored in air, water, dust, food, or drinking-water, the relative contributions of exposure sources in the general population are not well understood. Published studies of exposure have relied on synthesis of environmental measurements collected at varying times and places, often in different countries. These studies comprise the best available data, but are

typically based on convenience samples in one or few locations, and may not be representative of regional, national, or global exposures.

One such study has estimated that diet (including transfer of PFOA from food packaging) contributes 99% of total exposure to PFOA for adults in the general population in "western" countries, with negligible contributions from inhalation and ingestion of house dust and drinking-water (Fromme et al., 2009). Estimated adult mean PFOA intakes via indoor air, outdoor air, house dust, diet, and drinking-water were 0.053, 0.076, 0.986, 169, and 1.3 ng/day, respectively. The estimated dietary contribution was based on PFOA measurements in a 7-day duplicate-diet study of 31 participants aged 16–45 years in Germany (Fromme et al., 2007); estimated inhalation contributions were based on PFOA measurements at one indoor and four outdoor sites in Europe (Barber et al., 2007; the estimated house-dust ingestion contribution was based on measurements of PFOA from 67 homes in Ottawa, Canada, in the winter (Kubwabo et al., 2005), and the estimated contribution of drinking-water ingestion was based on river-water samples from the Rhine and its tributaries in Germany (Skutlarek et al., 2006). The contribution of house dust to PFOA exposure was based on a conservative estimate of 5% conversion of 8:2 FTOH to PFOA. Other precursor concentrations may actually exceed those of PFOA and 8:2 FTOH in house dust, but the extent of precursor metabolism in humans is unclear (De Silva et al., 2012).

Trudel et al. (2008) estimated that ingestion of food and house dust contributed > 90% of exposure to PFOA in adults, noting that PFOA-treated carpets and ingestion of dust may account for a larger proportion of exposure among children than adults. Typical uptake doses of PFOA for infants, toddlers, children, and teenagers/adults were estimated at 9.8, 7.6, 5.0, and 2.5–3.1 ng/kg body weight (bw) per day in North America and 6.0, 7.6, 6.7, and 2.8–4.1 ng/kg bw per day in Europe, respectively, based primarily on food concentrations of PFOA from data extracted from four previous studies covering 17 food categories with 1–131 samples each (for most food categories, measurements from Europe and North America were combined due to small sample sizes) and house-dust concentrations of PFOA from data from three small studies in Canada, Japan, and the USA (Moriwaki et al., 2003; Costner et al., 2005; Kubwabo et al., 2005). Infants may be exposed primarily through mother's milk (So et al., 2006; Kärrman et al., 2010; Kim et al., 2011a), for which the estimated perfluorooctanoate concentration was reported as 0.1 ng/g (Trudel et al., 2008).

However, drinking-water may have a larger contribution to exposure to PFOA in some populations. For example, Kim et al. (2011b) estimated that drinking-water ingestion contributes 30% of total exposure to PFOA in the Republic of Korea, where urban water supplies are often contaminated.

Drinking-water is thought to have been the predominant source of intake of PFOA for a highly exposed population near a production facility in West Virginia, USA, studied by the C8 Science Panel (Barry et al., 2013; Vieira et al., 2013a; Steenland et al., 2014), where both surface water and groundwater were contaminated by water and air emissions from the facility (Shin et al., 2011a).

Although residual amounts of PFOA (4–75 ng/g) and 8:2 FTOH are contained in non-stick cookware and can be released to the gas phase in small quantities when heated to normal cooking temperature, their contribution to exposure tends to decline with repeated use and is believed to be negligible compared with other exposure sources (Fromme et al., 2009). One study of four non-stick cookware items (with three samples each) reported emission rates of 19–287 and 42–625 pg/cm^2, for PFOA and 8:2 FTOH respectively, upon first heating after purchase; concentrations of gas-phase

PFOA were shown to decrease after repeated use (four times) for some cookware brands, but not for others. PFOA may be off-gassed at different rates from non-stick coatings, depending on how the non-stick coating was prepared and applied (Sinclair et al., 2007).

1.4 Regulations and guidelines

The EPA has a Provisional Health Advisory value of 0.4 µg/L for PFOA in drinking-water (EPA, 2009b).

The European Food Safety Authority (EFSA) recommended a tolerable daily intake (TDI) for PFOA of 1.5 µg/kg bw per day (EFSA, 2008).

The Environmental Agency of Norway has announced the following limits on PFOA in consumer products, which became effective as from 1 July 2014: 10 ppm in substances and mixtures; 1 mg/m^2 in textiles, carpeting, and other coated consumer products; and 1000 ppm in other consumer products. Food packaging, food contact materials, and medical devices are exempt from these limits in Norway (UL, 2014).

Germany has established an air quality control limit for PFOA of 0.15 g/hour and 0.05 mg/m^3 of dusts (including ammonium perfluorooctanoate) in exhaust gas (IFA, 2014).

PFOA and ammonium pentadecafluorooctanoate have been identified by the European Chemicals Agency as a Substance of Very High Concern under Article 57 (c) of the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulations as toxic for reproduction 1B and under Article 57 (d) as a substance that is persistent, bioaccumulative, and toxic, in accordance with the criteria and provisions set out in Annex XIII of the Regulations (ECHA, 2013).

2. Cancer in Humans

See Table 2.1, Table 2.2 and Table 2.3

Data on the occurrence of cancer in humans exposed to PFOA are available from epidemiological studies in three different types of populations: workers in chemical plants producing or using PFOA, communities surrounding a plant with environmental release of PFOA and contamination of public and private water supplies, and studies in the general population with background exposures. These studies have focused on cancers of the kidney, bladder, liver, pancreas, testes, prostate, thyroid, and breast because of initial findings from the epidemiological studies, or because of congruence with sites of toxicity identified in experimental studies in animals. Cancer incidence, rather than mortality, provides a stronger basis for inferring causation for these diseases because, except for cancers of the liver and pancreas, survival is relatively high (i.e. 5-year survival, > 70%) for these cancer types (SEER, 2014). Studies of incident cases of cancer of the prostate may also present challenges with respect to consideration of the influence of use of screening tests (e.g. prostate-specific antigen testing), and variation in use of these tests, among study participants.

2.1 Occupational exposure

See Table 2.1

Studies of occupational cohorts were conducted in plants in West Virginia (Leonard et al., 2008; Steenland & Woskie, 2012) and Minnesota (Gilliland & Mandel, 1993; Lundin et al., 2009; Raleigh et al., 2014), USA; results from the most recent general follow-up are summarized in Table 2.1. A study of workers producing tetrafluoroethylene (Consonni et al. (2013) also provides some potentially relevant information, but was not included in the tables because the study population overlapped with

Table 2.1 Cohort studies on cancer and occupational exposure to perfluorooctanoic acid (PFOA)

Reference, location, follow-up period	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Steenland & Woskie (2012) West Virginia, USA, 1950–2008	5791	JEM using 2125 serum samples collected in 1979–2004 to develop regression models to predict exposure by year for 8 job-category groups	Kidney	Dupont referent	12	1.28 (0.66–2.24)	SMR, unlagged; no covariates other than those used for rate standardization; two sets of analyses presented (Dupont plants – plants from 8 surrounding states, excluding study plant and US referents); similar patterns seen with 10- and 20-year lags Increased risk of mesothelioma (SMR, 2.85; highest quartile SMR, 6.27)
				US referent	12	1.09 (0.56–1.90)	
				By quartile (ppm-yrs)			
				0 to < 904	1	1.07 (0.02–3.62)	
				904 to < 1520	3	1.37 (0.28–3.99)	
				1520 to < 2720	0	0.0 (0.00–1.42)	
				≥ 2720	8	2.66 (1.15–5.24)	
			Bladder	Dupont referent	10	1.08 (0.52–1.99)	
				US referent	10	0.95 (0.46–1.75)	
			Liver	Dupont referent	10	1.07 (0.51–1.96)	
				US referent	10	0.77 (0.35–1.47)	
			Pancreas	Dupont referent	18	1.04 (0.62–1.64)	
				US referent	18	0.85 (0.51–1.35)	
			Breast	Dupont referent	4	0.65 (0.13–1.90)	
				US referent	4	0.79 (0.21–2.02)	
			Testis	Dupont referent	1	1.80 (0.05–10.03)	
				US referent	1	0.74 (0.02–4.12)	
			Prostate	Dupont referent	21	0.76 (0.47–1.16)	
				US referent	21	0.72 (0.45–1.10)	
			All cancers	Dupont referent	304	0.93 (0.83–1.04)	
				US referent	304	0.74 (0.66–0.83)	

Table 2.1 (continued)

Reference, location, follow-up period	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Raleigh et al. (2014) [update of Lundin et al., 2009 and Gilliland & Mandel, 1993] Minnesota, USA, 1947–2008	4668	JEM using personal and area samples collected in 1977–2000; 8-hour TWA for PFOA calculated for 23 departments and 45 job titles	Kidney [mortality]	Q1–Q2 (< 1.5×10^{-4} µg/m³-yr)	3	0.38 (0.11–1.23)	Time-dependent Cox regression (HR), by quartile of cumulative exposure, adjusted for year of birth and sex; referent was workers in St Paul, Minnesota (non-exposed; assigned general background exposure) Incidence analysis was limited to 1988–2008 Unexposed group from another plant in the area (St Paul, Minnesota) also included (n = 4359)
				Q3–Q4 (> 1.5×10^{-4} µg/m³-yr)	3	0.39 (0.11–1.32)	
			Kidney [incidence]	Q1 (< 2.9×10^{-5} µg/m³-yr)	4	1.07 (0.36–3.16)	
				Q2 (2.9×10^{-5} to 1.5×10^{-4} µg/m³-yr)	4	1.07 (0.36–3.17)	
				Q3 (1.5×10^{-4} to 7.9×10^{-4} µg/m³-yr)	4	0.98 (0.33–2.92)	
				Q4 (> 7.9×10^{-4} µg/m³-yr)	4	0.73 (0.21–2.48)	
			Bladder [mortality]	Q1–Q2 (< 1.5×10^{-4} µg/m³-yr)	3	1.03 (0.27–3.96)	
				Q3–Q4 (> 1.5×10^{-4} µg/m³-yr)	5	1.96 (0.63–6.15)	
			Bladder [incidence]	Q1 (< 2.9×10^{-5} µg/m³-yr)	7	0.81 (0.36–1.81)	
				Q2 (2.9×10^{-5} to 1.5×10^{-4} µg/m³-yr)	6	0.78 (0.33–1.85)	
				Q3 (1.5×10^{-4} to 7.9×10^{-4} µg/m³-yr)	15	1.50 (0.80–2.81)	
				Q4 (> 7.9×10^{-4} µg/m³-yr)	12	1.66 (0.86, 3.18)	
			Liver and biliary passages [incidence]	Q1–Q2 (< 1.5×10^{-4} µg/m³-yr)	6	2.09 (0.69–6.31)	
				Q3–Q4 (> 1.5×10^{-4} µg/m³-yr)	2	0.67 (0.14–3.27)	
			Pancreas [mortality] (ICD codes, NR)	Q1 (< 2.9×10^{-5} µg/m³-yr)	2	0.32 (0.08–1.35)	
				Q2 (2.9×10^{-5} to 1.5×10^{-4} µg/m³-yr)	5	0.89 (0.34–2.31)	
				Q3 (1.5×10^{-4} to 7.9×10^{-4} µg/m³-yr)	5	0.82 (0.32–2.12)	
				Q4 (> 7.9×10^{-4} µg/m³-yr)	6	1.23 (0.50–3.00)	
			Pancreas [incidence]	Q1–Q2 (< 1.5×10^{-4} µg/m³-yr)	1	0.13 (0.02–1.03)	
				Q3–Q4 (> 1.5×10^{-4} µg/m³-yr)	9	1.36 (0.59–3.11)	

Table 2.1 (continued)

Reference, location, follow-up period	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Raleigh et al. (2014) (cont.)			Prostate [mortality]	Q1 (< 2.9×10^{-5} μg/m³-yr)	5	0.34 (0.25, 1.60)	
				Q2 (2.9×10^{-5} to 1.5×10^{-4} μg/m³-yr)	8	1.12 (0.53–2.37)	
				Q3 (1.5×10^{-4} to 7.9×10^{-4} μg/m³-yr)	3	0.36 (0.11–1.17)	
				Q4 (> 7.9×10^{-4} μg/m³-yr)	8	1.32 (0.61–2.84)	
			Prostate [incidence]	Q1 (< 2.9×10^{-5} μg/m³-yr)	42	0.80 (0.57–1.11)	
				Q2 (2.9×10^{-5} to 1.5×10^{-4} μg/m³-yr)	42	0.85 (0.61–1.19)	
				Q3 (1.5×10^{-4} to 7.9×10^{-4} μg/m³-yr)	49	0.89 (0.66–1.21)	
				Q4 (> 7.9×10^{-4} μg/m³-yr)	55	1.11 (0.82–1.49)	
			Breast [mortality] (ICD codes, NR)	Q1–Q2 (< 1.5×10^{-4} μg/m³-yr)	8	0.61 (0.25–1.48)	
				Q3–Q4 (> 1.5×10^{-4} μg/m³-yr)	3	0.54 (0.15–1.94)	
			Breast [incidence] (ICD codes, NR)	Q1 (< 2.9×10^{-5} μg/m³-yr)	8	0.36 (0.16–0.79)	
				Q2 (2.9×10^{-5} to 1.5×10^{-4} μg/m³-yr)	8	0.65 (0.29–1.42)	
				Q3 (1.5×10^{-4} to 7.9×10^{-4} μg/m³-yr)	14	1.47 (0.77–2.80)	
				Q4 (> 7.9×10^{-4} μg/m³-yr)	4	0.85 (0.29–2.46)	
			All cancers [mortality]	SMR	332	0.87 (0.78–0.97)	SMRs calculated based on state (Minnesota) expected rates

CI, confidence interval; HR, hazard ratio; ICD, International Classification of Disease; JEM, job-exposure matrix; Q, quartile; NR, not reported; SMR, standardized mortality ratio; TWA, time-weighted average; yr, year

other studies, and the assessment of exposure to PFOA was limited. This study is reviewed in detail in the *Monograph* on tetrafluoroethylene, in the present volume. Two other studies examined workers at a plant producing perfluorooctanesulfonyl fluoride in a plant in Alabama, USA (Alexander et al., 2003; Alexander & Olsen, 2007). The manufacturing process produced PFOA as a by-product, and PFOA was also used in some other production processes and was manufactured at the plant beginning in 1998. The focus of the studies in this plant has been on perfluorooctanesulfonate (PFOS) exposure measures, which are higher than, but correlated with PFOA exposures (Olsen et al., 2003a); these studies are not discussed further here.

For each of these cohorts, plant operations began around 1950; the study in West Virginia included individuals who had worked at least 1 day (Steenland & Woskie, 2012), while the Minnesota cohort required at least 365 work days for inclusion (Raleigh et al., 2014). The proportion of women was approximately 20%, and each was a relatively young cohort. The studies included a cumulative-exposure indicator based on a job-exposure matrix developed using serum PFOA concentrations in workers or air-monitoring data, but differed in terms of the extent of available samples and modelling of exposure, with consideration of changes in exposure over time. Standardized mortality ratios (SMR) for all causes, all cancers, and heart disease ranged from 0.7 to 1.0.

Steenland & Woskie (2012) examined mortality risk in 5791 workers (1084 deaths) in a fluoropolymer-production plant in West Virginia, USA, with a mean follow-up of 30 years. Exposure assessment was based on 2125 blood samples collected from 1979 to 2004. These data were used to define eight job group-categories based on similarity of exposure (Woskie et al., 2012). The categories included three with direct exposure, four with intermittent direct exposure, and plant background. Restricted cubic spline regression was used to model serum levels within each job category over time. This analysis was used to develop cumulative exposure estimates for each worker, based on their job-history data. Trends of increasing risk of cancer of the kidney and mesothelioma with increasing exposure to PFOA (P = 0.02) were observed, with standardized mortality ratios of 2.66 (95% CI, 1.15–5.24; 8 cases) and 6.27 (95% CI, 2.04–14.63; 5 cases), respectively, in the highest quartile of PFOA exposure. There was no indication of increased risk for cancers of the bladder, liver, pancreas, breast, or prostate (Table 2.1). [A strength of this study was the detailed exposure analysis, while a limitation was the small numbers. The Working Group interpreted the association between PFOA exposure and risk of mesothelioma to be an indication of exposure to asbestos in these workers.]

Raleigh et al. (2014) examined mortality risk in 4668 workers (1125 deaths) in a plant manufacturing ammonium perfluorooctanoate in Minnesota, USA, with a mean follow-up of 34 years. Exposure assessment was based on 205 personal air samples and 659 area samples collected from production areas in 1977–2000; exposures before 1977 were estimated based on variation in annual production levels; procedures and tasks had not changed over this period. The exposure data were combined with job-history data (department, job title, work area, equipment, task and year) to estimate time-weighted average exposures, which were then used to estimate cumulative exposure estimates for individual workers. Mortality was analysed for the period 1960–2008. Incidence data, based on Minnesota and Wisconsin state cancer registries were also included, but were limited to cases occurring since 1988, when both of these registries were in operation. Workers at another plant in the area, manufacturing tape and abrasive products, were used as the referent group (n = 4359) for internal analyses of mortality and incidence. For mortality from cancer of the bladder, the relative risk estimate for the combined upper two

quartiles of exposure (compared with unexposed referents) was 1.96 (95% CI, 0.63–6.15; 5 cases); in the analysis of incidence of cancer of the bladder (40 exposed cases), the pattern across the four quartiles of cumulative exposure was 0.81, 0.78, 1.50, and 1.66, respectively (Table 2.1). Cancer of the kidney was not associated with exposure to PFOA in analyses of mortality (6 exposed cases) or incidence (16 exposed cases). Examination of incidence and mortality data in relation to cumulative exposure revealed little or no evidence of increased risk of cancer of the liver, pancreas, prostate, or breast. Risks were not analysed for cancers of the thyroid or testes. [The Working Group noted the reasonable quality of the exposure data. Another strength of this study was the use of incidence data, but this analysis covered only a 20-year period, which limited the number of observed cases for some cancers.]

[In summary, these studies conducted in two different occupational cohorts included some evidence of an association between PFOA exposure and cancer of the kidney (Steenland & Woskie, 2012) or bladder (Raleigh et al., 2014), with elevated risks seen at higher exposures in one (but not both) of the studies. Elevated risk of cancer of the liver, pancreas, or breast in relation to higher exposure was not seen in either study, and the initial report of an increased risk of cancer of the prostate (Lundin et al., 2009) was not substantiated in subsequent analyses (Steenland & Woskie, 2012; Raleigh et al., 2014). These studies did not provide a basis for examining cancer of the testes or thyroid, since an analysis of incidence data was not available for these cancers.]

2.2 Community studies of high exposure

See Table 2.2

An area along the Ohio River in West Virginia and Ohio, USA, surrounding one of the fluoropolymer production plants described in the previous section has been the site of a series of community health studies. Emissions from this plant resulted in contamination of public water systems and private wells with PFOA. Three studies examined cancer risk for multiple cancer types (Barry et al., 2013; Vieira et al., 2013b) or specifically for cancer of the colon (Innes et al., 2014). [The Working Group noted that Barry et al. (2013) and Vieira et al. (2013b) were overlapping, rather than independent studies, in that the same geographical areas and some of the same cases are included in both analyses.]

Using a case–control design, Vieira et al. (2013b) examined incident cancers occurring in 1996–2005, using West Virginia and Ohio state cancer registries. Cases living in 13 counties around the fluoropolymer production plant were identified; analyses were limited to 18 cancer types that were of a-priori interest, or that had at least 100 cases in each state. The controls for each analysis were all other cancer types, excluding cancers of the kidney, liver, pancreas, and testes. In one set of analyses, residence at time of diagnosis was used to assign study participants to specific water districts in Ohio and West Virginia (Vieira et al., 2010, 2013a). A more robust exposure assessment was used in the second set of case–control analyses, restricted to the Ohio data, where exposure was estimated based on street-level data. This information was combined with emission data, environmental characteristics, and pharmacokinetic data to estimate annual exposure from 1951 to date of diagnosis, assuming that residence at time of diagnosis was the residence for the previous 10 years (Shin et al., 2011a, b). Residence in a contaminated water district was not associated with a notable increase in the risk of any cancer. In analyses of cancer incidence in relation to estimated serum PFOA concentrations, elevated risks of cancer of the kidney (2.0; 95% CI, 1.0–3.9; 9 cases) and testes (2.8; 95% CI, 0.8–9.2; 6 cases), and more modestly increased risks for cancer of the prostate

Table 2.2 Community-based studies(high-exposure setting) of cancer and exposure to perfluorooctanoic acid (PFOA)

Reference, study location, period, design	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Vieira et al. (2013b) Ohio and West Virginia, USA; case–control study; incident cases and controls from 1996–2005, from state cancer registries	23 107 cancer cases (West Virginia, 17 238; Ohio, 7869	For Ohio participants (analysis presented here), serum PFOA concentration for 1951–2008 was estimated using geocoded residence, emissions data, environmental characteristics, water pipe installation, and pharmacokinetic data	Kidney [incidence]	*Estimated serum levels (μg/L) 10 yr before diagnosis (Ohio)*			Logistic regression, adjusted for age, sex, diagnosis year, insurance provider, smoking status, and race; unlagged models also examined, with similar results Controls had cancers other than kidney, liver, pancreas, and testis (numbers not reported) Another set of analyses included both West Virginia and Ohio participants, but was limited to water district-level exposure assessment (not presented here)
				Low: 3.7–12.8	11	0.8 (0.4–1.5)	
				Medium: 12.9– 30.7	17	1.2 (0.7–2.0)	
				High: 30.8– 109	22	2.0 (1.3–3.2)	
				Very high: > 110	9	2.0 (1.0–3.9)	
			Bladder [incidence]	Low: 3.7– 12.8	23	0.9 (0.6–1.4)	
				Medium: 12.9– 30.7	21	0.9 (0.6–1.4)	
				High: 30.8– 109	21	1.2 (0.8–2.0)	
				Very high: > 110	4	0.6 (0.2–1.5)	
			Liver [incidence]	Low: 3.7–12.8	4	1.1 (0.4–3.1)	
				Medium: 12.9–30.7	4	0.9 (0.3–2.5)	
				High: 30.8–109	3	1.0 (0.3–3.1)	
				Very high: > 110	0	Not estimated	
			Pancreas [incidence]	Low: 3.7–12.8	12	1.3 (0.7–2.3)	
				Medium: 12.9–30.7	10	0.9 (0.5–1.7)	
				High: 30.8–109	9	1.1 (0.6–2.3)	
				Very high: > 110	2	0.6 (0.1–2.5)	
			Prostate [incidence]	Low: 3.7–12.8	71	1.1 (0.8–1.5)	
				Medium: 12.9–30.7	65	0.8 (0.6–1.0)	
				High: 30.8–109	47	0.8 (0.5–1.1)	
				Very high: > 110	31	1.5 (0.9–2.5)	
			Testis [incidence]	Low: 3.7– 12.8	1	0.2 (0.0–1.6)	
				Medium: 12.9–30.7	3	0.6 (0.2–2.2)	
				High: 30.8–109	1	0.3 (0.0–2.7)	
				Very high: > 110	6	2.8 (0.8–9.2)	
			Thyroid [incidence]	Low: 3.7–12.8	5	0.9 (0.4–2.3)	
				Medium: 12.9–30.7	5	0.9 (0.4–2.3)	
				High: 30.8–109	3	0.7 (0.2–2.1)	
				Very high: > 110	2	0.8 (0.2–3.5)	

Table 2.2 (continued)

Reference, study location, period, design	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Vieira et al. (2013b) (cont.)			Breast, female [incidence]	Low: 3.7–12.8	72	0.9 (0.7–1.2)	
				Medium: 12.9–30.7	77	1.1 (0.8–1.5)	
				High: 30.8–109	45	0.7 (0.5–1.0)	
				Very high: > 110	29	1.4 (0.9–2.3)	
Barry et al. (2013) Ohio and West Virginia, USA Cohort analysis of participants in C8 Health Project (2005–2006); follow-up, 1992–2011	32 541 (28 541 community; 3713 workers)	Modelled estimates of serum PFOA for 1951–2008; for workers, workplace exposure based on JEM and modelling using serum samples and job history data	Kidney [incidence]	Cumulative serum PFOA concentration **Continuous*	105	1.10 (0.98–1.24)	Proportional hazards modelling, using time-varying cumulative exposure, adjusting for time-varying smoking, time-varying alcohol use, sex, education, 5-yr birth period; results presented are unlagged; 10-yr lag models gave similar results *Continuous analysis based on per unit ln-transformed cumulative serum concentrations
				By quartile, 0 lag (mid-point)			
				Q2 (515 ng/mL-yr)	NR	1.23 (0.70–2.17)	
				Q3 (3085 ng/mL-yr)	NR	1.48 (0.84–2.60)	
				Q4 (105 770 ng/mL-yr)	NR	1.58 (0.88–2.84)	
				Trend tests (by quartile medians; by continuous log-transformed)		$P = 0.18$; $P = 0.10$	

Table 2.2 (continued)

Reference, study location, period, design	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Barry et al. (2013) (cont.)			Bladder [incidence]	*Continuous	105	1.00 (0.89–1.12)	87 of the cases were from the community (non-worker sample): HR = 1.0, 1.34, 1.95, and 2.04 across quartiles (trend *P* value = 0.20); among the 18 worker cases, HR = 1.0, 0.84, 4.20, 0.83 (trend *P* value = 0.54)
			Liver [incidence]	*Continuous	9	0.73 (0.43–1.23)	
			Pancreas [incidence]	*Continuous	24	1.00 (0.78-1.29)	
			Prostate [incidence]	*Continuous	446	0.99 (0.93–1.04)	
			Testis [incidence]	*Continuous	17	1.34 (1.00–1.75)	
				By quartile (mid-point)			15 of the cases were from the community (non-worker) sample: HR =1.0, 0.80, 3.07 and 5.80 across quartiles (trend *P* value = 0.05)
				Q2 (513 ng/mL-yr)	NR	1.04 (0.26–4.22)	
				Q3 (2650 ng/mL-yr)	NR	1.91 (0.47–7.75)	
				Q4 (105 302 ng/mL-yr)	NR	3.17 (0.75–13.45)	
				Trend tests (by quartile medians; by continuous log-transformed)		*P* = 0.04; *P* = 0.05	
			Thyroid [incidence]	*Continuous	86	1.10 (0.95–1.26)	78 of the cases were from the community (non-worker sample): HR = 1.0, 1.54, 1.71, and 1.40 across quartiles (trend *P* value = 0.46); stronger patterns seen among the 8 worker cases: HR = 1.0, 4.64, 9.70, 14.7 (trend *P* value = 0.04)
				By quartile (mid-point)			
				Q2 (248 ng/mL-yr)		1.54 (0.77–3.12)	
				Q3 (1331 ng/mL-yr)		1.48 (0.74–2.93)	
				Q4 (104 251 ng/mL-yr)		1.73 (0.85–3.54)	
				Trend tests (by quartile medians; by continuous log-transformed)		*P* = 0.25; *P* = 0.20	
			Breast [incidence]	*Continuous	559	0.93 (0.88–0.99)	

Table 2.2 (continued)

Reference, study location, period, design	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Innes et al. (2014) Ohio and West Virginia, USA Case–control study among participants in C8 Health Project (see Barry et al., 2013)	208 prevalent cases (self-report with verification by chart review) and 47 151 controls (no reported history of cancer)	Serum PFOA, collected in 2005–2006	Colorectum	*By quartile* 13.5–27.8 ng/mL 27.9–71.2 ng/mL ≥ 71.3 ng/mL Trend tests (by quartiles; by continuous log-transformed)	 36 49 65 	 0.47 (0.31–0.74) 0.49 (0.33–0.74) 0.61 (0.42–0.89) $P = 0.001$; $P = 0.35$	Age, race, sex, education, income, employment status/ disability, marital status, smoking status, current alcohol consumption, vegetarian diet, exercise programme, BMI, menopausal status, self-report of 12 conditions, and current treatment for hypertension or hyperlipidaemia. Similar patterns seen in analyses stratified by sex or BMI, and in analyses limited to diagnosis within 6 yr with no change in residence since 1990 ($n = 71$ cases) or since 1990 ($n = 60$ cases)

BMI, body mass index; CI, confidence interval; HR, hazard ratio; ICD, International Classification of Disease; NR, not reported; yr, year

(1.5; 95% CI, 0.9–2.5; 31 cases), and breast (1.4; 95% CI, 0.9–2.3; 29 cases) were observed in the upper 10% of the exposure distribution. There was no indication of an increased risk of cancers of the bladder, liver, pancreas, or thyroid (Table 2.2). [A strength of this study was its use of incidence data. A limitation was that for the part of the sample residing in West Virginia, it was not possible to conduct the more detailed exposure assessment based on street addresses, reducing the sample size for these analyses. Another limitation was that the residential data were limited to only one residence (i.e. residence at time of diagnosis), rather than a more complete residential history.]

Barry et al. (2013) examined incident cancers occurring in 1992–2011 based on self-reported cancer diagnoses from questionnaires administered in 2005–2006 and 2008–2011 in a cohort identified as a result of a lawsuit brought by residents of the area surrounding the fluoropolymer production plant in West Virginia (the C8 Health Project cohort; Frisbee et al., 2009). Cancer diagnoses were verified through the state cancer registries or medical record review (Barry et al., 2013). The total sample size was 32 254, of whom 3713 (11.5%) had worked at some time in the production plant. Individual-level data on residential history, drinking-water source, and tap-water consumption were obtained from the questionnaires. Annual exposure from 1952 to date of diagnosis was estimated using models incorporating this questionnaire data, emission data, environmental characteristics, and pharmacokinetic (Shin et al., 2011a, b). For workers, workplace exposure based on serum samples and job-history data was also estimated. Barry et al. (2013) included exposure–response analyses based on cumulative exposure measures for cancers of the kidney, testes, and thyroid. In analyses with no exposure lag, the relative risks for cancer of the kidney (n = 105 cases) were 1.23, 1.48, and 1.58 in quartiles 2, 3, and 4, respectively, compared with the lowest quartile of exposure (P for trend, based on continuous variable measure, 0.10). For cancer of the testes (n = 17 cases), relative risks of 1.04, 1.91, and 3.17 across quartiles of exposure were observed (P for trend, 0.05). The trend P using another test (i.e. using median values of quartiles) was 0.04, and the two P values for trend in the 10-year lagged analysis were 0.02 and 0.10, respectively, for quartile and continuous analysis. For cancer of the thyroid, the relative risks by quartile were 1.54, 1.48, and 1.73 (P for trend, 0.20). Similar results were obtained with a 10-year exposure lag. There was no indication of increased risk for the other cancer sites (liver, pancreas, prostate, and breast) (Table 2.2). [The strengths of this study included its use of incidence data and individual-level exposure modelling using lifetime residential history, and the validation of the exposure modelling.]

Innes et al. (2014) conducted a case–control study of prevalent cases of cancer of the colorectum among 47 359 participants in the C8 Health Project (see Barry et al., 2013), using medical history and blood samples collected in the 2005–2006 survey. Self-reported cases of cancer of the colorectum, verified by chart review (n = 208) were compared to the 47 151 participants who did not report a history of any type of cancer. An inverse association was seen between serum PFOA concentrations and risk of cancer of the colorectum, including in analyses restricted to cases diagnosed within the past 6 years who had lived in the same residence for the previous 10 or 15 years (Table 2.2). [A limitation of this study was that the PFOA measurements were taken after diagnosis, and so may not have reflected the etiologically relevant exposure to PFOA.]

2.3 Studies in the general population

See Table 2.3

Three population-based case–control studies were available that examined PFOA serum concentrations in relation to various types of cancer (Eriksen et al., 2009; Bonefeld-Jorgensen et al., 2011; Hardell et al., 2014). Exposure levels in these studies were considerably lower than those seen in the community studies of high exposure or occupational studies described previously.

Eriksen et al. (2009) was a nested case–control study of cancers of the bladder (n = 332 cases), liver (n = 67 cases), pancreas (n = 128 cases), and prostate (n = 713 cases) among 57 053 people in Denmark aged 50–65 years at baseline; 772 controls selected from the cohort were frequency-matched to the sex distribution of the cases. Blood samples were taken at enrolment and stored for later analysis, with a median time between enrolment and diagnosis of 7 years. Median PFOA concentration among controls was 6.6 ng/mL. There was no association between variation in PFOA exposure in this population and risk of cancers of the bladder or liver (Table 2.3). For cancer of the pancreas, the rate ratio in the highest quartile was 1.55 (95% CI, 0.85–2.80), and for cancer of the prostate the corresponding rate ratio was 1.18 (95% CI, 0.84–1.65). PFOS was also measured in the blood samples; the correlation between PFOA and PFOS was r = 0.70. PFOS was not associated with cancers of the bladder, liver, or pancreas. For cancer of the prostate, however, the rate ratio for the highest quartile of PFOS exposure was 1.38 (95% CI, 0.99–1.93) [A strength of this study was that the PFOA measurements were based on samples collected before diagnosis, and thus are likely to reflect an etiologically relevant time-window of exposure; however, the number of cases of cancer of the liver was relatively small. Another limitation was the relatively high correlation between PFOA and PFOS, which hampered interpretation of the association with cancer of the prostate seen with each of these exposures.]

Hardell et al. (2014) examined risk of cancer of the prostate in relation to serum concentrations of PFOA in a case–control study in Sweden in 2007–2011 (n = 201 cases, 186 controls). PFOA concentration was measured in whole blood samples collected after enrolment (i.e. after diagnosis for cases); among controls, the median PFOA concentration was 1.9 ng/mL (range, 0.35–8.4 ng/mL). There was no association between PFOA concentration and cancer of the prostate in the analysis of the full sample, but a relative risk of 2.6 (95% CI, 1.2–6.0) was seen among individuals who reported a first-degree relative with cancer of the prostate, and who had a serum PFOA concentration that was above the median for controls (compared with individuals with no family history of cancer of the prostate and serum PFOA concentration that was greater than the median for controls) (Table 2.3). [A limitation of this study was that the PFOA measurements were taken after diagnosis, and so may not reflect a relevant time-window of exposure.]

Bonefeld-Jorgensen et al. (2011) examined risk of cancer of the breast in relation to PFOA exposure (and other environmental exposures, including polychlorinated biphenyls, organochlorine pesticides, and metals) in a small case–control study (31 cases and 115 controls) of incident cases of cancer of the breast in Greenland in 2002–2003. Serum PFOA concentrations were measured in samples taken at the time of diagnosis for cases, and at enrolment for controls; among controls, the median PFOA concentration was 1.6 ng/L (95% CI, 2.11–2.90). Only 7 cases and 69 controls were included in analyses adjusting for covariates (age, body mass index, pregnancy, cotinine, breastfeeding, and menopausal status) because of missing data (Table 2.3). [The Working Group considered this study to be uninformative because of the small sample size resulting from the high proportion of missing covariate data.]

Table 2.3 Case–control studies of cancer of the bladder, liver, prostate, pancreas, or breast and exposure to perfluorooctanoic acid (PFOA)

Reference, study location, period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Eriksen et al. (2009) Denmark Nested case–control study; initial cohort enrolled 1993–1997 and followed until 2006	1240 cases (332 bladder; 713 prostate; 67 liver; 128 pancreas) 772 controls	Cohort	Plasma sample taken at baseline	Bladder	*By quartile*			Smoking status, intensity, and duration, years of school, 9 occupations
					2	82	0.71 (0.46–1.07)	
					3	83	0.92 (0.61–1.39)	
					4	83	0.81 (0.53–1.24)	
					per 1 ng/mL increase	332	1.00 (0.95–1.05)	
				Prostate	*By quartile*			Years of school, BMI, dietary fat intake, fruit and vegetable intake
					2	178	1.09 (0.78–1.53)	
					3	178	0.94 (0.67–1.32)	
					4	178	1.18 (0.84–1.65)	
					per 1 ng/mL increase	713	1.03 (0.99–1.07)	
				Liver	*By quartile*			Smoking status, years of school, alcohol intake, occupation
					2	17	1.00 (0.44–2.23)	
					3	17	0.49 (0.22–1.09)	
					4	16	0.60 (0.26–1.37)	
					per 1 ng/mL increase	67	0.95 (0.86–1.06)	
				Pancreas	*By quartile*			Smoking status, intensity, and duration, dietary fat intake, fruit and vegetable intake
					2	32	0.88 (0.49–1.57)	
					3	32	1.33 (0.74–2.38)	
					4	32	1.55 (0.85–2.80)	
					per 1 ng/mL increase	128	1.03 (0.98–1.10)	

Table 2.3 (continued)

Reference, study location, period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Hardell et al. (2014) Sweden, 2007–2011	201 cases 186 controls	Population registry: matched on age and geographical area	Blood sample (collected at time of diagnosis	Prostate	Above vs below median in controls (1.9 ng/mL) Effect modification by family history (first-degree relative with prostate cancer):	108	1.1 (0.7–1.7)	Age, BMI, year of blood sampling
					Family history negative, PFOA ≤ median	77	1.0 (referent)	
					Family history positive, PFOA ≤ median	16	1.1 (0.5–2.6)	
					Family history negative, PFOA > median	84	1.0 (0.6–1.5)	
					Family history positive, PFOA > median	24	2.6 (1.2–6.0)	
Bonefeld-Jorgensen et al. (2011) Greenland, 2000–2003	31 cases 115 controls	Population: frequency matched by age and district from two studies on persistent organochlorines	Blood sample, collected at diagnosis for cases and enrolment for controls	Breast	Median in controls: 1.6 ng/mL	7	1.20 (0.77–1.88) per unit increase in ln-transformed serum PFOA	Age, BMI, pregnancies, and cotinine; because of missing data, only 7 cases and 69 controls were included in the adjusted analysis

BMI, body mass index; CI, confidence interval; ICD, International Classification of Disease; vs, versus; yr, year

3. Cancer in Experimental Animals

PFOA was tested for carcinogenicity by the oral route of exposure (in the feed) in two studies in rats. There were also four initiation–promotion studies: two studies in rats and two studies in rainbow trout. No studies of carcinogenicity in mice exposed to PFOA were available to the Working Group.

3.1 Rat

See Table 3.1

3.1.1 Oral administration

Two 2-year studies of carcinogenicity had been conducted with PFOA (specifically, ammonium perfluorooctanoate, or C8) in Sprague-Dawley rats.

The first study was conducted by a pharmaceutical company in the USA. Original reports of this study were submitted as regulatory documents to the EPA in 1983, and were not publicly available until Butenhoff et al. (2012a) published a report of this study. In this study, male and female Sprague-Dawley rats [Crl:COBS@CD(SD)BR] (age, 39–41 days) were given diets containing PFOA at a concentration of 0, 30, or 300 ppm, corresponding to an average daily dose of approximately 0, 1.3, and 14.2 mg/kg bw in males, and 0, 1.6, and 16.1 mg/kg bw in females. At 2 years, there was a significant treatment-related increase in the incidence of testicular Leydig cell adenoma in males at 300 ppm compared with concurrent controls, but not at 30 ppm. There was an increase in the incidence of fibroadenoma of the mammary gland in females at 30 and 300 ppm, but only the increase in the group at 300 ppm was significant compared with concurrent controls. There was an increase in the incidence of hepatocellular hypertrophy in males and females at the highest dose, and an increase in the incidence of liver cystic degeneration and portal mononuclear cell infiltrate in males at the highest dose (Butenhoff et al., 2012a). In 2005, a pathology working group was convened to review the original slides of the mammary glands and to provide a consensus diagnosis for the neoplasms of the mammary gland using current diagnostic criteria. The pathology working group concluded that several lesions originally diagnosed as lobular hyperplasia had features consistent with fibroadenoma of the mammary gland (mainly in slides from the control group), and that, consequently, PFOA did not induce neoplasms of the mammary gland (Hardisty et al., 2010). In a review of the pancreatic lesions from the male rats, using the same diagnostic criteria as those applied in the study by Biegel et al. (2001) (see below), a significant increase in the incidence of pancreatic acinar cell hyperplasia was identified at the highest dose (3/46, 1/46, 10/47) (Caverly-Rae et al., 2014). These hyperplastic lesions were considered to be proliferative lesions similar to the pancreatic acinar adenomas seen in the study by Biegel et al. (2001), and this supported the conclusion that the pancreas is a target of PFOA in male rats.

In the second study, designed to compare the carcinogenic effects of Wyeth-14643 with those of PFOA (specifically, ammonium perfluorooctanoate) (Biegel et al., 2001), there was a treatment group in which male Sprague-Dawley rats [Crl:CD BR (CD)] (age, 6 weeks) were given diet containing PFOA at a concentration of 300 ppm for 2 years. There was also a control group that was fed ad libitum, and a control group that received the same amount of food as the PFOA-treated group (pair-fed control group). The average daily doses of PFOA were 0, 0, and 13.6 mg/kg bw in the control group fed ad libitum, the pair-fed control group, and the treated group, respectively. There were initially 156 animals per group, but rats were killed at various interim time-points for measurements of cell proliferation, peroxisome proliferation, and hormone levels. [It was unclear how many rats were designated for pathological

Table 3.1 Studies of carcinogenicity with perfluorooctanoic acid (PFOA) in rats

Reference Species, strain (sex) Duration	Dosing regimen Animals/group at start	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Butenhoff et al. (2012a), Hardisty et al. (2010) Rat, Sprague-Dawley Crl: COBS CD(SD)BR (M) 24 mo	Diet containing 0, 30, 300 ppm [actual doses: 0, 1.3, and 14.2 mg/kg bw per day 65 control and high-dose groups, 50 low-dose group (15 rats from control and high-dose groups were killed at 1 year)	Leydig cell adenoma: 0/49, 2/50 (4%), 7/50 (14%)*	*$P < 0.05$	Ammonium perfluorooctanoate (purity, > 97.2%) No neoplasms at 1-year interim kill Survival: 35/50 (70%), 36/50 (72%), 44/50 (88%)
Butenhoff et al. (2012a), Hardisty et al. (2010) Rat, Sprague-Dawley Crl: COBS CD(SD)BR (F) 24 mo	0, 1.6, and 16.1 mg/kg bw per day	Mammary gland, fibroadenoma: 10/46 (22%), 19/45 (42%), 21/44 (48%)*	*$P < 0.05$	Survival: 25/50 (50%), 24/50 (48%), 29/50 (58%) No neoplasms at 1-year interim kill Peer review of the mammary gland data by a panel of pathologists (Hardisty et al., 2010) using contemporary diagnostic criteria generated the following incidence data (with no statistical significance): Mammary gland fibroadenoma: 16/50 (32%), 16/50 (32%), 20/50 (40%) Mammary gland fibroadenoma, multiple: 2/50 (4%), 6/50 (12%), 3/50 (6%)
Biegel et al. (2001) Rat, Sprague-Dawley Crl: CD BR (CD) (M) 24 mo	Diet containing PFOA at 0 (controls fed ad libitum), 0 (pair-fed controls), or 300 ppm [actual doses: 0, 0, 13.6 mg/kg bw per day] 156 rats/group	Hepatocellular adenoma: 2/80 (3%), 1/79 (1%), 10/76 (13%)* Hepatocellular carcinoma: 0/80, 2/79 (3%), 0/76 Hepatocellular adenoma or carcinoma (combined): 2/80 (3%), 3/79 (4%), 10/76 (13%)* Leydig cell adenoma: 0/80, 2/78 (3%), 8/76 (11%)* Pancreatic acinar cell adenoma: 0/80, 1/79 (1%), 7/76 (9%)* Pancreatic acinar cell carcinoma: 0/80, 0/79, 1/76 (1%) Pancreatic acinar cell adenoma or carcinoma (combined): 0/80, 1/79 (1%), 8/76 (11%)*	*$P < 0.05$	Ammonium perfluorooctanoate (purity, 98–100%) Survival: ~15%, ~33%, ~47% [estimated from a graph] Only the liver, testes, epididymides, pancreas, and organs with gross lesions were examined microscopically Leydig cell hyperplasia: 11/80 (14%), 26/78 (33%), 35/76 (46%)* Pancreatic acinar cell hyperplasia: 14/80 (18%), 8/79 (10%), 30/76 (39%)* Some rats were designated for interim kill for measurement of cell proliferation, hormone, and peroxisome proliferation, and unclear how many were designated for pathological evaluation at the 2 year time-point

Table 3.1 (continued)

Reference Species, strain (sex) Duration	Dosing regimen Animals/group at start	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Abdellatif et al. (1991) Rat, Wistar (ICO:WI IOPS AF/Han) (M) 12 mo	Initiation–promotion study NDEA given by single i.p. injection, PFOA and PB (positive control) in diet Initiation: 200 mg/kg bw NDEA (all 4 groups) Promotion: 0% (control), 0.05% PB, 0.015% PFOA, or 0.02% PFOA 10 rats/group	Hepatocellular carcinoma: 0/7, 2/7 (28%), 1/7 (16%), 5/9 (55%)*	*$P < 0.05$	Analytical-grade PFOA (purity, NR) Average daily dose of PFOA, NR Only the liver was collected for microscopic evaluation Survival: 7/10 (70%), 7/10 (70%), 7/10 (70%), 9/10 (90%) (no tumours found in rats that died early)
Abdellatif et al. (1990, 1991) Rat, Wistar (ICO:WI IOPS AF/Han) (M) 28 wk	Initiation–selection–promotion study NDEA given by single i.p. injection, 2-AAF administered in diet, CCl_4 given by gavage, PFOA and PB administered in diet Initiation: 200 mg/kg bw NDEA (all 3 groups) Selection: 2 wk after initiation, 0.03% 2-AAF for 2 wk; after 1 wk of 2-AAF treatment, rats received one dose of $CCl_{4\ at}$ 2 mL/kg bw in corn oil Promotion: 0% (control), 0.05% PB or 0.15% PFOA Control group: 7 rats; PB: 8 rats; PFOA-treated: 12 rats	Hepatic cancers (all): 0/7, 6/8 (75%)*, 4/12 (33%)**	*$P < 0.02$ **$P < 0.05$	Analytical-grade PFOA (purity, NR) Average daily dose of PFOA, NR Only the liver was collected for microscopic evaluation Hepatic cancers in phenobarbital-treated group were hepatocellular carcinomas. Three hepatic cancers in the PFOA-treated group were hepatocellular carcinomas and one was reported as "other" but was not further classified

2-AAF, 2-acetylaminofluorene; bw, body weight; CCl_4, carbon tetrachloride; F, female; i.p., intraperitoneal; M, male; mo, month; NDEA, *N*-nitrosodiethylamine; NR, not reported; PB, phenobarbital; wk, week; yr, year

evaluation at the 2-year time-point. Survival data were provided in graphic form only (the actual numbers were not reported); the Working Group estimated survival percentages from the graph presented.] At 2 years, exposure to PFOA significantly increased the incidence of hepatocellular adenoma, testicular Leydig cell adenoma, pancreatic acinar cell adenoma, and pancreatic acinar cell adenoma or carcinoma (combined). In the testis, there was also an increase in the incidence of Leydig cell hyperplasia in the treated group compared with concurrent controls (Biegel et al., 2001).

3.1.2 Initiation–promotion

In an initiation–promotion study, male Wistar rats were given *N*-nitrosodiethylamine (NDEA) at a dose of 200 mg/kg bw as a single intraperitoneal injection (initiation), followed 2 weeks later by diet containing 0.05% phenobarbital, 0.015% PFOA [analytical grade, purity not reported], or 0.02% PFOA, for 46 weeks (Abdellatif et al., 1991). A control group was initiated with NDEA and was fed untreated diet. There were 10 rats per group. The average daily doses of phenobarbital and PFOA were not reported. Survival in the initiated group was 7/10, 7/10, 7/10, and 9/10 in the control group, the phenobarbital-treated group, and the groups treated with 0.015% PFOA, and 0.02% PFOA, respectively. No tumours were identified in rats that died at an early stage of the experiment, all within the first 8 months of the study, with the cause of death reported to be pneumonia in all cases. At 12 months, there was a significant increase in the incidence of NDEA-induced hepatocellular carcinoma in the rats receiving 0.02% PFOA compared with the control group. No organs other than the liver were evaluated in this study. [The Working Group noted the small number of animals and the absence of liver tumours in the control group.]

In an initiation–selection–promotion study, male Wistar rats were initiated with NDEA at a dose of 200 mg/kg bw as a single intraperitoneal injection (Abdellatif et al., 1990, 1991). After 2 weeks, they were given diet containing 0.03% 2-acetylaminofluorene (2-AAF) for 2 weeks. After 1 week of treatment with 2-AAF, the rats received a single necrogenic dose of carbon tetrachloride (2 mL/kg bw) by gavage. One week after the cessation of treatment with 2-AAF, the rats were given diet containing 0.05% phenobarbital or 0.015% PFOA for 23 weeks. A control group were initiated with NDEA then received 2-AAF plus carbon tetrachloride, but was fed untreated diet. The average daily doses of 2-AAF, phenobarbital, or PFOA were not reported. There were 7 rats in the control group, 8 rats in the phenobarbital-treated group, and 12 rats in the PFOA-treated group. Survival was 100% in all groups. The incidences of hepatic cancers were 0/7, 6/8, and 4/12 in the control, phenobarbital-treated, and PFOA-treated groups, respectively. The incidences in the phenobarbital-treated and PFOA-treated groups were significantly increased compared with controls. The cancers reported were hepatocellular carcinomas in all cases, except for one in the PFOA-treated group, that was reported as "other histologic type" and not further classified. [The Working Group noted the small number of animals, the absence of liver tumours in the control group, and the large amount of 2-AAF and chloroform administered.]

3.2 Rainbow trout

See Table 3.2

Initiation–promotion

Rainbow trout have been used as a model of hepatic carcinogenesis for many years and are sensitive to several suspected human carcinogens, including the hepatic carcinogens aflatoxin B_1 (AFB_1) and polycyclic aromatic hydrocarbons

Table 3.2 Studies of carcinogenicity with perfluorooctanoic acid (PFOA) in the rainbow trout

Reference Species, strain (sex) Duration	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Benninghoff et al. (2012) Rainbow trout, Mount Shasta strain (M, F) 10 mo	Initiation–promotion study Treatment groups were as follows: – 0.01% EtOH (non-initiated sham control)/untreated diet; – 0.01% EtOH /promotion with 2000 ppm PFOA for 6 mo; – initiation with 10 ppb AFB_1 for 30 min/untreated diet; – initiation with 10 ppb AFB_1 for 30 min/promotion with 2000 ppm PFOA for 6 mo ~250 fish/group	Hepatic neoplasms (all): 0%, 0%, 13%, 62%*	*$P < 0.01$ (vs AFB_1/untreated feed group)	Analytical-grade PFOA (purity, NR) Untreated diet: OTD (semipurified, casein-based) Incidence values, NR (only percentages) Distribution of hepatic neoplasms for AFB1/control group: 26% hepatocellular adenomas, 23% hepatocellular carcinomas, 2% mixed adenomas, 47% mixed carcinomas, 2% cholangiocellular carcinomas Distribution of hepatic neoplasms for AFB1/PFOA group: 10% hepatocellular adenomas, 27% hepatocellular carcinomas, 1% mixed adenomas, 54% mixed carcinomas, 4% cholangiocellular adenomas, 5% cholangiocellular carcinomas Hepatic neoplasms were classified according to Hendricks et al. (1984)
Benninghoff et al. (2012) Rainbow trout, Mount Shasta strain (M, F) 10 mo	Initiation–promotion study Treatment groups were as follows: – 0.01% DMSO (non-initiated sham control)/untreated diet; – initiation with 35 ppm MNNG for 30 min/untreated diet; – initiation with 35 ppm MNNG for 30 min/promotion with 2000 ppm PFOA for 6 mo ~167 fish/group	Hepatic neoplasms (all): 0%, 51%, 86%*	$P < 0.001$ (vs MNNG/untreated diet group)	Analytical-grade PFOA (purity, NR) Untreated diet: OTD (semipurified, casein-based) Incidence values, NR (only percentages) Distribution of hepatic neoplasms for MNNG/control group: 25% hepatocellular adenomas, 28% hepatocellular carcinomas, 3% mixed adenomas, 39% mixed carcinomas, 2% cholangiocellular adenomas, 3% cholangiocellular carcinomas Distribution of hepatic neoplasms for MNNG/PFOA group: 26% hepatocellular adenomas, 11% hepatocellular carcinomas, 4% mixed adenomas, 55% mixed carcinomas, 3% cholangiocellular adenomas, 1% cholangiocellular carcinomas Hepatic neoplasms were classified according to Hendricks et al. (1984)

AFB_1, aflatoxin B_1; DMSO, dimethyl sulfoxide; EtOH, ethanol; F, female; M, male; min, minute; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; mo, month; OTD, Oregon test diet; NR, not reported; vs, versus

(Williams et al., 2003, Williams, 2012). The background incidence of hepatic neoplasms is reported to be approximately 0.1% at age 9–12 months (Williams et al., 2003).

In an initiation–promotion study in rainbow trout (Mount Shasta strain), one cohort of four groups (with approximately 250 fish per group) was exposed to either 0.01% ethanol (non-initiated sham control) or 10 ppb AFB_1 for 30 minutes by aqueous exposure at 10 weeks post-spawn. Another cohort of three groups (with approximately 167 trout per group) was exposed to either 0.01% dimethylsulfoxide (non-initiated sham control) or *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) at 35 ppm for 30 minutes by aqueous exposure at 10 weeks post-spawn. For the subsequent 4 weeks, the trout were fed untreated feed (Oregon Test Diet, or OTD, a semipurified, casein-based diet). Beginning at 14 weeks post-spawn (4 weeks after initiation), the trout were given feed containing PFOA at 2000 ppm for six months after which the trout were held for 3 months before necropsy. Control trout were fed untreated OTD. The average daily dose of PFOA was not reported.

In the first cohort, there were four groups: non-initiated sham control/untreated feed control, AFB_1/untreated feed control, non-initiated sham control/PFOA, and AFB_1/PFOA. Neither non-initiated group developed hepatic neoplasms. The group initiated with AFB_1 had an incidence of hepatic neoplasms of 13%, while the group initiated with AFB_1 and promoted with PFOA had an incidence of hepatic neoplasms of 62%, which was significant compared with the AFB_1/control group. In the second cohort, there were three treatment groups as follows: non-initiated sham control/untreated feed control, MNNG/untreated feed control, and MNNG/PFOA. While the control/control group did not develop hepatic neoplasms, both MNNG-initiated groups developed hepatic neoplasms. There was, however, a significant increase in the incidence of hepatic neoplasms in the MNNG/PFOA group (86%) compared with the MNNG/control group (51%) (Benninghoff et al., 2012).

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

An extensive database was available on the toxicokinetics of PFOA in humans, non-human primates, rodents and other species of experimental animal. Toxicokinetic studies have been conducted in adult animals, and also in pregnant or lactating dams, neonates and fetuses at various stages of development. In addition, several physiologically based pharmacokinetic models have been developed for humans and animals (primates and rodents), and for different life stages.

4.1.1 Absorption

PFOA is essentially completely absorbed after oral exposure, and is also absorbed dermally and by inhalation of dust.

(a) Humans

The only experimental data in humans were from a phase I clinical trial that used a purified straight-chain isomer of the ammonium salt of PFOA (compound CXR1002, United States patent application publication 2013/0029928) (Elcombe et al., 2013). A total of 43 subjects (all with tumours of varying tissue origin) were given an oral dose of 50–1200 mg of CXR1002 each week, for up to 6 weeks. Rapid absorption was observed and peak plasma concentrations were noted at ~1.5 hours. After repeated weekly doses, plasma levels increased in stepped increments in all subjects, indicating continued absorption and accumulation with repeated exposure. The study group comprised an approximately equal number of males and females, ranging in age

from age 39 to 78 years; no age or sex differences in the internal dose were found.

Percutaneous absorption of the ammonium salt of PFOA through human skin was shown in an in-vitro study, which reported the permeability coefficient to be $9.49 \pm 2.86 \times 10^{-7}$ cm/h (Fasano et al., 2005).

(b) Experimental systems

(i) Non-human primates

The pharmacokinetics of PFOA have been investigated in one set of experiments in non-human primates. Specifically, groups of four to six male cynomolgus monkeys were given daily (7 days per week) oral doses (0, 3, 10 or 20 mg/kg bw) of the ammonium salt of PFOA for 6 months, and pharmacokinetic data were collected (Butenhoff et al., 2002, 2004). While blood samples were collected only at approximately 2-week intervals, and considerable variability occurred, serum levels of PFOA reached steady state within 4–6 weeks after initiation of treatment. Mean serum PFOA values per group during treatment increased with, but were not linearly proportional to, dose. Incomplete absorption was suggested by lower observed steady-state serum PFOA concentrations from oral exposures than predicted from a single intravenous exposure (Butenhoff et al., 2004).

(ii) Rats

Oral bioavailability of the ammonium salt of PFOA in rats (males and females) is approximately 100% (Kennedy et al., 2004). For example, after a single oral dose of ^{14}C-labelled PFOA ammonium salt in male CD rats, 93% of the administered dose was absorbed within 24 hours (Gibson & Johnson, 1979). After PFOA administration by oral (up to 25 mg/kg bw) or intravenous (1 mg/kg bw) routes in male and female Sprague-Dawley rats, similar concentration–time profiles were observed in plasma, indicating 100% oral bioavailability (Kemper & Jepson, 2003). This study also reported that peak plasma concentrations were observed at 1.25 and 10.5 hours for females and males, respectively, after oral administration. However, it is likely that these different concentrations were due to sex differences in excretion (discussed below), rather than differences in absorption. A study by Cui et al. (2010) demonstrated that more than 92% of the dose was absorbed when male Sprague-Dawley rats were exposed to PFOA (0, 5, and 20 mg/kg bw per day once daily by gavage for 28 days).

In a study of inhalation exposure of PFOA (0, 1, 8, or 84 mg/m^3, 6 hours per day, 5 days per week for 2 weeks) in Crl:CD rats, absorption was found to be dose-dependent (Kennedy et al., 1986). Similar to the results from oral and intravenous exposures, peak blood levels of PFOA were observed at less than 1 hour and 8 hours for females and males, respectively (Kennedy et al., 2004). PFOA absorption after inhalation exposures to aerosols (0, 1, 10, or 25 mg/m^3) was studied in male and female Sprague-Dawley rats (Hinderliter, 2003). Effective absorption was shown in both sexes; however, the male C_{max} values were approximately 2–3 times higher than the female C_{max}. [The Working Group noted that this could be due to sex differences in elimination.]

As demonstrated by detection of PFOA in blood, the ammonium salt of PFOA (0, 20, 200, or 2000 mg/kg bw, 6 hours per day, 5 days per week for 2 weeks) in male Crl:CD rats was effectively absorbed after dermal administration; however, the rate of absorption was not estimated (Kennedy, 1985). Percutaneous absorption of ammonium salt of PFOA through rat skin was shown in an in-vitro study that reported the permeability coefficient to be $3.25 \pm 1.51 \times 10^{-5}$ cm/h (Fasano et al., 2005).

(iii) Mice

Rapid absorption of PFOA, as judged by the time of maximum observed concentration (4–8 hours), was observed in male and female

CD1 mice given single oral doses of PFOA at 1 and 10 mg/kg (Lou et al., 2009). The concentrations of PFOA in the liver and kidney followed a kinetic profile similar to that in blood. PFOA concentrations in the liver were found to be higher than those in sera, while both were substantially higher than in the kidney.

(iv) Other species

Indirect evidence of dermal absorption was provided by the demonstration of PFOA lethality in a study of male and female New Zealand White rabbits exposed dermally to PFOA at a dose of 100, 1000, or 2000 mg/kg bw per day for 14 days (O'Malley & Ebbins, 1981). No quantitative data were obtained on serum or tissue concentrations of PFOA; all animals died in the group at the highest dose, some died in the group at the intermediate dose, and none died in the group at the lowest dose.

4.1.2 Distribution

(a) Humans

The high solubility of PFOA in water suggests wide distribution in the body. Systemic availability of PFOA is expected, as it has been measured in human blood after environmental, occupational, and experimental clinical exposures (Calafat et al., 2007b; Olsen et al., 2007; Bartell et al., 2010; Elcombe et al., 2013). Some, but not all, human donor livers also contained quantifiable levels of PFOS, presumably due to environmental exposures (Olsen et al., 2003b). In a recent study of perfluorinated chemicals in five autopsy tissues from 20 individuals in Spain (Pérez et al., 2013), the largest amounts of PFOA (per g wet weight of tissue) were found in bone, followed by the lung, liver, and kidney. PFOA was not detected in the brain. PFOA was found in the kidney, albeit in smaller amounts, in 95% of subjects, while detectable levels in liver, bone and lung were observed in 42–55% of subjects. The median ratio of PFOA concentrations in cerebrospinal fluid versus blood was reported as 17.6 ($\times 10^{-3}$), suggesting that PFOA cannot pass freely through the blood–brain barrier (Harada et al., 2007). Yeung et al. (2013) reported detectable levels of PFOA in all matched samples of serum (range, 0.44–45.5 ng/mL) and liver (range, 0.10–2.3 μg/mL) from 66 subjects who underwent liver transplantation.

PFOA has been found in human breast milk (Kärrman et al., 2007; Tao et al., 2008; Völkel et al., 2008; von Ehrenstein et al., 2009; Llorca et al., 2010; Thomsen et al., 2010) and in umbilical cord blood (Apelberg et al., 2007a, b; Midasch et al., 2007; Monroy et al., 2008; Chen et al., 2012; Arbuckle et al., 2013), indicating that it can cross the placenta and partition into milk, exposing the fetus and neonate.

Multiple studies have demonstrated that PFOA can bind substantially to plasma proteins, potentially limiting distribution to tissues. In a study of human plasma protein fractions, albumin, β-lipoproteins, and α-globulin bound effectively to PFOA, with albumin being most efficient (> 96% binding); other human plasma proteins exhibited binding of < 10% (Kerstner-Wood et al., 2003). Analysis of PFOA distribution into serum lipoprotein fractions in humans found that 40% of the administered dose of PFOA can bind to β-lipoproteins in physiological saline. In human donor plasma lipoprotein fractions, however, most PFOA was found in lipoprotein-depleted plasma. Plasma density gradient fractionation suggested that only 1% or less of PFOA is distributed to lipoprotein-containing fractions (Butenhoff et al., 2012b). Overall, it has been estimated that more than 90% of PFOA would be bound to serum albumin in human blood (Han et al., 2003). Consistent with this estimate, another study with various concentrations of PFOA (1–500 ppm) observed > 99% protein binding in human plasma (Kerstner-Wood et al., 2003).

PFOA also has affinity for liver fatty acid-binding protein (L-FABP), but far less than that

of a natural ligand oleic acid (Luebker et al., 2002). Weiss et al. (2009) used a radioligand-binding assay to measure binding of PFOA and other perfluorinated compounds to serum human thyroid hormone transport protein, transthyretin; PFOA was found to have a high binding affinity for transthyretin and caused inhibition of binding of the natural ligand, thyroxine (T4).

(b) Experimental systems

(i) Non-human primates

Systemic availability of PFOA has also been demonstrated in non-human primates. A single intravenous dose of PFOA potassium salt of 10 mg/kg bw was administered to three male and three female cynomolgus monkeys that were aged approximately 3–4 years at the start of the study (Butenhoff et al., 2004). The monkeys were observed, and urine, faeces, and blood were collected for up to 123 days after the injection. The volume of distribution at steady-state was 181 ± 12 and 198 ± 69 mL/kg for males and females, respectively, which suggests distribution primarily in extracellular space (Butenhoff et al., 2004).

Data on tissue distribution in non-human primates were limited to the liver. In a study in male cynomolgus monkeys given the ammonium salt of PFOA by oral gavage (for up to 6 months), PFOA concentrations in the liver were less than those in either serum or urine, and did not increase in linear proportion to dose (Butenhoff et al., 2002, 2004). [The Working Group noted that the steady-state serum PFOA concentrations were lower than would have been predicted from the study of intravenous administration (Butenhoff et al., 2004) and suggested the existence of enterohepatic recirculation of PFOA.]

Plasma protein binding has also been observed in non-human primates. Greater than 99% protein binding was observed in monkey plasma at various concentrations of PFOA (1–500 ppm) (Kerstner-Wood et al., 2003).

(ii) Rats

Several studies on the tissue distribution of PFOA in rats suggested that most of the delivered dose is found in the blood, liver, and kidney (Johnson et al., 1984; Ylinen et al., 1990; Kemper & Jepson, 2003; Kennedy et al., 2004). In male rats given ^{14}C-labelled PFOA ammonium salt as a single gavage dose at 10 mg/kg bw, small amounts (5–10% of the administered dose) were found in the lungs, heart and skin, and trace amounts (0.5–3%) were found in the testes, muscle, fat, and brain 5 days after dosing (Kennedy et al., 2004). Female CD rats given ^{14}C-labelled PFOA ammonium salt as a single oral dose at 10 mg/kg bw had negligible amounts of the radioactive compound in organs and tissues collected 5 days after dosing (Hundley et al., 2006). The volume of distribution values in male and female rats were similar to those found in cynomolgus monkeys (Ohmori et al., 2003; Butenhoff et al., 2004).

In plasma from male and female rats, most PFOA (> 90%) was found to be in protein-bound form, and the primary PFOA-binding protein in rat plasma was serum albumin (Ylinen et al., 1990; Han et al., 2003; Ohmori et al., 2003). At various concentrations of PFOA (1–500 ppm), > 97% protein binding was observed with rat plasma (Kerstner-Wood et al., 2003). There was little evidence that PFOA binds to glutathione or other thiols such as coenzyme A (Kuslikis et al., 1992; Vanden Heuvel et al., 1992a).

PFOA is known to enter enterohepatic circulation in the rat, but this process is not a major elimination route (Johnson et al., 1984).

Transplacental transfer of PFOA was reported to occur in the rat. In a study in 19-day pregnant dams given ^{14}C-labelled PFOA as a single oral gavage dose at 10 mg/kg bw, PFOA was detected in fetuses with maternal blood:fetal ratio of

22:4.5 between 2 and 8 hours, respectively, after dosing (Kennedy et al., 2004).

The placental and lactational transport pharmacokinetics of PFOA in rats were studied by Hinderliter et al. (2005). In this study, time-mated female rats were given PFOA by oral gavage once daily at concentrations of 3, 10, or 30 mg/kg bw per day, starting on day 4 of gestation and continuing until termination. Steady-state concentrations of PFOA in breast milk were found to be 10 times less than those in maternal plasma. The concentration of PFOA in fetal plasma on day 21 of gestation was approximately half the steady-state concentration in maternal plasma. The concentrations in milk appeared to be generally similar to the concentrations in pup plasma. PFOA was also detected in placenta (days 15 and 21 of gestation), amniotic fluid (days 15 and 21 of gestation), embryo (days 10 and 15 of gestation), and fetus (day 21 of gestation).

(iii) Mice

The available data on distribution in mice were consistent with studies in humans, non-human primates, and rats. In male and female CD-1 mice given ^{14}C-labelled PFOA ammonium salt as single and repeated doses at 10 mg/kg bw, the largest amounts of radiolabelled compound were found in the blood and liver (Kennedy et al., 2004). Trace amounts (0.2–3% of the administered dose) were found in other tissues, including the kidneys, skin, lungs, heart, testes, muscle, fat, and brain. No sex difference in tissue distribution was observed.

Several studies in mice addressed exposure to PFOA in utero and in breast milk. In a single-dose study, maternal and pup fluids and tissues were collected over time after exposure to different doses of PFOA (0, 0.1, 1, or 5 mg/kg bw) administered on day 17 of gestation (Fenton et al., 2009). Serum PFOA concentrations were significantly higher in pups than their respective dams, and their body burden of PFOA increased after birth until at least postnatal day 8, regardless of dose, indicating exposure through milk. The distribution of PFOA in milk compared with serum was found to be in excess of 0.20. In a repeat-dosing study with PFOA administered on days 1–17 or 10–17 of gestation, high PFOA concentrations were found in the liver and serum of the offspring for up to 6 weeks after birth; brain concentrations were low, and became undetectable 4 weeks after birth (Macon et al., 2011). Although maternal exposures in this study ceased on day 17 of gestation, the body burden of PFOA in the pups continued to increase until day 14 after birth, which was indicative of breast milk-derived PFOA exposure in the newborns.

(iv) Other species

In male and female rabbits (New Zealand White) and male hamsters (BIO-15.16) given a single oral gavage dose of ^{14}C-labelled PFOA ammonium salt at 10 mg/kg bw, organs and tissues contained negligible amounts of radiolabel by 168 or 120 hours, respectively, after dosing (Hundley et al., 2006). Female hamsters in the same study had the highest concentrations of radiolabel (7–9%) in the blood, liver, and kidneys, followed by the lungs, heart, and skin (all 3–4%). Negligible amounts (< 2%) were found in the fat, muscle, and brain (Kennedy et al., 2004; Hundley et al., 2006).

4.1.3 Metabolism

Evidence from studies in humans and experimental animals (i.e. rats) shows that PFOA is not metabolized. D'eon & Mabury (2011) failed to detect any biotransformation products of PFOA in the faeces of rats exposed to polyfluoroalkyl phosphate esters. Moreover, no conjugation of PFOA to lipids or polar metabolites of PFOA in the urine or bile of male or female rats was detected (Vanden Heuvel et al., 1991). Despite PFOA being an organic acid and belonging to a diverse group of peroxisome proliferators that have been hypothesized to require activation by

Table 4.1 Species- and sex-specific differences in the elimination half-life of perfluorooctanoic acid (PFOA)

Species	Sex	Elimination half-life	Reference
Human	Mostly males	3.8 yr	Olsen et al. (2007)
	Males and females	2.3 yr	Bartell et al. (2010)
Monkey, cynomolgus	Male	21 ± 12.5 days (i.v.) 19.5–20.8 days (p.o.)	Noker (2003), Butenhoff et al. (2004)
	Female	32.5 ± 8.0 days (i.v.)	
Rat	Male	7–12 days	Kemper & Jepson (2003)
	Female	< 1 day	
Mouse	Male	19–21 days	Kudo & Kawashima (2003), Lou et al. (2009)
	Female	15–17 days	
Dog	Male	20–23 days	Hanhijärvi et al. (1988)
	Female	8–13 days	
Rabbit	Males and females	< 1 day	Kudo & Kawashima (2003)
Cattle	Male	< 1 day	Lupton et al. (2012)

i.v., intravenous; p.o., oral; yr, year

formation of a coenzyme A (CoA) thioester, no CoA derivative has been found (Kuslikis et al., 1992). Based on PFOA having a free carboxyl group, another potential metabolic pathway is glucuronidation. However, in-vitro studies in liver microsome preparations from rat and human liver, kidney, and intestines also failed to detect formation of PFOA–glucuronide (Kemper & Nabb, 2005). Fluorine-19 nuclear magnetic resonance (NMR) spectroscopy of various body fluids and livers of male Fischer 344 rats exposed to PFOA detected only the parent compound, and showed no evidence for any fluorine-containing metabolites (Goecke et al., 1992). The absence of metabolism seems to be accounted for by the extremely strong fluorocarbon bonds in the PFOA molecule (Ophaug & Singer, 1980; Vanden Heuvel et al., 1991).

4.1.4 Excretion

PFOA is eliminated primarily in the urine, with lesser amounts eliminated in the faeces (including as a result of biliary excretion) and expired air. Available data on elimination half-lives of PFOA by species and sex are summarized in Table 4.1. Renal clearance is the major determinant of the elimination rate, and is inversely correlated ($r^2 = 0.91$) with serum half-life across species (Han et al., 2012). Sex-specific differences in the elimination of PFOA have also been observed in some, but not all, species. For instance, male hamsters excrete PFOA more rapidly than female hamsters. In dogs, the half-life of PFOA is longer in males. In cynomolgus monkeys, the half-life of PFOA is somewhat longer in females. In contrast, sex-specific differences are not observed in mice or rabbits, or in humans. Renal transport processes have also been hypothesized to be determinants of overall renal clearance. The available data for different species are described below.

(a) Human

Two studies in humans were informative with regard to providing numerical estimates of the serum half-life of PFOA. In a study of 26 (24 male, 2 female) retired fluorochemical-production workers (at the time of initial blood collection, subjects had been retired for an average of 2.6 years), followed up for 5 years, the arithmetic mean serum half-life of PFOA was 3.8 years (Olsen et al., 2007). In a study of 200 people

(equal male/female participation) exposed via public water supplies and followed for 1 year after installation of filtration for the water supplies, the mean half-life was 2.3 years (Bartell et al., 2010). A clinical trial with CXR1002, a purified straight-chain isomer of the ammonium salt of PFOA, could not determine the elimination half-life due to the relatively short duration of the study (less than 6 weeks), other than to determine that it was greater than 6 weeks (Elcombe et al., 2013).

Biliary excretion of PFOA was significantly higher than serum clearance via the urine, but does not substantially contribute to overall elimination, due to high biliary reabsorption (Harada et al., 2007).

Of all species studied, humans have the highest estimated percentage of renal tubular reabsorption of PFOA – 99.94% – an observation that has been attributed to the high affinity of PFOA for human uptake transport proteins (Han et al., 2012). Two transporters on the basolateral membrane of human proximal tubular cells have been identified as contributing to renal secretion (i.e. uptake from blood into the cell) of PFOA, namely the organic anion transporter 1 (OAT1; solute carrier family 22 member 6 *SLC22A6*) and organic anion transporter 3 (OAT3; *SLC22A8*) (Nakagawa et al., 2008). This has been established by the use of human embryonic kidney HEK 293 cells expressing the specific transporter cDNAs. Both transporters are secondary active carriers that mediate the uptake of a broad range of organic anions in an electroneutral exchange for 2-oxoglutarate. Both carriers exhibited a reasonably high affinity for PFOA, with K_m values for OAT1 and OAT3 being 48 µM and 49.1 µM, respectively (Nakagawa et al., 2008). Human organic anion transporter 2 (OAT2; *SLC22A7*) does not participate in PFOA uptake across the basolateral membrane (Han et al., 2012). No transporters in human renal proximal tubular cells have been identified as being responsible for the efflux step, which involves transport of PFOA from the renal cell across the apical brush-border membrane (BBM) and into the tubular lumen.

Besides secretion, PFOA that undergoes glomerular filtration can also be reabsorbed by transport from the tubular lumen across the BBM and into the proximal tubular cell. Two human renal BBM carriers, the organic anion transporter 4 (OAT4; *SLC22A11*) and urate transporter 1 (URAT1; *SLC22A12*) have been identified as mediating the initial step in the reabsorption of PFOA (Nakagawa et al., 2009; Yang et al., 2010). OAT4, which is only expressed in human kidney, is thought to act primarily in the facilitated uptake of organic anions, and URAT1 similarly mediates the facilitated uptake of various organic anions including urate. While OAT4 exhibited a lower affinity for PFOA (172–310 µM) than the basolateral membrane carriers, the affinity of URAT1 for PFOA (64.1 µM) was similar to that of OAT1 and OAT3 (Yang et al., 2010). In addition to the various SLC22A family proteins on the BBM, human kidney also expresses carriers from the solute carrier organic anion (SLCO) family of organic anion-transporting polypeptides (OATPs). The major SLCO carrier in the BBM of human proximal tubular cells is OATP1A2 (*SLCO1A2*). Despite its broad specificity for catalysing uptake of organic anions and even some organic cations, OATP1A2 is not capable of transporting PFOA (Yang et al., 2010; Han et al., 2012).

Like the process of secretion, which ends with efflux across the BBM (i.e. cell to lumen), reabsorption ends with efflux across the basolateral membrane (i.e. cell to blood). Also similar to the process of secretion for PFOA, no specific carrier involved in the efflux step at the basolateral membrane has been identified in human proximal tubules. Thus while efflux clearly occurs and is carrier-mediated, no evidence is available for a role for any of the major efflux carriers (e.g. multidrug resistance-associated proteins) in PFOA transport.

(b) Experimental systems

(i) Non-human primates

In cynomolgus monkeys (three males and three females) given a single intravenous dose of PFOA potassium salt at 10 mg/kg bw, the range of serum PFOA elimination half-lives was 14–42 days, with a mean of 21 ± 12.5 days in males and 32.5 ± 8 days in females. The difference in elimination between the sexes was not statistically significant (Noker, 2003).

In male cynomolgus monkeys treated with repeated oral doses of the ammonium salt of PFOA, the serum PFOA elimination half-life was 19.5 days and 20.8 days for groups that received PFOA at 10 mg/kg bw or 20 mg/kg bw, respectively (Butenhoff et al., 2002, 2004). First-order elimination kinetics were reported for both doses. Elimination through the urine and faeces (via bile and enterohepatic recirculation) was reported with a much greater (at least three-fold) concentration of PFOA in the urine than in faeces at all doses tested, indicating that the amount of PFOA eliminated in the faeces was at least 25-fold lower than in the urine (per mg of PFOA excreted calculated using estimated faecal and urine quantities).

It was estimated that in Japanese macaque, the process of reabsorption of PFOA predominates over clearance, with 81% and 91% reabsorption in females and males, respectively (Han et al., 2012). However, it is not clear which renal transporters may be responsible for clearance and reabsorption of PFOA in non-human primates.

(ii) Rats

In a study in rats given ^{14}C-labelled PFOA intravenously, females excreted essentially 100% of the administered dose within the first 24 hours after dosing. In contrast, the males excreted only about 20% of the administered dose within 24 hours; by 36 days after dosing, male rats had excreted 83% via the urine and 5.4% via the faeces, and retained 2.8% and 1.1% of the total radiolabel administered in the liver and plasma, respectively, with detectable levels in other tissues (Johnson & Ober, 1980). Similar observations were made after oral administration. For example, in a study of CD rats given a single oral dose (10 mg/kg bw) of ^{14}C-labelled PFOA ammonium salt, substantial sex differences in excretion were observed (Hundley et al., 2006). Female rats excreted > 99% of the radiolabel within 120 hours after dosing, while the male rat excreted only 39% in the same time period.

In studies of oral and intravenous administration of PFOA, approximately 25% of the radiolabel in females and 10% in males was found in the faeces, representing either unabsorbed PFOA (in oral studies) or PFOA from biliary excretion. For example, in male Charles River CD rats, a single intravenous dose of ^{14}C-labelled PFOA ammonium salt (13.3 mg/kg bw) was eliminated in the urine and faeces, although elimination in the urine was about twofold higher than in the faeces after a 14-day observation period (Johnson et al., 1984).

Biliary excretion and faecal elimination of PFOA was reported to be a minor pathway in male and female rats (Kudo et al., 2001). Biliary excretion is slower in male than female rats (Kudo et al., 2001).

In a study that compared the rate of urinary excretion of PFOA (2 mg/kg bw, by oral gavage) in male and female Holtzman rats, female rats were found to excrete 76% of the administered dose in 24 hours, while male rats excreted only 9% (Hanhijärvi et al., 1982). This suggested that, in female rats, PFOA may be eliminated by an active secretion mechanism, because of the high PFOA:inulin clearance ratio, and the fact that PFOA clearance was inhibited by probenecid, an inhibitor of active renal secretion system, by over sevenfold in female rats; in males, the inhibition was less than twofold.

Sex-specific differences in the renal clearance of PFOA in the rat have been attributed to sex-hormone dependence. Testosterone was

shown to inhibit renal excretion of PFOA in male rats, but not females (Vanden Heuvel et al., 1992b). Conversely, estradiol increased urinary excretion of PFOA in castrated and intact male rats (Ylinen et al., 1989). Sex-specific differences in serum concentration, but not renal clearance, of PFOA were also reported in weanling rats, suggesting that the difference in renal clearance in adult rats may be a result of sexual maturation (Kojo et al., 1986). Indeed, the sex-specific difference in PFOA elimination is developmentally regulated and the ability of female rats to rapidly excrete PFOA develops at between age 3 and 5 weeks (Hinderliter et al., 2006).

It has been hypothesized that a saturable renal transport process (reabsorption) in the proximal tubule of the kidney is responsible for the long plasma half-lives of PFOA in male rats. In female rats, net secretion of PFOA predominates over net reabsorption, while in male rats the opposite is true, with the estimated percentage of reabsorption at 94% (Han et al., 2012). No evidence is available for the function of a specific rat basolateral membrane efflux carrier in PFOA reabsorption.

Transporter activity has been studied in rats, and several organic anion transporters have been found to mediate PFOA transport, including Oat1, Oat3, Urat1, and Oatp1a1 (Yang et al., 2009a; Weaver et al., 2010). K_m values that are similar to those of the human orthologues, in the range of 50–80 μM, have been reported for rat Oat1 and Oat3 (Nakagawa et al., 2008; Weaver et al., 2010). In models of heterologous expression, rat Oat3 exhibited a 1.5-fold higher V_{max} for PFOA than rat Oat1, suggesting that the former may play the larger role in PFOA uptake from the blood and in renal secretion (Weaver et al., 2010).

The transporters on the BBM of the proximal tubular cells that are involved in PFOA transport are very different in rats and humans. While OAT4 (only expressed in humans) and URAT1 are the carriers identified from the BBM of human proximal tubules, only the rat organic anion transporting polypeptide 1a1 (Oatp1a1; *Slco1a1*) has been confirmed to transport PFOA from the tubular lumen into the proximal tubular cell (Yang et al., 2009a). Neither Urat1 and Oat2 function in PFOA uptake across the rat renal BBM. Regarding efflux across the BBM, which is the critical last step in the renal secretion process, no specific carriers have been identified, but Mrp2 does not function in PFOA efflux (Han et al., 2012).

The sex-specific difference in rat elimination half-life for PFOA has been suggested to be due to differential expression of renal transporter proteins, in particular Oatp1a1 (Kudo et al., 2002; Weaver et al., 2010). It is not clear, however, whether sex differences in expression of other transporters may play a role in clearance of PFOA, as no differences between males and females were observed in studies with PFOA and probenecid, an inhibitor of both Oat1 and Oat3 (Kudo et al., 2002).

(iii) Mice

In a study of male and female CD-1 mice treated with ^{14}C-labelled PFOA ammonium salt as a single oral dose (10 mg/kg bw), both male and female mice excreted only 21% of the administered radiolabel by 120 hours after dosing (Hundley et al., 2006). The estimates of percentage of PFOA renal reabsorption in mice are > 95% in both males and females (Han et al., 2012). No information on the role of specific basolateral membrane carriers from mouse proximal tubule in PFOA uptake or efflux was available.

(iv) Other species

In a study of male and female hamsters and rabbits treated with a single oral dose of ^{14}C-labelled PFOA ammonium salt (10 mg/kg bw), the male hamster excreted > 99% of the radiolabel by 120 hours after dosing; conversely, the female hamster excreted only 60% of the radiolabel by 120 hours after dosing (Hundley et al., 2006). The male and female rabbits excreted

the radiolabel rapidly and completely within 168 hours after dosing. Indeed, renal tubular secretion of PFOA predominates over reabsorption (Han et al., 2012).

In a study of male and female Beagle dogs given PFOA (30 mg/kg bw) intravenously, no sex-specific differences in renal clearance were found, although some difference in the plasma half-life of PFOA was observed (Hanhijärvi et al., 1988). In male dogs, plasma half-life was about 21 days, while in female dogs it was approximately 11 days. Administration of probenecid had a significant effect in both sexes, indicating that elimination of PFOA occurs through an active renal secretion mechanism.

In a study of Angus cattle, ^{14}C-labelled PFOA administered as a single oral dose (1 mg/kg bw) was completely excreted in the urine within 9 days of dosing (Lupton et al., 2012).

4.2 Genotoxicity and related effects

No data on in-vivo genotoxicity in humans exposed to PFOA were available to the Working Group.

Table 4.2 summarizes the studies available investigating the genotoxic potential of PFOA in human cell lines in vitro, in mammalian systems in vitro and in vivo, in non-mammalian eukaryotic system in vitro, and in bacterial and other systems.

4.2.1 Human cell lines

In vitro, PFOA increased the levels of 8-hydroxydeoxyguanosine (8-OH-dG) and of reactive oxygen species (ROS) in cultured human hepatoma HepG2 cells in the absence of metabolic activation, and induced DNA strand breaks, as assessed by the comet assay (Yao & Zhong, 2005). [The Working Group noted that the genotoxic effects observed in HepG2 cells were probably due to oxidative DNA damage induced by intracellular ROS.]

In a study by Eriksen et al. (2010) in human HepG2 cells, PFOA did not induce strand breaks or formamidopyrimidine-DNA glycosylase-sensitive sites in the comet assay. Florentin et al. (2011) confirmed that PFOA induced neither DNA damage in the comet assay nor micronucleus formation in the micronucleus assay in human HepG2 cells. The study also showed a decrease in ROS generation.

PFOA did not cause chromosomal aberrations in human lymphocytes with or without metabolic activation (Murli, 1996a; NOTOX, 2000). Induction of micronuclei in human HepG2 cells was observed in the absence of metabolic activation (Yao & Zhong, 2005). In human–hamster hybrid (AL) cells (containing a standard set of CHO-K1 chromosomes and a single copy of human chromosome 11), PFOA (16 days at 200 μM) induced mutagenic effects (Zhao et al., 2011). No significant increase in the frequency of mutation was observed after shorter treatments of 1, 4, or 8 days. Intracellular ROS, superoxide anions ($O_2^{\bullet-}$), and nitric oxide (NO) levels were increased after 1 day of treatment with PFOA at 100 μM (41.5 μg/mL) (no further increase was observed at > 100 μM or with longer exposure time). On the other hand, no mutagenic effects and no increase in ROS or $O_2^{\bullet-}$ generation was observed in mitochondrial-DNA deficient human–hamster hybrid (ρ°AL) cells treated with PFOA for up to 16 days. ROS inhibitor decreased the PFOA-induced mutagenic effect observed in AL cells. Caspase activities in AL cells were increased by PFOA exposure, and suppressed by inhibitors of ROS or nitrogen species.

4.2.2 Other experimental systems

(a) Mammalian systems

(i) Gene mutation

Sadhu (2002) showed that PFOA did not induce gene mutation in hypoxanthine-guanine phosphoribosyl transferase *Hprt* locus when

Table 4.2 Studies of genotoxicity of perfluorooctanoic acid (PFOA) in human and mammalian cell lines in vitro, in mammalian systems in vivo, in non-mammalian eukaryotic systems in vitro, and in bacterial and other systems

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Humans in vitro				
8-hydroxydeoxyguanosine, human hepatoma HepG2 cells	+	NT	41.5	Yao & Zhong (2005)
DNA strand breaks (comet assay), human hepatoma HepG2 cells	+	NT	21	Yao & Zhong (2005)
DNA damage, comet assay (strand breaks and FPG-sensitive sites), human HepG2 cells	–	NT	165.6	Eriksen et al. (2010)
DNA damage, comet assay, human HepG2 cells	–	NT	165.6	Florentin et al. (2011)
Chromosomal aberrations, human lymphocytes	–	–	1510	Murli (1996a)
Micronucleus formation, human hepatoma HepG2 cells	+	NT	41.5	Yao & Zhong (2005)
Micronucleus formation, human HepG2 cells	–	NT	165.6	Florentin et al. (2011)
Gene mutation, normal human–hamster hybrid cells (A_L)[c]	+[d]	NT	83	Zhao et al. (2011)
Gene mutation, mitochondrial DNA-deficient human–hamster hybrid cells (ρ° A_L)	–	NT	83	Zhao et al. (2011)
Mammalian systems in vitro				
Gene mutation, *Hprt* locus, K-1 Chinese hamster ovary cells	–	–	39	Sadhu (2002)
Chromosomal aberrations, Chinese hamster ovary cells	–[e]	+	2500[f]	Murli (1996b)
Chromosomal aberrations, Chinese hamster ovary cells	(+)[g]	+	4970[h]	Murli (1996c)
Polyploidy, Chinese hamster ovary cells	–	+	2250[i]	Murli (1996b)
Polyploidy, Chinese hamster ovary cells	+[j]	+[k]	3740	Murli (1996c)
Cell transformation, C3H10T½ mouse embryo fibroblasts	–	NT	200	EPA (1981)
Mammalian systems in vivo				
Micronucleus, mouse bone marrow, polychromatic erythrocytes	–	NA	5000	Murli (1995)
Micronucleus formation, mouse bone marrow, polychromatic erythrocytes	–	NA	950 p.o. ×1	Murli (1996d)
8-hydroxydeoxyguanosine, male Fischer 344 rats, liver	+	NA	100 i.p. ×1	Takagi et al. (1991)
8-hydroxydeoxyguanosine, male Fischer 344 rats, kidney	–	NA	100 i.p. ×1	Takagi et al. (1991)
8-hydroxydeoxyguanosine, male Fischer 344 rats, liver	+	NA	0.02% diet, 2 wk	Takagi et al. (1991)
8-hydroxydeoxyguanosine, male Fischer 344 rats, kidney	–	NA	0.02% diet, 2 wk	Takagi et al. (1991)

Table 4.2 (continued)

Test system	Results[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Non-mammalian eukaryotic systems in vitro				
Gene mutation, *Saccharomyces cerevisiae*	–	–	500	Griffith & Long (1980)
DNA damage, comet assay (pH 13), paramecia *Paramecium caudatum*	+[l]	NT	41.5	Kawamoto et al. (2010)
DNA damage, comet assay (pH 12.1), paramecia *Paramecium caudatum*	–	NT	41.5	Kawamoto et al. (2010)
8-OHdG, paramecia *Paramecium caudatum*	–	NT	41.5	Kawamoto et al. (2010)
Prokaryote (bacteria)				
Gene mutation, *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538	–	–	1000	Griffith & Long (1980)
Gene mutation, reverse mutation *Salmonella typhimurium* TA98, TA100, TA1535, TA1537	–[m]	–	5000 µg/plate	Lawlor (1995, 1996)
Gene mutation, *Salmonella typhimurium* TA1535/pSK1002 (*hisG46*, *rfa*, *uvrB*), *umu* test	–	–	414	Oda et al. (2007)
Gene mutation, reverse mutation *Salmonella typhimurium* TA98, TA100, TA102, TA104	–	–	207	Fernández Freire et al. (2008)
Gene mutation, *Escherichia coli* WP2 *uvrA*	–	–	5000 µg/plate	Lawlor (1995, 1996)

[a] +, positive; (+), weakly positive; –, negative; NA, not applicable; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw per day; inh, inhalation; p.o., oral; i.p., intraperitoneal

[c] A_L cells contain a standard set of CHO-K1 chromosomes and a single copy of human chromosome 11. Chromosome 11 contains the *CD59* gene (also known as *M1C1*) at 11p13.5. This gene encodes the CD59 cell-surface antigen marker (formerly known as S1) that renders A_L cells sensitive to killing by monoclonal antibodies E7.1 in the presence of rabbit serum complement

[d] Levels of reactive oxygen species (ROS) increased after 1 day of treatment with PFOA at 100 µM (no further increases at concentrations > 100 µM or with longer exposure time). ROS inhibitor decreased the mutagenic effects of PFOA. PFOA increased intracellular ROS, NO, and $O2^-$ production in AL cells. Caspase activities in AL cells were increased by PFOA and suppressed by inhibitors of ROS/nitrogen species. Results suggested that mitochondria-dependent ROS plays an important role in PFOA mutagenic effects observed in AL cells

[e] After long treatment (18 or 42 hours) and harvest time 20 or 44 hours after initiation of treatment, respectively; tested up to 2000 µg/mL

[f] LED in 3-hour treatment with S9, and harvest time 20 hours after initiation of treatment

[g] After short treatment (3 hours) and harvesting 44 hours after initiation of treatment, only at highest dose of 3740 µg/mL

[h] LED in 3-hour treatment with S9, and harvesting 20 hours after initiation of treatment (this treatment caused a 70% decrease in cell confluence, but an acceptable 43% decrease in mitotic index). An increase in chromosomal aberrations observed at 3730 µg/mL was not reproducible

[i] LED after 44 hours treatment

[j] In 3-hour treatment and harvesting 44 hour after initiation of treatment; LED, 3740 µg/mL

[k] In 3-hour treatment with S9 and harvesting 44 hours after initiation of treatment; LED, 4970 µg/mL. Toxicity prevented scoring of chromosomal aberration at this concentration, for this treatment

[l] An increase in intracellular ROS generation was also observed. Addition of glutathione inhibited PFOA-induced ROS, but did not abolish the DNA damage observed

[m] A significant increase observed at one dose in TA1537 without S9-mix was not reproduced in a repeat experiment (Lawlor, 1996).

tested with or without metabolic activation in the K-1 line of Chinese hamster ovary (CHO-K1) cells in culture.

(ii) Chromosomal aberration

PFOA was tested twice for its ability to induce chromosomal aberrations in CHO cells (Murli, 1996b, c). In the first assay, PFOA induced chromosomal aberrations and polyploidy in the presence and absence of metabolic activation (Murli, 1996c), while in the second assay it induced chromosomal aberrations and polyploidy only in the presence of metabolic activation (Murli, 1996b). These effects were observed only at toxic concentrations, which caused up to 70% decrease in cell monolayer confluence, but acceptable decrease in mitotic index (Murli, 1996b, c).

(iii) Micronucleus formation

PFOA did not induce a significant increase in micronucleus formation when tested twice in an in-vivo micronucleus assay in bone marrow in mice at a single oral dose of 5000 mg/kg bw (Murli, 1995, 1996d).

(iv) DNA binding and other DNA damage

In-vivo administration of PFOA as a single intraperitoneal injection at 100 mg/kg bw in male Fischer 344 rats induced an increase in the levels of 8-OH-dG in liver DNA, but not in kidney DNA (Takagi et al., 1991). In male Fischer 344 rats, feeding with diets containing PFOA at concentrations of 0.02% for 2 weeks induced hepatomegaly and also increased the levels of 8-OH-dG in liver DNA, but not in kidney DNA (Takagi et al., 1991).

(v) Cell transformation

PFOA did not induce cell transformation in C3H10T½ mouse embryo fibroblasts (EPA, 1981).

(b) Non-mammalian eukaryotic system: DNA damage

Kawamoto et al. (2010) showed that PFOA induced DNA damage in the comet assay in paramecia *Paramecium caudatum* at pH 13, but not at pH 12.1, which suggested that the damage may be due to alkali-labile sites. The study also demonstrated an increase in ROS generation, while the level of 8-OHdG remained unchanged. Moreover, addition of glutathione inhibited the PFOA-induced ROS, but did not abolish the DNA damage observed.

(c) Bacterial and other systems: gene mutation

PFOA did not induce mutation in either *Salmonella typhimurium* or *Escherichia coli* when tested either with or without metabolic activation (Griffith & Long, 1980; Lawlor, 1995, 1996). PFOA was not mutagenic with or without metabolic activation in *S. typhimurium* strains TA98, TA100, TA102, and TA104 (Fernández Freire et al., 2008). PFOA was not mutagenic in *S. typhimurium* TA1535/pSK1002 (*hisG46*, *rfa*, *uvrB*) with or without metabolic activation, using the umu test (Oda et al., 2007). PFOA did not induce mutation in *S. cerevisiae* with or without metabolic activation (Griffith & Long, 1980).

4.3 Other mechanistic data relevant to carcinogenesis

4.3.1 Mammary gland

(a) Humans

No data were available to the Working Group.

(b) Experimental animals

Zhao et al. (2010b) exposed C57BL/6 wild-type and C57BL/6 PPARα null mice to deionized water (control) or to PFOA (5 mg/kg bw) by oral gavage once daily for 5 days per week, for 4 weeks, starting at age 21 days. Both wild-type and null mice had elevated levels of several

growth factors in the mammary gland, including epidermal growth factor, estrogen, and proliferating cell nuclear antigen. [The Working Group noted that these data illustrate that PFOA affects the mammary gland in a manner independent of peroxisome proliferator-activated receptor α (PPARα) involvement.]

4.3.2 Kidney

(a) Humans

No data were available to the Working Group.

(b) Experimental animals

Palmitoyl CoA oxidation and carnitine acetyl transferase activity, enzyme markers of peroxisome proliferation, were elevated in the kidney of male Wistar rats given PFOA as a single intraperitoneal injection at 75 mg/kg bw (Diaz et al., 1994). The same study also reported an increase in the activity, mRNA, and protein expression of the cytochrome CYP4A subfamily, which is an effect that is typical of peroxisome proliferating compounds in the kidney.

In the monkey kidney-derived Vero cell line, Fernández Freire et al. (2008) showed that high concentrations of PFOA (500 μM) cause oxidative stress, which was closely linked to cell cycle arrest and induction of apoptosis.

4.3.3 Liver

Numerous studies have suggested several mechanisms for the observed PFOA-induced toxicity in the liver in human cells, and in experimental animal models and cells, including PPARα activation (as measured by changes in PPARα-related gene expression), peroxisome proliferation (as represented by increases in enzymes associated with β-peroxisomal oxidation), and oxidative stress. The following section is arranged by these putative mechanisms.

(a) PPARα activation

(i) Humans

In-vitro studies in primary human hepatocytes or cell lines transfected with human PPARα have demonstrated that PFOA can activate human PPARα, as measured by changes in PPARα-related gene expression, but at doses higher than those required to activate rodent PPARα. Cultured human hepatocytes were exposed to various concentrations of PFOA (0–200 μM), and induction of several PPARα-related genes was observed (Bjork & Wallace, 2009; Bjork et al., 2011). Additionally, a study by Bjork et al. (2011) demonstrated that a relatively low dose (25 μM) was sufficient to induce PPARα-related genes. Of note was that these responses were not as pronounced as those observed in primary rat hepatocytes. Takacs & Abbott (2007) demonstrated that PFOA activated human PPARα, but at a concentration ~200% greater than the lowest effective concentration required to activate mouse PPARα. Wolf et al. (2012) reported similar results in a luciferase reporter assay with mouse and human PPARα. [The Working Group noted that these studies demonstrated that human PPARα may be activated by PFOA exposure, but at much higher concentrations than those required to activate rodent PPARα.] Additional data on various human PPAR transactivation assays are presented in Section 4.3.3(b).

(ii) Experimental animals

Rats

Ren et al. (2009) performed a meta-analysis of transcript profiles from published studies of rats exposed to PFOA and confirmed that exposure to PFOA activates PPARα in the rat liver. Bjork & Wallace (2009) and Bjork et al. (2011) exposed cultured rat hepatocytes to various concentrations of PFOA (up to 200 μM), and observed induction of PPARα-related genes. Additionally, a study by Bjork et al. (2011) demonstrated that

a relatively low dose of PFOA (25 μM) was sufficient to induce PPARα-related genes.

Mice

Lee et al. (1995) developed a transgenic mouse (Sv/129 × C57BL/6N) model with a disruption in the ligand-binding domain of PPARα. Male mice with this mutation fed diets containing peroxisome-proliferating chemicals (clofibrate, a pharmaceutical agent, and Wy-14 643) for 2 weeks had peroxisomes, but failed to display peroxisome proliferation. Rosen et al. (2008a) demonstrated that in wild-type and PPARα-null mice exposed to PFOA (1 or 3 mg/kg for 7 days), most genes whose transcripts were altered in the livers of wild-type mice were done so through PPARα activation; changes in livers of PPARα-null mice were likely to be attributable to other receptors, including other isoforms of PPAR. However, no clear data on an association between carcinogenesis and PPARα target genes were provided.

Nakamura et al. (2009) exposed 129/Sv wild-type, PPARα-null, and mice with a humanized PPARα to ammonium perfluorooctanoate at a oral dose of 0.1 or 0.3 mg/kg for 2 weeks. Expression of PPARα target genes or proteins in the livers of mice with a humanized PPARα was not altered by exposure. However, Nakagawa et al. (2012) reported that PFOA at a dose of 1 or 5 mg/kg was sufficient to activate PPARα in mice with a humanized PPARα, although the activation was less than that observed for mouse PPARα.

(b) Peroxisome proliferation

(i) Humans

No data were available to the Working Group.

(ii) Experimental animals

Non-human primates

Male cynomolgus monkeys were given daily oral doses of ammonium perfluorooctanoate at 0, 3, 10, or 20 mg/kg bw per day of for 26 weeks (Butenhoff et al., 2002). Livers from monkeys in the group at the highest dose had statistically significant increases in palmitoyl CoA oxidation.

Rats

Long-term exposure to PFOA in rats has been associated with increases in peroxisome proliferating enzymes. In a 2-year study designed to evaluate mechanisms associated with PFOA-induced tumour production, Biegel et al. (2001) fed male CD rats with PFOA at 300 ppm or Wy 14 643 (a known PPARα agonist) at 50 ppm. Hepatic β-oxidation was increased by exposure to Wy 14 643 and PFOA.

Short-term exposures to PFOA in rats also have been associated with increases in peroxisome proliferating enzymes. Male and female Wistar rats given diet containing PFOA at ~15 mg/kg for 2 or 26 weeks had elevated levels of peroxisomal β oxidation at both time-points (Kawashima et al., 1994). Elevation of peroxisomal enzymes in males was ~375% greater than in controls after 2 weeks of exposure; female levels were only ~50% greater than in controls. After 26 weeks of exposure, peroxisomal-enzyme levels in males were ~200% greater and in females were ~60% greater relative to controls. Males also had elevated microsomal content of cytochrome P450 (Kawashima et al., 1994). Male Wistar rats given diet containing PFOA at ~15 mg/kg for 2 or 26 weeks had a marked increase in peroxisomal β-oxidation at all administered doses (Uy-Yu et al., 1990). Females exposed to the same dose and duration had mild, but statistically significant, increases in peroxisomal β-oxidation only at the two higher doses administered (Uy-Yu et al., 1990).

Short-term exposures to PFOA in rats also have been associated with increases in peroxisome proliferating enzymes. Elcombe et al. (2010) gave male Sprague-Dawley rats diet containing ammonium perfluorooctanoate at 300 ppm (15 mg/kg) for 1, 7, or 28 days. Palmitoyl CoA oxidase activity was increased by approximately

8- and 10-fold after 7 or 28 days of exposure, respectively. Additionally, exposure at all durations led to increases in CYP4A1 protein levels (Elcombe et al., 2010). Peroxisomal enzymes in male Wistar rats fed diet containing PFOA at 3.75–60 mg/kg for 1 week increased in a dose-dependent manner (Kawashima et al., 1995). Induction of peroxisomal β-oxidation occurred in male Fischer 344 rats after a single dose of PFOA at 150 mg/kg bw by gavage; induction occurred rapidly after exposure and remained elevated up to 5 days after exposure in rats of various ages (Badr & Birnbaum, 2004). A study by Thottassery et al. (1992) demonstrated that a single oral dose of PFOA (150 mg/kg bw) administered to male Sprague-Dawley rats resulted in induction of peroxisome proliferation in centrilobular regions of the liver lobule; increases in cell proliferation were mostly periportal. Male Wister rats given a single intraperitoneal injection of PFOA at 75 mg/kg bw and killed three days after exposure had elevated palmitoyl CoA oxidation, elevated cartinine acetyl transferase activity, and increases in activity, mRNA, and protein expression of the cytochrome CYP4A subfamily (Diaz et al., 1994).

Additional long-term studies using initiation–selection–promotion protocols also demonstrated increases in peroxisome proliferating enzymes. In a pair of studies with initiation–selection–promotion protocols for induction of tumours of the liver, adult male Wistar rats given diet containing PFOA at 0.015% for 7 months showed increased levels of peroxisomal enzymes and peroxisomal β-oxidation (Abdellatif et al. 1990, 1991). Nilsson et al. (1991) used an identical initiation–selection–promotion protocol in male Wister rats, followed by diet containing 0.015% PFOA for 7 months and, in agreement with the studies by Abdellatif et al. (1990, 1991), reported that PFOA acted as a promoter of tumours of the liver, and that promotion was associated with increases in peroxisomal-enzyme activity. In a follow-up study, Abdellatif et al. (2003) evaluated the tumour-promoting activity of PFOA with a biphasic protocol (initiation followed by dietary PFOA at 0.005% or 0.02%, for 14 and 25 weeks) or a triphasic protocol (initiation, selection–promotion followed by dietary PFOA at 0.015%, for 25 weeks). PFOA exposure induced fatty acyl CoA oxidase, a peroxisomal-enzyme marker for PPARα activation.

Finally, a study in Rat Morris hepatoma 7800C1 cells (a rat liver cell line) exposed to culture medium containing PFOA at 500 µM for 7 days demonstrated induction of peroxisomal enzymes and CYP4A (Sohlenius et al., 1994).

Mice

Sohlenius et al. (1992a, b) evaluated the effects of dietary administration of PFOA (0.02–0.05%) in male and female C57BL/6 mice exposed for 5 or up to 10 days. Increases (> 1000% over control levels) in peroxisomal enzymes were observed for all groups of exposed mice; differences between responses in male and female mice were not observed. Five days of exposure to PFOA at 0.05% led to an increase in peroxisomal enzyme activity that persisted for up to 20 days after exposure.

Lee et al. (1995) developed a transgenic mouse (Sv/129 × C57BL/6N) model with a disruption to the ligand-binding domain of PPARα. Male mice with this mutation fed diets containing peroxisome proliferating chemicals for 2 weeks failed to display transcriptional activation of PPARα target genes.

(c) Activation of other nuclear receptors

(i) Humans

Cultured human hepatocytes exposed to PFOA at various concentrations (0–200 µM) showed an induction of the liver X receptor α (LXRα) (Bjork & Wallace, 2009; Bjork et al., 2011). This response was not as pronounced as that observed in primary rat hepatocytes. Of note was that similarly exposed rat hepatocytes showed induction of constitutive androstane

receptor (CAR) and pregnane X receptor (PXR), in addition to LXRα.

(ii) Experimental animals

Rats

Several studies have explored the involvement of additional nuclear receptors and/or transcription factors in carcinogenicity associated with exposure to PFOA. Elcombe et al. (2010) gave male Sprague-Dawley rats diets containing ammonium perfluorooctanoate at a concentration of 300 ppm (15 mg/kg) or Wy 14 643 at 50 ppm for 1, 7, or 28 days. Ammonium perfluorooctanoate caused increased expression of genetic markers of activation for CAR and CAR/PXR.

Bjork et al. (2011) exposed cultured rat hepatocytes to PFOA at various concentrations up to 200 μM, and observed robust induction of not only PPARα-related genes, but genes associated with the nuclear receptors CAR, PXR, and LXRα. These receptors, like PPARα, play a role in fatty acid metabolism.

Mice

Rosen et al. (2008a, b) compared transcript profiles of livers from mice exposed by gavage for 7 days to PFOA (1 or 3 mg/kg) or Wy 14 643 (50 mg/kg), including livers from PPARα-null mice. In wild-type mice, it appeared that expression of most genes was altered by PFOA through PPARα; however, in PPARα-null mice, a subset of genes appeared to be altered in expression by PFOA through CAR and possibly PPARγ.

Fish

Several long-term and short-term dietary studies with PFOA in trout (*Oncorhynchus mykiss*), a species that generally does not experience liver peroxisome proliferation, demonstrated concomitant induction of estrogen receptor-responsive genes and proteins in the liver, and tumours of the liver (Tilton et al., 2008; Benninghoff et al., 2011, 2012). Studies with other species of freshwater fish (rare minnow and tilapia) have demonstrated that short-term dietary exposure to PFOA induces estrogen receptor-responsive genes and proteins in the liver (Liu et al., 2007a; Wei et al., 2007, 2008).

(d) Oxidative stress

(i) Humans

Several studies in human cell lines or cells transfected with human receptors also have evaluated the ability of PFOA to induce oxidative stress. Panaretakis et al. (2001) exposed human HepG2 cells to PFOA at 200 or 400 μM (1.5–24 hours) and reported increased formation of ROS, with a peak at 3 hours. However, Eriksen et al. (2010) exposed human HepG2 cells to PFOA at varying concentrations (0.4–2000 μM) and reported a statistically significant, but relatively modest increase in ROS. Additionally, Florentin et al. (2011) exposed human HepG2 cells to PFOA at several concentrations (5–800 μM) and did not observe significant changes in ROS generation. A study with a human–hamster hybrid cell line reported induction of ROS after 16 days of exposure to PFOA at 200 μM (Zhao et al., 2011).

(ii) Experimental animals

Increased production of ROS

Several studies in rats and mice examined markers of increased production of ROS after exposure to PFOA. Male Wistar rats fed diets containing PFOA at a concentration of ~15 mg/kg (0.01%) for 26 weeks had an imbalance of metabolism of hydrogen and lipid peroxides (Kawashima et al., 1994). In male F344 rats given diet containing PFOA at a concentration of ~30 mg/kg (0.02%) for 2 weeks, 8-OH-dG levels were increased in liver DNA (Takagi et al., 1991). Similarly, 8-OH-dG levels in liver DNA were increased 1, 3, 5, or 8 days after a single intraperitoneal dose of PFOA of 100 mg/kg bw (Takagi et al., 1991). Male C57BL/6 mice given diet containing PFOA at a concentration of ~30 mg/kg (0.02%) for 2 weeks had increased

lipid peroxidation in liver microsomes, as measured by ADP-Fe^{3+}-NADPH-dependent consumption of oxygen (Cai et al., 1995).

Liu et al. (2007a) observed induction of oxidative stress in primary cultured liver cells from freshwater tilapia (*Oreochromis niloticus*) exposed 24 hours to 15 or 30 mg/L of PFOA.

Decreased antioxidant capacity

Badr & Birnbaum (2004) found that the effects of PFOA on oxidative stress in the liver in male F344 rats were modulated with age; after a single oral dose of 150 mg/kg bw of PFOA, the ratio of hepatic peroxisomal β-oxidation to liver catalase activity increased as animals aged.

In male Japanese medaka fish (*Oryzias latipes*), exposure to PFOA at a concentration of 50 or 100 mg/L caused decreases in the antioxidant activity of catalase in the liver, suggesting that PFOA may cause oxidative stress in the liver (Yang, 2010).

Mitochondrial dysfunction

Mitochondrial dysfunction also may contribute to oxidative stress associated with exposure to PFOA. In male Sprague-Dawley rats treated with PFOA at a dose of 30 mg/kg bw by gavage for 28 days, PFOA stimulated mitochondrial biogenesis or inhibited mitochondrial metabolism in the liver, which may contribute to metabolic imbalance (Walters & Wallace, 2010).

Male zebrafish (*Danio rerio*) exposed to PFOA at a concentration of 1 mg/L for 14 days had decreased liver mitochondrial electron-transport activity (Hagenaars et al., 2013).

4.3.4 Pancreas

No studies in humans and a single study in experimental animals have addressed biochemical and cellular effects in relation to pancreatic carcinogenicity associated with exposure to PFOA.

In a 2-year study in which male CD rats were fed ammonium perfluorooctanoate at 300 ppm or Wy 14 643 (a known PPARα agonist) at 50 ppm, pancreatic acinar cell proliferation was increased by ammonium perfluorooctanoate but not by Wy 14 643, although both compounds produced increases in acinar cell hyperplasia (Biegel et al., 2001).

4.3.5 Testes (Leydig cells)

Interference with steroidogenic enzymes is a putative mechanism that may result in testicular carcinogenesis.

(a) Humans

No studies examining interference with steroidogenic enzymes in humans exposed to PFOA were available to the Working Group.

(b) Experimental animals

(i) Non-human primates

Male cynomolgus monkeys were given ammonium perfluorooctanoate at daily oral doses of 0, 3, 10, or 20 mg/kg bw per day for 26 weeks (Butenhoff et al., 2002). Testicular cell proliferation, as measured by a proliferating cell nuclear antigen assay, was not affected by treatment with PFOA.

(ii) Rats

In a 2-year study in which male CD rats were fed diets containing ammonium perfluorooctanoate at a concentration of 300 ppm or Wy 14 643 (a known PPARα agonist) at 50 ppm, levels of serum estradiol and Leydig cell hyperplasia were increased by Wy 14 643 and PFOA (Biegel et al., 2001). Similarly, in a 14-day study, levels of serum estradiol in male CD rats given PFOA at a dose of 10, 25, or 50 mg/kg bw by gavage were elevated relative to levels in controls (Cook et al., 1992). In an additional group of rats exposed to ammonium perfluorooctanoate at a dose of 50 mg/kg bw for 14 days and challenged with human chorionic gonadotropin 1 hour before killing (to maximize increases in serum

testosterone), Cook et al. (1992) reported a 50% reduction in serum testosterone levels relative to those in controls. In a follow-up study, serum estradiol, transforming growth factor α (TGFα), and estradiol in testicular interstitial fluid were found to be elevated in rats given ammonium perfluorooctanoate at a dose of 25 mg/kg bw by gavage for 14 days, relative to pair-fed controls (Biegel et al., 1995). Additionally, liver aromatase activity was 4.5-fold that of pair-fed controls.

Biegel et al. (1995) also examined the effects of ammonium perfluorooctanoate on Leydig cells isolated from CD rats. Leydig cells were treated in vitro with PFOA at a concentration of 100–1000 μM for 2 hours. Leydig cells were also isolated from rats treated in vivo with PFOA at 25 mg/kg by gavage. Both sets of cells were stimulated with human chorionic gonadotropin; cells treated in vitro showed a dose-related decrease in testosterone production, while cell isolated from animals treated in vivo showed an increase in testosterone production. In another study, Zhao et al. (2010a) cultured Leydig cells from Sprague-Dawley rats for 24 hours with PFOA at 10 or 100 μM, and exposed testicular microsomes from Sprague-Dawley rats to PFOA at concentrations of up to 100 μM. Both exhibited inhibition of 3-beta-hydroxysteroid dehydrogenase and 17-beta-hydroxysteroid dehydrogenase 3, enzymes that are involved in testosterone biosynthesis. Additionally, Leydig cells that had been exposed to PFOA failed to produce increases in testosterone relative to stimulated control cells when stimulated with luteinizing hormone.

4.3.6 Other target organs

Although the bladder and prostate gland were identified as tumour sites targeted by exposure to PFOA, no studies on potential biochemical or cellular effects were available to the Working Group.

A limited number of epidemiological studies in humans have evaluated thyroid hormone concentrations, thyroid gland function, and thyroid disease associated with exposure to PFOA (discussed in Section 4.4.5). No studies in humans or experimental animals addressing biochemical and cellular effects in the thyroid gland were available to the Working Group.

4.3.7 Modulation of inflammatory pathways

It has been suggested that modulation of inflammatory pathways is a mechanism underlying PFOA-induced carcinogenesis. In one study, Qazi et al. (2009) reported increases in serum levels of interleukin-6 (IL-6) and tumour necrosis factor α (TNFα) – cytokines that induce inflammation– in mice fed diets containing PFOA (0.02%) for 10 days after stimulation of inflammation with lipopolysaccharide (100 ng/mL). However, in a series of in-vitro assays, Corsini et al. (2011, 2012) reported that PFOA (0.1–10 μg/mL) was the least potent of a suite of perfluoroalkyl substances to alter lipopolysaccharide-stimulated release of IL-6 and TNFα. PFOA binds to PPARα and a significant number of PPARα agonists have been shown to reduce inflammation (Griesbacher et al., 2008). [As markers of inflammatory processes, it would be expected that TNFα and IL-6 would decrease after exposure to PFOA. However differences in dose, rodent strain, cell type, and receptor affinity make it difficult to predict whether exposure to PFOA would lead to chronic inflammation and contribute to carcinogenicity risk via this pathway.]

4.3.8 Nuclear receptors

PFOA and its ammonium salt have been tested in a large number of high-throughput screening assays in the Toxicity Forecaster (ToxCast) and Toxicity Testing in the 21st Century (Tox21) research programmes of the government of the USA (Kavlock et al., 2012; Tice et al., 2013). The data from these programmes are publicly

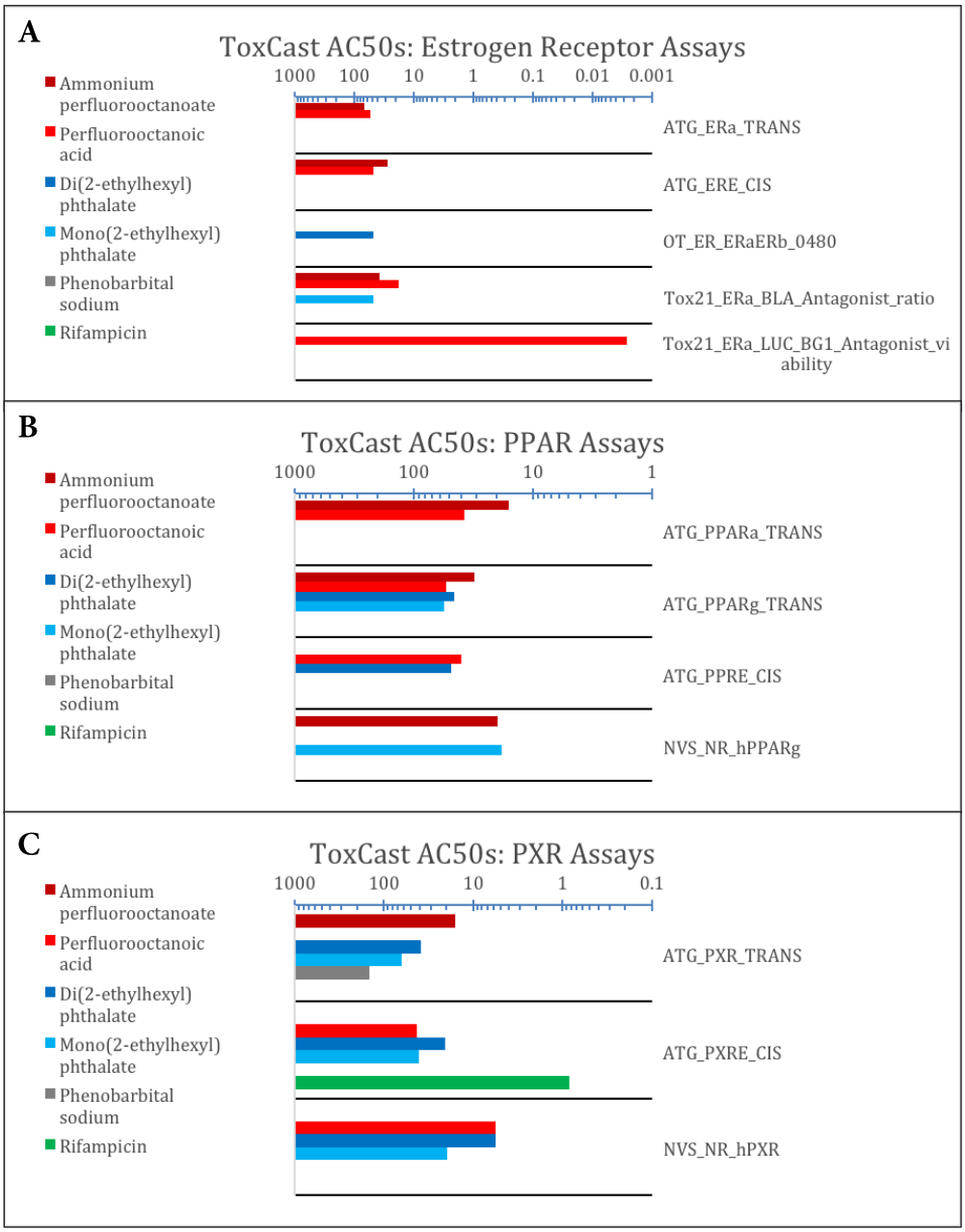
available through the iCSS dashboard (ToxCast, 2014) Specifically, data on 821 assays and 1858 chemicals were publicly available through the iCSS Dashboard v0.5 as of 1 June 2014. [The Working Group used this information to examine the molecular targets affected by PFOA and its ammonium salt, and to compare the molecular signatures with those of several prototypical nuclear receptor activators: rifampicin (CAS No. 13292-46-1; PXR), phenobarbital (CAS No. 57-30-7; CAR), and di(2-ethylhexyl)phthalate (CAS No. 117-81-7; peroxisome proliferator response elements) and mono(2-ethylhexyl)phthalate (MEHP) (CAS No. 4376-20-9; PPARs). Data on all assays for these six compounds were downloaded. Assays in which all of the six compounds were inactive (as indicated by an altering concentration [AC]50 value of 1000), or in which any of the compounds were not tested, were removed and the results of the remaining 37 assays (about 4.5% of the total) were analysed. These included cell-free enzymatic and ligand-binding high-throughput screening assays (labelled "NVS") (Sipes et al., 2013), cell-based nuclear receptors and transcription-factor response element assays (labelled "ATG") (Martin et al., 2010), and Tox21 robotic platform high-throughput assays (labelled "Tox21") (Attene-Ramos et al., 2013). Most of these assays were designed for human enzymes and transcription factors.

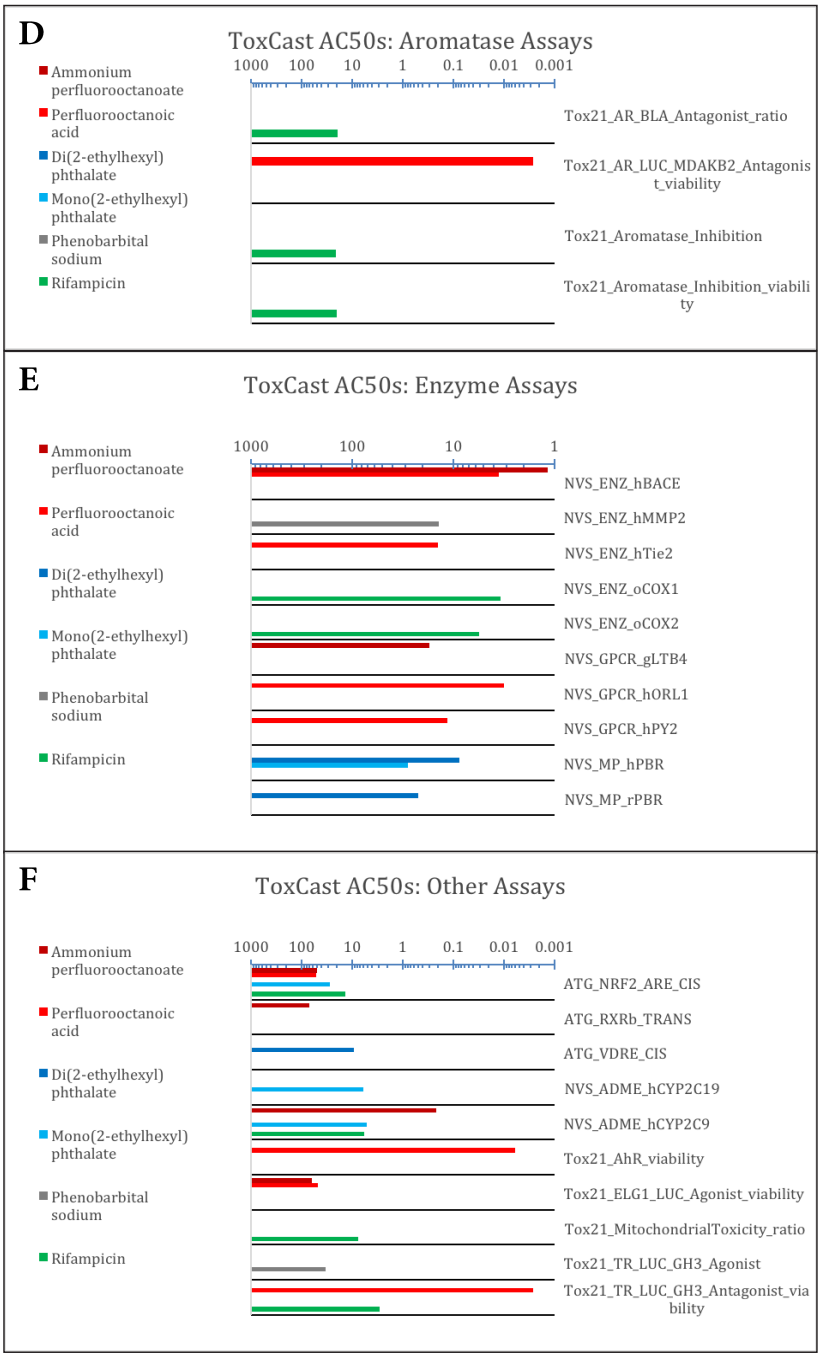
AC50 values downloaded from the database were derived from quantitative concentration–response modelling using Hill function based on 7–10 concentrations spanning several orders of magnitude, ranging from low nanomolar to ~200 μM. Each chemical and assay had one AC50 value (ranging from 1000 indicating "inactive", to 2.6 nM indicating the most potent response) that was used to create plots displayed in Fig. 4.1, Panels A–F. The data on 37 assays were subdivided into 6 groups by the molecular targets as follows: estrogen receptor assays (panel A), PPAR assays (panel B), PXR assays (panel C), aromatase assays (panel D), enzyme assays (panel E), and other (panel F).

[The Working Group interpreted the outcome of this analysis to indicate that great similarity exists across the assays in responses elicited by PFOA and its ammonium salt. At the same time, it was apparent that the responses of these two compounds are distinct from those of other prototypical activators of nuclear receptors CAR, PXR, and PPARs. This outcome is consistent with observations that multiple nuclear receptors are activated by PFOA in vivo in rodents (Rosen et al., 2008b; Elcombe et al., 2010). Additionally, the Working Group noted that, unlike the selected comparison compounds, PFOA and its ammonium salt appeared to be consistently active in estrogen receptor assays, in keeping with observations on effects on reproductive hormones and tissues (Cook et al., 1992; Biegel et al., 1995; Yang et al., 2009b; Zhao et al., 2010a, b).]

Additional studies have evaluated the ability of PFOA to activate estrogen receptors (including ERα and ERβ) in a variety of in-vitro assays. In a yeast two-hybrid assay with human ERα and ERβ, Ishibashi et al. (2007) reported that exposing these cells to PFOA did not increase transcriptional activity of ERs. However, in a separate study of PFOA-exposed human embryonic kidney (HEK-293T) cells, Benninghoff et al. (2011) reported induction of ERα gene reporter activity. Two studies with MCF-7 human breast cancer cells (Maras et al., 2006; Henry & Fair, 2013) demonstrated that PFOA was estrogenic via an E-SCREEN assay, an assay designed to use the estrogen sensitivity of MCF-7 to determine effects of exogenous agents on cell proliferation (Henry & Fair, 2013). Maras et al. (2006) also reported that PFOA induced a small upregulation in the expression of estrogen-responsive genes (Maras et al., 2006).

Fig. 4.1 Comparison of in-vitro screening results for perfluorooctanoic acid (PFOA) with those of several prototypical nuclear receptor-activating compounds





Specifically, each panel shows AC50s (micromolar concentrations) from in-vitro assays reported on the United States Environmental Protection Agency CSS Dashboard (http://actor.epa.gov/dashboard/) for PFOA or its ammonium salt and several prototypical nuclear receptor activators: rifampicin (pregnane X receptor, PXR), phenobarbital (constitutive androstane receptor, CAR), and di(2-ethylhexyl)phthalate (peroxisome proliferator-activated receptor response elements) and mono(2-ethylhexyl)phthalate (peroxisome proliferator-activated receptors, PPARs). All assays in which positive results were obtained for at least one of the six compounds are shown. Results were subdivided into six groups by the molecular targets as follows: estrogen receptor assays (panel A), PPAR assays (panel B), PXR assays (panel C), aromatase assays (panel D), enzyme assays (panel E), and other (panel F)
Compiled by the Working Group

4.4 Organ toxicity

4.4.1 Mammary gland

(a) Humans

No studies of toxicological effects relevant to carcinogenicity in the breast/mammary gland after exposure to PFOA in humans were available to the Working Group.

(b) Experimental animals

Two studies in experimental animals reported effects on the mammary gland after exposure to PFOA. Pre-pubertal C57BL/6 or BALB/c mice (age, 21 days) were exposed to PFOA at a dose of 1, 5, or 10 mg/kg bw by gavage once daily, 5 days per week, for 4 weeks (Yang et al., 2009b). PFOA inhibited mammary-gland development in BALB/c mice. In C57BL/6 mice, PFOA inhibited mammary-gland development at 10 mg/kg bw, and stimulated mammary-gland development at 5 mg/kg bw. PFOA increased numbers of terminal end buds and stimulated/enlarged terminal ducts, which is indicative of mammary epithelial-cell proliferation. In another study, female CD-1 mice given PFOA at a dose of 0, 0.01, 0.1, or 1 mg/kg bw for 3 days starting on postnatal day 18, showed an increased weight of the uterus at the lowest dose of PFOA, suggesting an estrogenic effect (Dixon et al., 2012).

4.4.2 Nephrotoxicity

(a) Humans

Several studies in humans have reported mixed results regarding serum concentrations of PFOA and serum markers of kidney damage. In a cross-sectional study of adults from a community in which the drinking-water was contaminated with PFOA from a chemical plant, higher serum concentrations of PFOA were associated with higher serum concentrations of uric acid, but the limits of the study prohibited conclusions of causality (Steenland et al., 2010). Two studies included in a review by Steenland et al. (2010) reported no significant association between exposure to PFOA and either urea nitrogen or creatinine in occupationally exposed subjects (Emmett et al., 2006; Costa et al., 2009). Using data from the National Health and Nutrition Examination Survey (NHANES), Shankar et al. (2011a, b) reported that elevated levels of serum uric acid and incidence of chronic kidney disease, defined as low glomerular filtration rate, were associated with increases in serum PFOA. Additional evaluations of data concerning associations between PFOA and the glomerular filtration rate in adolescents and children suggested that increases in serum PFOA may result from decreases in glomerular filtration rate rather than the opposite (Watkins et al., 2013).

(b) Experimental animals

One study in experimental animals reported kidney toxicity after exposure to PFOA. Male Sprague-Dawley rats given PFOA at a dose of 5 or 20 mg/kg bw by gavage for 28 days had signs of turbidity and tumefaction in the epithelia of the proximal convoluted tubule, including mild symptoms of congestion in the renal cortex and medulla, and enhanced cytoplasmic acidophilia (Cui et al., 2009).

4.4.3 Hepatotoxicity

(a) Humans

Several studies in humans have reported associations between serum concentrations of PFOA and serum markers of liver enzyme concentrations, which can be indicative of hepatocellular damage. Several such studies were included in a review by Steenland et al. (2010), and while they have reported some associations of changes to liver enzymes with serum PFOA concentrations (Emmett et al., 2006; Olsen & Zobel, 2007; Sakr et al., 2007; Costa et al., 2009; Lin et al., 2010), the changes in liver enzymes

were small, and the clinical significance of the reported changes was uncertain (Steenland et al., 2010). A more recent cross-sectional study of adults from a community in which drinking-water was contaminated with PFOA from a chemical plant also reported mild increases in serum PFOA and one liver enzyme (Gallo et al., 2012).

(b) Experimental animals

(i) Non-human primates

Male cynomolgus monkeys were given daily oral doses of ammonium perfluorooctanoate at 0, 3, 10, or 20 mg/kg bw per day for 26 weeks (Butenhoff et al., 2002). Histopathological evidence of liver injury was not observed in animals at 3 or 10 mg/kg bw; one moribund animal from the group at 20 mg/kg bw was killed on day 29, and was found to have mid-zonal and centrilobular hepatocellular degeneration and necrosis, diffuse hepatocellular vacuolation, and hepatocyte basophilia.

(ii) Rats

Several studies reported histopathology in the livers of rats exposed to PFOA either in the long-term, short-tem, or as a single dose. In a long-term dietary study, male and female Sprague-Dawley rats were fed diets containing ammonium perfluorooctanoate at 1.5 or 15 mg/kg for 2 years (Butenhoff et al., 2012b). After 1 year of exposure, histopathology was confined to the liver; male rats at 15 mg/kg had focal hepatocellular necrosis, portal mononuclear cell infiltration, and increased cytoplasmic volume in parenchymal cells. The cytoplasm had a finely granular appearance. Male and female rats at unscheduled or terminal necropsy also showed signs of non-neoplastic effects in the liver, including cystoid degeneration, portal mononuclear cell infiltration, and hepatocellular necrosis.

Similarly, the livers of male Sprague-Dawley rats given PFOA at a dose of 5 or 20 mg/kg bw by gavage for 28 days exhibited cytoplasmic vacuolation, focal or flakelike necrosis, fatty degeneration, angiectasis and congestion in the hepatic sinusoid or central vein, and acidophil lesion (Cui et al., 2009). Changes observed in livers of male Sprague-Dawley rats fed diet containing ammonium perfluorooctanoate at 15 mg/kg for 1, 7, or 28 days included hepatocellular hyperplasia, glycogen loss, and fatty vacuolation (Elcombe et al., 2010).

(iii) Mice

In male ICR mice exposed to drinking-water containing PFOA at a concentration of 0, 2, 10, 50, or 250 mg/L for 21 days, hepatic acidophilic cytoplasm was reported in the group with the highest exposure (Son et al., 2008). Both wild-type (129S4/SvlmJ) and PPARα null mice given PFOA at a dose of 0, 12.5, 25, or 50 μmol/kg per day by gavage for 4 weeks had numerous histological changes in the liver, including reduction in glycogen granules, degranulation and disruption of the rough endoplasmic reticulum, and increased numbers of mitochondria (Minata et al., 2010).

(c) Other experimental systems

Human hepatoblastoma HepG2 cells incubated with PFOA at 0–550 μM for 24 hours exhibited a dose-dependent increase in the frequency of apoptosis, starting at 200 μM. With PFOA at higher doses (400 and 500 μM), cells underwent primary and secondary necrosis (Shabalina et al., 1999). [The Working Group noted that these data were indicative of an antiproliferative response.] Additionally, a study with an ammonium salt of PFOA known as CXR1002 demonstrated that in various cell lines, CXR1002 could inhibit a protein kinase (PIM) that is anti-apoptotic when activated (Barnett et al., 2010). The antiproliferative effects of PFOA are the subject of a patent application for CSR1002 as an antineoplastic drug (Elcombe et al., 2013).

4.4.4 Male reproductive organs

(a) Humans

No studies of toxicological effects relevant to testicular/Leydig cell carcinogenicity after exposure to PFOA in humans were available to the Working Group.

(b) Experimental animals

Two studies in experimental animals reported toxicological effects on male reproductive organs after exposure to PFOA. In a 2-year study in which male CD rats were fed diets containing PFOA at 0 or 300 ppm, or Wy 14 643 (a known PPARα agonist) at 50 ppm, ad libitum, absolute testis weight was increased by exposure to PFOA or to Wy 14 643 at 24 months. No consistent changes were observed in the weights of epididymides or accessory sex organs (Biegel et al., 2001). Cook et al. (1992) reported that unit weight of accessory sex organs (combined ventral and dorsal lateral prostate, seminal vesicles, and coagulating glands) was decreased in CD rats exposed to ammonium perfluorooctanoate at 25 or 50 mg/kg bw by gavage for 14 days. Two separate studies in CD rats given ammonium perfluorooctanoate orally for 14 days or up to 2 years reported no changes in weight of the prostate gland (Cook et al., 1992; Biegel et al., 2001).

4.4.5 Thyroid gland

(a) Humans

A limited number of epidemiological studies in humans have evaluated thyroid hormone concentrations, thyroid gland function, and thyroid disease associated with exposure to PFOA. A large-scale study of children aged 1–17 years from a highly exposed population in the mid-Ohio Valley, USA, reported that increases in serum PFOA concentrations were correlated with increases in hypothyroidism, but that neither serum total T4, nor thyroid-stimulating hormone were associated with serum PFOA concentrations (Lopez-Espinosa et al., 2012). In an evaluation of adults from this mid-Ohio Valley population, increases in serum PFOA concentrations were associated with increases in serum T4 and a reduction in triiodotyronine (T3) uptake (Knox et al., 2011). Winquist & Steenland (2014) examined the association between PFOA and thyroid disease among community members and workers of a chemical plant in mid-Ohio River valley. Associations were observed for hyperthyroidism and hypothyroidism among women. Some subanalyses also suggested increased hypothyroidism among men (Winquist & Steenland, 2014). Finally, in evaluations of data from NHANES, Melzer et al. (2010) reported that self-reported incidence of current thyroid disease (not specified) increased with serum PFOA concentrations, and Wen et al. (2013) reported increases in serum T4 and T3 levels with increases in serum PFOA.

(b) Experimental animals

(i) Non-human primates

Male cynomolgus monkeys were given daily oral doses of ammonium perfluorooctanoate at 0, 3, 10, or 20 mg/kg bw per day for 26 weeks (Butenhoff et al., 2002). At the end of the dosing period, decreases in levels of free and total T3 and T4 were noted in monkeys in the group receiving the highest dose. Additionally, monkeys from the groups at 3 and 10 mg/kg bw had increases in levels of thyroid-stimulating hormone, and decreases in total T4.

(ii) Rats

Male Sprague-Dawley rats given PFOA at a dose of 30 mg/kg bw by gavage for 28 days showed reductions in levels of serum thyroid-stimulating hormone, total T4, and free T4 (Butenhoff et al., 2012c). Similarly exposed female Sprague-Dawley rats had normal levels of serum thyroid-stimulating hormone, but reductions in serum total and free T4. After a 3-week recovery period, all levels returned to those of the controls, except

for levels of serum total and free T4 in males (Butenhoff et al., 2012c).

4.4.6 Development

Abbott et al. (2007) found that PPARα was required, in part, for certain developmental effects induced by PFOA in the mouse. Postnatal lethality and delays in development occurred in similarly exposed 129S1/SvlmJ wild-type mice, but not in PPARα null mice exposed to PFOA from day 1 of gestation until day 17. In CD-1 mice exposed to PFOA day 1 of gestation until day 17, patterns of PPARα expression from gestation until age 28 days were tissue-specific and, in the liver, correlated with nutritional changes as the offspring matured (Abbott et al., 2012). As early as day 14 of gestation, exposure to PFOA affected PPARα and related genes associated with fatty acid biosynthesis, β-oxidation, and glucose metabolism, suggesting a role for these genes in poor postnatal survival and growth. An additional study by Albrecht et al. (2013), using the same exposure protocol, but in wild-type, PPARα-null, and human PPARα-transgenic mice, demonstrated that while human PPARα-transgenic mice had increases in hepatic markers of PPARα activation at day 18 of gestation, no effect was seen at postnatal day 20. Unlike wild-type mice, postnatal survival in human PPARα-transgenic mice was unaffected by exposure to PFOA, suggesting reduced sensitivity in mice expressing human PPARα.

4.4.7 Other target organs

No studies on the toxicological effects of exposure to PFOA in the bladder and pancreas were available to the Working Group. Several studies examined the effects of PFOA on the immune system.

Intraperitoneal administration of PFOA in male Sprague-Dawley rats inhibited induced oedema and thermal hypersensitivity in a dose-dependent manner (Taylor et al., 2002). Subsequent studies indicated that the anti-inflammatory properties of PFOA were not mediated through the release of endogenous glucocorticoids (Taylor et al., 2005), but possibly involved binding to the retinoid X receptor (RXR) α (Wan & Badr, 2006). Microarray analyses of liver from Sprague-Dawley rats treated with PFOA indicated anti-inflammatory properties of PFOA at the mRNA level, with the observation of reduced expression of genes regulating inflammatory mediators (Guruge et al., 2006). [The Working Group noted that studies evaluating cytokine responses after exposure to PFOA (Section 4.3.7) indicated that PFOA can be proinflammatory.]

4.5 Susceptible populations

4.5.1 Polymorphisms

PFOA is not metabolized in humans or other mammalian organisms (Lau, 2012; Post et al., 2012); thus it is unlikely that known genetic polymorphisms in xenobiotic metabolism genes that have been associated with genetic susceptibility to other toxicants would have relevance to PFOA as a human health hazard. It is clear, however, that species- and sex-specific differences in renal clearance of PFOA are largely attributable to the function of renal transporters. Depending on species and sex, excretion and reabsorption transporters were implicated as major determinants of the rate of elimination of PFOA (Han et al., 2012). Specifically, OAT4 and URAT1 were identified as transporters that are most likely to be responsible for efficient renal tubular reabsorption of perfluorinated compounds, including PFOA, due to their localization in the apical membrane of the proximal tubular cells in human kidney (Han et al., 2012).

No study has examined the role of transporter polymorphisms in PFOA-dependent effects in humans. However, several studies examined polymorphisms in OAT4 and URAT1.

Non-synonymous single nucleotide polymorphisms were reported that result in amino acid differences in OAT4 (Xu et al., 2005).

Functional URAT1 polymorphisms and transcription factor-dependent differences in expression have been reported. Loss-of-function mutations of URAT1 are the cause of familial idiopathic renal hypouricaemia (Enomoto & Endou 2005). Additional polymorphisms were detected in patients with renal hypouricaemia that were either silent or led to reduced urate transport (Burckhardt, 2012). HNF-1α and HNF-1β increase the promoter expression of human and mouse URAT1, and HNF-1α-deficient mice showed diminished expression of Urat1 in the kidney (Kikuchi et al., 2007). In addition, promoter methylation status is important for tissue-specific URAT1 expression (Kikuchi et al., 2007).

In a study of immortalized human lymphoblast cell lines from the Centre d'Etude du Polymorphisme Humain (CEPH) trios assembled by the HapMap Consortium, exposure to PFOA was shown to elicit the greatest degree of interindividual variability in cytotoxicity and induction of apoptosis (O'Shea et al., 2011). Notably, responses to PFOA and phenobarbital were highly correlated across the population of cell lines tested in the cytotoxicity assay. The genome-wide analysis showed suggestive evidence ($P < 10^{-6}$) for the loci on chromosomes 4 and 14. Within loci spanning 500 kb and flanking single nucleotide polymorphisms with highest association, there were several potential candidate genes associated with susceptibility to PFOA (O'Shea et al., 2011). On chromosome 4, FAT tumour suppressor homologue 1 (FAT1) is a human gene whose rat homologue has been shown to be responsive to PFOA treatment (in the liver) (Guruge et al., 2006; O'Shea et al., 2011). On chromosome 14, three genes were located in the candidate quantitative trait locus: solute carrier family 24 member 4 (SLC24A4); cleavage and polyadenylation specific factor 2 (CPSF2); and Ras and Rab interactor 3 (RIN3). SLC24A4 is a sodium/potassium/calcium exchange protein that is highly expressed in the kidneys. Although CPSF2 and RIN3 have not been shown in previous studies to be responsive to treatment with PFOA, they are tightly linked through a gene network to genes that have been observed as responsive to PFOA treatment in other species. Networks for CPSF2 and RIN3 showed the interactions with immunoglobulin heavy constant mu and RAB5A/B, member of RAS oncogene family (RAB5A and RAB5B), respectively, which are responsive to PFOA in rat and chicken liver (Guruge et al., 2006; Yeung et al., 2007).

4.5.2 Lifestage

As discussed in Section 4.4.6, several studies examined the effects of exposure to PFOA in early life. However, none of these studies evaluated the effects of these exposures on tumour production or carcinogenesis in adult animals, or compared different exposure periods to determine whether susceptibility to toxic events in later life was increased when exposure occurs early in life.

4.6 Mechanistic considerations

The toxicokinetics of PFOA are well established in animals and humans. PFOA is not metabolized in humans or experimental animals (D'eon & Mabury, 2011). Sex-specific differences in plasma half-life have been observed in rats (Kemper & Jepson, 2003). It is also evident that the plasma half-life in humans is much longer than in any experimental animal studied (Butenhoff et al., 2002, 2004; Noker, 2003; Hundley et al., 2006; Olsen et al., 2007; Bartell et al., 2010). These differences in half-lives were attributed to differences in renal reabsorption of PFOA (Han et al., 2012). While there are no direct data on genetic susceptibility, renal transporters that are involved in reabsorption of PFOA are polymorphic in

human populations, suggesting the potential for genetic susceptibility.

It is widely accepted that PFOA is not directly genotoxic. Associations between PFOA-induced oxidative stress and DNA damage or mutation have been reported in some studies (Yao & Zhong, 2005; Fernández Freire et al., 2008; Zhao et al., 2011), but not in others (Florentin et al., 2011). Overall, the role of PFOA-related oxidative stress in carcinogenicity remains unclear.

A wide array of experimental studies in animals and in vitro have been conducted with PFOA and show adverse health effects. Several potential mechanistic events have been identified as possible drivers of PFOA toxicity in multiple tissues. These include, but are not limited to, nuclear receptor activation, cytotoxicity, oxidative stress, alteration of inflammatory pathways, and alterations in hormone levels.

The liver is the most prominent target tissue of PFOA, with rats and mice being the most responsive species to liver-specific effects. Limited data are available indicating liver toxicity in non-human primates (Butenhoff et al., 2002). Additionally, serum levels of PFOA have been positively associated with serum markers of liver injury in humans (Sakr et al., 2007; Lin et al., 2010; Gallo et al., 2012). Liver toxicity observed in rodents has been associated with both PPARα-dependent and -independent mechanisms. The analysis by the Working Group of data from humans in vitro is consistent with multiple molecular pathways being in operation. Cytotoxicity, cell proliferation, and liver hypertrophy have also been observed in studies with PFOA in rodents, indicating that other mechanisms may also contribute.

PFOA modulates inflammatory pathways, such as those involving the production of cytokines. Additionally, PFOA alters hormone levels and activates hormone receptors. Changes in levels of thyroid hormones have been observed in rodents (Butenhoff et al., 2012b) and in non-human primates (Butenhoff et al., 2002). In studies in humans, PFOA increased levels of serum thyroid hormones (Knox et al., 2011; Lopez-Espinosa et al., 2012; Wen et al., 2013). In human cells in vitro, and in fish in vivo and in vitro, PFOA activated estrogen receptors (ToxCast research programme, see Section 4.3.8) (Maras et al., 2006; Liu et al., 2007b; Wei et al., 2007, 2008; Tilton et al., 2008; Benninghoff et al., 2011, 2012; Henry & Fair, 2013). In rodents, PFOA altered female reproductive hormones and tissues (Yang et al., 2009b; Zhao et al., 2010c), disrupted the estradiol/testosterone balance, and induced aromatase activity (Cook et al., 1992; Biegel et al., 1995, 2001). Prenatal and early-life exposures to PFOA also affect mammary-gland development in rodents. However, the importance of PFOA-induced modulation of the immune system or hormone levels in carcinogenesis is uncertain.

5. Summary of Data Reported

5.1 Exposure data

Perfluorooctanoic acid (PFOA) is a synthetic fluorinated carboxylic acid. There are two production methods: the electrochemical fluorination process results in a mixture of branched and straight-chain isomers of the ammonium salt, while the telomerization process, a method in use since the early 2000s, results in an isomerically pure, straight-chain product. PFOA and its salts have been mainly used as emulsifiers in the production of fluoropolymers such as polytetrafluoroethylene. PFOA has been used in metal cleaners, electrolytic-plating baths, self-shine floor polishes, cement, fire-fighting formulations, varnishes, emulsion polymerization, lubricants, gasoline, leather, and textile treatments and as non-stick coatings on cookware and in paper coatings such as food packaging. PFOA is persistent in the environment and has been detected in air, water, dust, and food. For most of the general population, the predominant sources of exposure

are food (including transfer of PFOA from food packaging) and dust. Serum concentrations of perfluorooctanoate of less than about 10 μg/L have been measured in the general population worldwide; serum concentrations increased over time until about 2000, and have since remained constant or decreased. In people living near industrial sources of perfluorooctanoate, mean serum concentrations have ranged from near-background concentrations to > 200 μg/L. In these groups, the predominant route of exposure was drinking-water. Occupational exposure, through inhalation and dermal contact, occurs during fluoropolymer production using PFOA, and mean serum concentrations in groups of workers with the highest exposure were measured as > 1000 μg/L.

5.2 Human carcinogenicity data

The literature on the epidemiology of cancer in relation to PFOA is relatively small and includes studies in three different types of populations: workers exposed in chemical plants producing or using PFOA, high-exposure communities (i.e. areas surrounding a plant with documented release of PFOA and contamination of public and private water supplies), and studies in the general population with background exposures.

5.2.1 Cancer of the testis

The only informative results on risk of cancer of the testis were from two studies of cancer incidence in a high-exposure community setting in West Virginia and Ohio, USA; there was some overlap in the cases examined in these studies. Both publications, using different study designs (i.e. a cohort study of incidence and a population-registry case–control study), observed an increased risk of incidence of cancer of the testis. In the highest quartile of exposure in both studies, the observed increase in risk was approximately threefold, with a significant trend in increasing risk with increasing exposure in the cohort study (no trend test was reported in the case–control study). The evidence for cancer of the testis was considered credible and unlikely to be explained by bias and confounding, however, the estimate was based on small numbers.

5.2.2 Cancer of the kidney

There were several publications that have examined PFOA and risk of cancer of the kidney. Three of these were conducted in West Virginia, USA, and included occupational and community exposure, and the fourth was conducted in a different occupational setting. In the exposure–response analysis of workers in West Virginia, 8 of the 12 deaths from cancer of the kidney were seen in the highest quartile of exposure, with an elevated standardized mortality ratio and a significant trend in increasing risk with increasing exposure. The other occupational cohort study reported no evidence for increased incidence. A modestly increased risk of incidence of cancer of the kidney was seen in a community population with high exposure. A study in a somewhat overlapping population also found elevated relative risks in the groups with high and very high exposure compared with the group with low exposure. The evidence for cancer of the kidney was considered credible; however, chance, bias, and confounding could not be ruled out with reasonable confidence.

5.2.3 Other cancer sites

The evidence regarding other cancer sites, including the urinary bladder, thyroid, prostate, liver, and pancreas was also evaluated. Some positive associations were observed for cancers of the bladder, thyroid, and prostate, but the results were inconsistent among studies and based on small numbers. The evidence for carcinogenicity for all of these sites was judged to be inadequate.

5.3 Animal carcinogenicity data

PFOA was administered in the feed in one study of carcinogenicity in male and female rats, and in another study in male rats. PFOA increased the incidence of testicular Leydig cell adenoma in males in both studies, and increased the incidences of hepatocellular adenoma and pancreatic acinar cell adenoma in the study in male rats only.

PFOA was also shown to promote hepatocarcinogenesis in two feeding studies in male rats and two feeding studies in rainbow trout.

5.4 Mechanistic and other relevant data

PFOA does not undergo metabolism in the experimental systems studied or in humans. It is readily absorbed via all routes of exposure and is excreted into the urine. Among the species studied, humans are unique in that the reabsorption of PFOA in the kidneys is highly efficient, leading to much longer retention in the body when compared with all other animals. Therefore, the body burden of PFOA experienced by humans is much greater than in experimental animals.

PFOA is not DNA-reactive, and gives negative results in an overwhelming number of assays for direct genotoxicity. Therefore, there is *strong* evidence that direct genotoxicity is not a mechanism of PFOA carcinogenesis. Some studies with PFOA indicate that indirect DNA damage may result from induction of oxidative stress, therefore there is *moderate* evidence that genotoxicity overall is not a mechanism of PFOA carcinogenesis.

Several studies in humans have examined the relationship between exposure to PFOA and toxicity, and suggest that PFOA may cause liver injury. In experimental animals, the liver is a well-established target for toxicity. Potential mechanisms for PFOA-induced toxicity and carcinogenicity in the liver include PPARα activation, involvement of other molecular pathways (i.e. constitutive androstane receptor, pregnane X receptor, estrogen receptor), and cytotoxicity. There is *moderate* evidence for these mechanisms, largely from studies in rats and mice. Based on the available evidence, human relevance of the liver findings in rodents cannot be excluded.

The effects of PFOA in other organs are not so well established, but modulation of inflammatory pathways and hormone levels has been reported. Studies in human cells, rodents, and fish, have documented perturbation of molecular pathways involving reproductive hormones and hormone receptors, such as activation of estrogen receptor, interference with testosterone/estradiol balance, and induction of aromatase, and effects on reproductive organs consistent with estrogenicity. Although there is *moderate* evidence that PFOA affects reproductive-hormone pathways, there is *weak* evidence for their relevance to PFOA-associated carcinogenesis.

Overall, there is *moderate* evidence for mechanisms of PFOA-associated carcinogenesis, including some evidence for these mechanisms being operative in humans.

6. Evaluation

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of perfluorooctanoic acid (PFOA). A positive association was observed for cancers of the testis and kidney.

6.2 Cancer in experimental animals

There is *limited evidence* in experimental animals for the carcinogenicity of perfluorooctanoic acid (PFOA).

6.3 Overall evaluation

Perfluorooctanoic acid (PFOA) is *possibly carcinogenic to humans (Group 2B).*

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TETRAFLUOROETHYLENE

Tetrafluoroethylene was reviewed previously by the Working Group in 1979, 1987, and 1998 (IARC, 1979, 1987, 1999). New data have since become available, and these have been incorporated, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

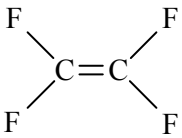
Chem. Abstr. Serv. Reg. No.: 116-14-3

Chem. Abstr. Serv. Name: Tetrafluoroethylene

IUPAC Systematic Name: 1,1,2,2-Tetrafluoroethene

Synonyms: Perfluoroethylene, Perfluoroethene, Ethylene tetrafluoro-, tetrafluoroethene

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: C_2F_4

Relative molecular mass: 100.01

1.1.3 Chemical and physical properties of the pure substance

From IFA (2014), unless otherwise indicated

Description: Colourless gas, odourless or sometimes described as having a faint sweetish odour; extremely flammable

Boiling point: –75.63 °C

Melting point: –131.15 °C (HSDB, 2014)

Density: 4216 kg/m^3 at 15 °C at 1 bar

Solubility: Slightly soluble in water, 159 mg/L at 25 °C (HSDB, 2014)

Vapour pressure: 2947 kPa and 20 °C

Stability: Decomposes into fluorine and fluorine compounds when heated (HSDB, 2014)

Reactivity: A terpene inhibitor (limonene) is generally added to the monomer to prevent spontaneous polymerization.

Risk of explosion in contact with air or in the absence of air at elevated temperatures and/or pressures (> 600 °C and 100 kPa). The stabilized monomer is flammable in air if ignited (flammability limits: lower, 11%; upper, 60%) producing soot and carbon tetrafluoride (Babenko et al., 1993; HSDB, 2014).

Incompatible with polymerization catalysts and peroxides. May react exothermically with

Table 1.1 Methods for the analysis of tetrafluoroethylene

Sample matrix	Sample preparation	Assay procedure	Limit of detection[a]	Reference
Air	Sample collected directly from the workplace	FTIR	0.17 ppm [≈ 0.7 mg/m³]	NIOSH (2003)
	Collection onto solid sorbents, such as activated charcoal, followed by solvent desorption	GC	0.18 ppm [≈ 0.7 mg/m³]	HSE (1997) ISO (2001)
	Air collected into a stainless steel container; sample analysed directly	GC/MS	NR	EPA (1999)

[a] Detection limit reported by ECETOC (2003)

FTIR, Fourier transform infra-red spectrometry; GC, gas chromatography; MS, mass spectrometry; NR, not reported

chloroperoxytrifluoromethane, sulfur trioxide and several other substances (HSDB, 2014). May react if in contact with aluminium, copper and their alloys, resulting in an uncontrolled exothermic reaction (ECHA, 2014).

Octanol/water partition coefficient (P): log P = 1.21 (estimated) (HSDB, 2014)

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), 1 mg/m³ = 4.09 ppm, calculated from mg/m³ = (relative molecular mass/24.45) × ppm.

1.1.4 Technical products and impurities

Industrial-grade tetrafluoroethylene generally has a purity of > 99.7%. Impurities may include various chloro-fluoro compounds (ECETOC, 2003). Limonene may be added to prevent spontaneous polymerization (HSDB, 2014).

1.1.5 Analysis

A range of sampling and analytical methods can be used to measure exposure to tetrafluoroethylene, although there is only one validated method from the United States National Institute of Occupational Safety and Health (NIOSH), based on using a Fourier transform infra-red (FTIR) spectrometer to directly detect tetrafluoroethylene. Selected available methods are summarized in Table 1.1.

Generic methods for the collection of volatile organic substances using solid sorbents such as activated charcoal, followed by analysis using gas chromatography (GC) have been used to measure occupational exposure. It is also possible to sample air contaminated with tetrafluoroethylene into a solid stainless steel container, and to then analyse the sample using gas chromatography-mass spectrometry (GC-MS).

1.2 Production and use

1.2.1 Production process

(a) Manufacturing processes

Tetrafluoroethylene is manufactured in a four-stage process involving the separate production of hydrogen fluoride and chloroform, which are subsequently reacted in the presence of antimony trifluoride to produce chlorodifluoromethane. The chlorodifluoromethane is pyrolysed at > 650 °C to produce tetrafluoroethylene (ECETOC, 2003; HSDB, 2014).

(b) Production volumes

Worldwide production of tetrafluoroethylene in 1977 was estimated at 15 000–20 000 tonnes (cited in IARC, 1999), and market growth has since been 3–5% per annum (Teng, 2012). The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) has estimated that the annual world production of

tetrafluoroethylene in 2001 was 100 000 tonnes (ECETOC, 2003).

In 2000, an estimated 10 000–50 000 tonnes of tetrafluoroethylene was produced in the European Union (European Chemicals Bureau, 2000). The Toxic Substances Control Act Inventory Update Rule of the United States Environmental Protection Agency (EPA) indicated that annual production of tetrafluoroethylene and importation into the USA totalled 50–100 million pounds [22 000–45 000 tonnes] from 1998 to 2006 (NTP, 2014).

1.2.2 Uses

Tetrafluoroethylene is used in the manufacture of oligomers, fluoroelastomers and fluoropolymers. The main use of tetrafluoroethylene is in the manufacture of polytetrafluoroethylene that is used as nonstick coatings on cookware, membranes for clothing that are both waterproof and breathable, electrical-wire casing, fire- and chemical-resistant tubing, and plumbing thread seal tape. It reacts with perfluoronitrosoalkanes to produce nitroso rubbers. It is also used in the production of compounds and intermediates of low relative molecular mass, including for the manufacture of iodoperfluoroalkanes (NTP, 2014).

1.3 Occurrence and exposure

1.3.1 Environmental occurrence

(a) Natural occurrence

Tetrafluoroethylene has been detected in very low concentrations in natural gas, and in gaseous emissions from volcanic vents (Gribble, 2010). There are no other known natural sources.

(b) Air and water

Emission of tetrafluoroethylene to air or water may occur from primary production, or from use in the manufacture of other products. Deliberate vent releases from industrial plants are generally destroyed by thermal oxidation (ECETOC, 2003).

Tetrafluoroethylene does not readily biodegrade in water, sediment, or soil, and has low potential to bioaccumulate in aquatic organisms (ECHA, 2014).

Gaseous tetrafluoroethylene degrades in the atmosphere by reaction with photochemically produced hydroxyl radicals, with a half-life of approximately 17 hours (HSDB, 2014). Modelling suggests that 99.99% of environmental emissions end in the air, with 0.008% in water (ECHA, 2014). An environmental survey realized by the government of Japan in 2012 detected tetrafluoroethylene in the air at 4 of the 10 sites tested, with concentrations up to 2.8 μg/m^3. Tetrafluoroethylene was not detected in water (Japanese Environmental Survey, 2012).

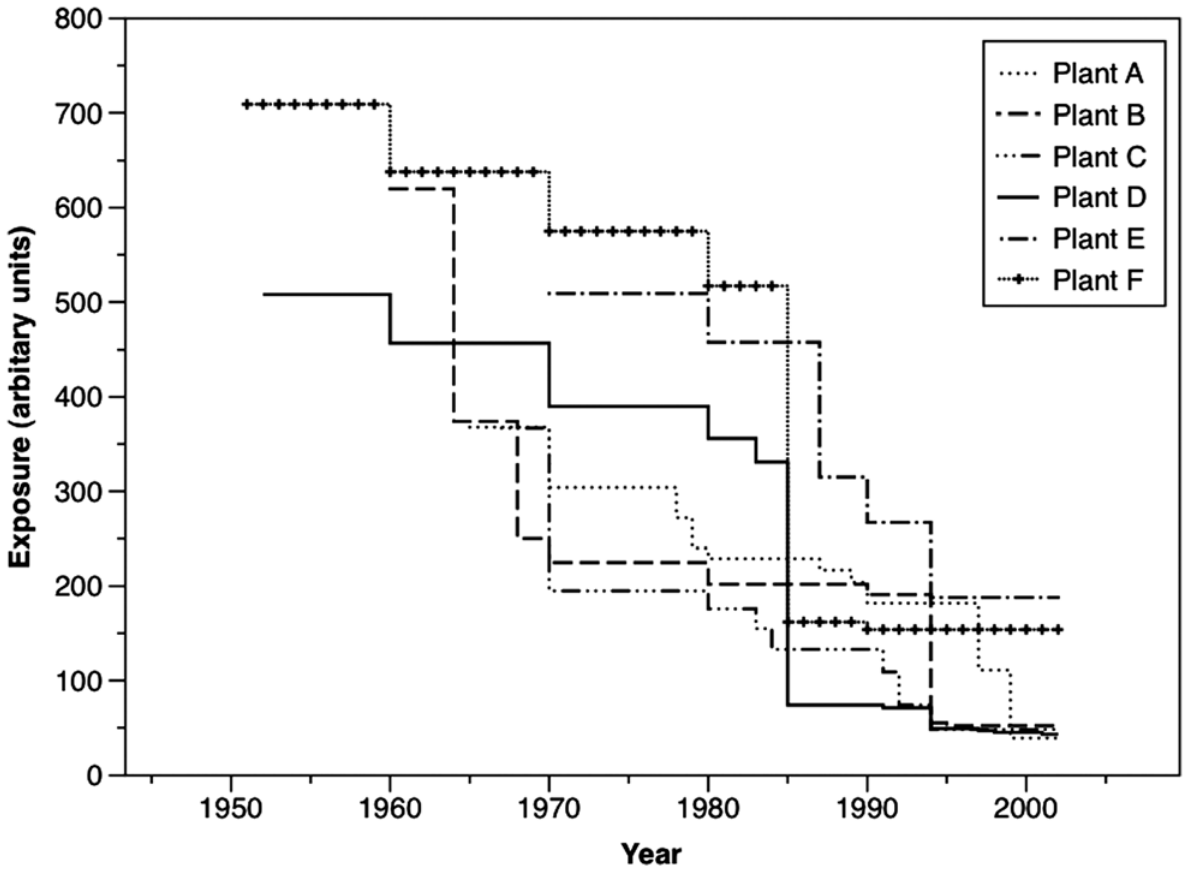
1.3.2 Occupational exposure

Occupational exposure occurs in the primary manufacture of tetrafluoroethylene and during the subsequent polymerization process.

Inhalation exposure has been measured in several European plants manufacturing tetrafluoroethylene. ECETOC (2003) reported levels of between 0.16 and 6 mg/m^3 in one plant, and between < 0.4 and 6.1 mg/m^3 (95% of samples, < 2 mg/m^3) in a second plant, in both data sets as an 8-hour time-weighted average. No other published data were available for workplace exposures to tetrafluoroethylene.

As part of an international epidemiological study of workers in six plants manufacturing polytetrafluoroethylene in Germany, the Netherlands, Italy, the United Kingdom, and the USA (New Jersey and West Virginia), Sleeuwenhoek & Cherrie (2012) made estimates of exposure to tetrafluoroethylene by inhalation using modelling methodology. The exposure reconstructions were made using descriptive information about the workplace environment

Fig. 1.1 Change in levels of exposure to tetrafluoroethylene for operators working in polymerization areas of six plants manufacturing polytetrafluoroethylene



Reproduced from Sleeuwenhoek & Cherrie (2012). with permission of The Royal Society of Chemistry
Note: Plants A–F were located in Germany, the Netherlands, Italy, the United Kingdom, and the USA (New Jersey and West Virginia)

and work processes, including changes over time. The methodology allowed for key changes in exposure modifiers such as local ventilation, use of respiratory protective equipment, working in a confined space, outdoor work, cleanliness, and the level of involvement of the workers in the process (e.g. operator or supervisor). There were very few measurements of exposure available from the plants (all unpublished), and so the exposure estimates were expressed on an arbitrary dimensionless scale. Two assessors made assessments independently and the results were then combined (Sleeuwenhoek & Cherrie, 2012).

In each plant, the highest estimated exposures for tetrafluoroethylene occurred in the polymerization area. The introduction of control measures, increasing process automation and other improvements, were judged to have resulted in exposures generally decreasing over time. In the polymerization area, the annual estimated decline in exposure to tetrafluoroethylene varied by plant from 3.8% to 5.7% (see Fig 1.1). The differences in the estimated exposure level for polymerization workers at any time were up to about fivefold. Part of these inter-plant differences can be explained by differences in technology and the work responsibilities of operators (Sleeuwenhoek & Cherrie, 2012). The biggest changes in exposure for polymerization workers were mainly due to the introduction of automatic

cleaning and automation at the autoclaves. Other improvements causing important declines in exposure levels were the introduction of localized ventilation and vacuum extraction at the end of the polymerization process (Sleeuwenhoek & Cherrie, 2012).

Operators in the monomer area always wore breathing apparatus when undertaking tasks where exposure to tetrafluoroethylene was possible, and so inhalation exposure for these workers would have been very low. In this area of the plants there were small decreases in estimated exposure levels due to general environmental improvements, such as the use of more efficient pumps and gaskets (Sleeuwenhoek & Cherrie, 2012).

Tetrafluoroethylene exposure for workers in the finishing areas of the plants was consistently low over the history of the plant. The decline in exposure levels was generally smaller in finishing areas than in other areas, and the changes were primarily due to improved general ventilation (Sleeuwenhoek & Cherrie, 2012).

Historically, workers in polytetrafluoroethylene production were potentially exposed to both tetrafluoroethylene and the ammonium salt of perfluorooctanoic acid (PFOA), which is also the subject of a *Monograph* in the present volume). Only a small number of jobs with lower exposure to tetrafluoroethylene had no possible exposure to ammonium perfluorooctanoate. Workers in most jobs were exposed to both chemicals, and there was a strong positive correlation between estimated exposure to tetrafluoroethylene and ammonium perfluorooctanoate ($r = 0.72$, $P < 0.001$) (Sleeuwenhoek & Cherrie, 2012).

[The Working Group considered that the limited quantity of data on measured occupational exposure suggested that in about 2000 the highest tetrafluoroethylene exposure levels in manufacturing plants were about 6 mg/m^3, and considering the temporal trends described above (average change over the history of production, about sixfold), it seems probable that the highest occupational average exposures to tetrafluoroethylene in the polytetrafluoroethylene-manufacturing industry in the 1950s and 1960s would have been < 40 mg/m^3.]

1.3.3 Exposure of the general population

No information was available about the levels of exposure to tetrafluoroethylene in the general population, although because of the necessity to contain the substance within an enclosed system due to its flammable nature, it is likely that any exposure is very low and localized around industrial facilities manufacturing or using tetrafluoroethylene. Tetrafluoroethylene is not detectable in its polymerized products, including polytetrafluoroethylene (analytical detection limit, < 0.05–0.01 mg/kg) (ECETOC, 2003). When heated to temperatures above those normally used for cooking, polytetrafluoroethylene-coated pans may emit tetrafluoroethylene, although the major hazard in such circumstances is particulate fumes, which can cause serious acute effects (NIOSH, 1977).

1.4 Regulations and guidelines

Major national regulatory occupational exposure limits for tetrafluoroethylene are given in Table 1.2.

Tetrafluoroethylene has been registered under the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) regulation of the European Union. All registered uses are under "PROC 1: Use in closed process, no likelihood of exposure" (ECHA, 2014).

The derived no-effect level (DNEL) under the REACH system for long-term exposure by inhalation based on systemic health effects is 6.4 mg/m^3, from the registration entry of the manufacturer/importer in data from the European Chemicals Agency (IFA, 2014).

Tetrafluoroethylene is categorized in Europe in carcinogenic category 1B, with H350 "may

Table 1.2 Regulations and guidelines for occupational exposure to tetrafluoroethylene

Country or region	Long-term average concentration (mg/m³)	Carcinogenicity
European Union (DNEL)[a]	6.4	Category 1B with H350 "may cause cancer"
USA (ACGIH)[b]	8.2	A3; confirmed animal carcinogen with unknown relevance to humans
USA (NTP)[c]	–	"Reasonably anticipated to be a human carcinogen"

[a] DNEL, derived no-effect level; data from the GESTIS DNEL database (IFA, 2014)
[b] Eight-hour time-weighted average (8-hour TLV-TWA); data from American Conference of Governmental Industrial Hygienists. Note that for all long-term threshold limit values (TLVs), excursions in exposure level may not exceed three times the 8-hour TLV-TWA for more than a total of 30 minutes during a workday, and under no circumstances should these excursions exceed five times the 8-hour TLV-TWA, provided that the TLV-TWA is not exceeded (ACGIH, 2013)
[c] Data from the United States National Toxicology Program (NTP, 2014)

Table 1.3 Acute exposure guideline levels (AEGLs) for tetrafluoroethylene

Type of AEGL	AEGL in ppm (mg/m³) for exposure duration				
	10 minutes	30 minutes	1 hour	4 hours	8 hours
AEGL-1[a] (non-disabling)	27 (110)	27 (110)	22 (89)	14 (56)	9 (37)
AEGL-2[b] (disabling)	69 (280)	6 (280)	55 (220)	34 (140)	23 (92)
AEGL-3[c] (lethal)	420 (1700)	420 (1700)	330 (1400)	210 (850)	100 (430)

[a] AEGL-1 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic non-sensory effects
[b] AEGL-2 is the concentration above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects, or have an impaired ability to escape
[c] AEGL-3 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening health effects or death
From NRC (2015)

cause cancer", under classification, labelling, and packaging Regulation (EC) No. 1272/2008) (ECHA, 2015).

In the USA, tetrafluoroethylene is classified as "reasonably anticipated to be a human carcinogen" by the National Toxicology Program (NTP) in its Report on Carcinogens (NTP, 2014).

Tetrafluoroethylene is included within the United States Toxics Release Inventory (TRI, 2016).

The Committee on Acute Exposure Guideline Levels of the United States National Research Council has set acute exposure guideline levels for tetrafluoroethylene (summarized in Table 1.3; NRC, 2015). Acute exposure guideline levels represent threshold exposure limits for the general public, and are applicable to emergency exposure periods ranging from 10 minutes to 8 hours. The American Industrial Hygiene Association has published emergency response planning guidelines for tetrafluoroethylene (AIHA, 2013).

2. Cancer in Humans

2.1 Cohort studies

See Table 2.1 for study details

Only one cohort study analysing cancer risk in relation to exposure to tetrafluoroethylene was available to the Working Group. Consonni et al. (2013) studied mortality from cancer and from selected non-malignant diseases in a

Table 2.1 Cohort studies of cancer and occupational exposure to tetrafluoroethylene

Reference, location, follow-up period	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	SMR (95% CI)	Covariates Comments
Consonni et al. (2013), six plants in Europe and the USA, 1950/1970–2001/2008	4773 exposed workers (Germany, 690; Italy, 415; the Netherlands, 658; United Kingdom 756; USA, 2254)	JEM based on semiquantitative exposure estimates on an arbitrary scale	All cancers	Overall	187	0.77 (0.67–0.89)	Men only; all were ever exposed, national reference rates were used
			Oesophagus		11	1.23 (0.62–2.21)	
			Liver		8	1.27 (0.55–2.51)	
			Pancreas (157)		13	1.15 (0.61–1.97)	
			Lung		59	0.73 (0.56–0.95)	
			Kidney and other urinary organs		10	1.44 (0.69–2.65)	
			Leukaemia		12	1.48 (0.77–2.59)	
			Liver	Cumulative exposure (unit-yrs)			Categorized into tertiles based on number of deaths from all causes based on cumulative exposure
				Low (< 80.5)	1	0.59 (0.01–3.28)	
				Medium (80.5–559)	2	0.95 (0.12–3.43)	
				High (≥ 560)	5	2.01 (0.65–4.70)	*P* for trend, 0.24
			Kidney and other urinary organs	Cumulative exposure (unit-yrs)			
				Low (< 80.5)	2	0.93 (0.11–3.37)	
				Medium (80.5–559)	6	2.58 (0.95–5.62)	
				High (≥ 560)	2	0.81 (0.10–2.93)	*P* for trend, 0.87
			Leukaemia	Cumulative exposure (unit-yrs)			
				Low (< 80.5)	4	1.47 (0.40–3.76)	
				Medium (80.5–559)	3	1.14 (0.24–3.34)	
				High (≥ 560)	5	1.83 (0.59–4.26)	*P* for trend, 0.77

CI, confidence interval; ICD, International Classification of Disease; JEM, job-exposure matrix; SMR, standardized mortality ratio; yr, year

cohort including workers in six polytetrafluoroethylene-production sites in Europe (Germany, the Netherlands, Italy, England) and the USA (New Jersey, West Virginia) from 1950 to 2002. Production of polytetrafluoroethylene involves the use of ammonium perfluorooctanoate, exposure to which was also analysed. Follow-up was from start of production between 1950 and 1970, until 2008. Of the 5879 men identified, 4773 who were potentially exposed were included in the analysis.

Semiquantitative estimates of individual exposure to tetrafluoroethylene and ammonium perfluorooctanoate were reconstructed based on a specifically developed job-exposure matrix (Sleeuwenhoek & Cherrie, 2012). Standardized mortality ratios (SMRs) were calculated using national mortality rates as comparison. Plant-specific results were not presented.

In the overall analysis, elevated risks were seen for all cancer sites of a-priori interest: liver, 1.27 (95% CI, 0.55–2.51); kidney, 1.44 (95% CI, 0.69–2.65); and leukaemia, 1.48 (95% CI, 0.77–2.59). No significant trends in risk with increasing exposure were observed with cumulative exposure to tetrafluoroethylene, or with duration of exposure or time since exposure for any of the cancer sites of interest (Table 2.1). A significant downward trend in the risk of cancer of the lung was observed with increasing exposure duration, but not with other exposure metrics. Additional analyses using regional comparison rates did not materially change risk estimates. Eighty-eight percent of workers were exposed to ammonium perfluorooctanoate as well as to tetrafluoroethylene. Analysis of patterns of mortality with ammonium perfluorooctanoate or tetrafluoroethylene as the exposure of interest gave very similar results.

[The results suggested an elevated risk of cancer of the liver and kidney, and leukaemia. Direct control for possible non-occupational confounders was not possible; however, based on analysis of mortality patterns in the cohort and general knowledge of exposures in the included plants, the Working Group judged that major confounding by alcohol, tobacco, hepatitis B virus, or vinyl chloride monomer was unlikely. The power of the study was, however, not sufficient to support a causal association with tetrafluoroethylene. The Working Group characterized this as a well-conducted study with thorough exposure assessment, which with a longer follow-up would be expected to have more deaths and hence more statistical power to detect any possible associations.]

2.2 Case–control studies

No case–control studies on cancer risk and exposure to tetrafluoroethylene were available to the Working Group.

3. Cancer in Experimental Animals

The carcinogenicity of tetrafluorethylene in experimental animals was reviewed previously by the Working Group (IARC, 1999). The Working Group at this time identified two studies of carcinogenicity in rodents treated with tetrafluoethylene by inhalation: one study in male and female mice, and one study in male and female rats.

3.1 Mouse

See Table 3.1

Groups of 48 male and 48 female B6C3F$_1$ mice (age, 7 weeks) were exposed to tetrafluoroethylene (purity, 98–99%) at a concentration of 0 (control), 312, 625, or 1250 ppm by inhalation for 6 hours per day, 5 days per week, for 95–96 weeks, with an observation period of 11 days after the final exposure. The study was terminated during week 96 because of reduced survival compared with controls. Mean body weights in

Table 3.1 Studies of carcinogenicity in mice exposed to tetrafluoroethylene by inhalation

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
$B6C3F_1$ (M) 95–96 wk + 11 days recovery NTP (1997)	0 (control), 312, 625, 1250 ppm for 6 h/day, 5 days/wk 48 mice/group	*Liver* Haemangioma[a]: 0/48, 10/48 (21%)**, 5/48 (10%)*, 2/48 (4%) Haemangiosarcoma[b]: 0/48, 21/48 (44%)**, 27/48 (56%)**, 37/48 (77%)** Haemangioma or haemangiosarcoma (combined)[c]: 0/48, 26/48 (54%)**, 30/48 (63%)**, 38/48 (79%)**	*$P < 0.05$ (Fisher exact test) **$P < 0.01$ (Fisher exact test)	Purity, 98–99% Surviving animals: 38, 11, 2, 1 Statistical analysis adjusted for survival
		Hepatocellular adenoma: 17/48 (35%), 17/48 (35%), 12/48 (25%), 20/48 (42%) Hepatocellular carcinoma: 11/48 (23%), 20/48 (42%)**, 33/48 (69%)**, 26/48 (54%)** Hepatocellular adenoma or carcinoma (combined)[c]: 26/48 (54%), 34/48 (71%)**, 39/48 (81%)**, 35/48 (73%)**		
		Histiocytic sarcoma (all organs)[d] 0/48, 12/48 (25%)*, 7/48 (15%)**, 7/48 (15%)**	*$P < 0.001$ (Fisher exact test) **$P < 0.01$ (Fisher exact test)	

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
B6C3F$_1$ (F) 95–96 wk + 11 days recovery NTP (1997)	0 (control), 312, 625, 1250 ppm for 6 h/day, 5 days/wk 48 mice/group	*Liver* Haemangioma[e]: 0/48, 5/48 (10%)*, 2/47 (4%), 1/47 (2%) Haemangiosarcoma[f]: 0/48, 27/48 (57%)**, 27/47 (58%)**, 34/47 (72%)** Haemangioma or haemangiosarcoma (combined): 0/48, 31/48 (65%)**, 28/47 (60%)**, 35/47 (73%)** Hepatocellular adenoma: 15/48 (31%), 17/48 (35%), 20/47 (43%)*, 15/47 (32%) Hepatocellular adenoma (multiple): 1/48 (2%), 7/48 (15%)**, 9/47 (19%)**, 7/47 (15%)** Hepatocellular carcinoma: 4/48 (8%), 28/48 (58%)**, 22/47 (47%)**, 20/47 (43%)** Hepatocellular carcinoma (multiple): 0.48, 5/48 (10%), 7/47 (15%), 7/47 (15%) Hepatocellular adenoma or carcinoma (combined)[g]: 17/48 (35%), 33/48 (69%)**, 29/47 (62%)**, 28/47 (60%)**	*$P < 0.05$ (Fisher exact test) **$P < 0.01$ (Fisher exact test)	Purity, 98–99% Surviving animals: 36, 4, 6, 4
		Histiocytic sarcoma (all organs)[h] 1/48 (2%), 21/48 (44%)*, 19/47 (40%)*, 18/48 (38%)*	*$P < 0.001$	

[a] Historical incidence for 2-year NTP inhalation studies with chamber control groups at all laboratories (mean ± standard deviation): 2/947 (0.2% ± 0.7%); range, 0–2%. Historical incidence for 2-year NTP inhalation studies with chamber control groups at Battelle Pacific North-western Laboratories: 1/448 (0.2% ± 0.7%); range, 0–2%

[b] Historical incidence at all laboratories: 12/947 (1.3% ± 1.7%); range, 0–6%. Historical incidence at Battelle Pacific North-western Laboratories: 2/448 (0.5% ± 0.9%); range, 0–2%

[c] Historical incidence at all laboratories: 358/947 (37.8% ± 12.5%); range, 11–60%. Historical incidence at Battelle Pacific North-western Laboratories: 186/448 (41.5% ± 9.2%); range, 30–60%

[d] For the liver, lung, spleen, mesenteric lymph node, bone marrow, and kidney, historical incidence for 2-year NTP inhalation studies with chamber control groups at all laboratories (mean ± standard deviation): 6/950 (0.6% ± 1.2%); range, 0–4%. Historical incidence for 2-year NTP inhalation studies with chamber controls at Battelle Pacific North-western Laboratories: 2/450 (0.4% ± 0.9%); range, 0–2%.

[e] Historical incidence at all laboratories: 1/937 (0.1% ± 0.5%); range, 0–2%. Historical incidence at Battelle Pacific North-western Laboratories: 0/446

[f] Historical incidence at all laboratories: 5/937 (0.5% ± 1.0%); range, 0–3%. Historical incidence at Battelle Pacific North-western Laboratories: 2/446 (0.5% ± 0.9%); range, 0–2%

[g] Historical incidence at all laboratories: 200/937 (21.3%)

[h] Historical incidence at all laboratories: 26/941 (2.8% ± 3.1%); range, 0–10%. Historical incidence at Battelle Pacific North-western Laboratories: 14/447 (3.1% ± 3.0%); range, 0–8%

h, hour; mo, month; wk, week

exposed groups were generally similar to those of the controls except at the end of the study, when body weight was decreased in mice at the highest dose. The survival rates of males in the group at 625 ppm (intermediate dose) and of all exposed groups of females were significantly less than those of the controls (NTP, 1997).

In male mice exposed to tetrafluoroethylene at a concentration of 0, 312, 625, or 1250 ppm, the incidence of liver haemangioma was significantly higher in the groups at the lowest and intermediate doses than in the control group. The incidences of haemangiosarcoma, and of haemangioma or haemangiosarcoma (combined), were significantly higher in all exposed groups than in the controls. The incidences of hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined), were significantly higher in all exposed groups. The incidence of eosinophilic foci in the liver was significantly higher in the groups at the intermediate and highest doses (1/48, 6/48, 7/48, 7/48).

The incidence of histiocytic sarcoma (in organs such as the liver, lung, spleen, mesenteric lymph node, bone marrow, and kidney) was significantly greater in all exposed groups than in the control group (NTP, 1997).

In female mice exposed to tetrafluoroethylene at a concentration of 0, 312, 625, or 1250 ppm, the incidence of liver haemangioma was significantly higher in the group at the lowest dose than in the controls. The incidences of haemangiosarcoma, and of haemangioma or haemangiosarcoma (combined), were significantly higher in all exposed groups. The incidence of hepatocellular adenoma was significantly higher in the group at the intermediate dose. The incidence of hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined), was significantly higher in all exposed groups. The incidence of eosinophilic foci of the liver was significantly higher in the groups at the lowest and intermediate dose (5/48, 13/48, 12/47, 7/47).

The incidence of histiocytic sarcoma (in organs such as liver, lung, spleen, mesenteric lymph node, bone marrow, and kidney) was significantly greater in all exposed groups than in the control group (NTP, 1997).

3.2 Rat

See Table 3.2

Groups of 50 male and 50 female F344/N rats (age, 7 weeks) were exposed to tetrafluoroethylene (purity, 98–99%) at a concentration of 0, 156 (males only), 312, 625, or 1250 (females only) ppm by inhalation for 6 hours per day, 5 days per week, for 104 weeks, with an observation period of 11 days after the final exposure. Mean body weights of exposed groups were generally similar to those of the controls except at the end of the study, when body weight was decreased in rats at the highest dose. The survival rates of males at 625 ppm (the highest dose) and of females in all exposed groups of were significantly less than those of the controls (NTP, 1997).

In male rats exposed to tetrafluoroethylene at a concentration of 0, 156, 312, or 625 ppm, the incidence of renal cell adenoma was significantly higher in the groups at the intermediate and highest dose than in the controls. The incidence of renal cell adenoma or carcinoma (combined) was significantly higher in the group at the highest dose. The incidence of hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined), was significantly higher in the group at the intermediate dose. The incidences of basophilic foci (22/50, 19/50, 33/50, 29/50), eosinophilic foci (3/50, 18/50, 22/50, 19/50) and mixed cell foci (5/50, 5/50, 16/50, 13/50) of the liver were significantly higher in the groups at the intermediate and highest dose (NTP, 1997).

The incidence of mononuclear cell leukaemia was significantly higher in males at the lowest and highest dose. There was a small but significant increase in the incidence of interstitial cell

Table 3.2 Studies of carcinogenicity in rats exposed to tetrafluoroethylene by inhalation

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance[g]	Comments
F344/N (M) 104 wk + 11 days NTP (1997)	0 (control), 156, 312, 625 ppm for 6 h/day, 5 days/wk, 104 wk 50 rats/group	*Kidney* Renal cell adenoma[c,d]: 2/50 (4%), 4/50 (8%), 9/50 (18%)*, 13/50 (26%)** Renal cell carcinoma[c]: 1/50 (2%), 1/50 (2%), 0/50, 0/50 Renal cell adenoma or carcinoma (combined)[c]: 3/50 (6%), 5/50 (10%), 9/50 (18%), 13/50 (26%)**	*$P < 0.05$ (Fisher exact test) **$P < 0.01$ (Fisher exact test)	Purity, 98–99% Surviving animals: 17, 12, 17, 1
		Liver Hepatocellular adenoma: 3/50 (6%), 6/50 (12%), 8/50 (16%), 5/50 (10%) Hepatocellular carcinoma: 1/50 (2%), 1/50 (2%), 10/50 (20%)*, 3/50 (6%)	* $P < 0.01$ (Fisher exact test) **$P = 0.005$ (Fisher exact test)	
		Hepatocellular adenoma or carcinoma (combined)[f]: 4/50 (8%), 7/50 (14%), 15/50 (30%)**, 8/50 (16%)		
		Mononuclear cell leukaemia[a] 34/50 (68%), 43/50 (86%)*, 38/50 (76%), 31/50 (62%)**	* $P < 0.05$ (Fisher exact test) **$P < 0.05$ (Life table test)	
		Testis Interstitial cell adenoma: 39/50 (78%), 40/50 (80%), 48/50 (96%)*, 47/50 (94%)**	*$P < 0.007$ (Fisher exact test) **$P < 0.020$ (Fisher exact test)	

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance[g]	Comments
F344/N (F) 104 wk NTP (1997)	0 (control), 312, 625, 1250 ppm 6 h/day, 5 days/wk, 104 wk 50 rats/group	*Kidney* Renal cell adenoma[c,e]: 0/50, 3/50 (6%), 3/50 (6%)*, 8/50 (16%)** Renal cell carcinoma[c]: 0/50, 0/50, 0/50, 3/50 (6%) Renal cell adenoma or carcinoma (combined)[c]: 0/50, 3/50 (6%), 3/50 (6%), 10/50 (20%)**	*$P < 0.05$ (Fisher exact test) **$P < 0.01$ (Fisher exact test)	Purity, 98–99% Surviving animals: 28, 16, 15, 18
		Liver Hepatocellular adenoma: 0/50, 4/50 (8%)*, 5/50 (10%)**, 6/50 (12%)** Hepatocellular carcinoma: 0/50, 4/50 (8%)*, 9/50 (18%)**, 2/50 (4%) Hepatocellular adenoma or carcinoma (combined)[h]: Overall rate: 0/50 (0%), 7/50 (14%)**, 12/50 (24%)**, 8/50 (16%)** Haemangiosarcoma[i]: 0/50 (8%), 0/50 (0%), 5/50 (10%)***, 1/50 (2%)	* $P < 0.05$ (Fisher exact test) **$P < 0.01$ (Fisher exact test) ***$P = 0.025$ (regression test)	
		Mononuclear cell leukaemia[b] 16/50 (32%), 31/50 (62%)*, 23/50 (46%)***, 36/50 (72%)**	*$P = 0.002$ (Fisher exact test) **$P < 0.001$ (Fisher exact test) ***$P = 0.008$ (lifetable test)	

[a] Historical incidence for 2-year NTP inhalation studies with chamber control groups at all laboratories (mean ± SD): 356/655 (54.4% ± 8.8%); range, 34–66%; at Battelle Pacific North-western Laboratories: 195/348 (56.0% ± 8.7%); range, 38–66%

[b] Historical incidence for 2-year NTP inhalation studies with chamber control groups at all laboratories (mean ± SD): 262/653 (40.1% ± 7.2%); range, 30–54%; at Battelle Pacific North-western Laboratories: 146/348 (42.0% ± 7.2%); range, 30–54%

[c] Single and step sections combined

[d] Historical incidence for 2-year NTP inhalation studies with chamber control groups at all laboratories (mean ± SD): 6/652; range, 0–4%; at Battelle Pacific North-western Laboratories: 5/347; range, 0–4%

[e] Historical incidence for 2-year NTP inhalation studies with chamber control groups at all laboratories (mean ± SD): 2/650; range, 0–2%; at Battelle Pacific North-western Laboratories: 2/346; range, 0–2%

[f] Historical incidence for 2-year NTP inhalation studies with chamber control groups at all laboratories (mean ± SD): 28/653; range, 2–9%; at Battelle Pacific North-western Laboratories: 11/347; range, 2–8%

[g] The logistic regression test regards neoplasms in animals dying before terminal kill as nonfatal. The lifetable test regards neoplasms as being the cause of death

[h] Historical incidence at all laboratories: 10/650; range, 0–6%; at Battelle Pacific North-western Laboratories: 7/346; range, 0–4%

[i] Historical incidence for all organs at all laboratories: 2/653; range, 0–2% (incidence in liver, 0/650)

h, hour; mo, month; SD, standard deviation; wk, week

adenoma of the testis in the groups at the intermediate and highest dose.

In female rats exposed to tetrafluoroethylene at a concentration of 0, 312, 625, or 1250 ppm, the incidence of renal cell adenoma or carcinoma (combined) was significantly higher in the group at the highest dose than in the controls. The incidence of haemangiosarcoma in the liver was significantly higher in the group at the intermediate dose. The incidence of hepatocellular adenoma was significantly higher in all exposed groups. The incidence of hepatocellular carcinoma was significantly higher in the groups at the lowest and intermediate dose. The incidence of hepatocellular adenoma or carcinoma (combined) was significantly higher in all exposed groups. The incidence of eosinophilic foci of the liver (1/50, 4/50, 5/50, 4/50) was significantly higher in the group at the intermediate dose, and the incidence of mixed cell foci (12/50, 14/50, 16/50, 18/50) was significantly higher in the group at the highest dose (NTP, 1997).

The incidence of mononuclear cell leukaemia was significantly higher in all exposed groups of females than in the controls.

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

Tetrafluoroethylene is a chemically unstable compound, and no studies on radioactively labelled tetrafluoroethylene were identified by the Working Group. Thus detailed, direct information on the degree of absorption, distribution and excretion of tetrafluoroethylene was not available. Tetrafluoroethylene is virtually insoluble in most solvents. Human exposures occur primarily through inhalation.

4.1.1 Absorption

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Indirect evidence for absorption of tetrafluoroethylene was available from several studies in experimental animals, including Dilley et al. (1974), who reported that in male Sprague-Dawley rats exposed to tetrafluoroethylene (3500 ppm) by inhalation for 30 minutes, fluoride excretion in the urine was significantly increased relative to controls.

Whole-body inhalational exposure to tetrafluoroethylene ("subacute", short term, or long term) in male and female B6C3F$_1$ mice (up to 1250 ppm for up to 96 weeks), or male and female Fischer 344 rats (up to 625 ppm for 104 weeks) resulted in toxicity in multiple organs, indicating absorption of tetrafluoroethylene in the lung (NTP, 1997). Additional evidence of absorption via inhalation included the observation of toxicity after single and long-term inhalational exposures to tetrafluoroethylene in mice, hamsters, guinea-pigs, and rabbits, as summarized in a review by Kennedy (1990). However, because toxicity or lethality after a single dose by inhalation in rats was observed only at very high concentrations (Clayton, 1967; Odum & Green, 1984), absorption via the lung is probably not very efficient, which is consistent with the very low solubility of tetrafluoroethylene. Low absorption in the lung was also confirmed by a study by Ding et al. (1980), who exposed rabbits to tetrafluoroethylene at 1000 ppm for 60 minutes via a face mask, and estimated alveolar absorption to be 6.8%.

No studies of oral or dermal exposure to tetrafluoroethylene were available to the Working Group.

4.1.2 Distribution

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

No data were available to the Working Group. Indirect evidence for distribution of tetrafluoroethylene to distal organs (kidney, liver, testes, etc.) after inhalation was available from several studies of toxicity after a single dose, or after long-term dosing, in experimental animals, as summarized above. In rats exposed by inhalation, metabolism of tetrafluoroethylene in the liver and kidney has been reported, suggesting distribution to these tissues (Odum & Green, 1984).

4.1.3 Metabolism

Unlike many other halogenated hydrocarbons, tetrafluoroethylene is not a substrate for cytochrome P450s (Odum & Green, 1984). However, tetrafluoroethylene is known to undergo metabolism, as shown by excretion of inorganic fluoride in the urine of male rats exposed to tetrafluoroethylene by inhalation (Dilley et al., 1974). Odum & Green (1984) have demonstrated that tetrafluoroethylene is metabolized to the glutathione conjugate *S*-(1,1,2,2-tetrafluoroethyl)glutathione (TFEG) in liver slices from Wistar rats.

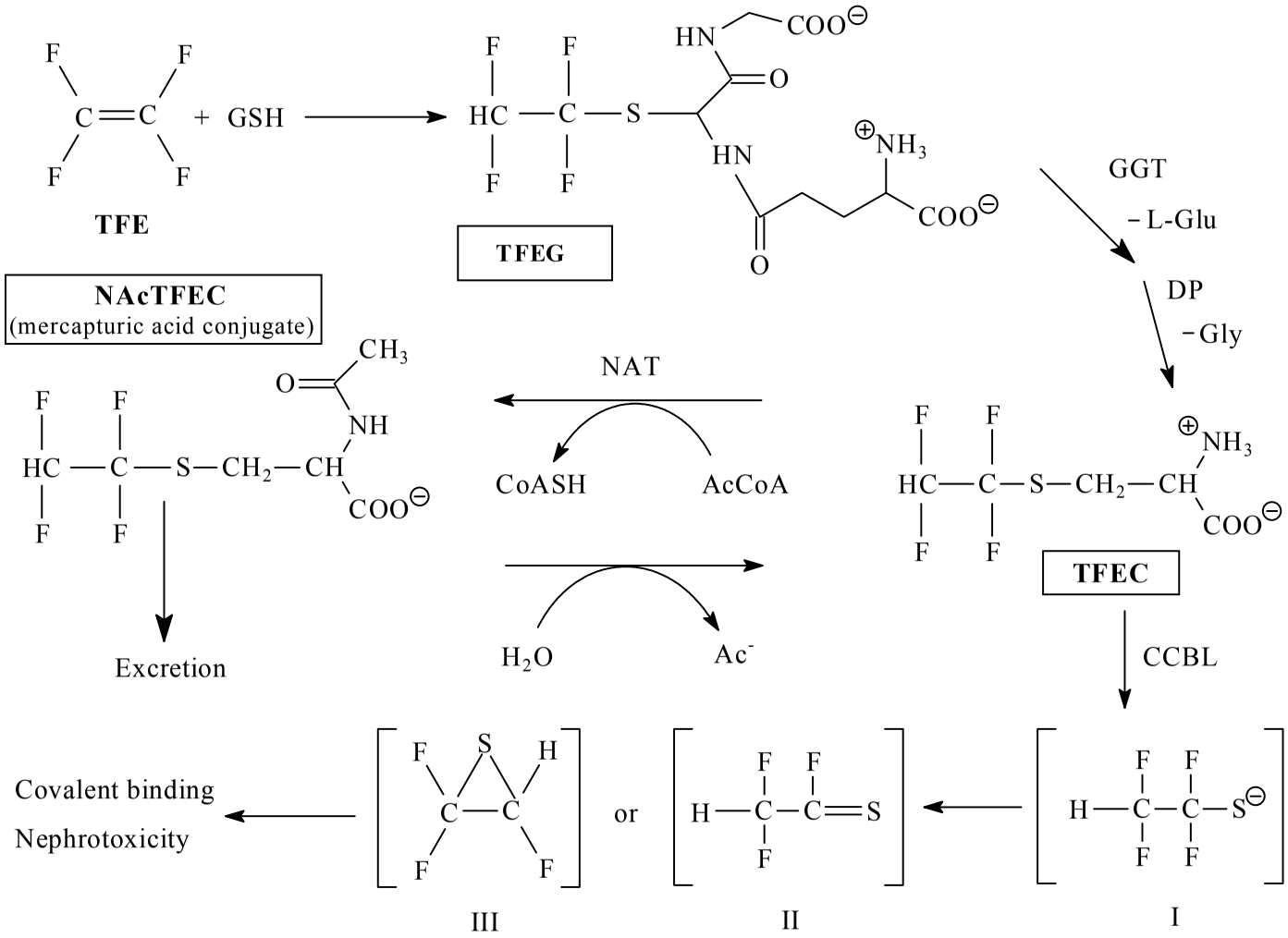
Based on analogy with other halogenated compounds (e.g. trichloroethylene and tetrachloroethylene, also known as perchloroethylene; Lash et al., 1988; Lash & Parker, 2001; Lash, 2005, 2007, 2011), it can be postulated that metabolism of tetrafluoroethylene follows the classical mercapturate pathway, as shown in Fig. 4.1 and Fig. 4.2. Although most of the glutathione (GSH) conjugation occurs in the liver, as catalysed by the abundant glutathione *S*-transferase (GST) activity in both hepatic cytoplasm and microsomes, it can also occur in the kidneys. Fig 4.1 details the chemical structures of three principal tetrafluoroethylene metabolites that have been detected – TFEG, *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine (TFEC) and *N*-acetyl-*S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine (NAcTFEC), as well as three putative metabolites thought to be reactive moieties formed from TFEC.

TFEG, whether formed in the liver or the kidney, can be sequentially degraded by gamma-glutamyltransferase (GGT) and cysteinylglycine dipeptidase on the external surface of the proximal tubular brush-border membrane of the kidney to yield the corresponding cysteine conjugate TFEC. TFEG formed in the liver can also be readily excreted into the bile, where it can undergo GGT- and dipeptidase-mediated degradation to form TFEC.

TFEC is a branching point in the tetrafluoroethylene metabolic pathway. TFEC may either be detoxified by the action of the cysteine conjugate *N*-acetyltransferase (NAT) to yield the mercapturate NAcTFEC, or may be bioactivated by one of the many enzymes with cysteine conjugate β-lyase (CCBL) activity to yield a reactive thiolate that ultimately produces nephrotoxicity (Commandeur et al., 1996). While TFEC, like many other cysteine *S*-conjugates of halogenated compounds (Krause et al., 2003), may also be a substrate for flavin-containing monooxygenases, generating a reactive sulfoxide, this possibility is not very likely because of the strength of the C–F bond relative to the C–Cl bond, and has never been tested.

The mercapturate NAcTFEC can be readily excreted in the urine, or may undergo deacetylation by aminoacylase III to regenerate the cysteine conjugate TFEC (Commandeur et al., 1989; Newman et al., 2007). The potent nephrotoxicity of NAcTFEC in rats, and its low recovery in urine suggested that a high ratio of *N*-deacetylation/*N*-acetylation activity exists (Commandeur et al., 1989). TFEC is a substrate for one of the many enzymes that possess CCBL activity, whose

Fig. 4.1 Metabolism of tetrafluoroethylene by the glutathione-conjugation pathway



AC⁻, acetate; AcCoA, acetyl-coenzyme A; CCBL, cysteine conjugate β-lyase; CoASH, coenzyme A; DP, dipeptidase; L-Glu, glutamate; Gly, glycine; GGT, gamma-glutamyltransferase; GSH, glutathione; GST, glutathione *S*-transferase; NAT, *N*-acetyltransferase; NAcTFEC, *N*-acetyl-*S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine; TFE, 1,1,2,2-tetrafluoroethylene; TFEC, *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine; TFEG, *S*-(1,1,2,2-tetrafluoroethyl)glutathione. Metabolites I, II, and III are putative reactive intermediates generated from the action of CCBL on TFEC. I, thiolate; II, difluorothionacyl fluoride; III thiirane

Compiled by the Working Group

catalytic action leads to formation of a reactive and unstable thiolate (metabolite I, see Fig. 4.1).

The β-lyase reaction mechanism forming reactive, thioacetylating species from cysteine *S*-conjugates can occur by either a direct β-elimination reaction, or a transamination reaction. The former cleaves the C–S bond. The latter, with a suitable α-keto acid cosubstrate, yields either a thiolate directly, or an unstable propionic acid derivative that rearranges to release the thiolate (Stevens et al., 1986; Elfarra et al., 1987). Multiple mammalian enzymes are known to be capable of catalysing the CCBL reaction (Cooper & Pinto, 2006); however, the relative importance of each of these activities in TFEC bioactivation is presently unknown. Therefore, it is unclear whether TFEC is converted to the thiolate (metabolite I, see Fig. 4.1) by both mechanisms or only by a direct β-elimination reaction. The addition of α-keto-γ-methiolbutyrate, a keto acid shown to stimulate renal CCBL activity (Elfarra et al., 1987), to incubations of purified cytosolic rat kidney CCBL with TFEC in the presence of pyridoxal-5′-phosphate did not stimulate activity (Abraham et al., 1995), suggesting that a direct β-elimination reaction may be more kinetically favourable for TFEC than for other substrates such as *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC).

Regardless of how the thiolate is formed, it is believed to subsequently rearrange to form either difluorothionoacyl fluoride (Fig. 4.1, metabolite II) or a thiirane (Fig. 4.1, metabolite III). It is these two putative reactive intermediates that form covalent adducts with various renal cellular proteins, leading to nephrotoxicity.

Although tetrafluoroethylene conjugation with GSH occurs primarily in the liver, it may also occur in the kidney. Hepatic TFEG is readily excreted into the bile, where it undergoes GGT- and dipeptidase-mediated degradation to form TFEC. Renal TFEG undergoes degradation to TFEC on the luminal or brush-border plasma membrane of renal proximal tubules. Regardless of where the initial and degradation reactions to form TFEC occur, all subsequent reactions leading to detoxification or bioactivated of TFEC occur in the kidney. These pathways of interorgan metabolism and transport are summarized schematically in Fig. 4.2.

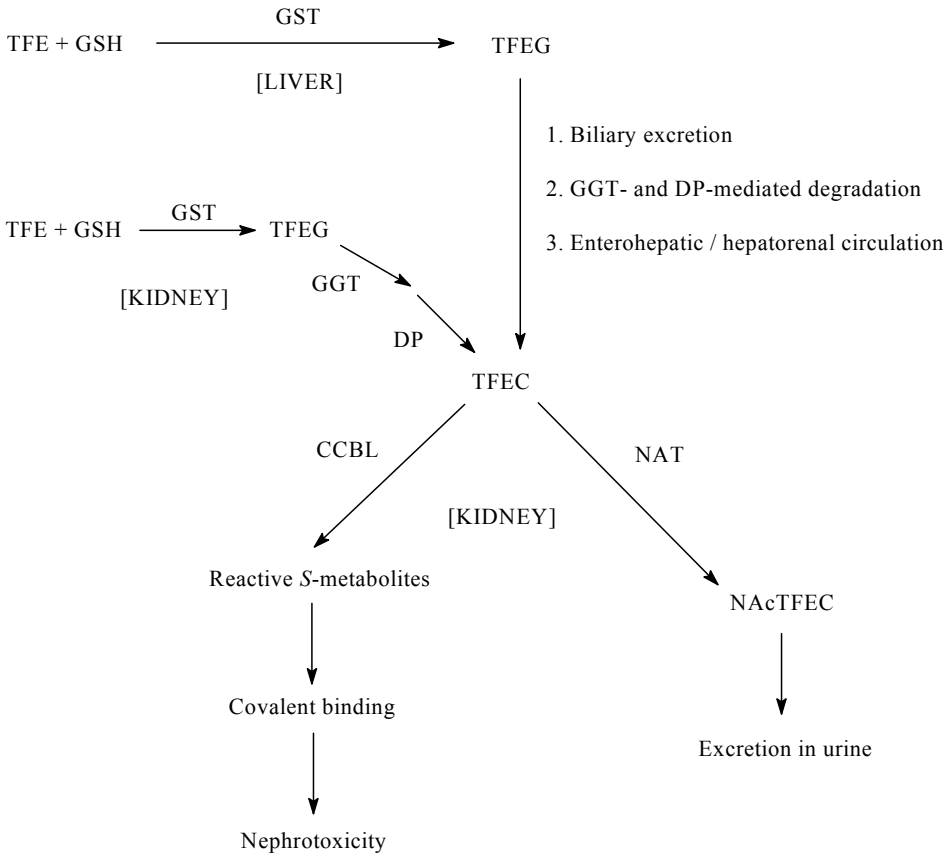
(a) Humans or human-derived tissues

No direct evidence for tetrafluoroethylene metabolism in humans was available to the Working Group, but one published study quantified CCBL activity with TFEC in samples of human kidney (McCarthy et al., 1994). In this study, the authors compared cytosolic CCBL activity in cytosolic samples of human kidney cortex, measuring release of pyruvate on incubation with cysteine conjugates of several halogenated aliphatic and aromatic hydrocarbons. Highest activities were reported for TFEC and DCVC (the cysteine conjugate of trichloroethylene), which were metabolized at similar rates by human CCBL.

(b) Rodents

Metabolism of tetrafluoroethylene in vivo was demonstrated in rats by measurement of fluoride ion excretion in urine (Dilley et al., 1974). Among the several fluorocarbons tested, which included hexafluoropropene, trifluoroethylene, vinylidene fluoride, vinyl fluoride, hexafluoroethane, and tetrafluoroethylene, some of the highest rates of fluoride ion excretion were observed in rats exposed to tetrafluoroethylene. However, no studies are available that report rates of GSH conjugation of tetrafluoroethylene in experimental systems, nor are there published reports of rates of degradation of TFEG to TFEC. Activities of GGT and dipeptidase in renal proximal tubules are not rate-limiting for metabolism and are typically well in excess of what is necessary to catalyse GSH-conjugate degradation. For this reason, one does not see accumulation of GSH conjugates in renal tissue. Rather, it is the cysteine or *N*-acetylcysteine conjugates that can accumulate.

Fig. 4.2 Scheme for interorgan metabolism of glutathione-derived metabolites of tetrafluoroethylene



CCBL, cysteine conjugate β-lyase; DP, dipeptidase; GSH, glutathione; GGT, gamma-glutamyltransferase; GST, glutathione *S*-transferase; NAT, *N*-acetyltransferase; NAcTFEC, *N*-acetyl-*S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine; TFE, 1,1,2,2-tetrafluoroethylene; TFEC, *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine; TFEG, *S*-(1,1,2,2-tetrafluoroethyl)glutathione
Compiled by the Working Group

Green & Odum (1985) compared metabolism of several cysteine conjugates of halogenated alkanes and alkenes by CCBL activity in rat kidney slices by measuring the release of pyruvate and ammonia. Among the conjugates tested as substrates, TFEC exhibited the fastest metabolism, with rates faster than those for well-established nephrotoxic and nephrocarcinogenic cysteine conjugates DCVC and *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC; cysteine conjugate of tetrachloroethylene).

MacFarlane et al. (1989) purified cytosolic CCBL activity (also known as glutamine transaminase K) from rat kidney and assayed activity during the course of purification with TFEC or DCVC (5 mM), and the non-nephrotoxic *S*-(2-benzothiazolyl)-L-cysteine (1 mM) as substrates. TFEC was by far the best CCBL substrate. Abraham et al. (1995) identified and partially purified a from rat kidney cytosol, and found that TFEC exhibited four- to fivefold higher activity than DCVC.

Cooper et al. (2001) co-purified mitochondrial heat shock protein 70 (HSP70) with a CCBL activity of high relative molecular mass, and demonstrated that TFEC was converted to a thioacylating species with associated release of pyruvate and ammonia. Three protein fractions

were identified that exhibited CCBL activity with TFEC as substrate. Thus multiple proteins in the rat kidney cortex are capable of activating TFEC to reactive species. In another study from the same group (Cooper et al., 2002), a mitochondrial aspartate aminotransferase was purified from rat liver and shown to catalyse CCBL activity with TFEC or DCVC as substrates. In this case, however, TFEC was a relatively poor substrate, exhibiting an apparent K_m of 25 mM and a V_{max} of 2 nmol/min per µg protein. In contrast, DCVC exhibited K_m and V_{max} values of 2.5 mM and 3 nmol/min per µg protein, respectively. In the same study, Cooper and colleagues also reported that TFEC underwent a β-elimination reaction to release pyruvate in the presence of cytosolic aspartate aminotransferase and alanine aminotransferase from pig heart (Cooper et al., 2002). These data emphasize that CCBL activity with TFEC as substrate is catalysed by multiple enzymes in multiple tissues. As explained above, it is the pattern of interorgan transport coupled with metabolism that determines the target-organ specificity of TFEC.

Although the putative reactive intermediates generated from TFEC by the catalytic action of CCBL (Fig. 4.1, metabolites I, II, and III) have not been isolated, their structure has been deduced by the known chemistry of these types of halocarbons and by isolation and identification of protein adducts. Hayden et al. (1991) demonstrated the formation of an N^{α}-acetyl-N^{ε}-(difluorothionoacetyl)lysine adduct by ^{19}F and ^{13}C nuclear magnetic resonance spectroscopy and mass spectrometry.

Commandeur et al. (1989) showed that TFEC was readily converted to NAcTFEC in the presence of either rat liver or kidney supernatants when acetyl-CoA was added. The rate of *N*-acetylation in rat kidney was fivefold higher than in rat liver. These authors also showed that NAcTFEC was deacetylated to form TFEC in both rat liver and kidney supernatants. Deacetylation activity was again much faster in rat kidney than in rat liver. This ability to readily deacetylate NAcTFEC in the target organ (i.e. the kidney) is likely a major factor in the potent cytotoxicity of NAcTFEC in vitro (Commandeur et al., 1989).

Kraus et al. (2000) purified NAT from porcine kidney microsomes and determined apparent kinetic parameters with several haloalkenyl cysteine conjugates. Among the conjugates tested as substrates, DCVC exhibited the lowest K_m (273 µM) and highest V_{max} (0.75 nmol/h). In contrast, TFEC was the poorest substrate, exhibiting a higher K_m (302 µM) and V_{max} (2.3 nmol/h) than DCVC. In agreement with the study by Commandeur et al. (1989), which showed a high ratio of deacetylation-to-*N*-acetylation activity in rat kidney, Newman et al. (2007) showed that NAcTFEC was a reasonably good substrate for mouse kidney aminoacylase III.

(c) Renal transport

As noted above, transport of *S*-conjugate metabolites across cellular membranes plays a critical role in the disposition of the various GSH-derived metabolites of tetrafluoroethylene. No direct evidence was available, however, on the membrane transport of either TFEG, TFEC, or NAcTFEC. Ample indirect evidence was available to conclude that several specific organic-anion and amino-acid carriers are likely involved. Pretreatment of rats with probenecid, the "classic" organic anion transport inhibitor, gave near complete protection from TFEC-induced nephrotoxicity (Lock & Ishmael, 1998). The presumption is that the presence of probenecid competitively inhibits the renal accumulation and subsequent bioactivation of TFEC.

Although there were no published studies on the transport of TFEG, TFEC, or NAcTFEC into renal proximal tubular cells, analogy with studies on the transport of the GSH-derived conjugates of trichloroethylene suggested that carrier proteins such as the organic anion transporter 1 and 3 (OAT1/3; soluble carrier *SLC22A6/8*) and possibly the sodium dicarboxylate carrier-3

Table 4.1 Studies of genotoxicity with tetrafluoroethylene and *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine

Test system	Results		Dose (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Tetrafluoroethylene				
Micronucleus test, $B6C3F_1$ mouse peripheral erythrocytes, in vivo	–	NT	5000 ppm, inhalation, 6 h/day, 5 days/wk, 13 wk	NTP (1997)
S-*(1,1,2,2-tetrafluoroethyl)-L-cysteine*				
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, or TA97, reverse mutation	–	–	250 mg/plate	Green & Odum (1985)

–, negative; HID, highest ineffective dose; h, hour; LED, lowest effective dose; NT, not tested; wk, week

(NaC3; *SLC13A3*) on the basolateral plasma membrane of renal proximal tubular cells may function (Lash, 2005, 2011; Lash et al., 2007). These presumptions have not been validated by studies specifically testing the transport function of these carriers with tetrafluoroethylene conjugates are required.

4.1.4 Excretion

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In a study of male Sprague-Dawley rats exposed to tetrafluoroethylene (3500 ppm) by inhalation for 30 minutes (Dilley et al., 1974), excretion of fluoride ion in the urine was monitored for up to 14 days after exposure, and fluoride excretion was significantly higher than in controls in exposed rats in the apparent cyclic excretion of fluoride ion 6 days after exposure and again at 13–14 days. However, the overall extent of excretion could not be determined.

Odum & Green (1984) reported biliary excretion of the GSH-conjugation-derived tetrafluoroethylene metabolite TFEC after inhalational exposure in rats, suggesting that faecal elimination of the products of tetrafluoroethylene metabolism is possible. However, the extent of reabsorption has not been determined, and no direct data on faecal elimination were available.

4.2 Genotoxicity and related effects

Table 4.1 summarizes the studies carried out to investigate the genotoxic potential of tetrafluoroethylene and TFEC in mammalian systems in vivo and in bacterial systems.

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

(a) Mammalian systems

(i) Gene mutation

No results from standard studies of mutagenicity in vivo were available to the Working Group. In $B6C3F_1$ mice, mutations in codon 61 of the H-*ras* oncogene occurred at a significantly lower frequency (15%) in tetrafluoroethylene-induced hepatocellular tumours than in spontaneous liver tumours (56–59%) (NTP, 1997). [The Working Group noted that this finding suggested that tetrafluoroethylene causes tumours of the liver via a *ras*-independent pathway.]

(ii) Chromosomal aberration

No data were available to the Working Group.

(iii) Micronucleus formation

Tetrafluoroethylene did not induce micronucleus formation in vivo in peripheral erythrocytes of male and female mice treated for 13 weeks at a concentration of 5000 ppm given via inhalation (NTP, 1997).

(iv) DNA binding and other DNA damage

No data were available to the Working Group.

(b) Bacterial systems: gene mutations

Cysteine conjugates of tetrafluoroethylene were not mutagenic in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA97, with or without metabolic activation with S9 fraction of rat kidney (Green & Odum, 1985).

4.3 Biochemical and cellular effects

The available studies in humans and experimental animals provided limited data on the biochemical and cellular effects of tetrafluoroethylene. One postulated non-genotoxic mechanism through which tetrafluoroethylene may induce tumour formation is via a cytotoxic GSH conjugate (Keller et al., 2000). Organ-specific toxicity data are reviewed below.

4.4 Organ toxicity

4.4.1 Kidney

(a) Humans

In comparison with national rates, observed mortality rates for nephritis and nephrosis were 25% lower than expected, according to standardized mortality ratios (SMR, 0.75; 95% CI, 0.21–1.93) in a cohort study of tetrafluoroethylene-production workers in Germany, Italy, the Netherlands, and the USA (Consonni et al., 2013); this decreased risk was similar to that seen for overall mortality (SMR, 0.77; 95% CI, 0.71–0.81). [The Working Group noted that because the number of deaths from nephritis or nephrosis (4 deaths) was a very small proportion of the total deaths observed (635 deaths; 0.63%), no conclusions about any association between nephritis or nephrosis and rates of mortality could be made.]

(b) Experimental animals

(i) Rats

In a 2-year study of carcinogenicity with tetrafluoroethylene, increases in the incidence of renal degeneration were observed in male Fischer F344/N rats exposed to tetrafluoroethylene at 156 ppm [640 mg/m^3], and in female F344/N rats at 625 ppm [2560 mg/m^3], and increases in the incidence of renal hyperplasia were observed in male and female rats at 625 ppm (NTP, 1997). Renal toxicity was also observed in 16-day and 13-week studies in F344/N rats treated with tetrafluoroethylene at concentrations of 625 ppm and higher; the damage was located predominantly at the corticomedullary junction. In addition, a review of data on the toxicity of tetrafluoroethylene indicated that rats exposed at 2500 ppm [10 250 mg/m^3] for 6 hours per day, 5 days per week, for 2 weeks, or at 2000 ppm [8200 mg/m^3] for 6 hours per day, 5 days per week, for 18 weeks, developed renal proximal tubule damage, which was more severe after 18 weeks than after 2 weeks (Kennedy, 1990). In study of toxicity in female F344 rats given tetrafluoroethylene by inhalation for up to 12 days, kidney weights were increased in rats exposed at 600 and 1200 ppm, and degeneration or necrosis of occasional tubule epithelial cells was reported in rats exposed at 1200 ppm (Keller et al., 2000). In male Alderley Park rats exposed to tetrafluoroethylene by inhalation at 6000 ppm [24 600 mg/m^3] for 6 hours, there was marked renal necrosis involving the pars recta of the proximal tubules, and an increase in levels of blood and urine markers of nephrotoxicity, including plasma area, urine volume,

glucose, alanine transaminase, *N*-acetyl-β-D-glucosaminidase, GGT, and alanine aminopeptidase (Odum & Green, 1984).

Tetrafluoroethylene metabolites

Keller et al. (2000) exposed female F344 rats to TFEC at oral doses of 5, 20, or 50 mg/kg for 9 days; severe changes were observed in the pars recta of the outer stripe of the outer medulla. When given TFEC as an oral dose at 100 mg/kg, male Alderley Park rats had increased blood and urine markers of nephrotoxicity, including increases in plasma urea, urine volume, glucose, protein, alanine transaminase, *N*-acetyl-β-D-glucosaminidase, GGT, and alanine aminopeptidase (Odum & Green, 1984). Lock & Ishmael (1998) reported renal tubular necrosis in male Alderley Park rats given a single intraperitoneal injection of TFEC. Rats given TFEC at a dose of 25 or 50 mg/kg had renal necrosis that included extensive necrosis seen as a band of damage in the outer stripe of the outer medulla with occasional tubular casts (25 mg/kg), or severe necrosis with a diffuse band involving the outer medulla and the inner cortex with many tubular casts (50 mg/kg). Similarly exposed female Alderley Park rats had extensive necrosis seen as a band of damage in the outer stripe of the outer medulla with occasional tubular casts at 25 mg/kg, and severe necrosis at 50 mg/kg, as in male rats (Lock & Ishmael, 1998).

Commandeur et al. (1988) suggested that difluorothionoacyl fluoride or difluorothioacetic acid, reactive intermediates of tetrafluoroethylene, induced nephrotoxicity specific to the proximal tubule, since necrosis in the region of the inner cortex was observed in male Wistar rats given a single intraperitoneal injection of NAcTFEC, the mercapturic acid of tetrafluoroethylene, at a dose of 112.5, 225, or 337.5 mg/kg.

(ii) Mice

In a 16-day study of toxicity preliminary to a study of carcinogenicity in B6C3F$_1$ mice, kidney weight increased in females exposed to tetrafluoroethylene at a concentration of 5000 ppm [20 500 mg/m^3] by inhalation (NTP, 1997). Renal tubule karyomegaly was observed, mainly in the inner cortex, of males and females exposed to 1250 ppm [5125 mg/m^3] or more. Karyomegaly was observed in the same region in the subsequent 13-week study with tetrafluoroethylene at the same concentrations. In the succeeding 2-year study of carcinogenicity, renal tubule karyomegaly was increased at 625 ppm in male mice, and at 1250 ppm in female mice. In a 12-day study of toxicity of female B6C3F$_1$ mice, cell necrosis was reported in mice exposed to tetrafluoroethylene at 1200 ppm (Keller et al., 2000).

Tetrafluoroethylene metabolites

Keller et al. (2000) also exposed female B6C3F$_1$ mice to TFEC at an oral dose of 5, 20, or 50 mg/kg for 9 days by gavage; moderate to severe changes were observed in the pars recta of the outer stripe of the outer medulla.

(iii) Other species

According to a review by Kennedy (1990), Syrian hamsters exposed to tetrafluoroethylene at 2500 ppm [10 250 mg/m^3] by inhalation for 6 hours per day, 5 days per week, for 2 weeks, or at 2000 ppm [8200 mg/m^3] for 6 hours per day, 5 days per week, for 18 weeks, showed no signs of renal toxicity, but testicular atrophy was reported.

4.4.2 Liver

(a) Humans

Mortality rates for cirrhosis of the liver were similar to national rates (SMR, 1.03; 95% CI, 0.65–1.54) in a cohort study of tetrafluoroethylene-production workers at six plants in Europe and the USA (observed deaths, 23; expected deaths, 22.4) (Consonni et al., 2013). An excess risk of cirrhosis of the liver was observed at one of these plants (observed deaths, 12; expected deaths, 2.4); these cases were classified in the

group with low exposure. In the remaining five plants, there were 11 observed deaths, and 20 expected deaths from cirrhosis of the liver.

(b) Experimental animals

(i) Rats

In a 13-week study in Fischer 344/N rats, liver weights were increased in males and females exposed to tetrafluoroethylene at a concentration of 5000 ppm [20 500 mg/m^3] by inhalation (NTP, 1997). In a 12-day study of toxicity in female F344 rats, liver weights were increased in rats exposed at 600 ppm [2460 mg/m^3] by inhalation (Keller et al., 2000).

(ii) Mice

In a long-term cancer bioassay, liver angiectasis was reported in male and female B6C3F$_1$ mice exposed to tetrafluoroethylene at concentrations at or above 312 ppm [1280 mg/m^3] by inhalation; there was also increased liver and spleen haematopoietic cell proliferation in female mice at these dose levels (NTP, 1997). In the 16-day study of toxicity (preliminary to a study of carcinogenicity) in B6C3F$_1$ mice , there were increases in liver weights of female mice exposed to tetrafluoroethylene at concentrations of 2500 ppm [10 250 mg/m^3] or more (NTP, 1997).

4.5 Susceptible populations

4.5.1 Polymorphisms

No data for tetrafluoroethylene specifically were available to the Working Group. Indirect evidence was available from data on other chemicals – methyl chloride and trichloroethylene – known to be metabolized through the same pathway. The predominant pathways for metabolism of tetrafluoroethylene are via GST in the liver, and via GGT and dipeptidase in the kidney (Odum & Green, 1984; Hayden et al., 1991; Keller et al., 2000); however, the GST isozyme(s) that may be involved in tetrafluoroethylene conjugation reactions have not been identified. It is possible that humans may conjugate tetrafluoroethylene at different rates owing to known genetic polymorphisms in GST and other metabolizing enzymes. The following data concern tetrafluoroethylene-related chemicals that undergo GST-mediated conjugation.

For methyl chloride, one study classified humans into "fast," "slow," or non-conjugators (non-metabolizers) (Nolan et al., 1985). Fast metabolism may lead to rapid production of the toxic cysteine metabolite, making this population more susceptible to kidney damage. However, among conjugators, the rate of conjugation of tetrafluoroethylene with GSH is expected to fall within a threefold range (Nolan et al., 1985; Mulder et al., 1999). In a study by Löf et al. (2000), glutathione *S*-transferase theta 1 (GSTT1) appeared to be the sole determinant of methyl chloride metabolism in humans; clearance of methyl chloride by metabolism, but not by exhalation, correlated well with GSTT1 activity.

For trichloroethylene, the role that polymorphisms in the genes encoding GST enzymes may play in cancer risk has been studied in several epidemiological studies. For example, Brüning et al. (1997) investigated the potential for an association between polymorphisms in glutathione *S*-transferase mu 1 (*GSTM1*) and *GSTT1* and risk of renal cell cancer in workers with high long-term occupational exposure to trichloroethylene. Among 45 patients with renal cell carcinoma, 27 carried at least one functional *GSTM1* gene, and 18 carried at least one functional *GSTT1* gene. The odds ratios for renal cell carcinoma were 2.7 for *GSTM1*+ individuals (95% CI, 1.18–6.33; $P < 0.02$), and 4.2 for *GSTT1*+ individuals (95% CI, 1.16–14.91; $P < 0.05$), respectively. The data from this cohort were re-evaluated by Wiesenhütter et al. (2007), who used data from additional control subjects to increase the size of the study population, finding that deletion polymorphisms in *GSTT1* and *GSTM1* had no

effect on the development of renal cell carcinoma attributable to trichloroethylene.

Moore et al. (2010) conducted a case–control study in central Europe (cases, 1097; controls, 1476) to assess the risk of renal cell carcinoma associated with occupational exposure to trichloroethylene (assessed from work history). Increased risk was observed among subjects who had ever been exposed to trichloroethylene [OR, 1.63; 95% CI, 1.04–2.54]. A significant association was found for trichloroethylene-exposed subjects with at least one intact *GSTT1* allele (active genotype; OR, 1.88; 95% CI, 1.06–3.33), but not for subjects with two deleted alleles (*GSTT1* null genotype; OR, 0.93; 95% CI, 0.35–2.44). Similar associations for all exposure metrics, including average intensity, were observed among *GSTT1*-active subjects (OR, 2.77; 95% CI, 1.01–7.58; P_{trend} = 0.02), but not among *GSTT1* null individuals (OR, 1.16; 95% CI, 0.27–5.04).

Among the transporter proteins known to be responsible for the uptake and cellular accumulation of tetrafluoroethylene conjugates, the influence of genetic polymorphisms has been best studied for OAT1 and OAT3 (Erdman et al., 2006; Lash et al., 2006; Urban et al., 2006). Expression and function of OATs and other organic-anion transporters have been shown to exhibit sex-dependent differences in humans and experimental animals (Gotoh et al., 2002; Kato et al., 2002; Kobayashi et al., 2002; Buist et al., 2003; Buist & Klaassen, 2004; Ljubojevic et al., 2004), suggesting that transport differences are a contributing factor to sex-specific differences in susceptibility to toxicity caused by tetrafluoroethylene metabolites.

4.5.2 Lifestage

No data were available to the Working Group.

4.6 Mechanistic considerations

The mechanisms by which tetrafluoroethylene causes toxicity are largely unknown, and most of the available information on this compound concerns observational studies on effects in the target organs, and metabolism.

Based on knowledge of tetrafluoroethylene metabolism, it is likely that GSH conjugation in the liver, followed by CCBL-mediated formation of a reactive thiol, is the main route of metabolism of tetrafluoroethylene. The mercapturic acid pathway of bioactivation of tetrafluoroethylene is similar to that of several halogenated solvents such as trichloroethylene and tetrachloroethylene, hence nephrotoxicity is expected to be mediated by reactive metabolites derived from a cysteine conjugate. The proximal nephrotoxic reactive intermediate of the tetrafluoroethylene cysteine conjugate is difluorothionoacetyl fluoride, which formed by α-elimination of a fluoride anion from the initial thiolate (see Fig. 4.1; Commandeur et al., 1996). In studies of acute and chronic effects of tetrafluoroethylene, kidney hypertrophy, proteinuria, renal tubular necrosis, and degeneration were observed in mice and rats (Odum & Green, 1984; NTP, 1997), and karyomegaly in mice (NTP, 1997; Keller et al., 2000). Tetrafluoroethylene caused increased proliferation and cellular hyperplasia in the rat kidney, and there was convincing evidence for kidney enlargement (NTP, 1997; Keller et al., 2000). Dose-dependent normocytic, normochromic, nonresponsive anaemia observed in rats and mice exposed to tetrafluoroethylene in a 13-week study was attributed to possible alterations in erythropoietin metabolism in the kidney due to the presence of renal lesions (NTP, 1997). Together, these changes suggest that cytotoxicity followed by compensatory proliferation may be the main non-genotoxic mechanism of carcinogenesis in the kidney, although no data were available to the Working Group to confirm this possibility.

Tetrafluoroethylene was not found to be genotoxic in the few standard assays available; however, because traditional bacterial mutagenesis assays use liver-derived S9 fraction to test bioactivation, data obtained from such studies are less informative than experimental evidence obtained with kidney homogenates or purified enzymes responsible for biotransformation of nephrotoxic haloalkenes to GSH conjugation-derived reactive electrophiles (Lash et al., 2014). The cysteine conjugate of tetrafluoroethylene has been tested in some genotoxicity assays with no positive results reported; however, reactive metabolites formed through GSH conjugation of tetrafluoroethylene and TFEC metabolite may still contribute to the carcinogenicity of tetrafluoroethylene in the kidney via a genotoxicity mechanism.

Little is known about potential mechanisms in the liver. Tetrafluoroethylene is thought not to be metabolized through cytochrome P450-mediated oxidation (Odum & Green, 1984). However, hepatomegaly has been observed in rats (NTP, 1997) and mice (Keller et al., 2000), suggesting that either cytotoxicity followed by compensatory proliferation, or nuclear receptor-mediated hypertrophy, may be involved. No study has examined these mechanisms in detail, and it is not known whether tetrafluoroethylene is a ligand for nuclear receptors, such as peroxisome proliferator-activated receptor α (PPARα). GSH conjugates of tetrafluoroethylene and other haloalkenes are not thought to be hepatotoxic or reactive, but no study tested potential hepatotoxicity of the GSH conjugate of tetrafluoroethylene, TFEG. Furthermore, it is not known what mechanism may lead to the formation of haemangiomas and haemangiosarcomas (very uncommon neoplasms in the mouse liver), which were observed in 2-year studies in mice (NTP, 1997).

The increased incidence of haematopoietic cell proliferation in female mice, and findings of mononuclear cell leukaemia in female rats have not been attributed to a specific mechanism of toxicity (NTP, 1997).

The only available relevant mechanistic data in humans concerned indirect evidence for absorption of tetrafluoroethylene by inhalation. Some data were also available to suggest that metabolism of TFEC by human enzymes is comparable in efficiency to that of DCVC.

5. Summary of Data Reported

5.1 Exposure data

Tetrafluoroethylene is a fluorinated monomer that is produced by the pyrolysis of chlorodifluoromethane. Estimated annual world production of tetrafluoroethylene is more than 100 000 tonnes. It is used mainly as an intermediate in the production of the polymer polytetrafluoroethylene, which is used in a wide range of industrial and consumer products, e.g. non-stick coatings and waterproof clothing. The occupational setting is the main source of concern regarding exposure to tetrafluoroethylene, predominantly during its production and use in polymerization. Exposure levels have decreased (estimated from plants in the USA and in Europe at < 40 mg/m^3 in the 1950s and 1960s, and now about < 6 mg/m^3).

5.2 Human carcinogenicity data

Only one study evaluating the possible carcinogenic effect of tetrafluoroethylene has been reported. Moderately but not statistically significantly elevated standardized mortality ratios were observed for all sites of a-priori interest, i.e. cancers of the liver and kidney, and leukaemia, based on small numbers of cases. The study was well conducted in terms of completeness and follow-up of the cohort and exposure assessment, but study precision was low and

the possible confounding from ammonium perfluorooctanoate could not be ruled out due to the high correlation between the two exposures.

5.3 Animal carcinogenicity data

There were two well-conducted studies of carcinogenicity with tetrafluoroethylene: one inhalation study in mice (males and females), and one inhalation study in rats (males and females). Tetrafluoroethylene increased the incidence of liver haemangioma and/or haemangiosarcoma, hepatocellular adenoma and/or carcinoma, and histiocytic sarcoma in male and female mice. In male and female rats, tetrafluoroethylene increased the incidence of renal cell adenoma or carcinoma (combined), and of hepatocellular adenoma and/or carcinoma. In female rats, tetrafluoroethylene caused an increase in the incidence of haemangiosarcoma of the liver. In rats, tetrafluoroethylene also caused increases in the incidence of mononuclear cell leukaemia in males and females, and testicular interstitial cell (Leydig cell) adenoma in males.

5.4 Mechanistic and other relevant data

Tetrafluoroethylene is a volatile, chemically unstable compound with poor solubility. Humans are primarily exposed through inhalation. Tissue distribution of tetrafluoroethylene is poorly characterized, but there is evidence for toxic effects at various tissues after exposure by inhalation. Urinary and faecal excretion of tetrafluoroethylene and its metabolites is likely, but elimination has not been studied in detail.

Unlike other halogenated compounds, tetrafluoroethylene is not metabolized by cytochrome P450 enzymes. Metabolism of tetrafluoroethylene is thought to primarily occur through the glutathione-conjugation pathway in the liver to the glutathione conjugate, which is further metabolized to the cysteine conjugate in the kidney. The resulting conjugate is an excellent substrate for cysteine conjugate β-lyase, which is known to form reactive electrophiles of cysteine conjugate metabolites of other halogenated compounds.

Limited data exist to characterize the potential genotoxicity of tetrafluoroethylene or its metabolites. No positive results were reported for either tetrafluoroethylene or its cysteine conjugate, but tests with kidney-derived metabolizing enzymes have not been performed.

Single, short-term, or long-term exposures to tetrafluoroethylene resulted in kidney toxicity in rats and mice. Both males and females were affected, although the effects in females occurred at a higher exposure level than in males. Liver enlargement and some evidence for liver toxicity have also been reported in studies with tetrafluoroethylene in rats and mice. Little is known about the mechanisms that may explain these adverse effects in the kidney and liver.

No study directly evaluated the potential role of genetic polymorphisms in the adverse health effects of tetrafluoroethylene. However, because of the major role that several glutathione *S*-transferase enzymes are likely to play in metabolism of tetrafluoroethylene, inter-individual variability in the formation of reactive electrophiles from the cysteine conjugate is plausible based on analogy to related chemicals. No studies were identified that explored whether lifestage susceptibility to tetrafluoroethylene exposure may exist.

Overall, the mechanistic data for tetrafluoroethylene are *weak* because the mechanistic events have not been directly established in humans or in experimental animals.

6. Evaluation

6.1 Cancer in Humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

6.4 Rationale

In the absence of adequate data on cancer in humans and adequate mechanistic data, the overall evaluation for the carcinogenicity of tetrafluoroethylene was upgraded from *Group 2B* to *Group 2A* based on unusual results in studies of cancer in experimental animals. Tetrafluoroethylene induced neoplasms at multiple sites, affecting cells of differing embryological origin, and were present in rats (renal cell adenoma or carcinoma combined, hepatocellular carcinoma, and mononuclear cell leukaemia) and mice (liver haemangiosarcoma, hepatocellular carcinoma, and histiocytic sarcoma) of both sexes. There was also a significant increase in the incidence of the rare liver haemangiosarcoma in female rats. Also, the tumour incidences are very high, especially liver haemangiosarcoma in mice, even at the lowest doses tested. This indicates that tetrafluoroethylene is a potent carcinogen.

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1,2-DICHLOROPROPANE

1,2-Dichloropropane was reviewed previously by the Working Group in 1986, 1987, and 1998 (IARC, 1987, 1999). New data have since become available, and these have been incorporated, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

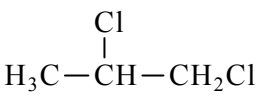
Chem. Abstr. Serv. Reg. No.: 78-87-5

Chem. Abstr. Serv. Name: 1,2-Dichloropropane

IUPAC Systematic Name: 1,2-Dichloropropane

Synonyms: Propylene dichloride; propylene bichloride; propylene chloride; dichloro-1,2 propane; chloromethylchloride

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_3H_6Cl_2$

Relative molecular mass: 112.99

1.1.3 Chemical and physical properties of the pure substance

From OECD/SIDS (2003), unless otherwise specified

Description: Colourless liquid with a chloroform-like odour

Boiling point: 96.4 °C (94.0 to 96.8)

Melting point: –100.4 °C

Density: d_4^{25} 1.159 (O'Neil et al., 2006)

Solubility: Slightly soluble (2800 g/m^3) in water at 25 °C; soluble in alcohol, ethyl ether (Bingham & Cohrssen, 2012)

Volatility: Vapour pressure, 6.62 kPa at 25 °C; relative vapour density (air = 1), 3.9 (Verschueren, 2001)

Stability: Vapour is highly flammable and explosive

Octanol/water partition coefficient (P): log P, 1.99 (Verschueren, 2001)

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), 1 mg/m^3 = 4.62 ppm; calculated from: mg/m^3 = (relative molecular mass/24.47) × ppm.

Table 1.1 Methods for the analysis for 1,2-dichloropropane

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Adsorb on charcoal; desorb with acetone/ cyclohexane	GC/HECD	0.1 µg/sample	NIOSH (1994)
	Air collected in specially-prepared canister; desorb on cold trap	GC/MS GC/ECD GC/FID GC/PID	0.21 ppm NR NR NR	EPA (1999a)
	Analyte collected on sorbent tube; thermally desorb to GC	GC/MS GC/ECD GC/FID GC/PID	NR NR NR NR	EPA (1999b)
Water	Purge with inert gas and trap; desorb to GC	GC/PID GC/ECD GC/MS GC/MS	NR 0.03 µg/L 0.088 µg/L 0.018 µg/L	EPA(1988) EPA (1995a) EPA (2013) EPA (2009)
	Purge with inert gas and trap; desorb to GC	GC/MS	0.04 µg/L	EPA (1995b)
	Add internal standard (isotope labelled dichloromethane); with inert gas and trap; desorb to GC	GC/ MS	10 µg/L	EPA (1996c)
Liquid and solid wastes	Purge with inert gas and trap	GC/PID GC/HECD	NR 0.006 µg/L	EPA (1996b)
	Purge with inert gas and trap; and various other methods	GC/MS	5 µg/kg (soil/sediment) 500 µg/kg (wastes) 5 µg/L (groundwater)	EPA (1996a)

ECD, electron capture detection; FID, flame ionization detection; GC, gas chromatography; HECD, Hall electrolytic conductivity detection; MS, mass spectrometry; NR, not reported; PID, photoionization detection

1.1.4 Technical products and impurities

Commercial 1,2-dichloropropane is marketed as a high-purity liquid (purity, 99–99.5%) for industrial use. Water and oxygenated organic impurities comprise a maximum of 0.05% and 0.1% of the product, respectively (Bayer AG, 1977). Trace amounts of chlorinated hydrocarbons of low relative molecular mass, such as chloropropenes and chloropropanes, are also present.

1.1.5 Analysis

Methods for the analysis of 1,2-dichloropropane have been reviewed by ATSDR (1989) and HSDB (2012). Selected methods for the analysis of 1,2-dichloropropane in various matrices are presented in Table 1.1. 1,2-Dichloropropane can be measured in the urine, blood, and exhaled air (ATSDR, 1989). There are no standardized analytical methods for the biological monitoring of exposure to 1,2-dichloropropane.

1.2 Production and use

1.2.1 Production

1,2-Dichloropropane, marketed as a solvent, is obtained as a by-product of the synthesis of propylene oxide by the chlorohydrin reaction (Mannsville Chemical Products Corp., 1984).

1,2-Dichloropropane is produced in North America, Europe, Asia, and South America. The total annual global production volume of 1,2-dichloropropane for 2001 was estimated to be 350 000 tonnes. In 2003, the estimated regional production percentages of 1,2-dichloropropane

were 64–69% in North America, 19–25% in Europe, 9–10% in South America, and 2–3% in Japan (OECD/SIDS, 2003).

Production of 1,2-dichloropropane in the USA decreased in the early 1980s since it was no longer used in paint strippers, furniture finishes, or as an insecticide (IARC, 1986; ATSDR, 1989; ACGIH, 2006). The amount manufactured and imported in countries of the European Union was between 1000 and 10 000 tonnes per year (ECHA, 2016). There were fewer data for the Asia–Pacific region, but the annual production of 1,2-dichloropropane in China was estimated as 45 000–68 000 tonnes (Chaoqun, 2008). In Japan, the annual production and import of 1,2-dichloropropane reported in 2011 was 1400 tonnes (METI, 2013).

1.2.2 Use

1,2-Dichloropropane is used primarily as a chemical intermediate in the production of other organic chemicals such as propylene, carbon tetrachloride, and tetrachloroethylene. It has been reported that co-product and raw material uses account for > 99.5% of the total production of 1,2-dichloropropane in the USA and Europe (OECD/SIDS, 2003).

Other uses for 1,2-dichloropropane include textile stain remover, oil and paraffin extractant, scouring compound, as a metal cleaner, and in insecticides (IARC, 1986). 1,2-Dichloropropane is used as a solvent or diluent in alkyd, acrylic, or polyurethane coatings and polyamide inks, as well as a metal degreaser in China (Chaoqun, 2008). According to the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) registration data, 1,2-dichloropropane is used in solvent-based degreasers and cleaning products, paint and stain removers, and glues and adhesives (ECHA, 2016).

1,2-Dichloropropane has been formulated with the active ingredient 1,3-dichloropropene for use as a grain and a soil fumigant, but has not been used in this way in the USA since 1989, or in the European Union since 2003. In Asia, the Organization for Economic Co-operation and Development (OECD) reports that soil fumigant use has been discontinued in Japan, but some agricultural uses may remain in other countries (OECD/SIDS, 2003).

In Japan, the major use of 1,2-dichloropropane is as a chemical intermediate, but it has also been used as cleaner in offset-printing processes (Kumagai et al., 2013). The use of 1,2-dichloropropane in the printing industry became common in Japan after the reduction in use of 1,1,1-trichloroethane because of the implementation of the Montreal Protocol and its amendment (UNEP, 2016). However, due to health concerns and legislative amendments, the use of 1,2-dichloropropane as a cleaning solvent in the printing industry has declined in Japan (MHLW, 2013a).

It is not known whether 1,2-dichloropropane has been used extensively in the printing industry in countries other than Japan. However, the United States Agency for Toxic Substances and Disease (ATDSR) toxicological profile for 1,2-dichloropropane, published in 1989, does not mention the use of 1,2-dichloropropane in the printing industry (ATSDR, 1989). Similarly, the WHO Environmental Health Criteria document on 1,2-dichloropropane, published in 1993, does not mention the use of 1,2-dichloropropane in the printing industry (IPCS, 1993).

1.3 Occurrence and exposure

1.3.1 Environmental occurrence

(a) Natural occurrence

1,2-Dichloropropane is not known to occur naturally.

(b) Air

Background concentrations of 1,2-dichloropropane in the air at isolated locations in the USA in 2003 were very low (mean, < 0.02 μg/m³) (McCarthy et al., 2006). Known environmental concentrations of 1,2-dichloropropane in the 1980s were summarized by WHO as: mean, 1.2 μg/m³ in Philadelphia, USA; between 0.02 and 0.04 μg/m³ after rain events in Portland, USA; and detectable at concentrations of 0.01–1.4 μg/m³ in a third of samples from 13 cities in Japan (IPCS, 1993).

(c) Water

Measurement of 1,2-dichloropropane in wells, groundwater, and surface water in the 1980s in the USA, the Netherlands, and Japan showed that 1,2-dichloropropane was only found in a minority of the water sources tested, and at concentrations that tended to be < 10 μg/L, although higher concentrations were occasionally reported (IPCS, 1993).

More recently, 1,2-dichloropropane has been found in 32 out of 324 samples of untreated ground water in Sicily, Italy, with the highest concentrations (up to 0.44 μg/L) being mainly located in plains where agricultural and industrial activity was most intense (Pecoraino et al., 2008). In the USA, 1,2-dichloropropane was detected in < 1% of 1207 samples of domestic well-water (Rowe et al., 2007). In three of these wells, the concentration of 1,2-dichloropropane exceeded the maximum contaminant level of 5 μg/L. In Cyprus, 1,2-dichloropropane was one of the most frequently detected volatile organic compounds contaminating surface water bodies, but concentrations were low (< 0.05 μg/L) (Fatta et al., 2007).

(d) Food

No data were available to the Working Group.

1.3.2 Occupational exposure

Occupational exposure to 1,2-dichloropropane may occur through inhalation and dermal contact. The main intake pathway is via the respiratory tract.

In small car-painting workshops in Italy, only one of the eight workshops investigated reported measurements of 1,2-dichloropropane that were above the level of detection (Vitali et al., 2006). In this particular shop, personal and stationary measurements of 5.3 mg/m³ were recorded during 5.5 hours of monitoring.

In another study in Italy, measurements of 1,2-dichloropropane in the breathing zone and the urine were reported for workers in plastic-product, paint-, and chemical-manufacturing industries (Ghittori et al., 1987). Most of the air concentrations were between 10 and 150 mg/m³, although two were > 400 mg/m³. Urinary concentrations (in μg/L) correlated very closely with the air concentrations.

Table 1.2 shows estimated levels of exposure to 1,2-dichloropropane (and dichloromethane) at a printing company in Osaka, Japan, following identification of a cluster of cancers of the biliary tract (cholangiocarcinoma) among company workers (Kumagai et al., 2013). The circumstances of exposure were quite specific in that the workers removed ink from rollers using volatile solvents between 300 and 800 times a day and the room had poor ventilation. There was co-exposure during several years to both dichloromethane and 1,2-dichloropropane (see the *Monograph* on Dichloromethane in the present volume). No exposure monitoring was undertaken at the time, so the Japanese National Institute of Occupational Safety and Health undertook a reconstruction experiment to estimate the exposure concentrations on the assumption that the exposure was proportional to the amount of chemical used. Additional details of the cluster investigation are given in Section 2 of this Monograph. Kumagai et al. (2013) reported

Table 1.2 Estimated exposure to 1,2-dichloropropane and dichloromethane of printers associated with cholangiocarcinoma clusters in Japan[a]

Location	Job classification and years	Number of workers	Estimated shift-TWA of dichloromethane (ppm)	Estimated shift-TWA of 1,2-dichloropropane (ppm)	Reference
Osaka	Proof printing (reconstruction)	50–100	130–360 at area estimate to the consumption at 0.938 L/h	60–210 at area estimate to the consumption at 0.812 L/h	JNIOSH (2012)
	1991–1993		80–210	120–430	Kumagai et al. (2013)
	1992–1998		190–540	100–360	
	1998–2006		NR	150–670	
Miyagi	Offset web printing 1992–2011	2	NR	80–170	Yamada et al. (2014) based on government survey data
Fukuoka	Offset web printing 1970–2008	3	0–150	62–200	Yamada et al. (2014) based on government survey data
				110–5200	Kumagai (2014)
Hokkaido	Proof printing 1985–1995	2	60–180	110–240	Yamada et al. (2014) based on government survey data
Aichi	Proof printing 1984–1995	1	240–6100	–	Kumagai (2014)

[a] The Working Group noted that the upper limits of these scenarios were estimated with the worst-case scenarios
h, hour; NR, not reported; ppm, parts per million; TWA, time-weighted average

that estimated concentrations of exposure to 1,2-dichloropropane in the proof-printing room were 120–430 ppm (mean, 220 ppm) [range, 416–1492 mg/m^3; mean, 763 mg/m^3] from 1991 to 1992/1993, 100–360 ppm (mean, 190 ppm) [range, 347–1249 mg/m^3; mean, 659 mg/m^3] from 1992/1993 to 1997/1998, and 150–670 ppm (mean, 310 ppm) [range, 520–2324 mg/m^3; mean, 1075 mg/m^3] from 1997/1998 to 2006. The front-room workers were estimated to be exposed to 1,2-dichloropropane at concentrations of 80 ppm [278 mg/m^3] from 1991 to 1992/1993, 70 ppm [243 mg/m^3] from 1992/1993, and 110 ppm [382 mg/m^3] from 1997/1998 to 2006 (Kumagai et al., 2013) (also see the *Monograph* on Dichloromethane, Section 1, Table 1.2, in the present volume).

A study of exposure to 1,2-dichloropropane by the Government of Japan showed that printers were still being exposed to 1,2-dichloropropane in 2012 (MHLW, 2013a).

1.3.3 Exposure of the general population

Very little information was available on exposure of the general population to 1,2-dichloropropane. Exposure may occur through inhalation of contaminated air, or through ingestion of contaminated water. In the United States National Health and Nutrition Examination Survey (NHANES) in 2003–2004, 1,2-dichloropropane was not detected in any of 1364 blood samples (CDC, 2009).

1.4 Regulations and guidelines

Limit values for occupational exposure to 1,2-dichloropropane in air vary from 10 ppm over 8 hours in Belgium, Ireland, Spain, and Japan, to 75 ppm over 8 hours in many other countries (Australia, Denmark, France, New Zealand, Singapore, Republic of Korea, Switzerland, USA).

Table 1.3 International limit values for occupational exposure to 1,2-dichloropropane

Country	Limit value, 8 hours	
	ppm	mg/m³
Australia	75	347
Belgium	10	47
Canada, Ontario	10	NR
Canada, Québec	75	347
Denmark	75	350
France	75	350
Hungary	NR	50
Ireland	10	46
Japan	10	NR
New Zealand	75	347
Poland	NR	50
Singapore	75	347
Republic of Korea	75	350
Spain	10	47
Switzerland	75	350
USA, Occupational Safety and Health Administration	75	350

NR, not reported; ppm, parts per million
From Working Environment Evaluation Standards (2013), IFA (2014)

Short-term limit values are 110 ppm in most jurisdictions (Table 1.3).

Dichloropropanes are included on the list of substances regulated under the European VOC Solvent Emissions Directive (European Commission, 1999; also described in Section 1.4 of the *Monograph* on Dichloromethane in the present volume.

In the USA, the Environmental Protection Agency (EPA) has regulated concentrations of 1,2-dichloropropane in drinking-water to < 5 ppb (EPA, 2014). WHO has set a provisional limit of 40 µg/L for 1,2-dichloropropane in drinking-water (WHO, 2011).

2. Cancer in Humans

Data on the association between cancer and exposure to 1,2-dichloropropane were available from several studies of cancer among printing workers in Japan (Kumagai et al., 2013; Kubo et al., 2014; Yamada et al., 2014), which were initiated after an unusual cluster of cholangiocarcinoma (cancer of the bile duct) was identified among workers in a printing plant in Osaka (Kumagai et al., 2013). Interpretation of these studies was challenging because the populations are small and workers were exposed not only to 1,2-dichloropropane, but also to more than 20 other chemicals, including dichloromethane, 1,1,1-trichloroethane, gasoline, kerosene and printing inks.

Three studies of broader groups of printing workers in Japan and other countries that were undertaken to follow up the initial findings in Japan, and that also reported data for cholangiocarcinoma were also reviewed (Okamoto et al., 2013; Vlaanderen et al., 2013; Ahrens et al., 2014). While previous studies have investigated cancer among printers (IARC, 1996), none have reported data for cholangiocarcinoma separately from all cancers of the liver, or provided data on 1,2-dichloropropane; these earlier studies on the printing industry were therefore not considered further by the Working Group.

2.1 Cholangiocarcinoma among printing workers in Japan

Three papers and a government report have presented findings concerning a cluster of cases of cholangiocarcinoma among workers at printing plants in Japan (Kumagai et al., 2013; MHLW, 2013a; Kubo et al., 2014; Yamada et al., 2014; see Table 2.1).

Table 2.1 Studies on cholangiocarcinoma and employment in the printing industry

Reference, location, follow-up period	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Japan							
Kumagai et al. (2013) Osaka, Japan, 1991–2011	62	Concentrations of 1,2-DCP and DCM estimated by simulation and mathematical modelling	Cholangiocarcinoma	All men	11	2900 (1100–6400)	Study initiated to investigate a cluster of cholangiocarcinoma in a single printing plant. Exposures were estimated but not used in the analysis. Women (n = 11) were excluded
MHLW (2013a) Osaka, Japan, 1991–2012	100	Concentrations of 1,2-DCP and DCM estimated by simulation and mathematical modelling	Cholangiocarcinoma	All workers	16	1226 (714–1963)	Follow-up investigation of the plant investigated by Kumagai et al. (2013), with more complete case finding and enumeration of the population. Women were included
Okamoto et al. (2013) Japan, 2009–2012	NR	Employment in the printing industry	Cholangiocarcinoma	All workers	76	1.28 (0.91–1.79)	Comparison of observed to expected insurance claims in "printing and related industry" to all other industries
			Intrahepatic (C22)		27	1.70 (0.91–3.15)	
			Extrahepatic (C24)		49	1.12 (0.75–1.69)	
			Cholangiocarcinoma	Men aged 30–49 yr	10	1.78 (0.63–5.00)	
			Intrahepatic (C22)		5	3.03 (0.52–17.56)	
			Extrahepatic (C24)		5	1.26 (0.34–4.71)	
Other countries							
Vlaanderen et al. (2013) Finland, Iceland, Norway, Sweden, 1961–2005	74 949	Job title	Intrahepatic cholangiocarcinoma (C22.1)	All printers and related workers, men	21	2.34 (1.45–3.57)	SIRs adjusted for country, age and period. Similar findings for women based on smaller numbers
				Typographers, men	11	2.01 (1.00–3.60)	
				Printers, men	6	3.54 (1.30–7.70)	
				Lithographers, men	2	3.91 (0.47–14.10)	

Table 2.1 (continued)

Reference, location, follow-up period	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates Comments
Vlaanderen et al. (2013) (cont.)			Extrahepatic cholangiocarcinoma (C23.9, C24.0, C24.1)	All printers and related workers, men	53	1.13 (0.85–1.48)	
				Typographers, men	34	1.09 (0.75–1.52)	
				Printers, men	9	1.37 (0.63–2.59)	
				Lithographers, men	0	0 (0.00–1.83)	
Ahrens et al. (2014) 9 European countries, 1995–1997	153 cases, 1421 population controls	Job title		Printing workers	5	2.42 (0.81–7.24)	ORs adjusted for country, birth year, gallstones and proxy interview
				Typesetters	3	5.78 (1.43–23.30)	

CI, confidence interval; DCM, dichloromethane; DCP, 1,2-dichloropropane; ICD, International Classification of Disease; OR, odds ratio; SIR, standardized incidence ratio; yr, year

2.1.1 *Workers at a printing plant in Osaka, Japan*

In a report on the initial investigation, Kumagai et al. (2013) described a cohort study of the relationship between occupational chemical exposure and incidence of cholangiocarcinoma (ICD10, C22.1, C24.0) among workers in the offset colour proof-printing section of a small printing company in Osaka, Japan. The study was initiated following the finding of a cluster of 11 cases of cholangiocarcinoma among workers in the printing section. The study population consisted of 62 men employed for at least 1 year between 1991 and 2006. Eleven women who were employed in the plant were excluded from the analysis as none had developed cholangiocarcinoma. Exposures were identified initially through worker interviews and company records. 1,2-Dichloropropane and dichloromethane had been used to remove ink from the transcription rubber roller (blanket), from approximately 1985 to 2006, and approximately 1985 to 1997, respectively. [The Working Group noted that a subsequent government investigation determined that exposure to dichloromethane had ended in 1996. A member of the Working Group involved with the Kumagai et al. (2013) study agreed that the government estimation was correct.]

All 62 workers had been exposed to 1,2-dichloropropane and 35 of them had also been exposed to dichloromethane. Solvent concentrations were estimated in a subsequent government investigation (MHLW, 2013b) by experimentally reconstructing past conditions in the plant (JNIOSH, 2012), as described in the Kumagai et al. (2013) report. The estimated airborne concentrations in the proof-printing room (51 workers) were 100–670 ppm [462–3090 mg/m^3] for 1,2-dichloropropane, and 80–540 ppm [278–1870 mg/m^3] for dichloromethane. In the front room (11 workers), the airborne concentrations were estimated to be 70–110 ppm [323–508 mg/m^3] for 1,2-dichloropropane and 50–130 ppm [173–451 mg/m^3] for dichloromethane.

Diagnoses for the 11 cases of cholangiocarcinoma were verified, and vital status of the cohort was ascertained from 1991 until 2011. Fourteen workers who could not be traced were assumed to be alive at the end of 2011. Age at diagnosis of cholangiocarcinoma was 25–45 years, and age at death for the six deceased individuals was 27–46 years. The primary cancer site was the intrahepatic bile duct for five patients, and the extrahepatic bile duct for six patients. All patients had been exposed to 1,2-dichloropropane for 7–17 years, and diagnosed with cholangiocarcinoma 7–20 years after their first exposure. Ten patients were also exposed to dichloromethane for 1–13 years. Known risk factors for cholangiocarcinoma were investigated among the cases, but none were found, with the exception of one patient with a silent biliary stone. The standardized mortality ratio (SMR) for cholangiocarcinoma was 2900 (expected deaths, 0.00204; 95% confidence interval, CI: 1100–6400) for all male workers combined, relative to the Japanese male population.

Kubo et al. (2014) reported on a further investigation of 111 former or current workers at the same Osaka plant as Kumagai et al. (2013), based on data from a subsequent government investigation (MHLW, 2013b) and clinical records from several hospitals. This report included 88 men and 23 women employed at any time between 1981 and 2012. Ten former workers could not be followed up. By the end of 2012, the number of cases of cholangiocarcinoma among the workers reached 17, all diagnosed before age 45 years.

At least 22 chemicals were reported to have been used at the plant during the study period. Use of dichloromethane and 1,2-dichloropropane reportedly began 1991, and ended in 1996 for dichloromethane and in 2006 for 1,2-dichloropropane, according to an investigation by the Ministry of Health, Labour and Welfare (MHLW, 2013b). Of the 17 patients with

cholangiocarcinoma, all had been exposed to 1,2-dichloropropane, 11 had been exposed to dichloromethane, and eight had been exposed to 1,1,1-trichloroethane. The period of exposure to chlorinated organic solvents ranged from 6 to 16 years. The amounts of other chemicals used were lower and the exposure period was shorter. No rate ratios comparing exposed and unexposed workers were presented.

A report of findings by an expert group assembled by the Japanese Ministry of Health, Labour and Welfare (MHLW, 2013b) provided further details on the epidemiological and industrial hygiene investigations of the cluster of cholangiocarcinoma cases at the Osaka plant (Kumagai et al., 2013; Kubo et al., 2014). The report states that various inks and solvents were used at the plant, but investigation of exposures focused on dichloromethane and 1,2-dichloropropane following a decision by a workers' compensation panel. The numbers of workers exposed to 1,2-dichloropropane and dichloromethane were the same as reported by Kubo et al. (2014). The estimated standardized incidence ratio (SIR) for cholangiocarcinoma among all workers in the Osaka plant was 1226 (95% CI, 714–1963), based on 16 observed cases in 100 employees followed until 2012. A 17th case identified later was not included in the counts of the numbers of cases exposed to each agent or in the overall SIR. Separate SIRs according to exposure were not presented. The report also described another case of cholangiocarcinoma from a different plant in Aichi Prefecture, who was exposed to dichloromethane only.

[Members of the Working Group who had been involved in the studies confirmed that the cohort and the 17 cases of cholangiocarcinoma described in MHLW (2013a) were the same as described by Kubo et al. (2014), and that the 17th case was a worker who had been hired in 1997 and was therefore unlikely to have been exposed to dichloromethane. Interpretation of the findings about cholangiocarcinoma in Japanese printers in the Osaka plant was challenging because workers were exposed to multiple chemicals, and complete information about the cohort and the agents to which it was exposed was not available to the Working Group. Enumeration and follow-up of the cohort were incomplete, and female workers were omitted from the initial study by Kumagai et al. (2013), although included in the later follow-up by Kubo et al. (2014). In addition, past exposures were assessed using interviews, company records, and experimental simulation of historical working conditions, resulting in some discrepancies between the various reports with respect to the dates and levels of exposure (e.g. specific months during which use of dichloromethane was discontinued). However, Working Group members who had been involved in all three studies on this plant agreed with the conclusion of MHLW (2013a) regarding the data on last use of dichloromethane (1996). Information about the distribution of exposures in the full cohort was also not reported. No cases were observed among women. A member of the Working Group confirmed lower exposure levels and shorter employment among women. Despite the limitations of these studies on the Osaka plant, it was clear that the risk of cholangiocarcinoma among workers in this plant was astonishingly high, and the universal exposure to solvents at concentrations far above current international limit values, the specificity of the outcome, the young ages at diagnosis and death, and the absence of other established risk factors among the cases are consistent with an occupational cause. Because the original reports did not include risk estimates for specific exposures, the Working Group attempted to estimate SIRs for cholangiocarcinoma according to exposure to the principal solvents used at the Osaka plant. Using information on the numbers of workers exposed reported by Kumagai et al. (2013), and case descriptions and the overall SIR reported by (MHLW, 2013a), the Working Group estimated that 43% of workers were exposed only to

1,2-dichloropropane, giving 0.0057 expected cases until 2012. Based on the information in Kubo et al. (2014) and MHLW (2013a) (6 cases were exposed only to 1,2-dichloropropane), the Working Group estimated the SIR for exposure to 1,2-dichloropropane only to be 6/0.0057 = 1053 (95% CI, 386–2291) and the corresponding SIR for exposure to both 1,2-dichloropropane and dichloromethane as 1487 (95% CI, 742–2660). It was not possible to estimate an SIR for exposure to dichloromethane only, because all of the workers in Osaka were exposed to 1,2-dichloropropane. Although these estimates were clearly very crude, they suggested that the relative risk for 1,2-dichloropropane only was extremely high, and it was not possible to determine which agent was responsible for the relative risk in the group exposed to both 1,2-dichloropropane and dichloromethane. The Working Group noted that new cases continue to accumulate, with five cases identified in 2012 alone.]

2.1.2 *Workers at other printing plants in Japan*

Kumagai (2014) described two additional cases from two different printing plants (not the original one in Osaka). One case in Fukuoka was also described by Yamada et al. (2014) (see below), while the second case, from Aichi Prefecture, had been exposed to dichloromethane and 1,1,1-trichloroethane, but not to 1,2-dichloropropane [Working Group members confirmed that the case exposed to dichloromethane only was the same case without exposure to 1,2-dichloropropane reported by MHLW (2013a) from the Aichi Prefecture.]

Yamada et al. (2014) reported on six workers with cholangiocarcinoma from three small printing plants with fewer than 50 workers each in Miyagi, Fukuoka and Hokkaido, Japan; these plants were separate from the Osaka printing company described above. All six workers had been exposed to 1,2-dichloropropane for 10–16 years. Using mathematical models, working-environment concentrations of 1,2-dichloropropane in the printing rooms were estimated to be 17–180 ppm [79–830 mg/m^3], and estimated exposure concentrations during the ink-removal operation were 150–620 ppm [690–2900 mg/m^3]. Shift time-weighted average (TWA) values were estimated to be 75–240 ppm [350–1100 mg/m^3]. Four of the six workers had also been exposed to dichloromethane at estimated working-environment concentrations of 0–98 ppm [0–340 mg/m^3] in the printing rooms, and 0–560 ppm [0–1900 mg/m^3] during the ink-removal operation. The two other workers had dichloromethane exposures of < 1 ppm. Shift TWA concentrations of dichloromethane were estimated to be 0–180 ppm [0–620 mg/m^3]. Other chlorinated organic solvents were also used in the ink-removal operation, but none of these exposures was common to all patients. [The Working Group noted that this study showed that there were other small printing companies with exposures similar to the Osaka plant studied by Kumagai et al. (2013), in which multiple cases of cholangiocarcinoma occurred, all of whom had long-term, high-level exposure to 1,2-dichloropropane, in addition to other chemicals and inks.]

Okamoto et al. (2013) conducted a study in Japan to assess the occurrence of cancer of the bile duct among workers in the printing industry. Medical insurance claims for cancer of the bile duct from April 2009 to March 2012 were compared for workers in the printing industry and for age-standardized controls in all other industries, using the claims database of the Japan Health Insurance Association. This association insures workers of small–medium-sized employers of all industries, but does not include employees of the previously investigated Osaka printing company. Among men aged 30–49 years in the printing industry, an elevated "standardized prevalence rate ratio" (SPRR) was reported for total cancer of the bile duct (SPRR, 1.78; 95%

CI, 0.63–5.00; 10 cases). The SPRR was higher for cancer of the intrahepatic bile duct (SPRR, 3.03; 95% CI, 0.52–17.56; 5 cases). [The Working Group noted that some of the cases reported by Yamada et al. (2014) might have also been included in the study by Okamoto et al. (2013), and that the cancers of the biliary tract in this study may not have been confirmed histologically. The "printing and related industries" category that served as the exposed group was broad, and it was not clear which types of workplaces and exposures were included. Furthermore, the study covered only a 3-year period after the use of dichloromethane and 1,2-dichloropropane had ceased. The Working Group was also uncertain as to the precise definition of the measure of association used in this study, and noted that it may be possible to interpret the SPRR as the ratio of incident claim rates.]

2.2 Cholangiocarcinoma among printing workers outside Japan

Following reports of excess cholangiocarcinoma among printing workers in Japan (Kumagai et al., 2013), data from two international studies of occupational exposure were analysed to determine whether a similar association existed in other countries (see Table 2.1).

Vlaanderen et al. (2013) conducted a cohort study using a database of four Nordic countries (Finland, Iceland, Norway, and Sweden) set up by linking occupational information from censuses to national cancer registry data using personal identity codes. Estimates of exposure to specific solvents were not used in the analysis, but dicholoromethane was known to have been used in the printing industry (Kauppinen et al., 2009). For men, elevated risks of cancer of the liver (standardized incidence ratio, SIR, 1.35; 95% CI, 1.14–1.60; 142 cases) and intrahepatic cholangiocarcinoma (SIR, 2.34, 95% CI, 1.45–3.57; 21 cases) were seen. SIRs for cancer of the liver were especially high among printers (SIR, 2.22, 95% CI, 1.44–3.28; 25 cases) and lithographers (SIR, 2.38, 95% CI, 1.03–4.70; 8 cases), and SIRs for intrahepatic cholangiocarcinoma were elevated among typographers (SIR, 2.01, 95% CI, 1.00–3.60; 11 cases) and printers (SIR, 3.54, 95% CI, 1.30–7.70; 6 cases). SIRs for extrahepatic cholangiocarcinoma were not increased (SIR, 1.13, 95% CI, 0.85–1.48; 53 cases). SIRs for women followed a similar pattern, but the number of cases was low.

Ahrens et al. (2014) reported associations between cancers of the extrahepatic bile duct and printing occupations in a multicentric study of rare cancers in Europe. Adjusted odds ratios were 2.42 (95% CI, 0.81–7.24; 5 cases) and 5.78 (95% CI, 1.43–23.29; 3 cases) for ever employment in a printing occupation or as a typesetter, respectively.

[The Working Group noted that there was some potential for overlap between Vlaanderen et al. (2013) and Ahrens et al. (2014). These studies suggested that an excess risk of cholangiocarcinoma among printing workers may to some extent be generalizable beyond Japan, but the studies did not provide risk estimates for specific agents.]

3. Cancer in Experimental Animals

The carcinogenicity of 1,2-dichloropropane in experimental animals was reviewed previously by the Working Group (IARC, 1999).

3.1 Mouse

There was one study in male and female mice given 1,2-dichloropropane by oral administration (gavage), and one study in male and female mice given 1,2-dichloropropane by inhalation.

See Table 3.1

Table 3.1 Studies of carcinogenicity with 1,2-dichloropropane in mice

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
NTP (1986) Mouse, B6C3F$_1$ (M) 104 wk	Oral administration (gavage) at a dose of 0, 125, or 250 mg/kg bw for 6 h/day, 5 days/wk 50 mice/group	Hepatocellular adenoma[a]: 7/50 (14%)*, 10/50 (20%), 17/50 (34%)** Hepatocellular carcinoma: 11/50 (22%), 17/50 (34%), 16/50 (32%) Hepatocellular adenoma or carcinoma (combined)[b]: 18/50 (36%)*, 26/50 (52%)[c], 33/50 (66%)**	*$P < 0.05$ (trend) **$P < 0.05$	Purity, > 99% Non-tumorous liver lesions were seen with an increased incidence in male mice at both dosing levels. Lesions included hepatomegaly, focal hepatocellular necrosis, and centrilobular necrosis
NTP (1986) Mouse, B6C3F$_1$ (F) 104 wk		Hepatocellular adenoma[a]: 1/50 (2%)*, 5/50 (10%), 5/50 (10%)** Hepatocellular carcinoma: 1/50 (2%), 3/50 (6%), 4/50 (8%) Hepatocellular adenoma or carcinoma (combined)[b]: 2/50 (4%)*, 8/50 (16%)**, 9/50 (18%)**	*$P < 0.05$ (trend) **$P < 0.05$	Purity, > 99% Mortality was increased in female mice at the highest dose
Matsumoto et al. (2013) Mouse, B6D2F$_1$ (M) 24 mo	Inhalation at a concentration of 0, 32, 80, or 200 ppm for 6 h/day, 5 days/wk 50 mice/group	Bronchiolo-alveolar adenoma: 5/50, 14/50*, 9/50, 12/50 Bronchiolo-alveolar carcinoma: 4/50, 6/50, 6/50, 8/50 Bronchiolo-alveolar adenoma or carcinoma (combined): 9/50, 18/50*, 14/50, 18/50* Histiocytic sarcoma: 1/50, 4/50, 7/50*, 0/50 Harderian gland adenoma: 1/50**, 2/50, 3/50, 6/50 Splenic haemangioma: 0/50, 1/50, 0/50, 1/50 Splenic haemangiosarcoma: 0/50, 3/50, 3/50, 5/50* Splenic haemangioma or haemangiosarcoma (combined): 0/50, 4/50, 3/50, 6/50* Mammary gland adenocarcinoma: 0/50, 0/50, 0/50, 1/50	*$P < 0.05$ **$P < 0.05$ (trend)	Purity, > 99.5%

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Matsumoto et al. (2013) Mouse, B6D2F$_1$ (F) 24 mo		Bronchiolo-alveolar adenoma: 1/50, 4/50, 4/50, 4/50 Bronchiolo-alveolar carcinoma: 1/50**, 1/50, 1/50, 4/50 Bronchiolo-alveolar adenoma or carcinoma (combined): 2/50**, 4/50, 5/50, 8/50* Histiocytic sarcoma: 0/50, 1/50, 0/50, 1/50 Harderian gland adenoma: 2/50, 2/50, 2/50, 2/50 Splenic haemangioma: 0/50, 0/50, 1/50, 0/50 Splenic haemangiosarcoma: 2/50, 0/50, 0/50, 0/50 Splenic haemangioma or haemangiosarcoma (combined): 2/50, 0/50, 1/50, 0/50 Mammary gland adenocarcinoma: 0/50, 0/50, 3/50, 1/50	*$P < 0.05$ **$P < 0.05$ (trend)	Purity, > 99.5%

[a] Historical controls (hepatocellular adenoma): males, 22/149 (14.7%); females, 8/148 (5.4%)

[b] Historical controls (hepatocellular adenoma or carcinoma, combined): males, 44/149 (29.5%); females, 11/148 (7.4%)

[c] As listed in the original report

F, female; h, hour; M, male; mo, month; ppm, parts per million; wk, week

3.1.1 Oral administration

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 7–9 weeks) were given 1,2-dichloropropane (purity, > 99%) in corn oil by gavage at a dose of 0, 125, or 250 mg/kg bw per day, 5 days per week, for 103 weeks. Mortality was increased in females at the highest dose. The incidence of liver adenoma [hepatocellular adenoma] and liver adenoma or carcinoma (combined) [hepatocellular adenoma or carcinoma (combined)] in treated groups of males and females was higher than that in the concurrent control groups. Non-tumorous liver lesions were seen with an increased incidence in males at both dose levels, and included hepatomegaly, focal hepatocellular necrosis, and centrilobular necrosis (NTP, 1986).

3.1.2 Inhalation

Groups of 50 male and 50 female B6D2F$_1$ mice (age, 6 weeks) were given 1,2-dichloropropane at a concentration of 0 (control), 32, 80, or 200 ppm (v/v) by whole-body inhalation for 104 weeks (Matsumoto et al., 2013). Exposure to 1,2-dichloropropane significantly increased the incidences of bronchiolo-alveolar adenoma, and bronchiolo-alveolar adenoma or carcinoma (combined) in males. There was also a significant positive trend in the incidence of adenoma of the Harderian gland in males. The incidence of bronchiolo-alveolar adenoma or carcinoma (combined) was significantly increased in females. In addition, there was a significant positive trend in the incidence of bronchiolo-alveolar carcinoma in females. Non-neoplastic lesions, including atrophy and respiratory metaplasia of the olfactory epithelium, and of the submucosal gland epithelium of the nasal cavity or respiratory epithelium were also significantly increased in females. There was a significant increase in the incidence of histiocytic sarcoma in males at the intermediate dose, and a significant increase in the incidence of splenic haemangiosarcoma in males at the highest dose.

3.2 Rat

There was one study in male and female rats given 1,2-dichloropropane by oral administration (gavage), and one study in male and female rats given 1,2-dichloropropane by inhalation.

See Table 3.2

3.2.1 Oral administration

Groups of 50 male and 50 female F344/N rats (age, 7–9 weeks) were given 1,2-dichloropropane (purity, > 99%) in corn oil by gavage at a dose of 0, 62, or 125 mg/kg bw per day, 5 days per week, for 103 weeks (NTP, 1986). Female rats in the group at the highest dose demonstrated decreased survival, and male and female rats at the highest dose also showed decreased body weight. The incidence of adenocarcinoma of the mammary gland was significantly increased in females at the highest dose (1/50, 2/50, and 5/50 in the control, low-dose, and high-dose groups, respectively). The report noted that three of the five adenocarcinomas of the mammary gland in the female rats were of low-grade malignancy, and may represent a variant of fibroadenoma. [The Working Group accepted the data from this study because of the rigorous pathology peer review described in the report.] There were no effects on tumour incidence in male rats exposed to 1,2-dichloropropane.

3.2.2 Inhalation

Groups of 50 male and 50 female F344 rats (age, 6 weeks) were given 1,2-dichloropropane at a concentration of 0 (control), 80, 200, or 500 ppm by whole-body inhalation for 104 weeks (Umeda et al., 2010). At 2 years, there was a significant increase in the incidence of papilloma of the nasal cavity in male and female rats at the

Table 3.2 Studies of carcinogenicity with 1,2-dichloropropane in rats

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
NTP (1986) Rat, F344/N (M, F) 103 wk	Oral administration (gavage) at a dose of 0, 62, or 125 mg/kg bw for 6 h/day, 5 days/wk 50 rats/group	Mammary gland fibroadenoma: 15/50 (30%), 20/50 (40%), 7/50 (14%) Mammary gland adenocarcinoma: 1/50 (2%)*, 2/50 (4%), 5/50 (10%)**	*$P < 0.05$ (trend) **$P < 0.05$	Purity, > 99% [No effects on tumour incidences in males]
Umeda et al. (2010) Rat, F344 (M) 104 wk	Inhalation at a concentration of 0, 80, 200, or 500 ppm for 6 h/day, 5 days/wk 50 rats/group	Nasal cavity papilloma: 0/50*, 0/50, 3/50 (6%), 15/50 (30%)** Esthesioneuroepithelioma [olfactory neuroblastoma]: 0/50, 2/50 (4%), 1/50 (2%), 0/50	*$P \leq 0.01$ (trend) **$P \leq 0.01$	Purity, > 99.5%
Umeda et al. (2010) Rat, F344 (F) 104 wk	Inhalation at a concentration of 0, 80, 200, or 500 ppm for 6 h/day, 5 days/wk 50 rats/group	Nasal cavity papilloma: 0/50*, 0/50, 0/50, 9/50 (18%)** Esthesioneuroepithelioma [olfactory neuroblastoma]: 0/50, 0/50, 0/50, 0/50		

F, female; h, hour; M, male; ppm, parts per million; wk, week

highest dose. There were three cases of esthesioneuroepithelioma [olfactory neuroblastoma] in exposed males. [The olfactory neuroblastoma is an uncommon neoplasm of the sinonasal tract.]

The total incidence of nasal tumours increased in a concentration-dependent manner. The incidences of hyperplasia of the transitional cell epithelium and squamous cell hyperplasia of the respiratory epithelium in this 2-year study also increased in a concentration-dependent manner, and these lesions were morphologically different from the hyperplasia of the respiratory epithelium, including goblet cell metaplasia, observed in a 13-week experiment reported in the study article. [These hyperplastic lesions may be preneoplastic.] In the 2-year study, there were significantly increased incidences of atrophy and respiratory metaplasia of the olfactory epithelium, and inflammation and squamous cell metaplasia of the respiratory epithelium. [It is known that olfactory sensory neurons differ between species (rat versus mouse) in terms of tissue-selective toxicity (Zhuo et al., 1999; Bozza et al., 2002).]

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

4.1.1 Absorption

(a) Humans

In workers exposed to 1,2-dichloropropane in air, there was a linear correlation between concentration in the breathing zone and concentration in the urine, indicating systemic absorption via the respiratory tract (Ghittori et al., 1987). No direct data on the absorption of 1,2-dichloropropane in humans exposed by oral or dermal administration were available. However, systemic toxicities after ingestion indicate oral absorption through the gastrointestinal tract (Chiappino & Secchi, 1968; Perbellini et al., 1985, Pozzi et al., 1985).

An estimate of the human blood:air partition coefficient of 10.7 ± 0.5 was obtained in vitro, indicating that under equilibrium conditions, respiratory uptake of 1,2-dichloropropane from inhaled air would be expected to be similar to

that for chlorinated compounds such as chloroform and trichloroethylene, all of which have partition coefficients of around 10 (Sato & Nakajima, 1979).

(b) Experimental systems

Hutson et al. (1971) gave male and female rats an oral dose of radiolabelled 1,2-dichloropropane at 4–5 mg/kg bw. After 24 hours, 74–95% of the radiolabel was recovered in the urine or expired air. Similarly, Timchalk et al. (1991) gave male and female rats a single oral dose of radiolabelled 1,2-dichloropropane at 1 or 100 mg/kg bw, and 1 mg/kg bw daily for 8 days. After 48 hours, more than 80% of the radiolabel was recovered in the urine or expired air, with less than 10% in the faeces. These studies indicated near complete systemic absorption of 1,2-dichloropropane via the oral route.

Timchalk et al. (1991) exposed male and female rats to air containing radiolabelled 1,2-dichloropropane at a concentration of 5, 50, or 100 ppm for 6 hours. After 48 hours, 80% or more of the radiolabel was recovered in the urine and expired air, with less than 10% in the faeces, indicating near complete systemic absorption via the inhalation route.

No direct data were available on dermal absorption. However, systemic effects, including death, have been observed after dermal administration of 1,2-dichloropropane in rabbits, indicating systemic absorption through the skin (Smyth et al., 1969).

4.1.2 Distribution

(a) Humans

No data on tissue distribution of 1,2-dichloropropane in humans were available to the Working Group. Meulenberg & Vijverberg (2000) used empirical regression models to predict human tissue:air partition coefficients based on measured saline:air and oil:air partition coefficients. Based on these predictions, tissue:blood partition coefficients in humans were estimated to range from 0.9 (kidney) to 28 (fat), depending on the lipid content of the tissue. These values suggested that 1,2-dichloropropane would be widely distributed to tissues after systemic delivery.

(b) Experimental systems

In rats exposed to 1,2-dichloropropane by inhalation, reported peak blood concentrations were 0.06 (0.06), 0.92 (1.00), and 3.87 (4.55) μg/g in males (females) exposed at 5, 50 and 100 ppm, respectively, indicating systemic delivery of 1,2-dichloropropane in blood via the circulatory system (Timchalk et al., 1991). No sex differences were found; peak concentrations were similar in males and females. No direct data on tissue distribution of 1,2-dichloropropane were available to the Working Group. However, Gargas et al. (1989) reported measured tissue:blood partition coefficients in the range of 0.64 (muscle) to 27 (fat), suggesting that 1,2-dichloropropane is widely distributed to tissues after systemic delivery.

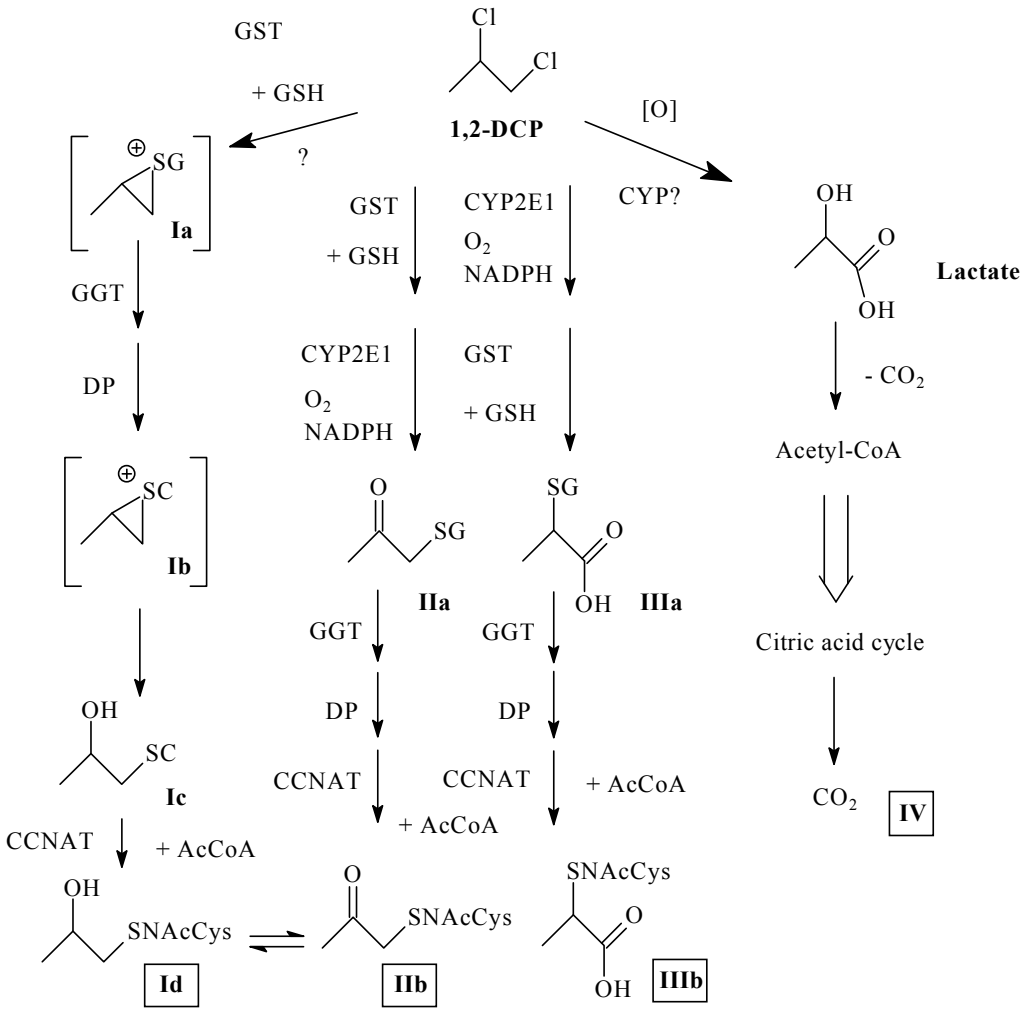
4.1.3 Metabolism

(a) Overview

There are four pathways for the metabolism of 1,2-dichloropropane (summarized in Fig. 4.1). The two best-characterized of these four pathways involve sequential action of cytochrome P450 (CYP) and glutathione *S*-transferase (GST); the other two pathways are less well characterized with respect to the enzymes involved, but do produce metabolites that have been isolated and identified. Some metabolites have been isolated (indicated in Fig. 4.1 by rectangles around their number designations), while others are presumed to occur based on the known chemistry of similar haloalkanes.

1,2-Dichloropropane undergoes sequential GST-mediated conjugation with glutathione (GSH) and then oxidative dehalogenation by cytochrome P450 (CYP) (or vice versa), to generate two GSH conjugates (see Fig. 4.1;

Fig. 4.1 Pathways for the metabolism of 1,2-dichloropropane



Adapted from Timchalk et al. (1991) and Bartels & Timchalk (1990)
Known and proposed pathways for the metabolism of 1,2-DCP (1,2-dichloropropane). Names of metabolites that have been recovered and identified are indicated in rectangles, whereas those that are chemically unstable and reactive are indicated in parentheses. Metabolites: Ia and Ib, glutathione (GSH) and cysteine-containing episulfonium ions; Ic, *S*-(2-hydroxypropyl)-L-cysteine; Id, *N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine; IIa, *S*-(2-oxopropyl)glutathione; IIb, *N*-acetyl-*S*-(2-oxopropyl)-L-cysteine; IIIa, *S*-(1-carboxylethyl)glutathione; IIIb, *N*-acetyl-*S*-(1-carboxyethyl)-L-cysteine; IV, carbon dioxide. Other abbreviations: AcCoA, acetyl-coenzyme A; CCNAT, cysteine conjugate *N*-acetyltransferase; CYP, cytochrome P450; DP, dipeptidase; GGT, γ-glutamyltransferase; GST, glutathione *S*-transferase

metabolites IIa [S-(2-oxopropyl)glutathione] and IIIa [*S*-(1-carboxyethyl)glutathione]). Guengerich et al. (1991) showed that CYP2E1 is very active in the metabolism of 1,2-dichloropropane and similar halogenated alkanes of low relative molecular mass, including dichloromethane. The two GSH conjugates are processed by the standard reaction pathway in the kidneys (Lash et al., 1988) to form the corresponding mercapturates (Fig. 4.1; metabolites IIb [*N*-acetyl-*S*-(2-oxopropyl)-L-cysteine] and IIIb [*N*-acetyl-*S*-(1-carboxyethyl)-L-cysteine]). In addition to these two mercapturates, which have been identified in the urine of rats exposed to 1,2-dichloropropane (Bartels & Timchalk, 1990), metabolite IIb (*N*-acetyl-*S*-(2-oxopropyl)-L-cysteine) can

be reduced to form metabolite Id (*N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine) (also called 2-hydroxypropyl-mercapturic acid), which has also been identified in the urine of rats exposed to 1,2-dichloropropane.

Alternatively, GSH conjugation of 1,2-dichloropropane has also been suggested to form an episulfonium ion (Fig. 4.1; metabolite Ia [GSH-containing episulfonium ion]), which should undergo spontaneous hydrolysis to produce the cysteine conjugate (Fig. 4.1; metabolite Ic [*S*-(2-hydroxypropyl)-L-cysteine]). This can in turn undergo *N*-acetylation to form metabolite Id [*N*-acetyl-*S*-(2-hydroxypropyl)-L-cysteine]. Metabolite Id has been identified in the urine of rats exposed to 1,2-dichloropropane, but this does not constitute definitive proof for this pathway, since it is also formed through sequential CYP–GST metabolism, as described previously. Based on studies with isotope-labelled 1,2-dichloropropane, Bartels & Timchalk (1990) have determined that formation of the mercapturate Ia through a GST-only pathway is negligible, and that it is most likely that the sequential CYP–GST pathway predominates.

A fourth presumed fate of 1,2-dichloropropane is oxidative dechlorination that leads to formation of lactate, and ultimately release of carbon dioxide. While this pathway is presumed to occur as indicated in Fig. 4.1, with carbon dioxide being detected as derived in part from 1,2-dichloropropane, the mechanism for conversion of 1,2-dichloropropane to lactate has not been determined (while a CYP enzyme is expected to be involved, this has not yet been demonstrated).

The CYP2E1 and GST reactions occur primarily in the liver, which is very efficient at excreting GSH conjugates (Fig. 4.1, metabolites Ia, IIa, and IIIa) into the bile. Because the biliary tract is a significant site of gamma-glutamyltransferase (GGT) and dipeptidase activities, some of the excreted GSH conjugates will be converted to the corresponding cysteine conjugates. These undergo enterohepatic and renal–hepatic circulation, ultimately forming the mercapturates (Fig. 4.1, metabolites Id, IIb, and IIIb). The GSH-conjugation reaction also occurs in the kidney, although the renal activity of CYP2E1 is relatively low, especially in humans. Formation of reactive episulfonium ions (Fig. 4.1, metabolites Ia and Ib) can occur via GSH conjugation, especially at higher concentrations of 1,2-dichloropropane when CYP2E1 is saturated. When this reaction occurs in the liver, excretion of these reactive metabolites into the biliary tract may be partly responsible for toxicity of 1,2-dichloropropane in the liver and/or the biliary tract.

(b) Humans or human-derived tissues

No data on the metabolism of 1,2-dichloropropane in humans were available to the Working Group.

The only published study of the metabolism of 1,2-dichloropropane in human-derived tissues was that of Guengerich et al. (1991), which demonstrated the key role of CYP2E1 in the metabolism of several small halogenated hydrocarbons. 1,2-Dichloropropane was found to be one of the better substrates among the chemicals tested with purified human liver CYP2E1 and human liver microsomes. Thus, while trichloroethane and chlorzoxazone were metabolized by the purified human liver CYP2E1 at rates of 1.6 and 3 nmol of product formed/minute per nmol CYP, respectively, the rate of metabolism of 1,2-dichloropropane was 1.1 nmol of product formed/minute per nmol CYP. This rate compared quite favourably to that of purified CYP2E1 with trichloroethylene, which was only slightly lower at 0.97 nmol of product formed/minute per nmol CYP. Further evidence that the metabolism of 1,2-dichloropropane by human liver microsomes is predominantly mediated by CYP2E1 came from studies of immunoinhibition with specific antibodies to CYP2E1.

(c) Experimental systems

Almost all of the published studies on 1,2-dichloropropane metabolism were either in vivo in rats or in various in-vitro preparations from rat liver tissue. Publications are listed in chronological order.

(i) In vivo

Hutson et al. (1971) exposed rats to ^{14}C-labelled 1,2-dichloropropane by stomach tube and examined products in the urine, faeces, and expired air for 96 hours. A relatively high proportion of the administered dose (approximately 20%) was recovered as carbon dioxide in the expired air during the first 24 hours. Little apparent difference was detected between males and females over the 96-hour collection period.

Jones & Gibson (1980) treated male Sprague-Dawley rats with 1,2-dichloropropane by either single intraperitoneal injection or daily oral dosing for 4 days. *N*-Acetyl-*S*-(2-hydroxypropyl)-L-cysteine (Fig. 4.1; metabolite Id) was the major urinary metabolite recovered over 96 hours. Another significant, although relatively minor, metabolite was β-chlorolactate; this finding provides support for carbon dioxide formation via the metabolic route shown in Fig. 4.1.

Timchalk et al. (1991) studied pharmacokinetics and metabolism in male and female Fischer 344 rats given ^{14}C-labelled 1,2-dichloropropane by oral administration or inhalation. By either route, metabolism was rapid, with three urinary mercapturates identified (Fig. 4.1; metabolites Id, IIb, IIIb), and radiolabelled carbon dioxide detected in expired air. As would be expected, the liver contained the highest proportion of radiolabel after oral exposure.

Bartels & Timchalk (1990) treated male and female Fischer 344 rats with radiolabelled 1,2-dichloropropane as a single oral dose at 100 mg/kg bw in corn oil, and measured metabolites in urine. As noted above, these studies were the first to demonstrate the recovery of three different mercapturates in vivo (Fig. 4.1; metabolites Id, IIb, and IIIb). Based on isotope labelling, Bartels & Timchalk (1990) also found no evidence of the pathway involving formation of an episulfonium ion being active.

Timchalk et al. (1991) followed up these studies with a more detailed analysis of the metabolism of ^{14}C-labelled 1,2-dichloropropane by exposing Fischer 344 rats both orally and by inhalation. Distribution of radioactivity in rats exposed to 1,2-dichloropropane at 5, 50, or 100 ppm by inhalation showed the predominance of urine as a route of recovery of metabolites. The concentration of carbon dioxide in expired air increased with 1,2-dichloropropane at 5 to 50 ppm, but decreased at 100 ppm, suggesting saturation of the metabolic pathway through lactate and the citric acid cycle. No sex-specific differences in pharmacokinetics or metabolism by either the oral or inhalation exposure route were observed.

(i) In vitro

The earliest study of the metabolism of 1,2-dichloropropane in vitro used rat liver microsomes (Van Dyke & Wineman, 1971). These authors examined the dechlorination of a series of chloroethanes and propanes. The dechlorination reaction was shown to require NADPH and oxygen, and be inducible by phenobarbital and benzo[*a*]pyrene, but not by methylcholanthrene. These results implicated the CYP monooxygenase system. However, this study also showed that a factor present in the supernatant was necessary for optimal activity. Among six different chlorinated propanes examined as substrates during the course of a 30-minute incubation, 1,1,2-trichloropropane was by far the best substrate (41% dechlorination). Of the dichlorinated propanes, 1,1-dichloropropane was by far the best substrate (25% dechlorination), whereas 1,2-dichloropropane was only a slightly better substrate than 2,2-dichloropropane (6% versus 2.5% dechlorination).

The dependence on a factor present in the supernatant for an optimal dechlorination reaction rate for 1,2-dichloropropane and the other chloropropanes was subsequently shown to be due to a requirement for GSH. Before isolation of mercapturates as the primary metabolites of 1,2-dichloropropane, Trevisan et al. (1989, 1993) and Imberti et al. (1990) showed an association between toxicity caused by 1,2-dichloropropane in rats and GSH status, and that exposure to 1,2-dichloropropane leads to depletion of GSH. Trevisan et al. (1993) further showed that blockage of oxidative metabolism with carbon monoxide prevented GSH depletion in the rat kidney, demonstrating the importance of the GSH-conjugation reaction in the metabolism of 1,2-dichloropropane.

Tornero-Velez et al. (2004) compared the kinetics of metabolism in rat liver microsomes of various dichlorinated and dibrominated alkanes, with carbon-chain lengths ranging from two to four. In general, metabolism was fastest with higher chain length and the presence of bromine rather than chlorine. 1,2-Dichloropropane exhibited a catalytic efficiency (i.e. V_{max}/K_m) that was approximately 25% of that of the most efficiently catalysed substrate, which was 1,3-dichloropropane.

Although most studies in mammals have suggested that CYP2E1 is the primary CYP enzyme that metabolizes 1,2-dichloropropane through the oxidative pathway, other CYPs also exhibit activity. For example, Lefever & Wackett (1994) studied the oxidation of several polychlorinated ethanes and 1,2-dichloropropane by cytochrome $P450_{CAM}$, which is now known as CYP101. Oxidation activity was highest with the more highly chlorinated ethanes (e.g. hexachloroethane and pentachloroethane); 1,2-dichloropropane was oxidized to chloroacetone at a rate that was only 25% of that of these two highly chlorinated ethanes and was only 5% of that of camphor. Nonetheless, these data suggest the possibility that other CYPs besides CYP2E1 may metabolize 1,2-dichloropropane.

4.1.4 Excretion

(a) Humans

Ghittori et al. (1987) measured 1,2-dichloropropane in the urine of men exposed occupationally, indicating that excretion of the parent compound occurs in urine.

(b) Experimental systems

In experimental animals, 1,2-dichloropropane is eliminated primarily as metabolites in urine and expired carbon dioxide, with lesser amounts expired as volatile organic compounds, and excreted in the faeces (Hutson et al., 1971; Timchalk et al., 1991). At 24 hours after oral administration in rats, Hutson et al. (1971) reported that 80–90% of the administered dose was eliminated in the faeces, urine, and expired air, of which urine accounted for 50.2%, carbon dioxide accounted for 19.3%, and expired volatiles accounted for 23.1%. Similarly, in rats exposed orally or by inhalation, Timchalk et al. (1991) reported 37–65% recovery in the urine, or 18–40% recovery in expired air, depending on dose. The amount expired as volatile organic compounds increased with dose or concentration, and in all cases the majority of the expired volatile organic material was found to be unchanged 1,2-dichloropropane (Timchalk et al., 1991). This dose-dependency is consistent with dose-dependent saturation of 1,2-dichloropropane metabolism (Timchalk et al., 1991). Overall, elimination is fairly rapid, with the majority of the administered dose excreted in the first 24 hours after exposure (Hutson et al., 1971; Timchalk et al., 1991).

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

See Table 4.1

The genetic toxicology of 1,2-dichloropropane has been reviewed previously by the Working Group (IARC, 1999). There is evidence for induction of base-pair mutation in two studies in *Salmonella typhimurium* (TA100, TA1535 [De Lorenzo et al., 1977, Principe et al., 1981]), with and without an exogenous metabolic system, but not in a third study (Haworth et al., 1983). Stolzenberg & Hine (1980) tested 1,2-dichloropropane at a lower dose, which may explain the negative results in that study. Results were negative in TA1537, TA1538, TA98, and TA1978 strains (De Lorenzo et al., 1977; Principe et al., 1981; Haworth et al., 1983). Results were also negative in one study in *Streptomyces coelicolor* (Principe et al., 1981). 1,2-Dichloropropane induced weak mutagenic effects, but no chromosomal effects in *Aspergillus nidulans* (Principe et al., 1981; Crebelli et al., 1984). It did not induce sex-linked recessive lethal mutation in *Drosophila melanogaster* (Woodruff et al., 1985). In Chinese hamster ovary cells in culture, 1,2-dichloropropane induced sister-chromatid exchange and chromosomal aberration, both with and without exogenous metabolic activation (Galloway et al., 1987; von der Hude et al., 1987).

The acute toxicity and mutagenicity of halogenated aliphatic compounds was assessed in a test for somatic mutation and recombination in *Drosophila melanogaster* (wing spot test). Compared with several structurally related compounds, the median lethal concentration (LC_{50}) of 1,2-dichloropropane was high (14.4 µg/L). At ½ LC_{50}, slight but statistically significant positive effects in terms of wing-spot number frequencies were noted (Chroust et al., 2007).

1,2-Dichloropropane was not mutagenic in the dominant-lethal assay in rats in a study by EPA (1989) in which male Sprague-Dawley rats were exposed to drinking-water containing 1,2-dichloropropane at a concentration of 0.024%, 0.10%, or 0.24% (w/v) for 14 weeks. The positive control, cyclophosphamide (100 mg/kg bw, single oral dose), induced a significant dominant lethal effect in the same study (EPA, 1989).

Male B6C3F_1 and *Gpt* Delta C57BL/6J mice were exposed to 1,2-dichloropropane (0, 150, 300, or 600 ppm), dichloromethane (400, 800, or 1600 ppm), or combinations of both solvents (1,2-dichloropropane plus dichloromethane at 150 plus 400 ppm and 300 plus 800 ppm), by inhalation (6 hours per day, 5 days per week, for 6 weeks for each agent, or for 4 weeks for the combination, respectively). Genotoxicity was assessed by *Pig-a* gene mutation and assays for micronucleus formation in peripheral blood, and by *Gpt* mutation and comet assays in the liver. *Pig-a* mutation frequencies and micronucleus incidences were not significantly increased by any exposure. In the liver, DNA damage as measured by the comet assay (tail intensity) was increased in a dose-dependent manner by 1,2-dichloropropane (being significant at 300 ppm), but not by dichloromethane (Suzuki et al., 2014). There was a significant increase in comet tail intensity at a lower dose of 1,2-dichloropropane (150 ppm) after co-exposure with dichloromethane (400 ppm) (Suzuki et al., 2014). *Gpt* mutations were not induced after exposure to 1,2-dichloropropane at 300 ppm, but were significantly increased after co-exposure to 1,2-dichloropropane (300 ppm) and dichloromethane (800 ppm) (Suzuki et al., 2014). [The Working Group noted that a plausible explanation for this result was that co-exposure to dichloromethane leads to saturation of CYP2E1, leading to greater bioactivation of 1,2-dichloropropane through the GSH pathway.]

4.3 Biochemical and cellular effects

In-vitro experiments using renal cortical slices from the kidneys of male Wistar rats showed that exposure to 1,2-dichloropropane caused loss of organic anion accumulation (a

Table 4.1 Studies of genotoxicity with 1,2-dichloropropane

Test system	Results[a]		Concentration[b] (LEC or HIC)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, reverse mutation	+	+	5 000	De Lorenzo et al. (1977)
Salmonella typhimurium TA100, reverse mutation	–	–	565	Stolzenberg & Hine (1980)
Salmonella typhimurium TA100, reverse mutation	+	+	2 900	Principe et al. (1981)
Salmonella typhimurium TA100, reverse mutation	(+)	–	5 000	Haworth et al. (1983)
Salmonella typhimurium TA1535, reverse mutation	+	+	5 000	De Lorenzo et al. (1977)
Salmonella typhimurium TA1535, reverse mutation	+	+	2 900	Principe et al. (1981)
Salmonella typhimurium TA1535, reverse mutation	(+)	–	5 000	Haworth et al. (1983)
Salmonella typhimurium TA1537, reverse mutation	–	–	5 800	Principe et al. (1981)
Salmonella typhimurium TA1537, reverse mutation	–	–	1 666	Haworth et al. (1983)
Salmonella typhimurium TA1538, reverse mutation	–	–	5 800	Principe et al. (1981)
Salmonella typhimurium TA98, reverse mutation	–	–	5 800	Principe et al. (1981)
Salmonella typhimurium TA98, reverse mutation	–	–	5 000	Haworth et al. (1983)
Salmonella typhimurium TA1978, reverse mutation	–	–	25 000	De Lorenzo et al. (1977)
Streptomyces coelicolor, forward mutation	–	NT	58 000	Principe et al. (1981)
Aspergillus nidulans, genetic crossing-over	–	NT	17 400	Crebelli et al. (1984)
Aspergillus nidulans, forward mutation	(+)	NT	58 000	Principe et al. (1981)
Drosophila melanogaster, sex-linked recessive lethal mutations	–	NR	7200 ppm, inhalation	Woodruff et al. (1985)
Chinese hamster ovary cells, sister-chromatid exchange, in vitro	+	+	113	Galloway et al. (1987)
Chinese hamster lung fibroblast V79 cells, sister-chromatid exchange, in vitro	+	+	370	von der Hude et al. (1987)
Chinese hamster ovary cells, chromosomal aberration, in vitro	(+)	(+)	660	Galloway et al. (1987)
Drosophila melanogaster larvae, wing spot test	+	NA	7.7 µg/L air	Chroust et al. (2007)
Male Sprague-Dawley rats, dominant-lethal assay	–[c]	NA	0.24% in drinking-water, 14 wk[d]	EPA (1989)

Table 4.1 (continued)

Test system	Results[a]		Concentration[b] (LEC or HIC)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Male B6C3F$_1$ mice, *Pig-a* mutant frequencies, blood, in vivo	–	NA	600 ppm (6 h/day, 5 days/wk, 6 wk), inhalation	Suzuki et al. (2014)
Male B6C3F$_1$ mice, micronucleus formation, reticulocytes, in vivo	–	NA	600 ppm (6 h/day, 5 days/wk, 6 wk), inhalation	Suzuki et al. (2014)
Male B6C3F$_1$ mice, DNA damage liver (comet assay, tail intensity, in vivo)	+	NA	300 ppm (6 h/day, 5 days/wk, 6 wk), inhalation	Suzuki et al. (2014)
Male B6C3F$_1$ mice, DNA damage liver (comet assay, tail intensity, in vivo)	+	NA	150 ppm (6 h/day, 5 days/wk, 6 wk), inhalation, with co-exposure to dichloromethane at 400 ppm	Suzuki et al. (2014)
Transgenic, *gpt* Delta C57BL/6J mice, gene mutation, Gpt in liver	–	NA	300 ppm (6 h/day, 5 days/wk, 4 wk), inhalation	Suzuki et al. (2014)
Transgenic *gpt* Delta C57BL/6J mice, gene mutation, Gpt in liver	+	NA	300 ppm (6 h/day, 5 days/wk, 4 wk) with co-exposure to dichloromethane at 800 ppm, inhalation	Suzuki et al. (2014)

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

[b] LEC, lowest effective concentration; HIC, highest ineffective concentration; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw per day

[c] Statistically significant increase was observed after 1 week of breeding in preimplantation losses and resorption rate at 0.024% and 0.24% treated group (not in 0.10 mid-dose) compared to concurrent controls. However, data from the second week showed no treatment-related statistical difference from concurrent controls

[d] 0.024%, 0.10%, 0.24% w/v continuous 14-week treatment corresponded to time-weighted average daily dosage of 28, 91, and 162 mg/kg bw per day, respectively

NA, not applicable; NT, not tested; wk, week

measure of renal function), release into the incubation medium of tubular enzymes, aspartate aminotransferase (AST) and lactate dehydrogenase, depletion of GSH, and increase in concentrations of malondialdehyde (Trevisan et al., 1993). Acivicin and aminooxyacetic acid, inhibitors of GGT and the cysteine conjugate β-lyase, respectively, partially prevented loss of organic anion accumulation (*p*-aminohippurate) and increases in malondialdehyde induced by exposure to 1,2-dichloropropane, suggesting that toxicity is at least partially related to the cysteine conjugate. Alpha-ketobutyrate, an activator of the cysteine conjugate β-lyase, enhanced the effects of 1,2-dichloropropane, suggesting that the toxicity of 1,2-dichloropropane is partially due to nephrotoxic thioalkanes formed from the cysteine conjugate activated by the β-lyase.

Another study investigated the effect of testosterone on the nephrotoxicity of 1,2-dichloropropane in naïve males, females, and castrated males with testosterone replacement (Odinecs et al., 1995). The nephrotoxicity was evaluated by measuring accumulation of an organic anion (*p*-aminohippurate) and release of AST into the incubation medium in renal cortical slices prepared from animals with differing hormonal status. 1,2-Dichloropropane decreased accumulation of *p*-aminohippurate by renal cortical slices and increased release of AST. This effect was the largest in the slices obtained from naïve male rats. Males were more susceptible

than females to the decreases in *p*-aminohippurate accumulation and increases in release of AST caused by exposure to 1,2-dichloropropane. Castration of males had a protective effect against the changes in *p*-aminohippurate uptake and AST release, but pretreatment with testosterone significantly increased the susceptibility of females for effects on *p*-aminohippurate accumulation only. This study showed that greater susceptibility to 1,2-dichloropropane-induced nephrotoxicity in males can be explained by CYP activity in the kidney, as treatment with testosterone leads to an increase of CYP activity in the kidneys of female and castrated males.

4.4 Organ toxicity

4.4.1 Liver

(a) Humans

Several studies show evidence for liver toxicity in humans exposed to 1,2-dichloropropane. A case report of a worker using stain-remover containing 1,2-dichloropropane described liver injury (indicated by elevations in AST and alanine aminotransferase (ALT), and reduced prothrombin activity). Hepatic biopsy revealed acute centrolobular necrosis characterized by pyknosis and a few "cellular shadows" (Lucantoni et al., 1992). Three cases of intoxication with 1,2-dichloropropane (one by ingestion, two by inhalation) were reported to present with clinical features of severe liver damage evident from elevation of serum enzymes (Pozzi et al., 1985).

Kumagai et al. (2014) reported indications of liver damage both during and after exposure to 1,2-dichloropropane in 10 printing workers later diagnosed with cholangiocarcinoma. Values for erythrocytes, haemoglobin, haematocrit (erythrocyte volume fraction), total cholesterol, triglycerides, and fasting plasma glucose were within the standard ranges during exposure to 1,2-dichloropropane for almost all patients, but GGT levels exceeded the standard range for six patients. Two of these six patients were diagnosed with cholangiocarcinoma during exposure, and the other four patients were diagnosed 1–9 years after termination of exposure. The remaining four patients had GGT levels that were within the standard range during exposure, but had increased GGT levels thereafter, and were diagnosed with cholangiocarcinoma 4–10 years after termination of exposure. AST and ALT levels were also elevated in exposed workers.

(b) Experimental systems

(i) Rats

In 13-week and 2-year studies in male and female F344 rats exposed to 1,2-dichloropropane by inhalation, absolute and relative liver weights were significantly increased in female rats exposed at 500 ppm and above, and swelling of centrilobular hepatocytes was observed in male and female rats exposed at 2000 ppm (Umeda et al., 2010). Centrilobular hepatic fatty degeneration with atrophy and necrosis of the liver was found in studies of shorter duration in rats (Heppel et al., 1946).

In studies in Sprague-Dawley rats given 1,2-dichloropropane by gavage for up to 13 weeks, morphological changes were reported in the liver, including moderate cytoplasmic condensation, necrosis of centrilobular hepatocytes, and mixed inflammatory cell infiltration (Bruckner et al., 1989). Another 13-week study in male and female F344N rats treated by oral gavage found centrilobular congestion of the liver, hepatic fatty changes, and centrilobular necrosis (NTP, 1986). Daily dosing of rats with 1,2-dichloropropane for 4 weeks resulted in a dose-dependent increase in the incidence of focal liver necrosis and steatosis (Trevisan et al., 1989).

Treatment of rats with buthionine sulfoximine, a glutathione-depleting agent, increased lethality of 1,2-dichloropropane (2 mL/kg bw, by gavage), while administration of *N*-acetylcysteine,

a glutathione precursor, decreased toxicity (Imberti et al., 1990).

(ii) Mice

In a 13-week study in B6D2F$_1$/Crlj mice given 1,2-dichloropropane by inhalation, swelling of centrilobular hepatocytes was found to be significantly increased in both male and female mice exposed at 300 ppm and above (Matsumoto et al., 2013). Other pathological observations included necrosis, fatty change, vacuolic change, and mineralization of centrilobular hepatocytes. Total bilirubin, AST, ALT, and lactate dehydrogenase were increased in male and female mice exposed at 400 ppm. Alkaline phosphatase activity was significantly increased in male mice exposed at 300 ppm and above. A study in C3H mice exposed to 1,2-dichloropropane at 400 ppm for up to 37 days (4–7 hours/day) found moderate to marked congestion and fatty degeneration of the liver, extensive centrilobular coagulation and necrosis of the liver. Some of the observations were made post mortem in mice that died during treatments (Heppel et al., 1948).

Several long-term bioassays in mice exposed to 1,2-dichloropropane by inhalation reported signs of liver histopathology (Heppel et al., 1946; Matsumoto et al., 2013). Dose-dependent increases in the incidences of hepatomegaly and hepatic necrosis (focal, not otherwise specified, and centrilobular combined) were also found in male mice, but not females, given 1,2-dichloropropane by gavage for 2 years, (NTP, 1986).

(iii) Other species

In a study by Heppel and colleagues, rabbits and dogs were exposed to 1,2-dichloropropane via inhalation (Heppel et al., 1946). Few animals were examined in each of these species, usually one per group. Mild steatosis was observed in two rabbits exposed for 1 or 2 weeks. Post-mortem (death due to 1,2-dichloropropane exposure at 1000 ppm for up to 96 days) pathological evaluation of the liver in dogs showed moderate to marked fatty degeneration of the liver. In a follow-up study in dogs treated with 1,2-dichloropropane at lower concentrations (400 ppm, 134 exposures, 4–7 hours per exposure) via inhalation, slight haemosiderosis was observed in the liver of one dog (Heppel et al., 1948).

4.4.2 Kidney

(a) Humans

Several case studies reported that exposure to 1,2-dichloropropane may cause acute renal failure in humans (Pozzi et al., 1985; Lucantoni et al., 1992; Fiaccadori et al., 2003).

(b) Experimental systems

(i) Rats

In male and female F344/DuCrj rats exposed to 1,2-dichloropropane for 13 weeks or 2 years, no exposure-related kidney lesions were reported (Umeda et al., 2010). In rats exposed to 1,2-dichloropropane for between 2 and 140 days, no kidney histological changes were observed (Heppel et al., 1948). No apparent nephrotoxicity was observed in male Sprague-Dawley rats treated with 1,2-dichloropropane by gavage for 1 day, 10 days, or 13 weeks (Bruckner et al., 1989), or in male and female F344 rats treated by gavage for 13 weeks or 2 years (NTP, 1986).

As mentioned above (see Section 4.3), in-vitro studies in which rat renal cortical slices were exposed to 1,2-dichloropropane showed that a depletion in GSH occurs, and that it can be prevented by carbon monoxide. It was also shown that the loss of organic anion accumulation (*p*-aminohippurate) can be partially inhibited by acivicin and aminooxyacetic acid, which are inhibitors of GGT and β-lyase activities, respectively (Trevisan et al., 1993).

(ii) Mice

Kidney toxicity has been observed in several studies in mice. In B6D2F1/Crlj mice exposed to 1,2-dichloropropane by inhalation for 2 years,

basophilic changes in the proximal tubules and mineralization of the cortex were reported in males (Matsumoto et al., 2013). No kidney pathology was found at the 13-week time-point in this study. Two additional studies in mice reported fatty degeneration of the kidney after a single lethal dose, or repeated dosing for 2–4 weeks (Heppel et al., 1946, 1948). In studies in male and female B6C3F$_1$ mice treated by gavage, no exposure-related lesions were reported in the kidney at either 13 weeks or 2 years (NTP, 1986).

(iii) Other species

In a study in guinea-pigs killed 6–8 months after exposure to 1,2-dichloropropane by inhalation for up to 4 months, renal cortical scarring, extensive renal fibrosis and amyloidosis, tubular atrophy and fatty degeneration alternating with dilated and occasionally cystic tubules were reported in some exposed animals (Heppel et al., 1948). A study in dogs exposed to 1,2-dichloropropane for up to 4 months observed scattered granulomatous lesions in the kidney, with no demonstrable acid-fast bacilli (Heppel et al., 1948).

4.4.3 Central nervous system

Depression of the central nervous system was reported in humans exposed to 1,2-dichloropropane at high concentrations (Perbellini et al., 1985; Imberti et al., 1987; Lucantoni et al., 1992). Depression of the central nervous system was observed in adult male Sprague-Dawley rats given 1,2-dichloropropane by gavage for 1 day, 10 days, or 13 weeks (Bruckner et al., 1989).

4.4.4 Haematotoxicity

Haemolytic anaemia has been observed in humans in two case reports of exposure to 1,2-dichloropropane (Pozzi et al., 1985; Lucantoni et al., 1992).

In experimental animals, haemolytic anaemia, accompanied by pathological changes of the spleen, was observed in B6D2F$_1$ mice and F344/DuCrj rats exposed to 1,2-dichloropropane by inhalation for 13 weeks (Umeda et al., 2010; Matsumoto et al., 2013), and in Sprague-Dawley rats exposed by gavage for 13 weeks (Bruckner et al., 1989).

4.4.5 Skin

In a case series of 10 subjects with contact allergic dermatitis, all demonstrated a positive response to 1,2-dichloropropane (Baruffini et al., 1989). In another case report, a woman exposed occupationally to 1,2-dichloropropane reported hand dermatitis that receded after changing work (Grzywa & Rudzki, 1981).

No data on experimental animals were available to the Working Group.

4.4.6 Respiratory system

No data on humans were available to the Working Group.

In mice exposed to 1,2-dichloropropane by inhalation for 13 weeks, treatment-related metaplasia and atrophy of the nasal cavity epithelium, and necrosis of the olfactory epithelium, were reported in males and females (Matsumoto et al., 2013). In rats exposed to 1,2-dichloropropane by inhalation for 13 weeks or 2 years, hyperplasia of the respiratory epithelium, and atrophy of the olfactory epithelium occurred in males and females (Umeda et al., 2010).

4.4.7 Adrenal gland

No data on humans were available to the Working Group.

In a study in rats exposed to 1,2-dichloropropane by inhalation for 13 weeks, the incidence of fatty changes in the adrenal gland was statistically significant in females (Umeda et al., 2010). In a study in dogs exposed by inhalation, marked congestion, atrophy, pigmentation and focal necrosis of the zona reticularis of the

adrenal gland was reported in one dog (Heppel et al., 1946).

4.5 Susceptible populations

4.5.1 Polymorphisms

No publications were available that had directly assessed the effects of 1,2-dichloropropane in potentially susceptible populations. However, the dependence of toxicity on the metabolism of 1,2-dichloropropane by CYP2E1 and GST suggests that genetic polymorphisms in these enzymes will modulate individual susceptibility to 1,2-dichloropropane. Specifically, it is expected that higher activities of CYP2E1 and certain GST isoforms would promote greater toxicity after exposure to 1,2-dichloropropane. Regarding the GST-dependent metabolism of 1,2-dichloropropane, the function of specific isoforms has not been determined.

4.5.2 Life stage

No studies providing data related to life-stage susceptibility to the carcinogenic effects of 1,2-dichloropropane were available to the Working Group.

4.6 Mechanistic considerations

Limited information was available on the toxicokinetics of 1,2-dichloropropane. However, the available data suggested that 1,2-dichloropropane behaves similarly to other halogenated alkanes, and is metabolized by CYP and GST-mediated conjugation with GSH. Available toxicokinetic data indicated that metabolism is extensive, with excretion of multiple urinary metabolites indicating that multiple metabolic pathways are active (Timchalk et al., 1991).

The best-studied metabolic pathways involve GSH conjugation in combination with CYP, leading to mercapturates that are excreted in the urine; GSH-conjugation alone, which may lead to formation of reactive metabolites; or CYP alone, which leads to formation of carbon dioxide that is exhaled. CYP2E1 plays a major role in CYP-mediated metabolism (Guengerich et al., 1991), although the evidence suggests that other CYPs can also be involved (Lefever & Wackett, 1994). Under conditions of higher exposure when CYP2E1 is saturated, it is plausible that GSH-only metabolism would predominate, but this has not been demonstrated. Alternatively, saturation of CYP without a shift to GSH-only metabolism would lead to increased excretion of the parent compound, which has been observed in rats (Timchalk et al., 1991). Moreover, a shift to GSH-only metabolism would lead to a change in the proportion of urinary mercapturates, but no such change was observed with increasing dose (Timchalk et al., 1991). Finally, based on isotope-labelling, Bartels & Timchalk (1990) found no evidence for activity of the GSH-only pathway. Overall, the Working Group concluded that, while plausible, there was insufficient direct evidence for the activity of a GSH-only pathway, leading to formation of reactive metabolites.

No data on the genotoxicity of 1,2-dichloropropane or its metabolites in humans were available to the Working Group. In experimental systems in vivo, no dominant-lethal effect was observed in one study (EPA, 1989). In another in-vivo study, no increases in the frequency of *Pig-a* mutation or micronucleus formation were observed with exposure to 1,2-dichloropropane, but DNA damage as measured by the comet assay was increased in a dose-dependent manner, with increases occurring at lower levels of exposure to 1,2-dichloropropane under conditions of co-exposure to dichloromethane (Suzuki et al., 2014). Genotoxicity with 1,2-dichloropropane has been observed in vitro, including mutation in *Salmonella*, and sister-chromatid exchanges in Chinese hamster ovary and lung fibroblast V79 cells, and chromosomal aberrations in Chinese hamster ovary cells, where results did not depend

on the presence or absence of exogenous metabolic activation (De Lorenzo et al., 1977; Principe et al., 1981; Galloway et al., 1987; von der Hude et al., 1987). While there is some evidence for genotoxicity with 1,2-dichloropropane in vivo and in vitro, the genotoxicity database contains mixed results and is not extensive.

1,2-Dichloropropane causes hepatic and renal toxicity, including fatty degeneration and necrosis, in humans (Perbellini et al., 1985; Pozzi et al., 1985; Lucantoni et al., 1992; Fiaccadori et al., 2003) and in experimental systems (Heppel et al., 1946, 1948; NTP, 1986). Damage is often extensive, and sometimes fatal. Haemolytic anaemia as a result of 1,2-dichloropropane exposure has also been consistently reported in studies in humans and experimental animals (Heppel et al., 1946; Pozzi et al., 1985; Lucantoni et al., 1992; Umeda et al., 2010; Matsumoto et al., 2013). Nasal, but not lung, toxicity has been reported in mice and rats exposed to 1,2-dichloropropane via inhalation, with effects observed including desquamation of the olfactory epithelium (Umeda et al., 2010; Matsumoto et al., 2013).

No direct data on susceptibility were available to the Working Group.

5. Summary of Data Reported

5.1 Exposure data

1,2-Dichloropropane is a synthetic, chlorinated solvent that is a by-product of the manufacture of propylene oxide. 1,2-Dichloropropane is used primarily as a chemical intermediate in the production of other organic chemicals, such as propylene, carbon tetrachloride, and tetrachloroethylene. It is also used as solvent in several uses including paint stripping. Until 2012, it was used as a printing-press cleaner in Japan. There are no data as to whether it has been used for this purpose in other countries. 1,2-Dichloropropane was formerly used as one component of a grain and soil fumigant, although this use is no longer permitted in Europe and the USA. Inhalation is the primary route of exposure in occupational settings, and dermal contact can also occur. Occupational exposures of > 1 g/m^3 have been estimated. Little information is available on exposure of the general population to 1,2-dichloropropane from environmental sources, but environmental air concentrations are likely to be very low.

5.2 Human carcinogenicity data

Investigations into the carcinogenicity of 1,2-dichloropropane were prompted by the recognition of a cluster of 17 cases of cancer of the biliary tract (identified histologically as cholangiocarcinoma) in a small offset-printing plant in Osaka, Japan. Subsequently, epidemiological and occupational hygiene investigations identified seven additional cases from four other small printing plants in Japan. Age of death or diagnosis for these cases was about 20–60 years; cancers of the biliary tract usually occur at later ages in the general population. Based on the results from the Osaka plant alone, the estimated relative risk for this rare and generally fatal cancer is extraordinarily high. Most workers at these plants were exposed to both dichloromethane and 1,2-dichloropropane at levels well above current international limit values, as well as to other solvents and inks. No studies of the association of cancer in humans with exposure to 1,2-dichloropropane in other countries or industries were available to the Working Group.

The exposure distribution of the full cohort in the Osaka plant was not described, but in the follow-up of about 100 workers until 2012, 17 cases were observed, of which 6 had no known exposure to dichloromethane. The Working Group estimated relative risks of approximately 900 for exposure to 1,2-dichloropropane. Seven additional cases of cancer of the bile duct were identified in subsequent reports from other

Japanese printing plants. Of these, one case was exposed to high levels of dichloromethane without exposure to 1,2-dichloropropane. The other six cases were all exposed to 1,2-dichloropropane, four to both dichloromethane and 1,2-dichloropropane, and two to 1,2-dichloropropane with only negligible exposure to dichloromethane (< 1 ppm).

Given the rarity of the outcome, the young ages at diagnosis, the absence of other known risk factors among the cases, and the very high relative risk, as well as the specificity and apparent intensity of the exposures, the finding of a large excess of cancer of the biliary tract among the printing workers is extremely unlikely to be the result of chance and very unlikely to be due to bias or confounding.

5.3 Animal carcinogenicity data

There were two studies of carcinogenicity with 1,2-dichloropropane in mice: one study of oral administration (gavage) in males and females, and one study of inhalation in males and females. 1,2-Dichloropropane increased the incidences of hepatocellular adenoma and/or carcinoma in male and female mice after oral administration, of bronchiolo-alveolar adenoma and/or adenocarcinoma in male and female mice exposed by inhalation, and of splenic haemangiosarcoma in male mice exposed by inhalation. 1,2-Dichloropropane induced histiocytic sarcoma and Harderian gland adenoma in male mice exposed by inhalation.

There were two studies of carcinogenicity with 1,2-dichloropropane in rats: one study of oral administration (gavage) in male and female rats and one study of inhalation in males and females. 1,2-Dichloropropane increased the incidence of adenocarcinoma of the mammary gland in female rats after oral administration, and of papilloma of the nasal cavity in male and female rats exposed by inhalation, and probably induced rare olfactory neuroblastoma in the nasal cavity of male rats exposed by inhalation.

5.4 Mechanistic and other relevant data

1,2-Dichloropropane is a volatile lipophilic compound that is readily absorbed after oral, inhalation, or dermal exposure. After absorption, 1,2-dichloropropane is extensively distributed systemically, and metabolized to mercapturates excreted in the urine and in carbon dioxide in exhaled breath. Multiple pathways involving cytochrome P450 (CYP) and glutathione *S*-transferase-mediated conjugation with glutathione (GSH), both individually and in combination, may be responsible for the metabolism of 1,2-dichloropropane. Metabolites formed through the combination of GSH conjugation and CYP oxidation, or through CYP alone, do not appear to be reactive. The pathway involving GSH conjugation alone is plausible, based on similarities to other halogenated hydrocarbons such as trichloroethylene and methyl chloride, and may lead to formation of reactive, genotoxic metabolites. However, there is no direct evidence for the activity of the GSH conjugation-only pathway for 1,2-dichloropropane.

Genotoxicity with 1,2-dichloropropane has been observed in vitro in some mammalian (e.g. Chines hamster ovary cells) and non-mammalian systems (some strains of *Salmonella*), as well as in some in-vivo experiments in mice. No data on genotoxicity in humans or human-derived cells were available. While there was some evidence of genotoxicity in vivo and in vitro, the data were mixed and limited in extent.

1,2-Dichloropropane causes hepatic and renal toxicity, and haemolytic anaemia, in humans and rodents. Nasal, but not lung, toxicity has been reported in mice and rats exposed to 1,2-dichloropropane via inhalation. These data suggest that the hepatic, renal, haematopoietic,

and respiratory systems are potential target tissues. Non-genotoxic mechanisms of carcinogenesis have not been identified.

Overall, given that there was some evidence for genotoxicity, the Working Group considered that the mechanistic evidence for 1,2-dichloropropane carcinogenesis is *moderate*.

6. Evaluation

6.1 Cancer in humans

There is *sufficient evidence* in humans for the carcinogenicity of 1,2-dichloropropane. 1,2-Dichloropropane causes cancer of the biliary tract (confirmed as cholangiocarcinoma).

The major challenge in evaluating the occurrence of cancer in the Japanese printing plants was to determine whether the observed excess of cholangiocarcinoma could be attributed to a specific agent, measured or unmeasured. Workers were exposed to numerous chemicals, but 1,2-dichloropropane was known to be common to all except one of the 24 cases of cholangiocarcinoma. Moreover, 6 of the cases had no exposure to dichloromethane and the Working Group's estimate of the relative risk for these cases was extremely high. Based on this evidence, the majority of the Working Group concluded that 1,2-dichloropropane is the causative agent responsible for the large excess of cholangiocarcinoma among the workers exposed to 1,2-dichloropropane, but not dichloromethane. However, a minority of the Working Group concluded that the association between 1,2-dichloropropane and cancer of the biliary tract was credible, but the role of exposure to other agents, principally dichloromethane, could not be separated with complete confidence, and noted that most of the evidence came from studies in a single plant.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

1,2-Dichloropropane is *carcinogenic to humans (Group 1).*

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DICHLOROMETHANE

Dichloromethane was reviewed previously by the Working Group in 1987 and 1998 (IARC, 1987, 1999). New data have since become available, and these have been incorporated, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

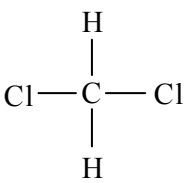
Chem. Abstr. Serv. Reg. No.: 75-09-2

Chem. Abstr. Serv. Name: Dichloromethane

IUPAC Systematic Name: Dichloromethane

Synonyms: Methane dichloride; methylene bichloride; methylene chloride; methylene dichloride

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: CH_2Cl_2

Relative molecular mass: 84.93

1.1.3 Chemical and physical properties of the pure substance

Description: Colourless liquid with penetrating ether-like odour (O'Neil et al., 2006; Haynes, 2010)

Boiling point: 40 °C

Melting point: –97.1 °C

Density: d_4^{20} 1.327 g/mL

Solubility: Slightly soluble (1.38 g/100 mL) in water at 20 °C; soluble in carbon tetrachloride; miscible in ethanol, diethyl ether, and dimethylformamide

Volatility: Vapour pressure, 58.2 kPa at 25 °C; relative vapour density (air = 1), 2.93 (Verschueren, 1996)

Stability: Vapour is nonflammable and is not explosive when mixed with air, but may form explosive mixtures in atmospheres with higher oxygen content (Sax, 1984)

Reactivity: Reacts vigorously with active metals (lithium, sodium, potassium) and with strong bases (potassium *tert*-butoxide) (Sax, 1984)

Octanol/water partition coefficient (P): log P, 1.25 (Hansch et al., 1995)

Table 1.1 Methods for the analysis of dichloromethane

Sample matrix	Sample preparation	Assay procedure[a]	Limit of detection	Reference
Air	Adsorb on charcoal; desorb with carbon disulfide	GC/FID	0.4 µg/sample	NIOSH (1998)
	Adsorb on charcoal; desorb with toluene	GC/ECD	0.002 µg/sample	
	Adsorb on charcoal; desorb with carbon disulfide	GC/FID	94 µg/m^3	OSHA (1990)
	Adsorb on carbon-based molecular sieve; desorb with 99:1 mixture of carbon disulfide/dimethylformamide in anhydrous sodium sulfate	GC/FID	697 µg/m^3	
	Air collected in specially prepared canister; desorb on cold trap	GC/MS	0.84–1.38 ppm [2.97–4.87 µg/m^3]	EPA (1999a)
		GC/ECD	NR	
		GC/FID	NR	
		GC/PID	NR	
	Analyte collected on sorbent tube; thermally desorb to GC	GC/MS	NR	EPA (1999b)
		GC/ECD	NR	
		GC/FID	NR	
		GC/PID	NR	
Water	Purge with inert gas and trap; desorb to GC	GC/PID	NR	EPA (1995a)
		GC/ECD	0.02 µg/L	EPA (2013)
		GC/MS	0.18 µg/L	EPA (2009)
		GC/MS	0.14 µg/L	
	Purge with inert gas and trap; desorb to GC	GC/MS	0.03 µg/L	EPA (1988)
	Add internal standard (isotope labelled dichloromethane); purge with inert gas and trap; desorb to GC	GC/ MS	10 µg/L	EPA (1996c)
Liquid and solid wastes	Purge with inert gas and trap	GC/PID	NR	EPA (1996b)
		GC/HECD	0.02 µg/L	
	Purge with inert gas and trap; and various other methods	GC/MS	5 µg/kg (soil/ sediment) 500 µg/kg (wastes) 5 µg/L (groundwater)	EPA (1996a)

ECD, electron capture detection; FID, flame ionization detection; GC, gas chromatography; HECD, Hall electrolytic conductivity detection; MS, mass spectrometry; NR, not reported; PID, photoionization detection

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), 1 mg/m^3 = 3.53 ppm; calculated from: mg/m^3 = (relative molecular mass/24.47) × ppm.

1.1.4 Technical products and impurities

Dichloromethane is available in several grades according to intended end use: technical grade; aerosol; vapour degreasing; special; urethane; and Food Chemicals Codex/ National Formulary (food and pharmaceutical applications). Purity, when reported, ranges from 99% to 99.99%. Acidity (as hydrochloric acid) may be up to 5 mg/kg. The maximum concentration of water in these grades of dichloromethane is 100 mg/kg (Rossberg et al., 1986; Holbrook, 1993; Dow Chemical Co, 1995; Vulcan Chemicals, 1995, 1996a, b, c, d).

Small amounts of stabilizers are often added to dichloromethane at the time of manufacture to protect against degradation by air and moisture. The following substances in the listed concentration ranges are the preferred additives

(wt%): ethanol, 0.1–0.2; methanol, 0.1–0.2; cyclohexane, 0.01–0.03; and amylene (2-methyl-2-butene), 0.001–0.01. Other substances have also been described as being effective stabilizers, including phenols (phenol, hydroquinone, *para*-cresol, resorcinol, thymol, 1-naphthol), amines, nitroalkanes (nitromethane), aliphatic and cyclic ethers, epoxides, esters, and nitriles (Rossberg et al., 1986; Holbrook, 1993).

1.1.5 Analysis

Methods for the analysis of dichloromethane in air, solids, liquids, water, and food have been reviewed by ATSDR (2000) and HSDB (2012). Selected methods for the analysis of dichloromethane in various matrices are presented in Table 1.1. Exposures to dichloromethane can also be monitored in air using a direct-reading infrared analyser, with a minimum detectable concentration of 0.7 mg/m^3 (0.2 ppm) (Goelzer & O'Neill, 1985).

Exposure to dichloromethane can be monitored in samples of blood, breath, or urine (ATSDR, 2000; WHO, 2000; SCOEL, 2009). Urinary concentrations of dichloromethane in humans are reported to correlate well with exposure concentrations in air (Di Vincenzo et al., 1972; SCOEL, 2009). The concentration of dichloromethane or carboxyhaemoglobin (COHb) levels are measured in blood (SCOEL, 2009). Since the relationship between alveolar carbon monoxide (CO) and COHb has not been well established for workers exposed to dichloromethane, breath analysis for CO cannot be considered as providing definitive quantitative information regarding exposure to dichloromethane (WHO, 2000).

1.2 Production and use

1.2.1 Production

Dichloromethane was first prepared in 1840 by the chlorination of methyl chloride in sunlight. It became an industrial chemical of importance during the Second World War (Rossberg et al., 1986). Two commercial processes are currently used for the production of dichloromethane: hydrochlorination of methanol and direct chlorination of methane (Rossberg et al., 1986; Holbrook, 1993; ATSDR, 2000).

Global production of dichloromethane increased from 93 000 tonnes in 1960 to an estimated 570 000 tonnes in 1980 (IARC, 1986), and is estimated to range from 764 000 to 814 000 tonnes per year from 2005 to 2010 (OECD/SIDS, 2011). In 2009, dichloromethane was produced by 26 manufacturers worldwide and was available from 133 suppliers (NTP, 2011). Production and imports of dichloromethane in the USA totalled 45 000–227 000 tonnes between 1996 and 2006 (NTP, 2011). In the European Union, the total tonnage band for dichloromethane was reported to be 100 000 to 1 000 000 tonnes per year (ECHA, 2016). The production and import of dichloromethane reported in Japan was 58 000 tonnes in 2011 (METI, 2013).

1.2.2 Use

Most of the applications of dichloromethane are based on its solvent properties (IARC, 1999). The principal uses worldwide comprise paint stripper (23–50%), aerosol solvents and propellants (10–25%), process solvent in the chemical and pharmaceutical industry (10–20%), and metal degreasing (8–13%) (WHO, 1996; IARC, 1999). The distribution of uses varies considerably among countries (OECD, 1994). Dichloromethane has also been used in the production of cellulose fibre, in the manufacture of photographic film, in textile manufacturing, for extraction of food flavourings and decaffeination

of coffee, as a blowing agent for polymer foams, in production of hydrofluorocarbon refrigerants, and in pesticides (OECD, 1994; IARC, 1999; NTP, 2011; EPA, 2012). Use of dichloromethane in Europe and the USA has been declining since the 1970s (Holbrook, 1993; WHO, 1996; EPA, 2012).

(a) Paint stripper

For use in paint strippers, dichloromethane is typically blended with other chemical components (Holbrook, 1993; WHO, 1996). Dichloromethane has been the major component of nearly all solvent-based paint stripper formulations for industrial, professional, and consumer use; the aircraft industry and military are important users (OECD, 1994). Alternative paint strippers have come onto the market (Joe et al., 2013), and paint-strippers containing dichloromethane are no longer permitted for professional or consumer use in Europe, although they remain available elsewhere (European Commission, 2009; Joe et al., 2013).

(b) Aerosols

Dichloromethane is used as propellant and solvent in aerosol products including paints, automotive products, adhesives, and hair sprays (WHO, 1996; ATSDR, 2000; NTP, 2011). The use of dichloromethane in consumer aerosol products has declined in the USA (ATSDR, 2000), and dichloromethane is no longer permitted for use in cosmetic products in the USA since 1989 (FDA, 1989).

(c) Process solvent

In chemical processing, dichloromethane is used in the manufacture of polycarbonate plastic, the manufacture of photoresist coatings, and as a solvent carrier for the manufacture of insecticides and herbicides. It is used by the pharmaceutical industry as a process solvent in the manufacture of steroids, antibiotics, vitamins and, to a lesser extent, as a solvent in the coating of tablets. Other uses include oil de-waxing, in inks and adhesives, and in plastics manufacture (Rossberg et al., 1986; Holbrook, 1993; IARC, 1999).

(d) Metal cleaning

In the metalworking industries, dichloromethane is used as a vapour degreasing solvent, or blended with petroleum and other hydrocarbons as a dip-type cleaner (IARC, 1999). In the manufacture of metal products, cleaning is needed before painting, plating, plastic coating, etc. Degreasing in the engineering industry is normally carried out with special equipment in which dichloromethane is used either in the liquid or vapour phase. Dichloromethane is also used in the electronics industry in the production of circuit boards and as a stripper for photoresists (OECD, 1994). In Japan and elsewhere, dichloromethane has widely been used for metal cleaning as an alternative solvent to 1,1,1-trichloroethane after the implementation of the Montreal Protocol on Substances that Deplete the Ozone Layer (OECD, 1994).

(e) Printing industry

Dichloromethane is a major ingredient of cleaning solvent used to remove printer ink during the offset printing process. For efficient manual wiping with a cloth, dichloromethane is often blended with other halogenated hydrocarbons or kerosene to adjust its evaporation rate. Almost all the dichloromethane in the solvent evaporates into the working environment. It is to be noted that offset printing is usually carried out indoors, sometimes with limited ventilation to ensure that temperature and humidity are kept constant (Kumagai et al., 2013). Offset proof printing requires frequent cleaning interventions, and offset web printing sometimes includes manual wiping under the machine, both of which lead to high concentrations of vapour in the breathing zone.

Ink for a three-dimensional printing process has been developed using a fast-drying thermoplastic solution comprising polylactic acid dissolved in dichloromethane (Guo et al., 2013).

(f) Other uses

Dichloromethane is used as feedstock in the production of hydrofluorocarbon-32 (HFC-32) refrigerant (difluoromethane). The demand for HFC-32 as a replacement chemical for HFC-22 (chlorodifluoromethane) may increase the use of dichloromethane in the USA (EPA, 2012).

1.3 Occurrence and exposure

1.3.1 Environmental occurrence

(a) Natural occurrence

Dichloromethane is not known to occur naturally.

(b) Outdoor air

Background levels from remote monitors in the USA in operation since 2003 have shown that the concentration of dichloromethane in air in isolated locations is very low (mean, 0.1 μg/m³) (McCarthy et al., 2006).

Levels of dichloromethane are higher in urban areas than in rural areas. For example, at 13 urban monitoring centres in the USA in 1996, the geometric mean concentration of dichloromethane varied from 0.05 to 0.24 ppb by volume (0.28 to 0.85 μg/m³) (Mohamed et al., 2002). In the 1990s, the concentration of dichloromethane at 22 urban sites in Canada was reported as being between 0.5 μg/m³ and 10 μg/m³ (Government of Canada, 1993).

There is also seasonal variation. In China, dichloromethane was one of the five most abundant volatile organic compounds measured in air at 14 sites in 9 cities in the south-eastern coastal region. The average concentration of dichloromethane in air was 50.2 μg/m³ in winter (range, 12.4–113 μg/m³) and 10.1 μg/m³ in summer (range, 6.3–22.8 μg/m³) (Tong et al., 2013).

Generally, the concentrations of dichloromethane in industrial areas tend to be much higher than those in residential and administrative areas. In a study of six different areas within Haicang, China, the mean levels of dichloromethane in two industrial areas were 102.0 μg/m³ and 219.1 μg/m³, in the harbour was 69.80 μg/m³, in surrounding residential and administration areas were 119.60 μg/m³ and 112.00 μg/m³, while in the background site in forests at a distance of 20 km, the level was 8.2 μg/m³ (Niu et al., 2012). Similarly, mean concentrations of dichloromethane were 42.5 μg/m³ in a biopharmaceutical plant in China and 3.5 μg/m³ in a residential area nearby (Pan et al., 2011).

(c) Indoor air

Eight-hour average concentrations of dichloromethane were measured in a range of indoor environments in China as follows: home, 1.0–1.3 μg/m³; office, 0.03 μg/m³; school, 0.1 μg/m³; restaurant, 3.3 μg/m³; shopping mall, 0.7 μg/m³; city train, 0.8 μg/m³; and bus, 0.4 μg/m³ (Guo et al., 2004).

A report from Canada quoted a study from 1988 that found that the mean concentration of dichloromethane in 757 homes was 16.3 μg/m³ (Government of Canada, 1993).

(d) Water

Dichloromethane has been detected in surface water and groundwater samples taken at hazardous waste sites and in drinking-water in Europe, the USA, Canada, and Japan. Concentrations in many water samples are below the limit of detection (ATSDR, 2000). Dichloromethane was measured in more than 5000 wells in the USA between 1985 and 2002; in 97% of samples, concentrations of dichloromethane were below maximum contaminant levels (MCLs). Dichloromethane was detected in 3% of samples, with concentrations ranging from 0.02 to 100 μg/L. These positive samples were

mainly collected in agricultural areas, which may be a result of transformation of carbon tetrachloride used as a grain fumigant (Moran et al., 2007).

A report on dichloromethane in Canada summarized a range of measurements, and found that mean concentrations of dichloromethane in municipal drinking-water supplies in Canada during the 1980s ranged from 0.2 µg/L to 2.6 µg/L (Government of Canada, 1993). Measurements in groundwater near known spills were extremely high. For example, 25 years after the rupture of a storage tank near Toronto, the measured dichloromethane in groundwater was 25 × 106 µg/L. Mean concentrations in surface water were low (generally < 1 µg/L).

(e) Food

In the 1970s, dichloromethane was detected in decaffeinated coffee and tea, with levels ranging from < 0.05 to 4.04 mg/kg in coffee, and < 0.05 to 15.9 mg/kg in tea (Page & Charbonneau, 1984). Because of concern over residual solvent, most decaffeinators no longer use dichloromethane (ATSDR, 2000).

In an investigation of several halocarbons in table-ready foods, 8 of the 19 foods examined contained dichloromethane at concentrations above the quantification limit (0.008 ppb), with the following ranges reported: butter, 1.1–280 µg/kg; margarine, 1.2–81 µg/kg; ready-to-eat cereal, 1.6–300 µg/kg; cheese, 3.9–98 µg/kg; peanut butter, 26–49 µg/kg; and highly processed foods (frozen chicken dinner, fish sticks, pot pie), 5–310 µg/kg (Heikes, 1987).

1.3.2 Occupational exposure

The principal route of exposure in occupational settings is inhalation (ATSDR, 2000).

Occupational exposure to dichloromethane may occur in several industries. Workers may be exposed during the production and processing of dichloromethane, or during use of products containing dichloromethane, particularly when the end product is sprayed or otherwise aerosolized (ATSDR, 2000).

Monitoring data for dichloromethane up to 1999 have been reviewed previously (IARC, 1999). More than 1.4 million workers in the USA and approximately 250 000 workers in Europe were estimated to be potentially exposed to dichloromethane in the 1980s and 1990s (IARC, 1999; NIOSH, 2013). Exposure occurred across a range of industries, levels varying widely by operation and within operation. Concentrations of dichloromethane exceeding 1000 mg/m^3 were recorded in paint stripping, in the printing industry, and in the manufacture of plastics and synthetic fibres. Full-shift exposures to dichloromethane at concentrations exceeding 100 mg/m^3 were thought to have occurred in furniture-stripping shops, and in certain jobs in the aeronautical, pharmaceutical, plastic, and footwear industries (IARC, 1999).

In 2012, the United States Environmental Protection Agency (EPA) reviewed available historical studies that had monitored dichloromethane concentrations in workers stripping paint (EPA, 2012). Many of the studies included a very small numbers of exposed workers, and the results may not be generalizable. Exposure levels varied widely. For example, aircraft refinishing was reported to result in 8 hour time-weighted average (TWA) exposures of 86–3802 mg/m^3 (25–1096 ppm) in different studies between 1994 and 2002. Workers stripping paint from metal, wood, or aircraft and furniture refinishing were all potentially exposed to 8 hour TWA exposures exceeding 1000 mg/m^3.

Many of the industries in the EPA report do not now use dichloromethane (see Section 1.4). Data published since 2000 are summarized in Table 1.2. Levels now tend to be lower than earlier reports, with measured values in printing, polyurethane manufacture, and automotive and aircraft maintenance tending to be lower than 150 ppm. Studies in furniture-stripping

Table 1.2 Measured occupational exposures to dichloromethane

Industry (location)	Job classification	Concentration	Reference
Printing workers (USA)	Cleaning presses	7 ppm	Lee et al. (2009)
Furniture stripping (USA)	Stripping and rinsing using tank	39–332 ppm 6 ppm (with controls installed)	Estill et al. (2002)
	Spray stripping using compressed air	44–647 ppm TWA < 2 ppm (with controls installed)	Fairfax & Grevenkamp (2007)
Automotive industry, technicians (USA)	Chemical paint stripping	26–120 ppm TWA	Enander et al. (2004)
Polyurethane manufacture (USA)	Mix and heat ingredients in oven	8 ppm TWA	Fairfax & Porter (2006)
Aircraft maintenance (Taiwan, China)	Paint stripping	4 hours average varied from 14–84 ppm	Uang et al. (2006)
Laboratory workers (Japan)	No details given	Below LOD (about 1 ppm)	Nomura et al. (2006)

LOD, limit of detection; ppm, parts per million; TWA, time-weighted average

plants showed that the installation of exposure surveillance was effective in reducing exposures to below 10 ppm (Estill et al., 2002; Fairfax & Grevenkamp, 2007).

A new concern has been identified in connection with bathtub refinishing. In 2012, the United States Occupational Safety and Health Administration identified 13 fatalities associated with stripping agents containing dichloromethane that had been investigated in nine states during 2000–2011. These deaths occurred when products containing between 60% and 100% of dichloromethane were used to refinish bathtubs in bathrooms with inadequate ventilation and without use of respiratory protective equipment. Autopsy specimens showed blood concentrations of dichloromethane ranging from 18 to 223 mg/L in the six decedents for whom values were recorded; a concentration of < 2 mg/L is expected in a person working within the allowable air standard for the USA. Air concentrations of dichloromethane associated with such work were estimated to exceed 100 000 ppm (Chester et al., 2012).

Levels of exposure to dichloromethane were estimated in a printing company in Osaka, Japan, after the identification of a cluster of cancers of the biliary tract among workers at the plant (Kumagai et al., 2013). The circumstances of exposure were quite specific in that the workers removed ink from rollers using volatile solvents between 300 and 800 times per day, and the room was poorly ventilated. There was co-exposure for several years to both dichloromethane and 1,2-dichloropropane (see the *Monograph* on 1,2-Dichloropropane in the present volume). No monitoring was undertaken at the time, so the Japanese National Institute of Occupational Safety and Health undertook a reconstruction experiment to estimate exposure concentrations on the assumption that the exposure was proportional to the amount of chemical used. Estimated values of exposure to dichloromethane in the room where proofs were printed ranged from 80 to 210 ppm (278–728 mg/m^3], with a mean of 140 ppm (486 mg/m^3) in 1991–1992 and were higher in later years (mean, 360 ppm, equal to 1249 mg/m^3) (Table 1.3). The estimated exposures in the front room were estimated to be 50 ppm (173 mg/m^3) in 1991–1993, and 130 ppm (451 mg/m^3) in 1992–1998 (Kumagai et al., 2013).

In another case series of printing workers with cholangiocarcinoma in Japan, estimated concentrations of dichloromethane were modelled for the jobs in which the cases had worked (Yamada et al., 2014). The estimated shift TWA for two of

Table 1.3 Estimated exposure to dichloromethane and 1,2-dichloropropane among printers associated with clusters of cholangiocarcinoma in Japan[a]

Location	Job classification and years	Number of workers	Estimated shift-TWA of dichloromethane (ppm)	Estimated shift-TWA of 1,2-dichlorophenol (ppm)	Reference
Osaka	Proof printing (reconstruction)	50–100	130–360	60–210	JNIOSH (2012)
	1991–1993		80–210	120–430	Kumagai et al. (2013)
	1992–1998		190–540	100–360	
	1998–2006		NR	150–670	
Miyagi	Offset web printing 1992–2011	2	NR	80–170	Yamada et al. (2014) based on government survey data
Fukuoka	Offset web printing 1970–2008	3	0–150	62–200	Yamada et al. (2014) based on government survey data
				110–5200	Kumagai (2014)
Hokkaido	Proof printing 1985–1995	2	60–180	110–240	Yamada et al. (2014) based on government survey data
Aichi	Proof printing 1984–1995	1	240–6100	–	Kumagai (2014)

[a] The Working Group noted that the upper limits of these scenarios were estimated with the worst-case scenarios.
h, hour; NR, not reported; ppm, parts per million; TWA, time-weighted average

the six workers was below 1 ppm. The other four workers were exposed to estimated shift TWAs of between 28 ppm (97 mg/m^3) and 180 ppm (620 mg/m^3). The highest levels were estimated for years before 1995. Additional details of the Japanese case-series studies are given in Section 2 of the *Monograph* on 1,2-Dichloropropane in the present volume.

1.3.3 Exposure of the general population

There are few data on exposure levels to dichloromethane of the general population. People may be exposed to dichloromethane from air, water, food, or during the use of consumer products containing dichloromethane (ATSDR, 2000). Exposure of the general population to dichloromethane may be much higher from indoor air than from outdoor air, especially from spray painting or use of other aerosols or consumer products containing dichloromethane as a solvent (ATSDR, 2000).

In the United States National Health and Nutrition Examination Survey (NHANES) study in 2003–2004, only 7 of the 1165 blood samples (0.6%) collected showed detectable levels of dichloromethane (CDC, 2009).

1.4 Regulations and guidelines

Several jurisdictions have acted to reduce the use and release of various volatile organic compounds, including dichloromethane. The California Air Resources Board was one of the first jurisdictions to regulate dichloromethane; in 1995, it limited the levels of total volatile organic compounds (VOCs) contained in aerosol coating products. Subsequent regulations prevented manufacture, sale, supply, or application of any aerosol coating product containing dichloromethane (Air Resources Board, 2001). California has also prohibited the manufacture, sale, or use of automotive cleaning and degreasing products containing dichloromethane.

In Japan, the environmental quality standards for dichloromethane state that outdoor air levels shall not exceed 0.15 mg/m³ (Ministry of the Environment Government of Japan, 2014).

A guideline value of 3 mg/m³ for 24-hour exposure is recommended by WHO. In addition, the weekly average concentration should not exceed one seventh (0.45 mg/m³) of this 24-hour guideline (WHO, 2000).

In the European Union, the VOC Solvent Emissions Directive (Directive 1999/13/EC) was implemented for new and existing installations on 31 October 2007 (European Commission, 1999). The Directive aims to reduce industrial emissions of VOCs from solvent-using activities, such as printing, surface cleaning, vehicle coating, dry cleaning, and manufacture of footwear and pharmaceutical products. Installations conducting such activities are required to comply either with emission limit values or with a reduction scheme. Reduction schemes allow the operator to reduce emissions by alternative means, such as by substituting products with a lower solvent content or changing to solvent-free production processes. The Solvents Directive was implemented in 2010 into the Industrial Emission Directive 2010/75/EU (IED).

The European Union has also restricted the use of paint strippers containing dichloromethane as of 2009 (Decision 455/2009/EC of the European Parliament amending Council Directive 76/769/EEC) as regards restrictions on the marketing and use of dichloromethane (European Commission, 2009). As noted above, dichloromethane-based paint strippers are banned for consumer and professional use. They may still be used in certain industrial applications with improved labelling and safety measures.

In the USA, the EPA National Emission Standards for Hazardous Air Pollutants (NESHAP) in 2008 adopted specific management practices to minimize emissions of dichloromethane in area sources that engage in paint stripping and spray application of coatings (EPA, 2008).

Occupational exposure limits for dichloromethane in air tend to be 50 ppm [176.5 mg/m³] over 8 hours, with United Kingdom permitting up to 100 ppm [353 mg/m³] (Table 1.4).

Table 1.4 International limit values for occupational exposure

Country	Limit value (8 hours)	
	ppm	mg/m³
Australia	50	174
Austria	50	175
Belgium	50	177
Canada, Québec	50	174
China	NR	200
Denmark	35	122
France	50	178
Germany, AGS	75	260
Hungary	NR	10
Ireland	50	174
Japan[a]	50	NR
Latvia	NR	150
New Zealand	50	174
Poland	NR	88
Singapore	50	174
Republic of Korea	50	175
Spain	50	177
Sweden	35	120
Switzerland	50	180
USA, OSHA	25	NR
United Kingdom	100	350

From GESTIS (2014)

[a] Notification on Standards for Work Environment Evaluation (No. 79 issued in 1988, amended in 2004) http://jaish.gr.jp/horei/hor1-18-2-1-2.html

AGS, Committee on Hazardous Substances (Ausschuss für Gefahrstoffe); NR, not reported; OSHA, Occupational Health and Safety Administration; ppm, parts per million

Biological monitoring regulations and recommendations

SCOEL (2009) recommended a biological monitoring limit value for dichloromethane in blood of 1 mg/L, and for dichloromethane in urine of 0.3 mg/L, both for samples collected at

the end of a working shift. These figures were considered comparable to an 8-hour limit value of 100 ppm (353 mg/m^3) for dichloromethane in air. The ACGIH recommended a Biological Exposure Index of 0.3 mg/L in urine at the end of a shift (ACGIH, 2012).

The Swiss authorities recommended a limit of 0.5 mg/L in blood (Suva, 2014). The Deutsche Forschungsgemeinschaft has provided the correspondence between concentrations in air and dichloromethane in blood (DFG, 2012).

2. Cancer in Humans

2.1 Introduction

Information about the risk of cancer associated with exposure to dichloromethane is available from cohort studies of occupational exposure among workers producing cellulose triacetate fibres and films, a cohort study of aircraft workers exposed to multiple solvents including dichloromethane, and case–control studies of several different cancers and occupational exposure to solvents. In addition, several studies have been conducted to investigate the occurrence of cancer of the liver among workers in the printing industry in Japan who were exposed to dichloromethane, 1,2-dichloropropane, and other solvents. Those studies are reviewed in the *Monograph* on 1,2-Dichloropropane in the present volume. While some other studies have been conducted in facilities where dichloromethane was mentioned as having been used (Ott et al., 1985; Shannon et al., 1988), only studies that reported estimates of association specifically for cancer and dichloromethane are reviewed here.

Only the cohort studies of cellulose-triacetate facilities provide quantitative measures of exposure to dichloromethane. While the availability of such information on exposure is the principal strength of these studies, the relatively small number of exposed workers is an important limitation. Among the case–control studies, most investigated cancers of the lymphohaematopoietic system, or cancers of the brain and central nervous system. The case–control studies typically assessed exposure to multiple solvents, including dichloromethane, in a semi-quantitative or qualitative manner, using expert judgment, job-exposure matrices or occupational titles. These studies consequently have limited ability to evaluate exposure–response patterns. However, those case–control studies that involved interviews with the subjects may provide improved ability to developed detailed work histories and account for non-occupational risk factors, to the extent those are relevant.

2.2 Occupational cohort studies of workers exposed to dichloromethane

Table 2.1 summarizes cohort studies of workers exposed to dichloromethane.

Lanes et al. (1993) conducted a cohort study of mortality among workers employed in the production of cellulose triacetate fibre in the USA who were potentially exposed to dichloromethane, extending earlier analyses by Ott et al. (1983a, b) and Lanes et al. (1990). The cohort consisted of 1271 workers employed between 1954 and 1976, and followed until 1990. Based on a combination of personal and area samples, median exposure levels (8-hour TWA) in 1977 were reported to be 140, 280, and 475 ppm [486, 971, 1650 mg/m^3] in three main work areas, but no dose–response analysis was performed. The workers had been also exposed to acetone and methanol. Standardized mortality ratios (SMRs) were elevated for cancer of the liver and biliary tract (SMR, 2.98; 95% CI, 0.81–7.63; 4 cases). Each of the deaths due to cancers of the liver and biliary tract occurred among employees with ≥ 10 years of employment and ≥ 20 years since

Table 2.1 Cohort studies on cancer and occupational exposure to dichloromethane

Reference, location, follow-up period	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Lanes et al. (1993) USA, 1954–1990	1271 (551 men and 720 women)	Workers from a plant producing cellulose triacetate fibre, employed for ≥ 3 mo in 1954–76	Malignant neoplasms	Overall	39	0.82 (0.77–1.04)	Results based on mortality records; adjusted for age, sex, race and calendar period Co-exposures: acetone, methanol
			Biliary passages and liver	Overall	4	2.98 (0.81–7.63)	
				≥ 10 yrs of employment, ≥ 20 yrs since first exposure	4	5.83 (1.59–14.9)	
			Bronchus, trachea, and lung	Overall	13	0.80 (0.43–1.37)	
Gibbs et al. (1996) USA, 1970–1989	3211 white workers (2187 men and 1024 women)	Workers from a plant producing cellulose triacetate fibre, employed for ≥ 3 mo in 1970–81	Malignant neoplasms	Men, high (350–700 ppm)	57	0.75 (0.57–0.98)	Results based on mortality records; adjusted for age, sex, race, and calendar period Co-exposures: acetone, methanol
				Women, high	5	1.09 (0.35–2.53)	
				Men, low (50–100 ppm)	64	0.91 (0.70–1.17)	
				Women, low	37	0.83 (0.58–1.14)	
				Men, none	23	0.82 (0.52–1.23)	
				Women, none	2	0.48 (0.06–1.74)	
			Biliary tract and liver (155–56)	Men, high (250–750 ppm)	1	0.81 (0.02–4.49)	
				Women, high	0	(0–374)	
				Men, low (50–100 ppm)	1	0.75 (0.02–4.20)	
				Men, none	0	(0.0–6.88)	
				Women, none	0	(0–35.50)	
			Bronchus, trachea, and lung	Men, high (250–750 ppm)	15	0.55 (0.31–0.91)	
				Women, high	2	2.29 (0.28–8.29)	

Table 2.1 (continued)

Reference, location, follow-up period	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Gibbs et al. (1996) USA, 1970–1989 (cont.)				Men, low (50–100 ppm)	20	0.78 (0.48–1.20)	
				Women, low	9	1.09 (0.50–2.07)	
				Men, none	6	0.59 (0.22–1.29)	
				Women, none	0	(0–4.92)	
			Cervix	Women, high	1	5.40 (0.14–30.10)	
				Women, low	5	3.00 (0.96–6.92)	
Hearne & Pifer (1999) USA, 1964–1994	1311 male white workers	Workers from a plant producing cellulose triacetate film, engaged for ≥ 1 yr in one of three areas in which dichloroethane was used (roll coating, doping, distilling) in 1946–70	All malignant neoplasms	Overall	93	0.88 (0.71–1.08)	Referent population (mortality) from New York, excluding New York City Co-exposures: acetone, methanol, 1,2-dichloropropane, 1,2-dichloroethane
				< 150 ppm	20	0.67 [0.41–1.03]	
				150–349 ppm	19	0.93 [0.56–1.45]	
				350–799 ppm	28	0.95 [0.63–1.37]	
				≥ 800 ppm	26	1.00 [0.65–1.47]	
			Liver and biliary ducts (155–156)	Overall	1	0.42 (0.01–2.36)	
			Lymphatic tissue, overall (200–203)	Overall	5	0.75 (0.24–1.76)	
			NHL (200 202)	Overall	2	0.49 (0.06–1.78)	
			Hodgkin disease (201)	Overall	2	1.82 (0.20–6.57)	
			Multiple myeloma (203)	Overall	1	0.68 (0.01–3.79)	
			Leukaemia (204–208)	Overall	8	2.04 (0.88–4.03)	
				< 150 ppm	2	1.61 [1.20–5.83]	
				150–349 ppm	0	0.00	
				350–799 ppm	1	0.98 [0.03–5.46]	
				≥ 800 ppm	5	5.89 [1.89–13.6]	
			Brain and other CNS	Overall	6	2.16 (0.79–4.69)	
				< 150 ppm		1.10 [0.03–6.12]	
				150–349 ppm		1.77 [0.05–9.95]	
				350–799 ppm		3.99 [0.83–11.7]	
				≥ 800 ppm		1.78 [0.05–9.95]	

Table 2.1 (continued)

Reference, location, follow-up period	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Hearne & Pifer (1999) USA, 1964–1994 (cont.)			Trachea, bronchus, and lung	Overall	27	0.75 (0.49–1.09)	
				< 150 ppm	5	0.52 [(0.17–1.21)]	
				150–349 ppm	6	0.90 [(0.33–1.96)]	
				350–799 ppm	9	0.86 [(0.57–2.37)]	
				≥ 800 ppm	7	0.77 [(0.31–1.59)]	
Tomenson (2011) England, 1946–2006	1785 male employees	Workers producing cellulose triacetate film base in 1946–88, and exposed to dichloromethane; the reference group comprised 312 male workers unexposed to dichloromethane	All cancers	All exposed	120	0.70 (0.58–0.83)	Age, calendar period Co-exposures: acetone, methanol
				< 400 ppm-yr	54	0.61 [(0.53–1.58)]	
				400–700 ppm-yr	12	0.82 [(0.54–2.59)]	
				≥ 800 ppm-yr	11	0.87 [(0.55–2.80)]	
				1000 ppm-yr	77	1.23 (0.71–2.11)	
			Biliary passages and liver (155–156)	Overall	0		
			Lymphatic and haematopoietic (200–208)	All exposed	11	0.89 (0.44–1.59)	
			Leukaemia (204–208)	All exposed	5	1.11 (0.36–2.58)	
			Brain and CNS	All exposed	8	1.83 (0.79–3.60)	
				< 400 ppm-yr	4	1.56 [(0.43–3.99)]	
				400–700 ppm-yr	2	7.21 [(0.87–26.1)]	
				≥ 800 ppm-yr	0	NR	
				1000 ppm-yr	6	1.11 (0.12–10.4)	
			Bronchus, trachea, and lung	All exposed	27	0.48 (0.31–0.69)	
				< 400 ppm-yr	10	0.35 [(0.17–0.64)]	
				400–700 ppm-yr	4	0.80 [(0.22–2.05)]	
				≥ 800 ppm-yr	1	0.24 [(0.01–1.34)]	
				1000 ppm-yr	15	1.04 (0.28–3.78)	

Table 2.1 (continued)

Reference, location, follow-up period	Total subjects	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Radican et al. (2008) USA, 1952–2000	1222 workers	Employees from Hill Air Force Base; exposure to 21 solvents and chemicals assessed by job and organization combinations	NHL (200 202 & C82-C85)	Overall, men	8	2.02 (0.76–5.42)	Age, race Internal comparison of deaths Co-exposures: several organic solvents, in particular trichloroethylene, and other occupational exposures
				Overall, women	0		
			Multiple myeloma (203 & C90)	Overall, men	7	2.58 (0.86–7.72)	
				Overall, women	0		
			Breast	Overall, women	6	2.35 (0.98–5.65)	

CI, confidence interval; CNS, central nervous system; ICD, International Classification of Diseases; mo, month; NHL, non-Hodgkin lymphoma; NR, not reported; ppm, parts per million; yr, year

first employment (SMR, 5.83; 95% CI, 1.59–14.92). Three out of these four deaths were attributed to cancer of the biliary tract, with durations of exposure to dichloromethane of < 1 to 28 years. These four cases were also observed in the initial analysis by Lanes et al. (1990) with an SMR of 5.75 (95% CI, 1.82–13.8) for cancers of the liver and biliary tract combined; the SMR estimated for cancer of the biliary tract alone was 20 (95% CI, 5.2–56) compared with a national referent population. [Although some of the subjects were also exposed to acetone and methanol, the Working Group considered these to be unlikely explanations for the observed risks because they were not known to be linked to cancer of the liver.] Results for other cancers were unremarkable; no results were reported for non-Hodgkin lymphoma (NHL).

Gibbs et al. (1996) conducted a cohort study of mortality among cellulose-triacetate fibre workers exposed to dichloromethane at a facility in the USA similar to that reported by Lanes et al. (1993). The cohort consisted of 3211 white workers who had been employed between 1970 and 1981 and followed until 1989. Comparisons were made with county mortality rates. The cohort was divided into three exposure groups; none; low (50–100 ppm [174–347 mg/m^3]) and high (350–700 ppm [1215–2430 mg/m^3]) based on the working area and exposure levels reported by Ott et al. (1983a, b). The workers had been also exposed to acetone and methanol. The risk of mortality from cancers of liver and biliary tract was not increased [SMR, 0.78; 95% CI, 0.09–2.81, for high and low exposure combined]. The two deaths in the group "liver and biliary tract cancer" were actually cancers of the biliary tract. Except for cancer of the prostate, for which there was a non-significant excess, SMRs for other cancers were < 1.0 for all exposure categories among men. The SMRs for women were based on very small numbers and were unstable. No data were reported for NHL. [The exposures observed in the studies by Lanes et al. (1993) and Gibbs et al. (1996) were higher than in other cohort studies. The proportion of cancers of the liver that occurred in the biliary tract in this study population was larger than would normally be expected (between 5% and 10% based on current data for the USA). While Gibbs et al. (1996) did not report an SMR for cancer of the biliary tract, if the value were to be computed, it might be higher than that reported for liver and biliary tract combined.]

Hearne & Pifer (1999) reported on mortality among a cohort of 1311 workers at a plant producing cellulose triacetate film base, in the USA. The cohort consisted of male workers who began working in the roll coating, or doping and distilling departments between 1946 and 1970, and were followed until 1994. Dichloromethane was introduced before the mid-1940s. Exposure to dichloromethane (8-hour TWA) was 0–520 ppm [0–1800 mg/m^3] in 1946–1965, 0–300 ppm [0–1040 mg/m^3] in 1966–1985, and 0–100 ppm [0–347 mg/m^3] in 1986–1994. Workers may have also been exposed to methanol, 1,2-dichloropropane, 1,2-dichloroethane, acetone, and benzene, but exposure levels were not reported for these agents. Malignant neoplasms with elevated SMRs were cancer of brain and central nervous system (SMR, 2.16; 95% CI, 0.79–4.69; 6 cases), leukaemia (SMR, 2.04; 95% CI, 0.88–4.03; 8 cases), and Hodgkin disease (SMR, 1.82; 95% CI, 0.20–6.57; 2 cases). Mortality from leukaemia increased with cumulative exposure among four exposure categories: for the group with the highest cumulative exposure, the SMR for leukaemia was 5.89 (95% CI, [1.89–13.6]; 5 cases) (Table 2.1). Three of the eight cases of leukaemia had also been exposed to benzene in the past. SMRs for cancer of the liver and NHL were less than unity, based on very small numbers (one and two cases, respectively). [The small numbers of exposed cases, which hampers analysis of exposure–response patterns, were an important limitation of this study.]

The above article (Hearne & Pifer, 1999) also reported on mortality among 1013 male workers who had been employed in the roll-coating department at any time between 1964 and 1970, and were followed until 1994. This superseded earlier analyses by Friedlander et al. (1978), Hearne & Friedlander (1981), Hearne et al. (1987), and Hearne et al. (1990). Because about 70% of the subjects in this cohort were also included in the larger cohort of cellulose triacetate workers, the description of this subcohort was omitted from this review.

Tomenson (2011) performed a cohort study of mortality among workers at a plant producing cellulose triacetate film base, in England. This extended earlier analyses by Tomenson et al. (1997). The cohort comprised 1785 male workers who had been employed at the site at any time between 1946 and 1988, and followed until 2006, of whom 1473 had been employed in jobs with potential exposure to dichloromethane. Exposure levels were estimated from area samples according to time period and work group. TWA exposures were estimated to range from 2 to 20 ppm [7–69 mg/m^3] before 1960, 6 to 127 ppm [21–441 mg/m^3] during the 1960s, 10 to 165 ppm [35–573 mg/m^3] during the 1970s, and 7 to 88 ppm [24–305 mg/m^3] during the 1980s Tomenson et al. (1997). The workers had been also exposed to acetone and methanol. Four exposure categories were established based on cumulative exposure, but 30% of the exposed could not be classified because employment histories were insufficiently precise. Only for cancer of the brain and central nervous system (SMR, 1.83; 95% CI, 0.79–3.60, among exposed workers) was the number of deaths more than 1.2 times that expected. No cancers of the liver were observed among exposed or unexposed workers (expected, 3.3 cases), and there was a significant deficit of cancer of the lung. Data for NHL were reported. Analysis of cumulative exposure for four cancer sites, including brain, did not show any significant trends with the level of exposure to dichloromethane. [The major weakness of this study was the small number of deaths, which limited the ability to conduct exposure–response analysis.]

Radican et al. (2008) performed a retrospective cohort study of mortality among workers at a military-aircraft maintenance facility in the USA, updating earlier studies (Spirtas et al., 1991; Blair et al., 1998). The cohort consisted of civilian employees employed between 1952 and 1956, and followed until 2000. Workers were exposed to numerous chemicals. Exposure was assessed quantitatively for trichloroethylene, and qualitatively (ever/never) to other agents including dichloromethane. The number of workers exposed to dichloromethane was 1222 (Stewart et al., 1991). Exposure to dichloromethane was associated with increased risks (hazard ratio, HR) of NHL (HR, 2.02; 95% CI, 0.76–5.42; 8 exposed cases) and multiple myeloma (HR, 2.58; 95% CI, 0.86–7.72; 7 exposed cases) for male workers, and cancer of the breast (HR, 2.35; 95% CI, 0.98–5.65; 6 exposed cases) for female workers. Results for other cancer sites in relation to dichloromethane exposure were not reported. [The strengths of this study included a large number of the subjects and a long follow-up period; however, because the primary analysis was for trichloroethylene, the exposure assessment and analysis for dichloromethane were limited.]

2.3 Case–control studies

Table 2.2 summarized case–control studies on the relationship between occupational exposure to dichloromethane and cancer.

2.3.1 Cancers of the lympho-haematopoietic system

Miligi et al. (2006) conducted a case–control study in Italy to evaluate the association between risk of lymphoma and exposure to dichloromethane and nine other organic solvents. The

Table 2.2 Case–control studies on lympho-haematopoietic cancer and exposure to dichloromethane

Reference, location, and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Miligi et al. (2006) Italy, 1991–1993	NHL, 1428 cases Controls, 1530	Population	Person-to-person interview, structured questionnaire, and industrial hygiene experts who assessed exposure to eight specific organic solvents	NHL	Very low/low	23	0.9 (0.5–1.6)	Sex, age, education and area Co-exposures: benzene, tetrachloroethylene, trichloroethylene, 1,1,1-trichloroethane OR not reported for follicular NHL, diffuse NHL, and other NHL
					Medium/high	13	1.7 (0.7–4.3) *P* for trend, 0.46	
					≤ 15 yr	8	1.4 (0.5–4.4)	
					> 15 yr	4	NR	
				Small lymphocytic NHL	Medium/high, excluding proxy respondents	8	3.2 (1.0–10.1)	
Seidler et al. (2007) Germany, 1999–2003	Malignant lymphoma, 710 cases Controls, 710	Population	Interview; exposure to eight organic solvents assessed by one industrial physician		*Exposure (ppm-yrs)*			Smoking and alcohol Co-exposure: trichloroethene, tetrachloroethylene, carbon tetrachlorine, benzene, toluene, xylene and styrene
				Lymphoma	0	681	1	
					> 0–≤ 26.3	8	0.4 (0.2–1.0)	
					> 26.3– ≤ 175	9	0.8 (0.3–1.9)	
					> 175	5	2.2 (0.4–11.6)	
							P for trend, 0.40	
				Hodgkin lymphoma	> 0–≤ 26.3	2	0.7 (0.2–3.6)	
					> 26.3–≤ 175		NR	
					> 175		NR	
				B-cell NHL	> 0–≤ 26.3	6	0.4 (0.2–1.1)	
					> 26.3–≤ 175	8	0.9 (0.3–2.3)	
					> 175	5	2.7 (0.5–14.5)	
				T-cell NHL	> 0–≤ 26.3		NR	
					> 26.3–≤ 175	1	1.2 (0.1–10.9)	
					> 175		NR	

Table 2.2 (continued)

Reference, location, and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Costantini et al. (2008) Italy, 1991–1993	Leukaemia, 586 cases Controls, 1278	Population	Person-to-person interview, structured questionnaire, and industrial hygiene experts who assessed exposure to eight specific organic solvents	Leukaemia (204–208)	Very low/low	7	0.7 (0.3–1.7)	Sex, age, education and area Co-exposures: benzene, tetrachloroethylene, trichloroethylene, 1,1,1-trichloroethane
					Medium/high	2	0.5 (0.1–2.3)	
				Acute myeloid leukaemia (205.0)	Very low/low	3	NR	
					Medium/high	0	NR	
				Chronic lymphocytic leukaemia (204.1)	Very low/low	2	0.4 (0.1–2.0)	
					Medium/high	2	1.6 (0.3–8.6)	
	Multiple myeloma, 263 cases Controls, 1100			Multiple myeloma (203)	Very low/low	4	NR	
					Medium/high	0	NR	
Gold et al. (2011) USA, 2000–2002	Multiple myeloma, 180 cases	Population	Interview and JEM	Multiple myeloma (ICD-O-2/3: 9731:9732)	Primary analysis *Ever exposed*	47	1.5 (0.9–2.3)	Age, race, study site, and years of education
					1–4 yr	9	1.2 (0.5–2.9)	
					5–11 yr	11	1.8 (0.8–4.1)	
					12–29 yr	17	1.8 (0.9–3.5)	
					30–51 yr	10	1.1 (0.5–2.6)	
					P for trend	0.35		
					Cumulative exposure score			
					1–318	7	1.2 (0.5–2.9)	
					319–2218	17	2.2 (1.1–4.6)	
					2219–7793	7	0.8 (0.3–1.9)	
					7794–57 000	14	1.6 (0.8–3.4)	
					P for trend	0.27		
					Cumulative exposure score, 10-yr lag			
					1–311	8	1.4 (0.6–3.3)	
					312–2089	12	1.6 (0.7–3.6)	
					2090–7285	10	1.2 (0.5–2.8)	
					7286–50 000	12	1.5 (0.7–3.2)	
					P for trend	0.39		

Table 2.2 (continued)

Reference, location, and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Gold et al. (2011) USA, 2000–2002 (cont.)					Secondary analysis *Ever exposed*	37	2.0 (1.2–3.2)	In secondary analyses, jobs assessed with low confidence are considered unexposed
					1–4 yr	8	2.0 (0.8–5.1)	
					5–7 yr	6	1.1 (0.4–3.1)	
					8–24 yr	13	2.7 (1.1–6.5)	
					25–47 yr	10	2.1 (0.9–5.2)	
					P for trend	0.01		
					Cumulative exposure score			
					1–102	5	1.6 (0.5–4.7)	
					103–1122	13	2.8 (1.2–6.6)	
					1123–5493	8	1.6 (0.6–3.8)	
					5494–57 000	10	2.4 (1.0–5.9)	
					P for trend	0.08		
					Cumulative exposure score, 10-yr lag			
					1–71	4	1.3 (0.4–4.4)	
					72–437	10	2.9 (1.1–7.5)	
					438–3903	9	1.9 (0.7–5.0)	
					3904–49 500	10	2.4 (1.0–6.1)	
					P for trend	0.06		

Table 2.2 (continued)

Reference, location, and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Wang et al. (2009) USA, 1996–2000	601 female cases 717 female controls	Population	Person-to person interview, structured questionnaire, and JEM	NHL	Ever	52	1.5 (1.0–2.3)	Adjusted for age, family history of haematopoietic cancer, alcohol consumption, and race Co-exposures: benzene, formaldehyde, chloroform, carbon tetrachloride, dichloroethane, trichloroethylene
					Low intensity	37	1.5 (0.9–2.4)	
					Medium high intensity	15	1.6 (0.7–3.3)	
					P for trend	0.11		
					Low probability	48	1.6 (1.0–2.4)	
					Medium high probability	4	1.2 (0.3–4.4)	
					P for trend	0.34		
					Low intensity and medium and high probability	1		
					Medium and high intensity and medium and high probability	3	0.9 (0.2–3.8)	
Barry et al. (2011) USA, 1996–2000	NHL, 518 cases Diffuse large B-cell lymphoma, 161 cases Follicular lymphoma, 119 cases Controls, 597	Population	Person-to person interview, structured questionnaire, and JEM	NHL	Ever	47	1.7 (1.1–2.7)	Women from the study by Wang et al. (2009) who provided a blood or buccal cell sample for genotyping; adjusted for age and race Co-exposures: benzene, formaldehyde, chloroform, carbon tetrachloride, dichloroethane, trichloroethylene
				Diffuse large B-cell lymphoma	Ever	33	2.10 (1.15–3.85)	
				Follicular lymphoma	Ever	19	1.27 (0.58–2.76)	
				NHL	Ever	30	4.42 (2.03–9.62)	
				Diffuse large B-cell lymphoma	Ever (with *CYP2E1* rs2070673 TT)	11	4.71 (1.85–12.0)	
				Follicular lymphoma	Ever (with *CYP2E1* rs2070673 TT)	5	2.67 (0.86–8.30)	
				NHL	Ever (with *CYP2E1* rs2070673 *TT + AA)*	13	0.80 (0.36–1.75)	
				Diffuse large B-cell lymphoma	Ever (with *CYP2E1* rs2070673 *TT + AA)*	6	1.12 (0.40–3.19)	
				Follicular lymphoma	Ever (with *CYP2E1* rs2070673 *TT + AA)*	4	0.96 (0.29–3.20)	

Table 2.2 (continued)

Reference, location, and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Christensen et al. (2013) Canada, 1979–85	215 cases 2341 controls			NHL (200 202)	Never exposed to chlorinated solvents	155	1 (reference)	Adjustment by age, census tract median income, educational attainment (yrs), ethnicity (French Canadian vs others), questionnaire respondent (self vs proxy), smoking (cigarettes-yrs) using only population controls. For bladder additionally: coffee intake, exposure to aromatic amines
					Any	3	0.6 (0.2–2.2)	
					Substantial	0	NR	

CI, confidence interval; ICD-O, International Classification of Diseases for Oncology; JEM, job-exposure matrix; NHL, non-Hodgkin lymphoma; NR, not reported; OR, odds ratio; ppm, parts per million; vs, versus; yr, year

study included 1428 cases of NHL (including 285 with small lymphocytic lymphoma, 308 with diffuse lymphoma, 100 with follicular lymphoma, and 315 with other lymphomas), and 1530 controls. Information about occupational history and other potential risk factors was obtained by in-person interview, and probability and intensity of occupational exposure to individual chemicals and chemical classes were assigned by expert assessment. Odds ratios were adjusted by area, sex, age, and education, excluding subjects with low probability of exposure. The odds ratio (OR) for NHL in the category for combined medium- and high-intensity exposure to dichloromethane was 1.7 (95% CI, 0.7–4.3; 13 cases; *P* for trend, 0.46). Among the NHL subtypes, an odds ratio for dichloromethane was reported only for small lymphocytic NHL: for medium or high exposure, the odds ratio was 3.2 (95% CI, 1.0–10.1). The study also included cases of Hodgkin lymphoma, but odds ratios for exposure to dichloromethane were not reported.

Seidler et al. (2007) conducted a case–control study to examine the relationship between malignant lymphoma and exposure to eight organic solvents including dichloromethane. The study included 710 cases (including 554 cases with B-cell NHL, 35 cases with T-cell NHL, and 1 case with combined B-cell and T-cell NHL), and 710 general-population controls matched for area, sex, and age collected from six areas in Germany. In-person interview obtained occupational history, medical history, and lifestyle. Exposure was assessed for several chlorinated solvents, with metrics of intensity, frequency, and confidence assigned by an industrial hygienist, and cumulative exposure was calculated. Odds ratios were adjusted for smoking and alcohol consumption. The odds ratio for high cumulative exposure to dichloromethane was 2.2 (95% CI, 0.4–11.6; *P* for trend, 0.40) for all lymphomas, and 2.7 (95% CI, 0.5–14.5; *P* for trend, 0.29) for B-cell NHL.

Costantini et al. (2008) conducted a case–control study of 586 cases of leukaemia and 1278 controls from seven areas in Italy, to evaluate the risks associated with exposure to ten organic solvents including dichloromethane. In-person interviews obtained occupational history, other exposures to chemicals, and other potential risk factors. Exposure was assessed by expert rating to assign metrics of probability and intensity of exposure to several solvents. Subjects with a low probability of exposure were excluded from the analysis and odds ratios were adjusted by area, sex, age, and education. No associations between acute leukaemia or myeloma and dichloromethane were seen. Four cases of chronic lymphocytic leukaemia (now classified as a type of NHL) were observed, with a non-significant odds ratio of < 1 for very low/low exposure, and an odds ratio of 1.6 (95% CI, 0.3–8.6) for medium/high exposure.

Gold et al. (2011) conducted a case–control study to evaluate the associations between risk of multiple myeloma and exposure to dichloromethane and other chlorinated solvents. During 2000–2002, 180 cases were collected from Seattle–Puget Sound region of Washington and Detroit metropolitan area of Michigan in the USA and 481 controls were collected from the general population in the same areas. In-person interviews obtained occupational history and additional job-specific modules were applied when solvent exposure was likely. Exposure metrics of probability, frequency, intensity, confidence, and cumulative exposure were assigned using a job-exposure matrix. Odds ratios were adjusted by area, race, sex, age, and education. Ever-exposure to dichloromethane entailed elevated risk of multiple myeloma (OR, 1.5; 95% CI, 0.9–2.3). Significant trends with exposure duration were observed when occupations that had low confidence scores were included in the unexposed category: the odds ratio for ever exposure was 2.0 (95% CI, 1.2–3.2) and odds ratios of 2.7 (95% CI, 1.1–6.5), and 2.1 (95% CI, 0.9–5.2), were observed for workers employed for 12–29 years

and 30–51 years, respectively (*P* for trend, 0.01). No such trend was seen for cumulative exposure.

Wang et al. (2009) conducted a case–control study to examine the association between NHL and exposure to nine organic solvents including dichloromethane. The study included 601 female cases, and 717 controls, matched for age, collected from the general population in Connecticut, USA. Information about occupational history and other potential risk factors was obtained by in-person interview and probability and intensity of exposure to solvents were assigned using a previously developed job-exposure matrix. Odds ratios were adjusted by race, age, family history of haematopoietic cancer, and alcohol consumption. Subjects ever-exposed to dichloromethane had an increased risk of NHL (OR, 1.5; 95% CI, 1.0–2.3). Analyses by intensity and probability of exposure indicated elevated ORs, but trends were not statistically significant.

Barry et al. (2011) conducted a further study in a subset of the population studied by Wang et al. (2009) to evaluate whether genetic variation in four genes involved in metabolism (*CYP2E1*, *EPHX1*, *NQO1*, *MPO*) modifies associations between exposure to organic solvents and risk of NHL or five major histological subtypes of NHLL (diffuse large B-cell lymphoma, follicular lymphoma, chronic lymphocytic leukaemia/small lymphocytic lymphoma, marginal zone lymphoma, and T-cell lymphoma). Ever-exposure to dichloromethane entailed elevated risk of NHL (OR, 1.69; 95% CI, 1.06–2.69). The risk associated with ever-exposure to dichloromethane was higher (OR, 4.42; 95% CI, 2.03–9.62) among women with the TT genotype for *CYP2E1* rs2070673. In contrast, no effects with dichloromethane was observed among women with the TA or AA genotype (OR, 0.80; 95% CI, 0.36–1.75). Similar patterns were observed for diffuse large B-cell lymphoma and follicular lymphoma. No interactions with other single-nucleotide polymorphisms (SNPs) in the studied genes, including *CYP2E1*, *EPHX1*, *NQO1*, or *MPO*, were statistically significant. [The Working Group noted that the functional role of the CYP2E1 polymorphism is unclear.]

2.3.2 Cancers of brain and central nervous system

See Table 2.3

Heineman et al. (1994) examined associations between astrocytic cancer of the brain and exposure to six chlorinated solvents including dichloromethane in a study of 300 men who died from astrocytic cancer of the brain in Louisiana and Pennsylvania, USA, and 320 men who died from other causes not associated with occupational exposure to chlorinated hydrocarbons. Information including occupational history and risk factors for cancer of the brain was obtained by interview of next-of-kin and exposure estimates were assigned using a job-exposure matrix. After adjusting for age at death and study area, significant trends in risk were observed with increasing probability and intensity of exposure, as well as with increasing exposure duration and cumulative exposure when the probability of exposure was high. [The reliability of the exposure assessment was judged to be relatively low because occupational information was obtained from the next of kin.]

Cocco et al. (1999) conducted a case–control study to examine associations between mortality from the cancer of the brain and other parts of central nervous system and exposure to 11 factors including dichloromethane. Cases were 12 980 women who died due to cancer of central nervous system in 24 states of the USA. Controls were 51 920 randomly selected women who died from non-malignant diseases, excluding neurological disorders. Probability and intensity of exposure were assigned using occupation and industry titles from subjects' death certificates and a job-exposure matrix. After adjusting for age at death, marital status, and socioeconomic status, the odds ratio for the association of exposure to

Table 2.3 Case–control studies of cancer of the brain and central nervous system and exposure to dichloromethane

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Heineman et al. (1994) Louisiana, New Jersey, and Philadelphia, USA, 1979–81	Cases, 300 white men from death certificates Controls, 320 white men	Death certificates from men who died from causes other than brain cancer	Next-of-kin interview and JEM	Brain or other CNS (ICD-9 191, 192, 225, 239.7)	All, ever	108	1.3 (0.9–1.8)	Age, study area Covariates: organic solvents, carbon tetrachloride, methyl chloroform, tetrachloroethylene, trichloroethylene
					Low probability, ever	71	1.0 (0.7–1.6)	
					Medium probability, ever	21	1.6 (0.8–3.0)	
					High probability, ever	10	2.4 (1.0–5.9)	
					P trend < 0.05			
					All, 2–20 yrs	80	1.2 (0.8–1.7)	
					Low probability, 2–20 yr	49	1.0 (0.6–1.6)	
					Medium probability, 2–20 yr	22	1.5 (0.7–3.2)	
					High probability, 2–20 yr	9	1.8 (0.6–6.0)	
					All 21+ yr	24	1.7 (0.9–3.6)	
					Low probability 21+ yr	12	1.2 (0.5–3.0)	
					Medium probability 21+ yr	4	1.5 (0.3–9.0)	
					High probability 21+ yr	8	6.1 (1.1–43.8)	
					P trend < 0.01 for duration (high probability)			
					All, low cumulative exposure	37	0.9 (0.5–1.5)	
					Low probability, low cumulative exposure	24	0.7 (0.4–1.3)	

Table 2.3 (continued)

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Heineman et al. (1994) Louisiana, New Jersey, and Philadelphia, USA, 1979–81 (cont.)					Medium probability, low cumulative exposure	9	1.3 (0.4–3.8)	
					High probability, low cumulative exposure	4	2.0 (0.3–16.7)	
					All, medium cumulative exposure	48	1.9 (1.1–3.2)	
					Low probability, medium cumulative exposure	29	1.6 (0.8–3.0)	
					Medium probability, medium cumulative exposure	13	2.3 (0.8–7.0)	
					High probability, medium cumulative exposure	6	4.2 (0.7–31.4)	
					All, high cumulative exposure	19	1.2 (0.6–2.5)	
					Low probability, high cumulative exposure	8	0.9 (0.3–2.5)	
					Medium probability, high cumulative exposure	4	0.9 (0.2–4.0)	

Table 2.3 (continued)

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Heineman et al. (1994) Louisiana, New Jersey, and Philadelphia, USA, 1979–81 (cont.)					High probability, high cumulative exposure	7	2.5 (0.6–11.0)	
					P trend < 0.05 for cumulative exposure (high probability)			
					Low-medium average intensity, total	76	1.1 (0.7–1.6)	
					High intensity, total	28	2.2 (1.1–4.4)	
Cocco et al. (1999) 24 states in USA, 1984–92	Cases, 12 980 women Controls, 51 920 women who died from non-malignant diseases	Death certificates	Usual occupation or industry from death certificate and JEM	Brain and other CNS (191, 192)	Any	867	1.2 (1.2–1.3)	State, race Co-exposures: electromagnetic fields, solvents, chlorinated aliphatic hydrocarbons, benzene, lead, nitrosamines, polyaromatic hydrocarbons, insecticides and fungicides, herbicides, contact with the public
					Low probability	756	1.2 (1.1–1.3)	
					Medium probability	83	1.2 (1.0–1.6)	
					High probability	28	1.3 (0.9–1.3)	
					Low intensity	370	1.3 (1.1–1.5)	
					Medium intensity	345	1.2 (1.1–1.4)	
					High intensity	152	1.0 (0.8–1.2)	
				Meningioma	Any	13	1.2 (0.7–2.2)	
De Roos et al. (2001) USA and Canada, 1 May 1992, and 30 April 1994	Cases, 538 from hospitals in the USA and Canada	Population controls from random-digit dialling	Self-reported exposure by parents and review by industrial hygienists	Neuroblastoma	Self-reported by parent (paternal exposure)	10	0.7 (0.3–1.6)	Adjusted for child's age, maternal race, maternal age, and maternal education
					Industrial hygienists reviewed exposure	4	0.7 (0.2–0.8)	

Table 2.3 (continued)

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Neta et al. (2012) Arizona, Massachusetts and Pennsylvania, USA, 1994–98	Cases, 489 glioma, 197 meningioma, Controls, 799	Hospital	Personal interviews and expert assessment	Glioma or other neuroepitheliomatous neoplasm (ICD-O-2 9380–9473 and 9490–9506)	Possible, all	126	0.8 (0.6–1.1)	Age group, race sex, hospital site and proximity of residence to hospital
					Probable, all	21	0.5 (0.3–0.9)	
					Possible, men	90	0.7 (0.5–1.0)	
					Probable, men	16	0.4 (0.2–0.8)	
					Possible, women	36	1.1 (0.7–1.1)	
					Probable, women	5	1.0 (0.3–2.9)	
					Unexposed, all	534	1	
					Years exposed, low	9	0.4 (0.2–0.8)	
					Years exposed, high	12	0.7 (0.3–1.4)	
					Cumulative low	11	0.5 (0.2–1.0)	
					Cumulative high	10	0.5 (0.2–1.1)	
					Average weekly exposure low	15	0.7 (0.3–1.3)	
					Average weekly exposure high	6	0.3 (0.1–0.8)	
					Highest exposure low	12	0.5 (0.3–1.1)	
					Highest exposure high	9	0.5 (0.2–1.0)	
				Meningioma (ICD-O-2 9530–9538) or acoustic neuroma (ICD-O-2 9560)	Possible	42	1.6 (0.7–3.5)	Age, race, sex, hospital and proximity to hospital and all other solvents
					Probable	8	0.8 (0.2–3.0)	

Table 2.3 (continued)

Reference, study location and period	Total cases Total controls	Control source (hospital, population)	Exposure assessment	Organ site (ICD code)	Exposure categories	Exposed cases	Relative risk (95% CI)	Covariates and comments
Ruder et al. (2013) Iowa, Michigan, Minnesota, Wisconsin, USA, 1995–97	798 cases 1175 controls	Population	Personal interview and industrial hygienist evaluation	Glioma (9380–948)	All	304	0.80 (0.66–0.97)	Age, education, sex
					Men	222	0.88 (0.69–1.13)	
					Women	82	0.69 (0.50–0.95)	
					Cumulative exposure (ppm-yrs), including unexposed participants, including proxy-only interviews	798	0.98 (0.97–0.99)	
					Cumulative exposure (ppm-yrs), excluding unexposed participants, including proxy-only interviews	304	0.96 (0.89–1.03)	

CI, confidence interval; CNS, central nervous system; ICD-O, International Classification of Diseases for Oncology; JEM, job-exposure matrix; NHL, non-Hodgkin lymphoma; NR, not reported; OR, odds ratio; ppm, parts per million; yr, year

dichloromethane and all cancer of the central nervous system was 1.2 (95% CI, 1.1–1.3). Odds ratios were generally similar for all categories of probability and intensity of exposure. [Because this study, like others using similar methods, assessed exposure from occupational information from death certificates, the specificity for dichloromethane was poor.]

De Roos et al. (2001) analysed occupations of 405 case fathers and 302 control fathers to identify paternal occupational exposures associated with an increased risk of cancer of the brain in children. When considering paternal exposure to dichloromethane as assessed by an industrial hygienist, the odds ratio for neuroblastoma was 0.70 (95% CI, 0.2–2.8; 4 exposed cases; adjusted by age, maternal race, maternal age, and maternal education).

Neta et al. (2012) conducted a hospital-based case–control study to examine associations between glioma and meningioma and exposure to six chlorinated solvents including dichloromethane. Cases were 484 patients with glioma and 197 patients with meningioma diagnosed in Massachusetts, Pennsylvania, and Arizona, USA. Controls were 797 patients admitted to the same hospitals for non-malignant conditions and were frequency-matched to cases by sex, age, race, hospital, and proximity to the hospital. Exposure to solvents was assessed by an industrial hygienist based on detailed occupational histories collected by interview. Odds ratios adjusted for the matching factors did not show any association between glioma or meningioma and overall exposure to dichloromethane or other metrics, including duration, intensity, and cumulative exposure.

Ruder et al. (2013) conducted a population-based case–control study to examine associations between glioma and exposure to six chlorinated solvents including dichloromethane. Cases were 798 patients with intracranial glioma in Iowa, Michigan, Minnesota, and Wisconsin, USA, and controls were 1175 residents selected from the same area. Lifetime occupational histories were obtained by interview and several exposure metrics were assigned by an industrial hygienist. Odds ratios adjusted for the frequency-matching variables (age group and sex), and for age and education. There were no associations between glioma and overall exposure to dichloromethane, or exposure probability and cumulative exposure.

In a multicentre case–control study of meningioma conducted in seven countries (INTEROCC) and including 1906 cases and 5565 controls, there were no subjects classified as exposed to dichloromethane after assessment of lifetime occupational histories using a modified version of the Finnish national job-exposure matrix (INTEROCC-JEM) (McLean et al., 2014).

2.3.3 Other cancer sites

The Working Group also reviewed case–control studies on dichloromethane and several other cancer sites. These included a case–control study on cancer at many sites (Christensen et al., 2013), and studies on cancer of the breast (Cantor et al., 1995), pancreas (Kernan et al., 1999), kidney (Dosemeci et al., 1999), and lung (Vizcaya et al., 2013). However, no remarkable excess of cancer was reported in these studies and the evidence for these cancer sites was regarded as inadequate.

2.3.4 Case report from Japan

In a case report, Kumagai (2014) described two cases of cholangiocarcinoma in workers employed in two different printing plants in Japan. One of the two cases had been exposed to dichloromethane and 1,1,1-trichloroethane, and the other had been exposed to 1,2-dichloropropane.

2.4 Meta-analysis

Liu et al. (2013) conducted a meta-analysis to examine the relationship between occupational exposure to dichloromethane and risk of cancer, with a focus on NHL and multiple myeloma. However, the population for one of the included studies on NHL was a subset of another (Wang et al., 2009; Barry et al., 2011) and one potentially informative study on multiple myeloma (Costantini et al., 2008) was not reviewed. The meta-analysis was consequently not considered further.

3. Cancer in Experimental Animals

The carcinogenicity of dichloromethane in experimental animals was reviewed previously by the Working Group (IARC, 1999).

3.1 Mouse

There were six studies of carcinogenicity with dichloromethane in mice (dichloromethane was administered orally in two studies, by inhalation in three studies, and by intraperitoneal injection in one study).

See Table 3.1

3.1.1 Oral administration

Groups of 50–200 male and female B6C3F$_1$ mice (age, 7 weeks) were given drinking-water containing dichloromethane (purity, 99%) at a dose of 0 (first control group), 0 (second control group), 50, 125, 185, or 250 mg/kg body weight (bw) per day for 104 weeks (Serota et al., 1986a). Two vehicle-control groups were run simultaneously. No significant exposure-related trend in survival was found in males; in females, a significant trend towards longer survival in exposed groups was reported. In male mice, there was an increased incidence of hepatocellular carcinoma at the highest dose compared with the first control group. A dose-related increase in the incidence of hepatocellular adenoma or carcinoma (combined) was also observed.

Groups of 50 male and 50 female Swiss mice (age, 9 weeks) were given dichloromethane (purity, > 99.9%) at a dose of 100 or 500 mg/kg bw in olive oil by gavage once per day, for 4 or 5 days per week, for 64 weeks (Maltoni et al., 1988). Groups of 60 male and 60 female mice were given olive oil only (vehicle controls). The mice were then kept under observation for their lifespan. Excess mortality was observed in male and female mice exposed to dichloromethane at the lowest and the highest dose. An increase in mortality appeared after week 36 of treatment and led to withdrawal of the treatment at week 64. In mice that died by week 78, the incidence of pulmonary adenoma or adenocarcinoma (combined) was significantly increased in the group at the highest dose. At the end of the experiment, the cumulative incidences of pulmonary adenoma or carcinoma (combined) in males were 5/50, 5/50, and 9/50. No treatment-related increase in the incidence of any tumour type in females, or of any type of tumour other than pulmonary in males was reported. [The Working Group noted the short period of exposure and the high numbers of animals lost due to mortality and thus not available for examination at the end of the experiment.]

3.1.2 Inhalation

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 8–9 weeks) were exposed to dichloromethane (purity, > 99%) at concentrations of 0, 2000, or 4000 ppm (0, 6940 or 13 900 mg/m^3) by whole-body inhalation for 6 hours per day, 5 days per week, for up to 102 weeks and were killed after 104 weeks (NTP, 1986). The final body weights of male mice at the highest dose and of female mice at the lowest and highest dose were 10–17% lower than those of the respective

Table 3.1 Studies of carcinogenicity with dichloromethane in mice

Reference Strain (sex) Duration	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Serota et al. (1986a) B6C3F$_1$ (M) 24 mo	Drinking-water 0, 0, 60, 125, 185, 250 mg/kg bw per day in deionized drinking-water continuously for 104 wk. Controls received deionized water 60–200 mice/group	Hepatocellular adenoma: 6/60 (10%), 4/65 (6%), 20/200 (10%), 14/100 (14%), 14/99 (14%), 15/125 (12%) Hepatocellular carcinoma: 5/60 (8%), 9/65 (14%), 33/200 (17%), 18/100 (18%), 17/99 (17%), 23/125 (18%)* Hepatocellular adenoma or carcinoma (combined): 11/60 (18%), 13/65 (20%), 51/200 (26%), 30/100 (30%), 31/99 (31%), 35/125 (28%)	NS[a] *P = 0.0114 (250 mg/kg vs control 1) NS	Purity, 99% Two vehicle -control groups were run concurrently No significant exposure-related trend in survival. Historical controls for hepatocellular adenoma or carcinoma (combined): mean, 32.1%; range, 7–58%
Serota et al. (1986a) B6C3F$_1$ (F) 24 mo	Drinking-water 0, 0, 60, 125, 185, 250 mg/kg bw per day in deionized drinking-water continuously for 104 wk. Controls received deionized water 50–100 mice/group	NR	NS	Purity, 99% Two vehicle-control groups were run concurrently Significant trend towards longer survival
Maltoni et al. (1988) Swiss (M) Lifetime	0, 100, 500 mg/kg bw by gavage in olive oil, once per day, 4–5 days/wk, for 64 wk Kept under observation for lifespan 60 or 50 mice/group	Pulmonary adenomas or adenocarcinomas (combined) in mice that died at 78 weeks: 1/14 (7%), 4/21 (19%), 7/24 (29%)* Pulmonary adenomas or adenocarcinomas (combined) at end of experiment: 5/50 (10%), 5/50 (10%), 9/50 (18%)	*P < 0.05	Purity, 99.9% Excess mortality (P < 0.01) was observed in male mice exposed to the lowest and highest dose Histopathology of tumours not further specified
Maltoni et al. (1988) Swiss (F) Lifetime	0, 100, 500 mg/kg bw by gavage in olive oil, once per day, 4–5 days/wk, for 64 wk 60 or 50 mice/group	NR	NS	Purity, 99.9% Excess mortality was observed in female mice at the lowest and highest dose
NTP (1986) B6C3F$_1$ (M) 24 mo	0, 2000, 4000 ppm by inhalation, 6 h/day, 5 days/wk for 102 wk 50 mice/group	Bronchiolo-alveolar adenoma: 3/50 (6%)*, 19/50 (38%)**, 24/50 (48%)** Bronchiolo-alveolar carcinoma: 2/50 (4%)*, 10/50 (20%)***, 28/50 (56%)** Hepatocellular adenoma: 10/50 (20%), 14/49 (29%), 14/49 (29%) Hepatocellular carcinoma: 13/50 (26%), 15/49 31%), 26/49 (53%)*** Hepatocellular adenoma or carcinoma (combined): 22/50 (44%)*, 24/49 (49%), 33/49 (67%)***	*P < 0.001 (trend)[a] **P < 0.001 ***P < 0.05	Purity, 99%, Survival: 78%, 48%, 22%, 40%

Table 3.1 (continued)

Reference Strain (sex) Duration	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
NTP (1986) B6C3F$_1$ (F) 24 mo	0, 2000, 4000 ppm by inhalation, 6 h/day, 5 days/wk for 102 wk 50 mice/group	Bronchiolo alveolar adenoma: 2/50 (4%)*, 23/48 (48%)**, 28/48 (58%)** Bronchiolo alveolar carcinoma: 1/50 (2%)*, 13/48 (26%)**, 29/48 (58%)** Hepatocellular adenoma: 2/50 (4%)*, 6/48 (13%), 22/48 (46%)** Hepatocellular carcinoma: 1/50 (2%)*, 11/48 (23%)***, 32/48 (67%)** Hepatocellular adenoma or carcinoma (combined): 3/50 (6%)*, 16/48 (33%)***, 40/48 (83%)**	*$P < 0.001$ (trend)[a] **$P < 0.001$ ***$P < 0.004$	Purity, 99%, Survival: 50%, 50%, 16%, 40%
Kari et al. (1993) B6C3F$_1$ (F) 24 mo	Inhalation, 6 h/days, 5 days/wk: 0 ppm, for 104 wk 2000 ppm, 26 wk/0 ppm, 78 wk 0 ppm, 78 wk/2000 ppm, 26 wk 2000 ppm, 52 wk/0 ppm, 52 wk 0 ppm, 52 wk/2000 ppm, 52 wk 2000 ppm, 78 wk/0 ppm, 26 wk 0 ppm, 26 wk/2000 ppm, 78 wk 2000 ppm, 104 wk 68 mice/group	Bronchiolo alveolar adenoma: 1/67 (1%), 8/68 (12%), 0/67, 12/63 (19%), 5/67 (7%), 19/68 (28%), 7/67 (10%), 18/67 (27%) Bronchiolo alveolar carcinoma: 4/67 (6%), 17/68 (25%), 3/67 (4%), 36/63 (57%), 6/67 (9%), 25/68 (37%), 7/67 (10%), 31/67 (46%) Bronchiolo alveolar adenoma or carcinoma (combined): 5/67 (7%), 21/68 (31%)*, 3/67 (4%), 40/63 (63%)*, 10/67 (15%), 38/68 (56%)*, 13/67 (19%)**, 42/67 (63%)* Hepatocellular adenoma: 8/67 (12%), 16/68 (24%), 16/67 (24%), 14/64 (22%), 9/67 (13%), 28/68 (41%), 17/67 (25%), 24/68 (35%) Hepatocellular carcinoma: 11/67 (16%), 14/67 (21%), 13/67 (19%), 18/64 (28%), 12/67 (18%), 25/68 (37%), 20/67 (30%), 35/68 (51%) Hepatocellular adenoma or carcinoma (combined): 18/67 (27%), 27/67 (40%), 23/67 (34%), 28/64 (44%)**, 21/67 (31%), 42/68 (62%)*, 32/67 (48%)**, 47/68 (69%)*	*$P < 0.01$[b] **$P < 0.05$	Purity, > 99% Survival: 59%, 47%, 54%, 34%, 59%, 35%, 47%, 40% Histopathological examination of the lung and liver only Statistical analysis applied to combined incidence only

Table 3.1 (continued)

Reference Strain (sex) Duration	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
JBRC (2000a), Aiso et al. (2014) Crj:BDF$_1$ (M) 24 mo	0, 1000, 2000, 4000 ppm by inhalation, 6 h/day, 5 days/wk, for 104 wk 50 mice/group	Bronchiolo alveolar adenoma: 7/50 (14%)*, 3/50 (6%), 4/50 (8%), 14/50 (28%) Bronchiolo-alveolar carcinoma: 1/50 (2%)*, 14/50 (28%)**, 22/50 (44%)**, 39/50 (78%)** Bronchiolo-alveolar adenoma or carcinoma (combined): 8/50 (16%)*, 17/50 (34%)***, 26/50 (52%)**, 42/50 (84%)** Hepatocellular adenoma: 10/50 (20%)*, 13/50 (26%), 14/50 (28%), 15/50 (30%) Hepatocellular carcinoma: 10/50 (20%)*, 9/50 (18%), 14/50 (28%), 20/50 (40%)*** Hepatocellular adenoma or carcinoma or hepatoblastoma (combined): 15/50 (30%)*, 20/50 (40%), 25/50 (50%)***, 29/50 (58%)*** Liver haemangioma: 0/50, 4/50 (8%), 3/50 (6%), 5/50 (10%)*** Adrenal gland pheochromocytoma: 1/50 (2%)****, 0/50, 1/50 ((2%), 3/50 (6%) Haemangioma (all organs): 1/50 (2%)****, 5/50 (10%), 6/50 (12%), 7/50 (14%)***	*$P < 0.001$ (trend)[c] **$P < 0.001$ ***$P < 0.05$ ****$P < 0.05$ (trend)	Purity, 99.9% Survival: 76%, 70%, 52%, 40% (statistical analysis, NR) The incidence of haemangioma (all organs) in males at the highest dose did not exceed the upper limit of the historical controls of the laboratory
JBRC (2000a), Aiso et al. (2014) Crj:BDF$_1$ (F) 24 mo	0, 1000, 2000, 4000 ppm by inhalation, 6 h/day, 5 days/wk 50 mice/group	Bronchiolo-alveolar adenoma: 2/50 (4%), 4/50 (8%), 5/49 (10%), 12/50 (24%)** Bronchiolo-alveolar carcinoma: 3/50 (6%)*, 1/50 (2%), 8/49 (16%), 20/50 (40%)** Bronchiolo-alveolar adenoma or carcinoma (combined): 5/50 (10%)*, 5/50 (12%), 12/49 (24%)***, 30/50 (60%)**	*$P < 0.001$ (trend)[c] **$P < 0.001$ ***$P < 0.05$ ****$P < 0.01$ (trend)	Purity, 99.9% Survival: 52%, 52%, 34%, 42% (statistical analysis, NR)

Table 3.1 (continued)

Reference Strain (sex) Duration	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
JBRC (2000a), Aiso et al. (2014) Crj:BDF$_1$ (F) 24 mo (cont.)		Hepatocellular adenoma: 1/50 (2%)*, 7/49 (9%)***, 4/49 (8%), 16/50 (32%)** Hepatocellular carcinoma: 1/50 (2%)*, 1/49 (2%), 5/49 (10%), 19/50 (38%)** Hepatocellular adenoma or carcinoma (combined): 2/50 (4%)*, 8/49 (16%)***, 9/49 (18%)***, 30/50 (60%)** Liver haemangioma or haemangiosarcoma (combined): 3/50 (6%)****, 2/49 (4%), 0/49, 7/50 (14%)		
Theiss et al. (1977) A/St (M) 24 wk	0, 160, 400, 800 mg/kg bw by intraperitoneal injection, 3 × per wk; 24, 17, 17 or 16 times 50 or 20 mice/group	Multiplicity of bronchiolo-alveolar tumours: 0.27, 0.94, 0.80, 0.50	NS	Purity, > 95% No tumour incidence provided Histopathological examination of the lung only. Full histopathology not performed Survival: 47/50, 18/20, 5/20, 12/20

[a] Incidental tumour test
[b] Likelihood ratio score test
[c] Peto test, Fisher exact test
bw, body weight; F, female; h, hour; M, male; mo, month; NR, not reported; NS, not significant; ppm, parts per million; wk, week

controls. The survival of exposed male rats was comparable to that of the controls. The survival of exposed male mice and of female mice at the highest dose was reduced relative to that of the controls. The incidences of bronchiolo-alveolar adenoma and of bronchiolo-alveolar carcinoma were significantly increased in exposed males and females. The incidence of hepatocellular adenoma was significantly increased in females at the highest dose, and the incidence of hepatocellular carcinoma was significantly increased in males and females at the highest dose.

Groups of 68 female B6C3F$_1$ mice (age, 8–9 weeks) were given dichloromethane (purity, > 99%) at a concentration of 0 ppm (control) or 2000 ppm [6940 mg/m^3] by whole-body inhalation for 6 hours per day on 5 days per week for various lengths of time over a 104-week period (Kari et al., 1993). Only the lung and liver were evaluated histopathologically. Survival was reduced compared with controls in groups exposed to dichloromethane for 52, 78, or 104 weeks. The incidences of bronchiolo-alveolar adenoma, bronchiolo-alveolar carcinoma, and adenoma or carcinoma (combined), and the incidences of hepatocellular adenoma, hepatocellular carcinoma, and adenoma or carcinoma (combined) were significantly increased in all groups in which exposure was begun during the first 26 weeks of the study. [The Working Group noted that statistical analyses were reported only for the combined tumour incidences.]

Groups of 50 male and 49 or 50 female Crj:BDF$_1$ mice (age, 6 weeks) were exposed to dichloromethane (purity, > 99.9%) at a concentration of 0, 1000, 2000, or 4000 ppm [0, 3470, 6940, or 13 900 mg/m^3] by whole-body inhalation for 6 hours per day on 5 days per week for up to 104 weeks (JBRC, 2000a; Aiso et al., 2014). Survival rates and body weights of both males and females exposed to 2000 and 4000 ppm were decreased [no statistical analysis reported]. The incidences of bronchiolo-alveolar carcinoma were significantly increased in exposed males and females. The incidences of bronchiolo-alveolar adenoma or carcinoma (combined) were significantly increased in exposed males and females. The incidences of hepatocellular carcinoma were significantly increased in males and females at the highest dose. The incidence of hepatocellular carcinoma, hepatoblastoma, or hepatocellular adenoma (combined) was significantly increased in exposed males, and the incidence of hepatocellular adenoma or carcinoma (combined) was significantly increased in females at the highest dose. The incidence of liver haemangioma was increased in males at the highest dose. The incidence of liver haemangioma or haemangiosarcoma (combined) was significantly increased in females at the highest dose. In males, the incidence of pheochromocytoma of the adrenal gland was increased with a positive trend. Hyperplasia in the terminal bronchiole of the lung [this lesion may be classified as a preneoplastic lesion capable of developing into bronchiolo-alveolar adenoma and carcinoma] and peripheral vacuolar change in the liver were increased in males and females at 4000 ppm.

3.1.3 Intraperitoneal injection

In a screening assay based on the production of bronchiolo-alveolar adenoma in strain A mice, groups of 20 male mice (age, 6–8 weeks), were given reagent-grade dichloromethane (purity, > 95%; impurities unspecified) at a dose of 0, 160, 400, or 800 mg/kg bw in tricaprylin by intraperitoneal injection three times per week for a total of 16–17 injections (total doses, 2720, 6800, and 12 800 mg/kg bw in the treated groups, respectively) (Theiss et al., 1977). After 24 weeks, 18, 5, and 12 mice were still alive in the three treated groups, respectively; these and 15 out of 20 surviving mice in the vehicle-control group were killed. Lungs were examined for macroscopic nodules. No significant increase was found in the multiplicity of bronchiolo-alveolar adenoma in exposed mice. [The Working Group

noted that histopathology was not performed on all of those nodules, and multiplicity was the only type of data reported in this study.]

3.2 Rat

There were seven studies of carcinogenicity with dichloromethane in rats (dichloromethane was administered orally in two studies, and by inhalation in five studies).

See Table 3.2

3.2.1 Oral administration

Groups of 25–85 male and female Fischer 344 rats, (age, 7 weeks) were given drinking-water containing dichloromethane (purity, 99%) at a dose of 0 (control group 1), 0 (control group 2), 5, 50, 125, 250 (highest dose), or 250 (recovery group) mg/kg bw per day for 104 weeks (Serota et al., 1986b). Interim terminations were carried out at 26, 52, and 78 weeks in control group 1 and in the groups at the lowest, intermediate, and highest dose, such that 50 males and 50 females per group received treatment for 104 weeks. There was no significant difference in survival between the exposed and control groups. In females, the incidence of hepatocellular carcinoma after 104 weeks was: 0/85, 0/50, 0/85, 2/83, 0/85, 2/85, and 0/25; the incidence of neoplastic nodules [hepatocellular adenomas] was: 0/85, 0/50, 1/85, 2/83, 1/85, 4/85, and 2/25; and the incidence of neoplastic nodules [hepatocellular adenomas] or hepatocellular carcinoma (combined) was: 0/85, 0/50, 1/85, 4/83, 1/85, 6/85, and 2/25 in the seven groups, respectively. This increasing trend was statistically significant (the recovery group was excluded). In male rats, no increased incidence of hepatocellular tumours was observed at 104 weeks. No other significant increase in tumour incidence was found.

Groups of 50 male and 50 female Sprague-Dawley rats (age, 12 weeks), were given dichloromethane (purity, > 99.9%) at a dose of 100 or 500 mg/kg bw in olive oil by gavage once per day, 4 or 5 days per week, for 64 weeks (Maltoni et al., 1988). A group of 50 males and 50 females was given olive oil only (vehicle controls) and additional groups of 20 males and 26 females were kept untreated (controls). The rats were then kept under observation for their lifespan. Excess mortality was observed in male and female rats given dichloromethane at the highest dose. An increase in mortality started to appear after 36 weeks of treatment and led to cessation of exposure after 64 weeks [details on mortality not reported]. There was no significant increase in tumour incidence associated with exposure. [The Working Group noted the short period of treatment and the inadequate reporting of the data.]

3.2.2 Inhalation

Groups of approximately 95 male and 95 female Sprague-Dawley rats (age, 8 weeks) were given dichloromethane (purity, > 99%) at a concentration of 0, 500, 1500, or 3500 ppm [0, 1740, 5200, or 12 100 mg/m^3] by whole-body inhalation for 6 hours per day, 5 days per week, for 104 weeks (Burek et al., 1984; EPA, 1985). The numbers of rats per group still alive at the end of the study were 14, 14, 6, 7 for males, and 21, 24, 13, 4 for females, respectively. From the 18th month onwards, the mortality among females at the highest dose was significantly increased. There was no significant increase in the incidence of benign or malignant tumours of the mammary gland; however, the total number of benign tumours of the mammary gland [type not specified] showed a small dose-related increase in males, and a dose-related increase in females [statistics not reported]. The incidence of sarcoma located around the salivary glands was increased in males at the highest dose (1/92, 0/95, 5/95, and 11/97). [The Working Group noted the reported occurrence of sialodacryoadenitis of the salivary

Table 3.2 Studies of carcinogenicity with dichloromethane in rats

Reference Strain (sex) Duration	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Serota et al. (1986b) F344 (M) 24 mo	0 (control), 0 (control), 5, 50, 125, 250 (highest dose), 250 (recovery group) mg/kg bw per day in drinking-water for 104 wk 50–85 rats/group	Liver neoplastic nodules [hepatocellular adenoma]: 4/85 (5%), 5/50 (10%), 2/85 (2%), 3/84 (3%), 3/85 (3%), 1/85 (1%), 4/25 (16%) Hepatocellular carcinoma: 2/85 (2%), 2/50 (4%), 0/85, 0/84, 1/85 (1%), 0/25 Liver neoplastic nodules [hepatocellular adenoma or hepatocellular carcinoma (combined)]: 6/85 (7%), 7/50 (14%), 2/85 (2%), 3/84 (3%), 3/85 (3%), 2/85 (2%), 4/25 (16%)	NS[a]	Purity, 99% Two vehicle-control groups were run concurrently. No significant exposure-related trend in survival was found in males. The recovery group was exposed for 78 wk
Serota et al. (1986b) F344 (F) 24 mo	0 (control), 0 (control), 5, 50, 125, 250 (highest dose), 250 (recovery group) mg/kg bw per day in drinking-water for 104 wk 25–85 rats/group	Liver, neoplastic nodules [hepatocellular adenoma]: 0/85, 0/50, 1/85 (1%), 2/83 (2%), 1/85 (1%), 4/85 (4%), 2/25 (8%) Hepatocellular carcinoma: 0/85, 0/50, 0/85, 2/83 (2%), 0/85, 2/85 (2%), 0/25 Liver, neoplastic nodules [hepatocellular adenoma] or hepatocellular carcinoma (combined): 0/85*, 0/50, 1/85 (1%), 4/83 (5%)**, 1/85 (1%), 6/85 (7%)**, 2/25 (8%)**	NS[a] NS *$P = 0.0041$ (trend) **$P \leq 0.05$	Purity, 99% Two vehicle-control groups were run concurrently. No significant exposure-related trend in survival was found in females. The recovery group was exposed for 78 wk Incidences within the range of historical controls
Maltoni et al. (1988) Sprague-Dawley (M) Lifetime	0 (untreated control), 0 (olive oil), 100, 500 mg/kg bw by gavage in olive oil, 4–5 days/wk, for 64 wk 20 or 50 rats/group	No significant differences in tumour incidence between control and treated rats	NS	Purity, 99.9% Excess mortality was observed in male rats at the highest dose ($P < 0.01$) [The period of treatment was short and reporting of data was inadequate]
Maltoni et al. (1988) Sprague-Dawley (F) Lifetime	0 (untreated control), 0 (olive oil), 100, 500 mg/kg bw, by gavage in olive oil, 4–5 days/wk for 64 wk 26 or 50 rats/group	No significant differences in tumour incidence between control and treated rats	NS	Purity, 99.9% Excess mortality was observed in female rats at the highest dose [The period of treatment was short and reporting of data was inadequate]
Burek et al. (1984), EPA (1985) Sprague-Dawley (M) 24 mo	0, 500, 1500, 3500 ppm, by inhalation, for 6 h/day, 5 days/wk, for 104 wk 92–97 rats/group	Salivary gland sarcoma: 1/92 (1%), 0/95, 5/95 (5%), 11/97 (11%)* Total number of benign mammary gland tumours: 8, 6, 11, 17	*$P = 0.002$[b] NR	Purity, > 99% No exposure-related effect on mortality

Table 3.2 (continued)

Reference Strain (sex) Duration	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Burek et al. (1984), EPA (1985) Sprague-Dawley (F) 24 mo	0, 500, 1500, 3500 ppm, by inhalation, 6 h/day, 5 days/wk, for 104 wk 95–97 rats/group	Total number of benign mammary gland tumours: 165, 218, 245, 287	NR	Purity, > 99% Mortality among females at the highest dose was significantly increased
NTP (1986) F344 (M) 24 mo	0, 1000, 2000, 4000 ppm, by inhalation, 6 h/day, 5 days/wk, for 102 wk 50 rats/group	Mammary gland adenoma or fibroadenoma (combined): 0/50*, 0/50, 2/50 (4%), 5/50 (10%)** Subcutis, fibroma or sarcoma (combined): 1/50 (2%)***, 1/50 (2%), 2/50 (4%), 5/50 (10%)	*$P < 0.001$ (trend)[c] **$P = 0.023$ ***$P = 0.026$ (trend)	Purity, 99% Survival: 32%, 32%, 34%, 18%
NTP (1986) F344 (F) 24 mo	0, 1000, 2000, 4000 ppm, by inhalation, 6 h/day, 5 days/wk, for 102 wk 50 rats/group	Mammary gland adenoma or fibroadenoma (combined): 5/50 (10%), 11/50 (22%), 13/50 (26%), 23/50 (26%)	$P < 0.001$ (trend)[c] $P < 0.001$ (high dose) $P < 0.05$ (mid-dose) $P < 0.05$ (low dose)	Purity, 99% Survival: 60%, 44%, 44%, 30%
Maltoni et al. (1988) Sprague-Dawley (F) Lifetime	0, 100 ppm, by inhalation, 4 h/day, 5 days/wk, for 7 wk, then 7 h/day, 5 days/wk, for 97 wk Start at age 13 wk (breeders) 60, 54 rats/group	No significant differences in tumour incidence between control and treated rats	NS	Purity, 99.9% No excess in mortality was found in the exposed group [Low exposure concentration and inadequate reporting of data]
Maltoni et al. (1988) Sprague-Dawley (M) Lifetime	0, 60 ppm, by inhalation, 4 h/day, 5 days/wk, for 7 wk, then 7 h/day, 5 days/wk, for 97 wk; or 7 h/day, 5 days/wk, for 8 wk Start at day 12 of gestation 158 or 60 rats/group	No significant differences in tumour incidence between control and treated rats	NS	Purity. 99.9% No excess in mortality was found in the exposed groups [Low exposure concentration and inadequate reporting of data]
Maltoni et al. (1988) Sprague-Dawley (F) Lifetime	0, 60 ppm, by inhalation, 4 h/day, 5 days/wk for 7 wk, then 7 h/day, 5 days/wk for 97 wk or 7 h/day, 5 days/wk for 8 wk Start at day 12 of gestation 149, 69 rats/group	No significant differences in tumour incidence between control and treated rats	NS	Purity, 99.9% No excess in mortality was found in the exposed groups [Low exposure concentration and inadequate reporting of data]

Table 3.2 (continued)

Reference Strain (sex) Duration	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Nitschke et al. (1988) Sprague-Dawley (M) 20 mo	0, 50, 200, 500 ppm by inhalation, 6 h/day, 5 days/wk 90 rats/group	No significant differences in tumour incidence between control and treated rats	NS	Purity, > 99.5% No exposure-related adverse effect on body weight or mortality was observed
Nitschke et al. (1988) Sprague-Dawley (F) 24 mo	0, 50, 200, 500 ppm, by inhalation, 6 h/day, 5 days/wk 108 rats/group Fifth group: 500 ppm for 12 mo, then to 0 ppm for 12 mo, 30 rats/group Sixth group: 0 ppm for 12 mo, then to 500 ppm for 12 mo, 30 rats/group	Mammary gland adenoma or fibroadenoma: 52/70 (74%), 58/70 (82%), 61/70 (71%)*, 55/70 (78%), 23/30 (77%), 23/30 (77%)	*$P < 0.05$[b]	Purity, > 99.5% No exposure-related adverse effect on body weight or mortality was observed
JBRC (2000b), Aiso et al. (2014) F344/DuCrj (M) 24 mo	0, 1000, 2000, 4000 ppm, by inhalation, 6 h/day, 5 days/wk, for 104 wk 50 rats/group	Subcutis fibroma: 1/50 (2%), 4/50 (8%), 7/50 (14%), 12/50 (24%) Mammary gland fibroadenoma: 1/50 (2%), 2/50 (4%), 3/50 (6%), 8/50 (16%) Peritoneal mesothelioma: 3/50 (6%), 1/50 (2%), 0/50, 7/50 (14%)	$P < 0.001$ (trend), $P < 0.001$ (high dose), $P < 0.05$ (mid-dose)[d] $P < 0.001$ (trend), $P < 0.05$ (high dose)[b] $P < 0.05$ (trend)[d]	Purity, 99.9% Survival: 64%, 86%, 76%, 56%
JBRC (2000b), Aiso et al. (2014) F344/DuCrj (F) 24 mo	0, 1000, 2000, 4000 ppm, by inhalation, 6 h/days, 5 days/wk, for 104 wk 50 rats/group	Mammary gland fibroadenoma: 7/50 (14%), 7/50 (14%), 9/50 (18%), 14/50 (28%)	$P < 0.01$ (trend)[d]	Purity, 99.9% Survival: 90%, 80%, 86%, 60%

[a] Cochran-Armitage, χ^2 test
[b] Fisher exact test
[c] Incidental tumour test
[d] Peto test, Fisher exact test
bw, body weight; F, female; h, hour; M, male; mo, month; NR, not reported; NS, not significant; ppm, parts per million; wk, week

gland early in the study. The effect of this viral infection on tumour formation is unknown.]

Groups of 50 male and 50 female Fischer 344/N rats (age, 7–8 weeks) were exposed to dichloromethane (purity, 99%) at a concentration of 0, 1000, 2000, or 4000 ppm (0, 3470, 6940, or 13 900 mg/m^3) by whole-body inhalation for 6 hours per day, 5 days per week, for 102 weeks and were killed after 104 weeks (NTP, 1986). Mean body weights of control and dosed males and females were similar throughout the study. Survival of treated males was similar to that of controls. Survival at termination of the study was reduced in females at the highest dose compared with controls. Significantly increased incidences of benign tumours of the mammary gland (all fibroadenoma, except for one adenoma in the group at the highest dose) were observed in treated females (5/50, 11/50, 13/50, 23/50). In males, there was a positive trend in the incidences of adenoma or fibroadenoma (combined) of the mammary gland, and of fibroma or sarcoma (combined) of the subcutis. There was no difference in the distribution of other types of tumours in the control and treated groups.

Groups of 54–70 male and female Sprague-Dawley rats (age, 13 weeks), were given dichloromethane (purity, > 99.9%) at a concentration of 100 ppm [347 mg/m^3] or 60 ppm [208 mg/m^3] by whole-body inhalation for 7 hours per day, 5 days per week (Maltoni et al., 1988). The exposure was started in female breeders, and male and female offspring (12-day embryos). The breeders and a first group of offspring were exposed for 104 weeks, and a second group of offspring was exposed for 15 weeks only. Control groups were composed of 60 female rats (untreated breeders controls), and 158 males and 149 females (untreated offspring controls). The rats were observed for their lifespan. No excess in mortality was found in the exposed groups. No significant increase in the incidence of any tumour type was noted. [The Working Group noted the low concentration of exposure.]

Groups of 90 male and 108 female Sprague-Dawley rats [age unspecified] were given dichloromethane (technical-grade; purity, > 99.5%) at a concentration of 0, 50, 200, or 500 ppm [0, 174, 694, or 1740 mg/m^3] by whole-body inhalation for 6 hours per day, 5 days per week, for 20 (males) or 24 (females) months (Nitschke et al., 1988). An additional group of 30 female rats was exposed to dichloromethane at 500 ppm for the first 12 months and to room air for the last 12 months of the study. An additional group of 30 female rats was exposed to room air for the first 12 months, followed by dichloromethane at 500 ppm for the last 12 months of the study. Subgroups of five rats per sex per exposure level were scheduled for interim termination after 6, 12, 15, or 18 months of exposure to dichloromethane. No exposure-related adverse effect on body weight or mortality was observed. In females, the incidences of benign tumours of the mammary gland (adenomas and fibroadenomas, combined) were 52/70, 58/70, 61/70 [significant increase], and 55/70 in the control group, and the groups at the lowest, intermediate, and highest dose, respectively. No significant increase in the incidence of any other tumour type was seen in the exposed groups. There was no significant increase in the incidence of any tumour type in males.

Groups of 50 male and 50 female F344/DuCrj rats (age, 6 weeks) were exposed to dichloromethane (purity, 99.9%) at concentrations of 0, 1000, 2000, or 4000 ppm (0, 3470, 6940, or 13 900 mg/m^3) by whole-body inhalation for 6 hours per day, 5 days per week, for 104 weeks (JBRC, 2000b; Aiso et al., 2014). Survival rates of females exposed to dichloromethane at 4000 ppm were decreased compared with the controls [no statistical analysis reported]. The incidence of fibroma of the subcutis was significantly increased in exposed males. The incidence of fibroadenoma of the mammary gland was significantly increased in males at the highest dose and with a positive trend in females. The incidence

Table 3.3 Studies of carcinogenicity with dichloromethane in hamsters

Reference Strain (sex) Duration	Route, dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Burek et al. (1984), EPA (1985) Syrian golden (Ela:Eng) (M) 24 mo	0, 500, 1500, 3500 ppm, by inhalation, 6 h/day, 5 days/wk, for 104 wk 95 hamsters/group	No significant differences in tumour incidence between control and treated hamsters	NS	Purity, > 99%
Burek et al. (1984), EPA (1985) Syrian golden (Ela:Eng) (F) 24 mo	0, 500, 1500, 3500 ppm, by inhalation, 6 h/day, 5 days/wk, for 104 wk 95 hamsters/group	Lymphosarcoma [malignant lymphoma]: 1/91 (1%), 6/92 (6%), 3/91 (3%), 7/91 (8%)*	$P < 0.05$[a]	Purity, > 99% Survival at the end of experiment: 0, 4, 10, 9

[a] Fischer exact test
F, female; h, hour; M, male; mo, month; NS, not significant; ppm, parts per million; wk, week

of peritoneal mesothelioma was significantly increased with a positive trend in males.

3.3 Hamster

There was one study of carcinogenicity in hamsters treated with dichloromethane by inhalation.

See Table 3.3

Groups of 95 male and 95 female Syrian golden hamsters (*Mesocricetus auratus*) (age, 8 weeks), were given dichloromethane (purity, > 99%) at a concentration of 0, 500, 1500, or 3500 ppm (0, 1740, 5200, or 12 100 mg/m^3) by whole-body inhalation for 6 hours per day, 5 days per week, for 104 weeks (Burek et al., 1984; EPA, 1985). The numbers of hamsters surviving to the end of the study were 16, 20, 11, and 14 in males, and 0, 4, 10, and 9 in females. The incidence of lymphosarcoma [malignant lymphoma] was significantly higher in females at the highest dose than in controls (1/91, 6/92, 3/91, and 7/91). [The Working Group noted that the higher survival in treated hamsters may have contributed to this non-dose-dependent result for which historical control data were not available.]

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

4.1.1 Absorption

(a) Humans

Dichloromethane is a lipophilic solvent of low relative molecular mass, which can readily cross biological membranes. Pulmonary uptake is rapid, approaching steady state within a few hours after the start of exposure (Riley et al., 1966; DiVincenzo et al., 1971, 1972; Astrand et al., 1975; DiVincenzo & Kaplan, 1981). Measured values of pulmonary uptake are about 55–75% at rest and 30–40% during physical exercise (Astrand et al., 1975; DiVincenzo & Kaplan, 1981). The blood:air partition coefficient for dichloromethane describes the ratio of the concentrations in the two media at steady state, and is a factor in determining pulmonary uptake. The partition coefficient has been measured in vitro using vial equilibrium methods. Mean reported values range from around 8 to 10 for humans (Sato & Nakajima, 1979; Gargas et al., 1989; Meulenberg & Vijverberg, 2000). However, these data might have been influenced

by the presence of glutathione *S*-transferase T1 (GSTT1) in human erythrocytes (Schröder et al., 1996).

Data on oral absorption in humans are limited to case reports of accidental ingestion, and suggest that dichloromethane is also readily absorbed by this route of exposure (Hughes & Tracey, 1993; Vetro et al., 2012). Quantitative estimates of oral bioavailability in humans are not available because the ingested amounts are not known precisely.

Ursin et al. (1995) report that the permeability of human skin to dichloromethane is 24 g/m^2 per hour. No other information on human dermal absorption of dichloromethane was available to the Working Group.

(b) Experimental systems

Inhalation studies in experimental animals provide clear evidence that dichloromethane is readily absorbed via the lungs into the systemic circulation (Carlsson & Hultengren, 1975; Anders & Sunram, 1982; McKenna et al., 1982; Andersen et al., 1991). The blood:air partition coefficient for dichloromethane, measured in vitro using vial equilibrium methods, has been reported to range from 19 to 23 for rodents (Gargas et al., 1989; Marino et al., 2006).

Absorption from the gut after oral doses is rapid and nearly complete, according to reports of several studies with radiolabel in mice and rats (McKenna & Zempel, 1981; Angelo et al., 1986a, b). For instance, Angelo et al. (1986b) reported that on average 97% of the radiolabel was recovered in expired air as dichloromethane, CO, and carbon dioxide (CO_2) in the 24 hours after each repeated oral dose of 50 or 200 mg/kg per day in rats. Angelo et al. (1986a) reported absorption in mice to be more rapid (but equally extensive) with an aqueous vehicle than with an oil-based vehicle, consistent with studies on other chlorinated solvents.

No studies of dermal uptake of dichloromethane in experimental animals were available to the Working Group.

4.1.2 Distribution and body burden

(a) Humans

Once absorbed, dichloromethane enters blood circulation and undergoes rapid systemic distribution to tissues. The highest concentrations are expected in adipose tissue and other fatty tissues, due to the lipophilicity of the compound. Engström & Bjurström (1977) detected dichloromethane in fat biopsy specimens obtained from men exposed to dichloromethane for 1 hour during light exercise. Other data in humans on tissue distribution in vivo are limited to tissues taken from autopsies after accidental fatalities, which showed wide systemic distribution in blood and across all tested tissues, including the fat, lung, liver, heart, kidney, spleen, and brain (Moskowitz & Shapiro, 1952; Winek et al., 1981; Shinomiya & Shinomiya, 1985; Manno et al., 1989; Leikin et al., 1990; Kim et al., 1996; Goullé et al., 1999). Goullé et al. (1999) and Leikin et al. (1990) measured the largest number of tissues, and found the highest concentrations in brain, spleen, and fat.

Engström & Bjurström (1977) also measured an in-vitro partition coefficient of 51 between adipose tissue and air using a vial equilibrium method. This value is about five times the blood:air partition coefficient, consistent with the lipophilicity of dichloromethane. Partition-coefficient measurements for other human tissues were not available to the Working Group.

(b) Experimental systems

Studies in experimental animals provide clear evidence that dichloromethane distributes widely to all tissues of the body. After in-vivo oral and/or intravenous exposures in mice and/or rats, dichloromethane has been measured in the liver, kidney, lung, and whole carcass, with

the highest concentrations in the liver (Angelo et al., 1986a, b). Several inhalation experiments with radiolabeled dichloromethane detected the presence of radiolabel in all tissues, including the liver, kidney, adrenals, brain, fat, lung, muscle, and testes (Carlsson & Hultengren, 1975; McKenna et al., 1982). While part of the radiolabel is likely to be metabolites, it is likely that a substantial portion also represents dichloromethane. Experiments in animals show that dichloromethane readily crosses the blood–brain barrier and the placenta (Savolainen et al., 1981; Anders & Sunram, 1982).

Tissue:air partition coefficients have also been measured in vitro for several tissues in rats and mice, including fat, liver, muscle, skin, kidney, and brain (Andersen et al., 1987; Gargas et al., 1989; Clewell et al., 1993). The highest reported values are for fat (60–120), with values for the remaining tissues ranging from 8 to 40, as compared with blood:air partition coefficients of around 20.

4.1.3 Metabolism

(a) Overview

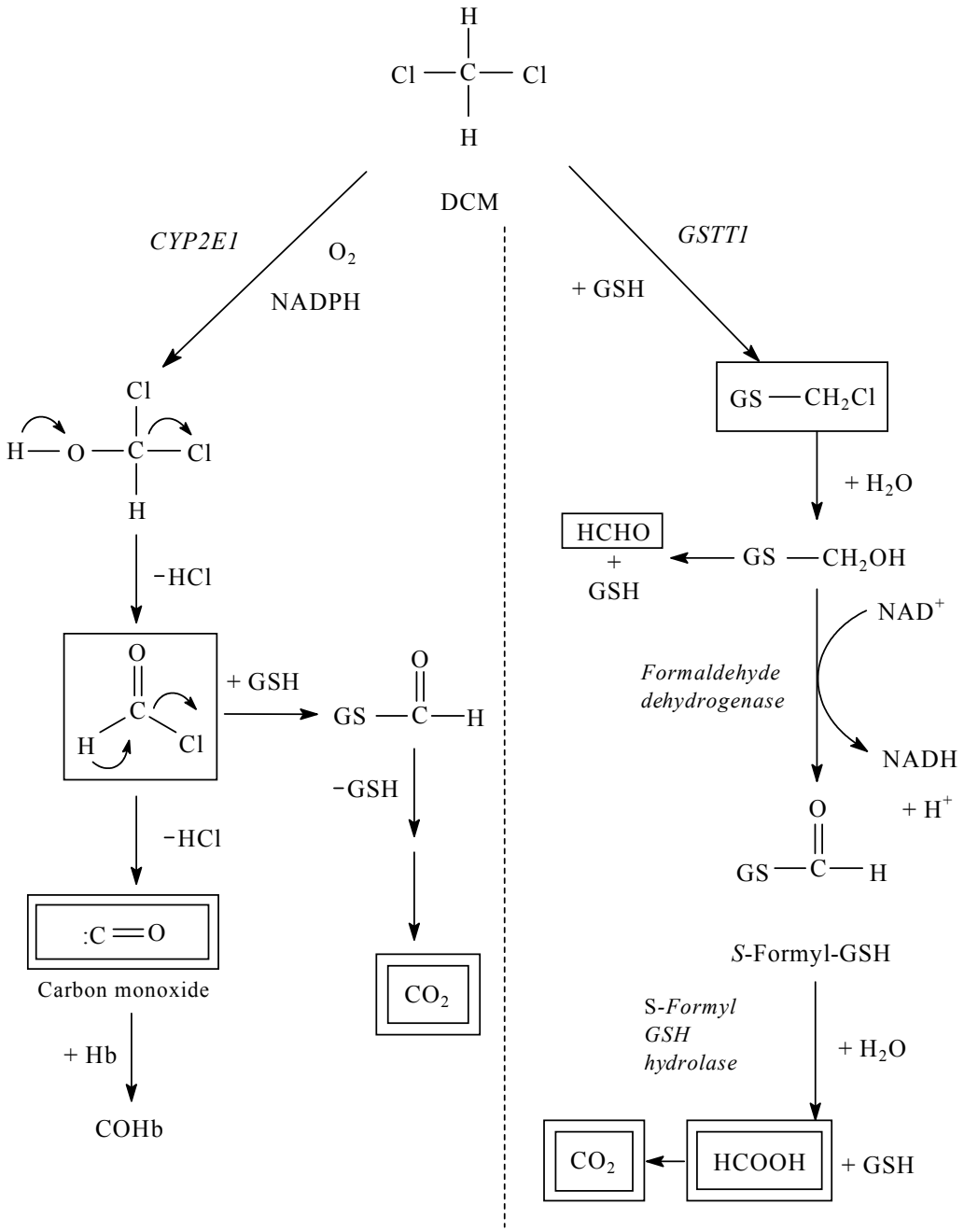
The pathways for metabolism of dichloromethane were initially characterized nearly 40 years ago in the mid-1970s and are widely considered to be well established (Kubic & Anders, 1975, 1978; Ahmed & Anders, 1976, 1978). Dichloromethane is metabolized by either of two pathways, as summarized in Fig. 4.1.

One pathway, a reductive dehalogenation that is a mixed-function oxygenation, was subsequently shown to be catalysed by cytochrome P450 2E1 (CYP2E1) (Guengerich et al., 1991), and ultimately generates CO and CO_2 as stable end products. The initial product of the reaction, chloromethanol, spontaneously rearranges to form formyl chloride, which is reactive and can spontaneously generate CO or react with nucleophiles such as glutathione (GSH) to generate formylglutathione; the latter rearranges to release CO_2. CO avidly reacts with haemoglobin, displacing oxygen and forming COHb.

The other pathway for dichloromethane metabolism involves conjugation with GSH, forming *S*-chloromethyl GSH. The conjugation is catalysed by GSTs, with the GSTT1 isoform being the most active (Mainwaring et al., 1996; Sherratt et al., 1997). *S*-Chloromethyl GSH is reactive and is believed to be one of the dichloromethane metabolites responsible for DNA binding and mutagenicity (Graves & Green, 1996). Alternatively, *S*-chloromethyl GSH can be hydrolysed to form hydroxymethyl GSH, which can either decompose to release formaldehyde or be oxidized by formaldehyde dehydrogenase to form *S*-formyl GSH. The latter is subsequently hydrolysed to release formic acid and GSH. Formic acid further decomposes to release CO_2. Thus, while both the CYP and GST pathways can generate CO_2, only the CYP pathway produces CO from dichloromethane. Although both pathways can generate reactive and unstable metabolites that are mechanistically linked to dichloromethane-induced genotoxicity and carcinogenesis, it is thought that these come primarily from the GST pathway (Andersen et al., 1987).

Despite the wealth of data over more than three decades from in-vivo and in-vitro studies in humans and experimental animals, which supports the function of both CYP2E1 and GSTT in dichloromethane metabolism, Evans & Caldwell (2010a) proposed a different explanation for dichloromethane metabolism that involves only CYP2E1. As precedent for this alternative metabolic pathway, the authors cited studies by Harrelson and colleagues (Harrelson et al., 2007, 2008) and Tracy (2006) and two studies by Guengerich and colleagues (Watanabe & Guengerich, 2006; Watanabe et al., 2007). Their conclusion was that the available data support a limited role for GST-dependent metabolism. Anders et al. (2010) criticized this proposal by noting that the authors misinterpreted the data from Watanabe & Guengerich (2006) and

Fig. 4.1 Pathways for the metabolism of dichloromethane



Dichloromethane is metabolized by both cytochrome P450 (CYP) and glutathione (GSH) conjugation. Reaction products surrounded by single rectangles are chemically reactive and are believed to be mechanistically linked to dichloromethane-induced mutagenesis and carcinogenesis. Reaction products surrounded by double rectangles are stable end products

CoHb, carboxyhaemoglobin; CDNB, 1-chloro-2,4-dinitrobenzene; CYP, cytochrome P450; CYP2E1, cytochrome P450 2E1; DCM, dichloromethane; GSH, glutathione; GSTT1, glutathione S-transferase theta-1; Hb, haemoglobin; MOA, mode of action; PBPK, physiologically based pharmacokinetic

Based on Ahmed & Anders (1978) and Kubic et al. (1974)

Adapted from Andersen et al. (1987) with permission from Toxicology and applied pharmacology, Vol 87, Andersen ME, Clewell HJ 3rd, Gargas ML, Smith FA, Reitz RH, Physiologically based pharmacokinetics and the risk assessment process for methylene chloride, Page Nos 185–205, Copyright Elsevier (1987)

Watanabe et al. (2007), for the limited in-vitro data supporting the alternative mechanism, for dismissing the wealth of data on the role of GSTT in dichloromethane metabolism, mutagenicity, and carcinogenicity, and for rejecting without any sound basis the several well-established and validated physiologically based pharmacokinetic models of dichloromethane metabolism in humans and rodents. Although Evans & Caldwell (2010b) maintained the validity of their interpretations, no data supporting metabolism of dichloromethane that exclude GST, particularly at higher dichloromethane concentrations, were identified by the Working Group.

Specific studies on dichloromethane metabolism and mechanisms in humans and human-derived tissues and in experimental systems are summarized below.

(b) Humans or human-derived tissues

Oxidative metabolism of dichloromethane to CO was first demonstrated in occupationally exposed humans (Stewart et al., 1972a, b; Ratney et al., 1974; Astrand et al., 1975). DiVincenzo & Kaplan (1981) measured dichloromethane metabolism using COHb in nonsmoking volunteers exposed to dichloromethane vapour at concentrations of up to 200 ppm for 7.5 hours (once, or daily for 5 days). Dose-dependent COHb formation was readily demonstrated, with the single-day exposures resulting in peak CoHb saturations of 1.9%, 3.4%, 5.3%, and 6.8%, respectively, at 0, 50, 100, and 200 ppm. A comparative study of the effects in humans exposed to either CO or dichloromethane up to concentrations that produce 5% COHb saturation was performed; both substances impaired performance (Putz et al., 1979), this was consistent with evidence that about 70% of dichloromethane at relatively low doses is metabolized to CO (Andersen et al., 1991).

Metabolic parameter estimates made by Clewell (1995) show that the oxidative pathway in human liver has a capacity of 100- to 200-fold that of the GST pathway, although in-vitro studies by Reitz et al. (1989) generally showed a much more modest difference in capacity of the two pathways, with the CYP pathway having a two- to fourfold higher capacity than the GST pathway in most of the human liver samples studied.

Bogaards et al. (1993) measured GST activity with dichloromethane and 1-chloro-2,4-dinitrobenzene (CDNB) in nine human liver cytosol samples, finding three distinct activity groups. Specifically, with dichloromethane, two exhibited no detectable activity, four exhibited relatively low activity (0.2–0.4 nmol/min per mg protein), and three exhibited relatively high activity (0.9–1.1 nmol/min per mg protein). Interestingly, although metabolic activity with CDNB as substrate also exhibited an approximately five-fold variation among the nine samples, there were no apparent null variants and the pattern of metabolism with CDNB and dichloromethane did not coincide. While CDNB is a substrate for multiple GST isoforms (Habig et al., 1974), it is now widely accepted that dichloromethane is selectively metabolized by GSTT (see below).

Mainwaring et al. (1996) determined mRNA and protein expression of GSTT1 in cells from human liver and lung, both of which are target organs for dichloromethane in the mouse. While expression of GSTT1 was readily detected in the liver, very low levels were detected in the lungs. Furthermore, GSTT1 activity with dichloromethane was measured in three samples of lung at 0.06, 0.21, and 0.23 nmol/min per mg protein, which was about one order of magnitude less than that in human liver.

Casanova et al. (1997) detected RNA–formaldehyde adducts in human hepatocytes with functional GST genes and incubated with dichloromethane, which is evidence that formaldehyde is formed in human cells as a metabolite of dichloromethane.

GST activity in human liver was further related to carcinogenic risk with dichloro-

methane in studies of GSTT1 polymorphism (El-Masri et al., 1999; Sherratt et al., 2002; Olvera-Bello et al., 2010). Although the importance of genetic polymorphisms in determining carcinogenic risk is discussed elsewhere (see Section 4.5.1), it is mentioned here as providing further evidence of the presence and importance of GST activity in dichloromethane metabolism.

In addition to absolute levels of GSTT protein expression in target organs, another important issue is the subcellular localization of the expressed enzyme. While GSTT11 in mouse liver is readily found in cytoplasm and nuclei of hepatocytes, it is found at lower levels in nuclei of bile-duct epithelial cells, and in cytoplasm and nuclei of some human hepatocytes (Sherratt et al., 2002). This less intense nuclear localization is thought to be of significance for carcinogenic risk because less *S*-chloromethyl GSH and formaldehyde will be generated near DNA.

GST is also present in human erythrocytes and is thought to play a role in toxicity of dichloromethane in lymphocytes (Hallier et al., 1993, 1994). Erythrocyte GSTT is polymorphic, as further discussed in Section 4.5.

(c) Experimental systems

(i) Rat

The metabolism of dichloromethane has been extensively studied in several experimental systems, predominantly those derived from rodents. This is particularly important in that mouse liver and lung have been identified as prominent target organs for dichloromethane, and toxicity has been clearly linked to metabolism. Some of the earliest studies that established the basic outlines of dichloromethane metabolism were conducted in rat liver microsomes (Kubic & Anders, 1975, 1978), rat liver cytosol (Ahmed & Anders, 1976, 1978), and rat lung microsomes (Kubic & Anders, 1975).

As noted above, the CYP-dependent oxidative pathway is considered to be a high-affinity, low-capacity pathway for dichloromethane metabolism, while the GST pathway is a low-affinity, high-capacity pathway. An in-vivo study of metabolism after oral administration of ^{14}C-labelled dichloromethane in rats showed dose-dependent metabolism primarily to CO and CO_2, with clear evidence of saturation (McKenna & Zempel, 1981). While rats given a dose of dichloromethane at 1 mg/kg metabolized approximately 88% of the administered dose over 48 hours, those given dichloromethane at 50 mg/kg only metabolized about 28% of the administered dose over the same period. Saturation of dichloromethane metabolism after inhalation in rats was also demonstrated by Kurppa & Vainio (1981), who showed that blood COHb levels stabilized at dichloromethane exposures of 500 ppm.

Gargas et al. (1986) measured COHb levels in rats given dichloromethane or other dihalomethanes by inhalation in a closed-atmosphere exposure system. The bromine-containing dihalomethanes exhibited the highest activities, while fluorine-containing dihalomethanes exhibited no detectable activity. Maximal rates of COHb formation from dibromomethane, chlorobromomethane, and dichloromethane were 72, 54, and 47 μmol/kg per hour, respectively. Pretreatment with pyrazole, which inhibits microsomal oxidation, abolished production of CO. Depletion of GSH with 2,3-epoxypropanol increased the steady-state levels of COHb generated from dichloromethane.

Takano & Miyazaki (1988) applied dichloromethane to perfused livers of male Wistar rats previously given phenobarbital to induce CYP, and examined spectral changes by scanning reflectance spectrophotometry. Both with and without addition of exogenous CO, a type-I spectral change with a peak at 450 nm was observed, demonstrating CYP-dependent metabolism of dichloromethane to CO in the intact rat liver.

Kim & Kim (1996) further explored the role of CYP2E1 in dichloromethane metabolism by

Table 4.1 Reaction rates for dichloromethane metabolism by CYP and GST in liver and lung tissue from different species

Enzyme activity	Organ	Concentration of dichloromethane (mM)	Reaction rate (nmol product formed/min per mg protein)			
			Mouse	Rat	Hamster	Human[a]
CYP	Liver	1	5.87	2.40	7.18	1.57
		5	11.4	4.10	14.5	3.90
		10	14.4	4.91	18.2	4.69
	Lung	5	4.62	0.16	0.99	< 0.1
GST	Liver	10	7.24	1.11	0.31	–
		25	18.5	3.19	0.76	2.41
		50	33.2	6.17	1.24	3.73
		100	48.6	12.1	2.64	4.34
	Lung	40	7.3	1.0	0.0	0.37

[a] Average value from two samples of human tissue for liver, and value from a single sample of lung

CYP, cytochrome P450; GST, glutathione *S*-transferase

Adapted from Toxicology Letters, Volume 43, issue 1–3, Reitz et al. (1988). Incorporation of in vitro enzyme data into the physiologically-based pharmacokinetic (PB-PK) model for methylene chloride: implications for risk assessment, pp. 97–116, Copyright (1988), with permission from Elsevier

examining the effect of prior administration of organic solvents that induce CYP2E1 on COHb levels in adult female rats after intraperitoneal administration of dichloromethane (3.0 mmol/kg). Peak COHb levels in blood reached 21%, 16%, and 23% in rats pretreated with benzene, toluene, or m-xylene, respectively, compared with only about 10% in rats given dichloromethane alone. The selective CYP2E1 inhibitor disulfiram (3.4 mmol/kg) blocked the elevations in COHb. No effects on hepatic GSH levels were observed with the single administration of the solvents, indicating no involvement with changes in the GST pathway in the observed responses.

(ii) Mouse

Reitz and colleagues analysed dichloromethane metabolism by the CYP and GST pathways in the liver and lung of male B6C3F$_1$ mice, F344 rats, Syrian golden hamsters, and humans (Table 4.1; Reitz et al., 1988). Several striking species-dependent differences are clearly evident from the data. First, mice exhibit similar rates of CYP-dependent metabolism in liver as hamsters and nearly threefold higher rates than rats or humans. Second, in lung tissue CYP-dependent metabolism of dichloromethane in mice was ~30-fold higher than in rats and ~5-fold higher than in hamsters. No CYP-dependent metabolism was detected in the human lung sample. Third, even greater species-dependent differences in addition to interindividual differences were observed in the liver and lung for GST-dependent dichloromethane metabolism. In this case, rates of GSH conjugation in mouse liver were ~4-fold faster than in rats, ~20-fold faster than in hamsters, and ~10-fold faster than in humans. Finally, perhaps the greatest species-dependent metabolic difference was observed for GST metabolism in the lung. Here, rates in mice were ~7-fold faster than in rats and ~20-fold faster than in humans. These metabolic differences have been interpreted to explain species-dependent differences as well as interindividual differences in target-organ specificity and sensitivity to dichloromethane-induced mutagenesis and carcinogenicity (Green, 1990; Starr et al., 2006). Furthermore, data on tumour incidence across species show a correlation with

the amount of dichloromethane metabolized by GST but not by CYP (Andersen et al., 1987).

Ottenwälder et al. (1989) gave two specific CYP inhibitors (i.e. pyrazole, 320 mg/kg, and diethyldithiocarbamate, 300 mg/kg) to male B6C3F$_1$ mice also exposed to dichloromethane at 1000 or 3000 ppm, or a mixture of dichloromethane at 1000 ppm and methyl chloride at 1000 ppm. For those mice given only dichloromethane, uptake by inhalation was markedly decreased by the CYP inhibitors. In contrast, CYP inhibitors had no effect on the uptake of methyl chloride by inhalation. Because methyl chloride is metabolized solely by GSTs, these results showed that even at relatively high exposures, dichloromethane is predominantly metabolized by CYP. These results contrasted with those of Andersen et al. (1987) described above, who concluded that GST-dependent, rather than CYP-dependent, metabolism was critical for dichloromethane-induced liver tumorigenesis.

The in-vivo metabolism of dichloromethane by CYP was further demonstrated by Casanova et al. (1992), who pre-exposed male B6C3F$_1$ mice to dichloromethane at 4000 ppm for 6 hours per day for 2 days, and then on day 3 to ^{14}C-labelled dichloromethane at a declining concentration (4500–2500 ppm). DNA–protein cross-links and incorporation of ^{14}C derived from dichloromethane into DNA was observed in the liver of these mice.

Foster et al. (1994) also showed that modulation of pulmonary CYP activity can also alter responses of the lung to dichloromethane.

4.1.4 Excretion

(a) Humans

In humans, the main route of excretion of dichloromethane is by exhalation of the parent compound and its primary metabolites CO_2 and CO, with lesser amounts as dichloromethane excreted in the urine (DiVincenzo et al., 1971, 1972; DiVincenzo & Kaplan, 1981). DiVincenzo & Kaplan (1981) estimated that only 5% of absorbed dichloromethane is exhaled unchanged, 25–34% excreted converted as CO, and the balance excreted as CO_2. After cessation of exposure, the half-life of dichloromethane in the blood has been estimated to be about 40 minutes, with concentrations of parent and metabolites returning the preexposure levels within a few days (DiVincenzo et al., 1972; DiVincenzo & Kaplan, 1981). Urinary excretion occurs mostly during and/or within the first hour after cessation of exposure, and in total accounts for less than 0.1% of uptake (DiVincenzo et al., 1971, 1972).

(b) Experimental systems

As in humans, the main route of excretion of dichloromethane in experimental animals is by exhalation of the parent compound and its primary metabolites CO_2 and CO, with lesser amounts excreted in the urine. As exposure levels increase, the percentage excreted as unchanged parent compound increases, reflecting saturation of metabolism. For instance, McKenna et al. (1982) reported that in rats exposed to dichloromethane at 50 ppm via inhalation, elimination in expired air consists of about 5% parent compound, and 26% and 27% CO_2 and CO, respectively. At 500 and 1500 ppm, elimination of parent compound increased to 30% and 55%, respectively, with declines in the amount of CO_2 and CO expired. Similarly, for oral doses of 1 mg/kg, McKenna & Zempel (1981) reported that rats exhaled 12% of the administered dose as parent compound, and 35% and 31% as CO_2 and CO, respectively. At higher oral doses (50 mg/kg or greater), rats and mice exhale greater amounts as parent compound (60–80%), and lesser amounts as CO_2 and CO (McKenna & Zempel, 1981; Angelo et al., 1986a, b).

Overall, experimental studies in rodents have found that > 90% of absorbed dichloromethane is eliminated within 24 or 48 hours of exposure, regardless of dose. McKenna et al. (1982) reported

Table 4.2 Studies of genotoxicity with dichloromethane in human cell lines in vitro

Test system	Results[a]		Concentration (LEC or HIC)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Single-strand breaks, human primary hepatocytes	–	NT	5100	Graves et al. (1995)
DNA–protein cross-links, human hepatocytes (expressing GSTT1)	–	NT	425	Casanova et al.(1997)
Unscheduled DNA synthesis, human AH fibroblasts	–	NT	65 000	Jongen et al. (1981)
Micronucleus test, human MCL-5 and h2E1 lymphoblastoid cells	+[c]	NT	200	Doherty et al. (1996)
Micronucleus test, human AHH-1 lymphoblastoid cells	–	NT	850	Doherty et al. (1996)
Sister-chromatid exchanges, human lymphocytes	+[d]	NT	290	Hallier et al. (1993)
Sister-chromatid exchange, human peripheral blood mononuclear cells	+	NT	60 ppm	Olvera-Bello et al. (2010)

[a] +, positive; (+), weakly positive; –, negative; NT, not tested
[b] LEC, lowest effective concentration; HIC, highest ineffective concentration; in-vitro tests, µg/mL (in bacterial tests, cells were exposed to dichloromethane vapour, so dose = µg /mL in atmosphere)
[c] Induction of kinetochore-positive and -negative micronuclei
[d] Positive results were reported in lymphocytes from donors lacking GST activity

that after inhalation exposure in rats, a low percentage of the initial body burden of dichloromethane remained at 48 hours. After a single intravenous dose in mice, Angelo et al. (1986a) reported 92–94% recovery within 4 hours after dosing. After repeated oral exposures in mice, Angelo et al. (1986a) reported 90–96% recovery of within 24 hours after each dose.

4.2 Genetic and related effects

Dichloromethane has been studied for genotoxic potential in a variety of assays. The genotoxicity of dichloromethane has been reviewed previously by the Working Group (IARC, 1999).

4.2.1 Humans

(a) In vivo

No data were available to the Working Group.

(b) In vitro

See Table 4.2

Dichloromethane did not induce DNA single-strand breaks in human primary hepatocytes (Graves et al., 1995). There was no induction of DNA–protein cross-links in vitro in human hepatocytes with functional *GSTT1* genes (Casanova et al., 1997) or unscheduled DNA synthesis in AH fibroblasts (Jongen et al., 1981) after treatment with dichloromethane in vitro.

In a study by Doherty et al. (1996), dichloromethane induced the formation of kinetochore-staining micronuclei (which are indicative of aneuploidy) and kinetochore-negative micronuclei in human MCL-5 cells that stably express cDNA encoding human CYP1A2, CYP2A6, CYP3A4, CYP2E1, and epoxide hydrolase and in h2E1 cells, which contains a cDNA for CYP2E1. The increased frequency of micronucleus formation is combined with the fact that MCL-5 and h2El cell lines showed the capacity to produce metabolites in the presence of dichloromethane. AHH-1 cells, constitutively expressing CYP1A1, showed no increase in the total frequency of

micronucleus formation or in the frequency of kinetochore-staining micronuclei.

Hallier et al. (1993) showed that sister-chromatid exchanges were induced in human peripheral blood lymphocyte cultures from non-conjugator donors lacking GST activity, but not in those from conjugators. This study did not provide details on the type of GST activity that was monitored. Sister-chromatid exchanges were also induced by dichloromethane in vitro in human peripheral blood mononuclear cells (Olvera-Bello et al., 2010). This study also demonstrated that the group with high GSTT1 activity showed a larger increase in the frequency of sister-chromatid exchanges induced by dichloromethane than did the groups with low and medium GSTT1 activity.

4.2.2 Experimental systems

(a) Mammalian systems

See Tables 4.3 and 4.4

(i) DNA damage

Exposure of B6C3F_1 mice to dichloromethane by inhalation induced DNA single-strand breaks in the lung and liver (Graves et al., 1995). Prior treatment of the mice with buthionine sulfoximine (a depletor of GSH) immediately before exposure to dichloromethane reduced the amount of DNA damage to control levels.

Dichloromethane induced DNA single-strand breaks in vivo in AP rat primary hepatocytes and B6C3F_1 mouse hepatocytes (Graves et al., 1994b), and in Clara cells (Graves et al., 1995). DNA damage was reduced in Clara cells co-treated with buthionine sulfoximine. DNA single-strand breaks were not observed in the liver or lung of AP rats treated by inhalation (Graves et al., 1994b, 1995), but were induced in the liver of CD rats treated by gavage (Kitchin & Brown, 1994), and in the liver of B6C3F_1 mice treated by inhalation (Graves et al., 1994b). Dichloromethane did not cause DNA damage as measured by the comet assay in male B6C3F_1 mice exposed by inhalation for 6 weeks (6 hours per day, 5 days per week) at 400, 800, or 1600 ppm (Suzuki et al., 2014).

The frequency of DNA single-strand breaks was increased in vitro in Chinese hamster ovary cells cultured with dichloromethane in the presence, but not in the absence, of an exogenous metabolic activation system (Graves et al., 1994b). DNA single-strand breaks were also induced in Chinese hamster ovary cells exposed to dichloromethane with or without exogenous metabolic activation, the effect being stronger with metabolic activation (Graves & Green, 1996). Conversely, DNA single-strand breaks were not induced in Syrian hamster hepatocytes (Graves et al., 1995).

Hu et al. (2006) performed the standard and proteinase K-modified comet assay to measure DNA damage and DNA–protein crosslinks in untreated V79 cells and in V79 cells transfected with the murine *GSTT1* gene (V79 mGSTT1). Dichloromethane induced DNA damage in both cell types. However, the study showed the presence of dichloromethane-induced DNA–protein crosslinks in the V79 mGSTT1 cell line and not in standard V79 cell line, which indicates that *GSTT1* was instrumental for the induction of DNA–protein crosslinks. Moreover, dichloromethane formed significantly higher amounts of cytosolic formaldehyde in V79 in GSTT1 cells.

No DNA binding was observed in vivo in the liver or kidney of male rats or male and female mice after intraperitoneal administration of dichloromethane (Watanabe et al., 2007). Covalent binding of dichloromethane to DNA was not observed in the liver, kidney, or lung of rats or mice exposed by inhalation, although metabolic incorporation of ^{14}C was found in normal deoxyribonucleosides in both species (Ottenwälder & Peter, 1989).

DNA–protein cross-links were induced in vivo in the liver, but not the lung of B6C3F_1/CrlBR mice exposed to dichloromethane (Casanova

Table 4.3 Studies of genotoxicity with dichloromethane in mammalian systems in vivo

Test system	Results[a]	Dose (LED or HID)	Reference
DNA single-strand breaks, B6C3F$_1$ mouse liver	+	4831 ppm, inh., 6 h	Graves et al. (1994b)
DNA single-strand breaks, AP rat liver	–	4527 ppm, inh., 6 h	Graves et al. (1994b)
DNA single-strand breaks, CD rat liver	+	1275 µg/mL, po × 1	Kitchin & Brown (1994)
DNA single-strand breaks, B6C3F$_1$ mouse liver	+[c]	4000 ppm, inh., 6 h	Graves et al. (1995)
DNA single-strand breaks, B6C3F$_1$ mouse lung	+[c]	2000 ppm, inh., 3 h	Graves et al. (1995)
DNA single-strand breaks, AP rat lung	–	4000 ppm, inh., 3 h	Graves et al. (1995)
DNA damage, male B6C3F$_1$ mouse liver, comet assay	–	1600 ppm, inh., 6 h/day, 5 days/wk, 6 wk	Suzuki et al. (2014)
DNA binding, rats (male) or mice (male and female), liver or kidney	–	5 mg/kg bw per day, ip	Watanabe et al. (2007)
DNA binding, rat or mouse liver, lung, or kidney	–	NR, inh.	Ottenwälder & Peter (1989)
DNA–protein cross-links, B6C3F$_1$/CrlBR mouse liver	+[b]	4000 ppm, inh., 6 h/day, 2 days	Casanova et al. (1992)
DNA–protein cross-links, Syrian hamster liver and lung	–	4000 ppm, inh., 6 h/day, 2 days	Casanova et al.(1992)
DNA–protein cross-links, B6C3F$_1$/CrlBR mouse liver	+	498 ppm, inh., 6 h/d, 2 days	Casanova et al. (1996)
DNA–protein cross-links, Syrian golden hamster liver	–	3923 ppm, inh., 6 h/d, 2 days	Casanova et al.(1996)
Sister-chromatid exchange, B6C3F$_1$ mouse lung cells	+[d]	2000 ppm, inh., 6 h/day, 5 days/wk 12wk	Allen et al. (1990)
Sister-chromatid exchange, B6C3F$_1$ mouse bone marrow	–	5000 µg/mL, sc × 1	Allen et al. (1990)
Sister-chromatid exchange, C57BL/6J mouse bone marrow	–	1500 µg/mL, ip × 1	Westbrook-Collins et al. (1990)
Unscheduled DNA synthesis, F344 rat hepatocytes	–	1000 µg/mL, po × 1	Trueman & Ashby (1987)
Unscheduled DNA synthesis, F344 rat hepatocytes	–	4000 ppm, inh., 6 h	Trueman & Ashby (1987)
Unscheduled DNA synthesis, B6C3F$_1$ mouse liver	–	4000 ppm, inh., 6 h	Trueman & Ashby (1987)
Gene mutation, *Pig-a* assay, male B6C3F$_1$ mouse, erythrocytes	–	1600 ppm, inh., 6 h/day, 5 days/wk, 6 wk	Suzuki et al. (2014)
Gene mutation, transgenic rodent, male *Gpt* Delta C57BL/6J mouse liver	–	800 ppm, inh., 6 h/day, 5 days/wk, 4 wk	Suzuki et al. (2014)
Chromosomal aberrations, B6C3F$_1$ mouse bone marrow	–	5000 µg/mL sc × 1	Allen et al. (1990)
Chromosomal aberrations, C57BL/6J mouse bone marrow	–	1500 mg/kg ip × 1	Westbrook-Collins et al. (1990)
Chromosomal aberrations, B6C3F$_1$ mouse bone marrow	(+)	8000 ppm, inh., 6 h/day, 5 days/wk, 2 wk	Allen et al. (1990)
Chromosomal aberrations, Sprague-Dawley rat bone marrow	–	3500 ppm, inh., 6 h/day, 5 days/wk, 2 yr	Burek et al. (1984)
Chromosomal aberrations, B6C3F$_1$ mouse lung cells	(+)	8000 ppm, inh., 6 h/day, 5 days/wk, 2 wk	Allen et al. (1990)
Micronucleus test, NMRI mouse bone marrow	–	1700 mg/kg, ip × 2	Gocke et al. (1981)
Micronucleus test, C57BL/6J/Alpk mouse bone marrow	–	4000 mg/kg, po × 1	Sheldon et al. (1987)

Table 4.3 (continued)

Test system	Results[a]	Dose (LED or HID)	Reference
Micronucleus test, CD-1 mouse bone marrow	–	1720 mg/kg, ip × 1	Morita et al. (1997)
Micronucleus test, $B6C3F_1$ mouse erythrocytes	(+)[e]	2000 ppm, inh., 6 h/day, 5 days/wk, 12 wk	Allen et al. (1990)
Micronucleus test, male $B6C3F_1$ mouse reticulocytes and normochromatic erythrocytes	–	1600 ppm, inh., 6 h/days, 5 days/wk, 6 wk	Suzuki et al. (2014)

[a] +, positive; (+), weakly positive; –, negative
[b] Negative in mouse lung
[c] Pre- or co-treatment with buthionine sulfoximine, a GSH-depleting agent, caused a decrease in DNA damage
[d] The highest dose tested (8000 ppm, 6 hours per day, 5 days per week, for 2 weeks) gave positive results in erythrocytes and lung cells, but negative results in bone marrow
[e] Negative in lung cells at this dose; positive in erythrocytes after exposure to 8000 ppm for 6 hours per day [10 000 mg/kg bw], 5 days per week, for 2 weeks
h, hour; HID, highest ineffective dose; inh., inhalation; ip, intraperitoneal; LED, lowest effective dose; NR, not reported; NT, not tested; po, oral; ppm, parts per million; sc, subcutaneous; wk, week; yr, year

et al., 1992). No DNA–protein cross-links were detected in Syrian hamster liver or lung after inhalation of dichloromethane (Casanova et al., 1992). DNA–protein cross-links were not induced in the liver of Syrian golden hamsters, but were observed in the liver of $B6C3F_1$/CrlBR mice treated with dichloromethane by inhalation (Casanova et al., 1996).

Dichloromethane induced DNA–protein cross-links in vitro in hepatocytes of male $B6C3F_1$ mice, but not in hepatocytes of Fischer 344 rats or Syrian hamsters (Casanova et al., 1997). DNA–protein cross-links were also induced in Chinese hamster ovary cells exposed to dichloromethane with or without exogenous metabolic activation, with DNA damage being greater in the presence of metabolic activation (Graves & Green, 1996). Using the proteinase K-modified comet assay, it was demonstrated that dichloromethane induced DNA–protein cross-links in V79 cells transfected with the murine *GSTT1* gene, but not in standard V79 cells (Hu et al., 2006). [The Working Group noted that this suggests a key role for GST in genotoxicity induced by dichloromethane.]

In a study in vivo, mice treated with dichloromethane at 2000 ppm [6940 mg/m^3] for 6 hours per day, 5 days per week, for 12 weeks showed an increased frequency of sister-chromatid exchange in lung cells (Allen et al., 1990). Exposure to higher concentrations (8000 ppm [27 800 mg/m^3] for 2 weeks) also induced an increase in the frequency of sister-chromatid exchange in peripheral blood erythrocytes. Dichloromethane did not induce sister-chromatid exchange in bone marrow of mice treated by intraperitoneal or subcutaneous injection (Westbrook-Collins et al., 1990; Allen et al., 1990). Dichloromethane did not increase the frequency of sister-chromatid exchange in Chinese hamster ovary cells in the presence or absence of an exogenous metabolic system (Thilagar & Kumaroo, 1983; Anderson et al., 1990). When tested in Chinese hamster lung V79 cells in the absence of exogenous metabolic activation, dichloromethane induced a slight increase in the frequency of sister-chromatid exchange (Jongen et al., 1981).

Dichloromethane did not induce unscheduled DNA synthesis in vivo in Fischer 344 rats treated by gavage or inhalation, or in $B6C3F_1$ mouse hepatocytes treated by inhalation (Trueman & Ashby, 1987).

Table 4.4 Studies of genotoxicity with dichloromethane in mammalian systems in vitro

Test system	Results[a]		Concentration[b] (LEC or HIC)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA–protein cross-links, B6C3F$_1$ mouse hepatocytes	+	NT	43	Casanova et al. (1997)
DNA–protein cross-links, F344 rat hepatocytes	–	NT	425	Casanova et al. (1997)
DNA–protein cross-links, Syrian hamster hepatocytes	–	NT	425	Casanova et al. (1997)
DNA–protein crosslinks, V79 cells	–	NT	850	Hu et al. (2006)
DNA–protein cross-link, murine GSTT1 transfected V79 cells	+[c]	NT	212	Hu et al. (2006)
DNA–protein cross-links, Chinese hamster ovary cells	(+)	+	3975	Graves & Green (1996)
DNA single-strand breaks, B6C3F$_1$ mouse hepatocytes	+	NT	34	Graves et al. (1994b)
DNA single-strand breaks, AP rat hepatocytes	+	NT	2550	Graves et al. (1994b)
DNA single-strand breaks, Chinese hamster ovary cells	–	+	5100	Graves et al. (1994b)
DNA single-strand breaks, Syrian hamster hepatocytes	–	NT	5100	Graves et al. (1995)
DNA single-strand breaks, B6C3F$_1$ mouse lung Clara cells	+[d]	NT	425	Graves et al. (1995)
DNA single-strand breaks, Chinese hamster ovary cells	(+)	+	3975	Graves & Green (1996)
DNA damage, V79 cells, comet assay	+[e]	NT	425	Hu et al. (2006)
DNA damage, murine *GSTT1* transfected V79 cells, comet assay	+[f]	NT	212	Hu et al. (2006)
Unscheduled DNA synthesis, Chinese hamster lung V79 cells	–	NT	65 000	Jongen et al. (1981)
Sister-chromatid exchange, Chinese hamster V79 cells	(+)	NT	13 000	Jongen et al. (1981)
Sister-chromatid exchange, Chinese hamster ovary cells	–	–	13 000	Thilagar & Kumaroo (1983)
Sister-chromatid exchange, Chinese hamster ovary cells	–	–	5000	Anderson et al. (1990)
Gene mutation, Chinese hamster ovary cells, *Hprt* locus	–	NT	65 000	Jongen et al. (1981)
Gene mutation, Chinese hamster ovary cells, *Hprt* locus	–	+	3975	Graves & Green (1996)
Gene mutation, Chinese hamster lung V79 cells, *Hprt* locus	–	NT	52 000	Jongen et al. (1981)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus	?	?	3300	Myhr et al. (1990)
Chromosomal aberrations, Chinese hamster ovary CHO cells	+	+	6500	Thilagar & Kumaroo (1983)
Chromosomal aberrations, Chinese hamster ovary CHO cells	–	–	5000	Anderson et al. (1990)
Cell transformation, RLV/Fischer rat	+	NT	14	Price et al. (1978)
Cell transformation, SA7/Syrian hamster embryo cells	+	NT	73	Hatch et al. (1982)

[a] +, positive; (+), weakly positive; –, negative; ?, inconclusive; NT, not tested
[b] LEC, lowest effective concentration; HIC, highest ineffective concentration; in-vitro tests, µg/mL
[c] DNA–protein crosslinks were demonstrated by increase in DNA migration following post-treatment with proteinase K
[d] Pre- or co-treatment with buthionine sulfoximine, a GSH-depleting agent, caused a decrease in DNA damage
[e] Concentration-dependent increase in DNA migration
[f] Concentration-dependent decrease in DNA migration; post-incubation with proteinase K increased DNA migration

(ii) Chromosomal aberration

Dichloromethane did not cause chromosomal aberration in vivo in bone marrow of mice treated by intraperitoneal or subcutaneous injection (Westbrook-Collins et al., 1990; Allen et al., 1990). A small increase in the frequency of chromosomal aberration in mouse bone marrow and lung cells was reported after exposure to dichloromethane at 8000 ppm by inhalation for 6 hours per day, 5 days per week, for 2 weeks (Allen et al., 1990). In a study by Burek et al. (1984), dichloromethane gave negative results in an assay for chromosomal aberration in rat bone marrow.

Dichloromethane induced chromosomal aberration in vitro in Chinese hamster ovary cells in the presence and absence of an exogenous metabolic system in one of two studies (Thilagar & Kumaroo, 1983; Anderson et al., 1990).

(iii) Micronucleus formation

Dichloromethane did not induce micronucleus formation in vivo in bone marrow of mice treated by gavage or intraperitoneal injection (Gocke et al., 1981; Sheldon et al., 1987; Morita et al., 1997). Mice treated with dichloromethane at 2000 ppm [6940 mg/m^3] for 6 hours per day, 5 days per week, for 12 weeks showed an increased frequency of micronuclei in peripheral blood erythrocytes (Allen et al., 1990). The highest dose tested (8000 ppm, 6 hours per day, 5 days per week, for 2 weeks) gave positive results in erythrocytes and lung cells, but negative results in bone marrow. On the other hand, dichloromethane did not cause micronucleus formation in male B6C3F$_1$ mice exposed at 400, 800 and 1600 ppm by inhalation for 6 weeks (6 hours per day, 5 days per week) (Suzuki et al., 2014).

(iv) Mutagenicity

Dichloromethane did not cause gene mutation in two inhalation experiments in vivo: a Pig-a assay in male B6C3F$_1$ mice exposed to dichloromethane at 400, 800, or 1600 ppm for 6 weeks (6 hours per day, 5 days per week); and a transgenic rodent gene mutation assay on *Gpt* Delta C57BL/6J mouse liver treated for 4 weeks (6 hours per day, 5 days per week) with dichloromethane at 800 ppm (Suzuki et al., 2014).

In vitro, dichloromethane was mutagenic in Chinese hamster ovary cells at the *Hprt* locus in one study, in the presence of exogenous metabolic activation (Graves & Green, 1996), and gave equivocal results in the mouse lymphoma Tk$^{+/-}$ assay in another study (Myhr et al., 1990). DNA sequence analysis of the *Hprt* mutants of Chinese hamster ovary cells treated with dichloromethane indicated that most mutations were GC→AT transitions (4 out of 8), with two GC→CG transversions and two AT→TA transversions. This pattern was more similar to that of 1,2-dibromoethane (ethylene dibromide) (IARC, 1999) (7 out of 9 being GC→AT transitions) than that of formaldehyde, a metabolite of dichloromethane that has been identified in vitro (see Section 4.1), for which all mutations were single-base transversions and 5 out of 6 arose from AT base pairs (Graves et al., 1996). When tested in Chinese hamster lung fibroblast V79 cells in the absence of exogenous metabolic activation, dichloromethane did not induce gene mutations at the *Hprt* locus (Jongen et al., 1981).

(v) Cell transformation

Virus-infected Fischer rat and Syrian hamster embryo cells were transformed after treatment with dichloromethane in vitro (Price et al., 1978; Hatch et al., 1982).

(b) Bacterial and other systems

See Table 4.5

Mutagenicity

Gene mutations were induced in *Salmonella typhimurium* strains TA100, TA1535, and TA98 exposed to dichloromethane vapour in a closed chamber with or without exogenous metabolic activation (JETOC, 1997).

The relationship between the metabolism of dichloromethane and mutagenicity has been examined in several studies with various assays for bacterial mutation. For example, Jongen et al. (1982) showed that while dichloromethane was directly mutagenic in *S. typhimurium* TA100, mutagenic activity was enhanced by addition of rat liver microsomes or cytosolic fraction (this implicated enhanced metabolism of dichloromethane by CYP and GST, respectively). In contrast, Green (1983) tested the mutagenicity of dichloromethane in the same *S. typhimurium* strain and observed an increase in mutagenic activity only when rat liver post-mitochondrial S9 fraction was added and not rat liver microsomes.

To further illustrate the complexities of how the two metabolic pathways interact to promote mutagenesis, Dillon and colleagues examined the involvement of endogenous and exogenous GSH using wild-type *S. typhimurium* TA100 and a GSH-deficient strain (NG54) that contains approximately 25% of the GSH content as the wild-type strain (Dillon et al., 1992). The influence of addition of rat liver S9 fraction, microsomes, or cytosol fractions was also studied. The NG54 strain was slightly less responsive to dichloromethane exposure, addition of rat liver cytosol marginally increased the mutagenic response to dichloromethane, but addition of GSH had little effect (Dillon et al., 1992).

DeMarini and colleagues assessed dichloromethane mutagenicity by using a *Salmonella* TA1535 strain that had been modified by the cloning of the rat gene for GSTT11 into its genome (DeMarini et al., 1997). This modified strain, called RSJ100, showed a positive mutagenic response to dichloromethane that was predominantly (96–100%) due to mutations that were GC→AT transitions. Interestingly, only 15% of the mutations were GC→AT transitions in the TA100 strain, a homologue strain that lacks the rat GSTT11 gene. These results suggested that different reactive metabolites are formed in the two strains, which leads to different mutations.

Studies using the liquid plate incorporation assay gave negative results (e.g. Zeiger & Dellarco, 1990), with the exception of one study reporting positive results in strain TA1535 transfected with rat Gstt1 (Thier et al., 1993). Dichloromethane also induced mutation in *Escherichia coli* (Dillon et al., 1992; Zielenska et al., 1993; Graves et al., 1994a; JETOC, 1997) and gene conversion and mutation in *Saccharomyces cerevisiae* (Callen et al., 1980). In *Drosophila melanogaster* dichloromethane did not induce sex-linked recessive lethal mutations (Gocke et al., 1981; Kramers et al., 1991).

4.3 Other mechanistic data relevant to carcinogenicity

Few experimental studies have examined the potential for non-genotoxic mechanistic events to play a role in carcinogenesis caused by dichloromethane in tissues that are targets for carcinogenesis in studies in experimental animals. In long-term studies of dichloromethane exposure in mice, elevations in liver-cell proliferation were not observed (Foley et al., 1993; Casanova et al., 1996). In the mouse lung, exposure to dichloromethane results in toxicity to Clara cells, which are secretory cells in the primary bronchioles. Acute exposure to dichloromethane produces vacuolization of Clara cells, which is not sustained with long-term exposure (Foster et al., 1992).

One recent genomics study in vitro compared the effects of dichloromethane and other volatile organic solvents (benzene, toluene, o-xylene, ethylbenzene, and trichloroethylene) on gene expression in human promyelocytoc leukaemia HL-60 cells (Sarma et al., 2010). Equi-toxic concentrations of all solvents were used in studies of gene expression (80% and 50% cell viability). Based on the overall changes in gene expression, dichloromethane exhibited a response that was distinct from other solvents; however, common signatures were identified. These included

Table 4.5 Studies of genotoxicity with dichloromethane in non-mammalian systems in vitro

Test system	Results[a]		Concentration[b] (LEC or HIC)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Prokaryotes (Bacteria)				
Salmonella typhimurium BA/3, forward mutation, Ara resistance	+	(+)	325	Roldán-Arjona & Pueyo (1993)
Salmonella typhimurium TA100, reverse mutation	+	NT	14	Simmon et al. (1977)
Salmonella typhimurium TA100, reverse mutation	+	+	19	Jongen et al. (1978)
Salmonella typhimurium TA100, reverse mutation	+	+	18	Gocke et al. (1981)
Salmonella typhimurium TA100, reverse mutation	+	+	23	Jongen et al. (1982)
Salmonella typhimurium TA100, reverse mutation	+	+	95	Green (1983)
Salmonella typhimurium TA100, reverse mutation	(+)	NT	6 800	Osterman-Golkar et al. (1983)
Salmonella typhimurium TA100, reverse mutation	(+)[c]	NT	3 700	Hughes et al. (1987)
Salmonella typhimurium TA100, reverse mutation	+[c]	+	150	Zeiger & Dellarco (1990)
Salmonella typhimurium TA100, reverse mutation	+	+	8.5	Dillon et al. (1992)
Salmonella typhimurium TA100, reverse mutation	+	NT	17 667	Graves et al. (1994a)
Salmonella typhimurium TA100, reverse mutation	+	+	34	JETOC (1997)
Salmonella typhimurium TA1535, reverse mutation	+	NT	300	McGregor (1979)
Salmonella typhimurium TA1535, reverse mutation	– [d]	NT	170	Thier et al. (1993)
Salmonella typhimurium TA1535 transfected with rat GST 5-5, reverse mutation	+ [d]	NT	42	Thier et al. (1993)
Salmonella typhimurium TA1535, reverse mutation	+	+	170	JETOC (1997)
Salmonella typhimurium TA1537, reverse mutation	–	–	340	JETOC (1997)
Salmonella typhimurium TA98, reverse mutation	+	+	19	Jongen et al. (1978)
Salmonella typhimurium TA98, reverse mutation	+	+	72	Gocke et al. (1981)
Salmonella typhimurium TA98, reverse mutation	?[c]	?	1500	Zeiger & Dellarco (1990)
Salmonella typhimurium TA98, reverse mutation	+	+	34	JETOC (1997)
Escherichia coli NR3835, forward mutation	+	NT	26 500	Zielenska et al. (1993)

Table 4.5 (continued)

Test system	Results[a]		Concentration[b] (LEC or HIC)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Escherichia coli K12, forward mutation, Rif resistance	–	(+)[e]	5100	Graves et al. (1994a)
Escherichia coli WP2 uvrA, reverse mutation	+	+	170	IETOC (1997)
Escherichia coli WP2 uvrA/pKM101, reverse mutation	+	+	21	Dillon et al. (1992)
Escherichia coli WP2 uvrA/pKM101, reverse mutation	+	+	170	IETOC (1997)
Saccharomyces cerevisiae, gene conversion	+	NT	13 300	Callen et al. (1980)
Saccharomyces cerevisiae, homozygosis	+	NT	13 300	Callen et al. (1980)
Saccharomyces cerevisiae, reverse mutation	+	NT	13 300	Callen et al. (1980)
Insects				
Drosophila melanogaster, sex-linked mutation	–	NT	52 600	Gocke et al. (1981)
Drosophila melanogaster, sex-linked mutation	–	NT	19.2	Kramers et al. (1991)
Plants				
Tradescantia species, gene mutation	+	NT	100	Schairer &Sautkulis (1982)

[a] +, positive; (+), weakly positive; -, negative; ?, inconclusive; NT, not tested
[b] LEC, lowest effective dose; HIC, highest ineffective dose; in-vitro tests, µg/mL (in bacterial tests, cells were exposed to dichloromethane vapour, so dose = µg /mL in atmosphere).
[c] Negative in liquid plate incorporation assay
[d] Liquid plate incorporation assay
[e] Positive with mouse liver S9, negative with rat liver S9

induction of the immune response, apoptosis, cell cycle regulation, and transport pathways. Select transcripts from these pathways were tested by real-time polymerase chain reaction (PCR) in two other cell lines, human erythromyeloblastoid leukaemia K562 and human leukaemic monocyte lymphoma U937. [The Working Group noted that these data were difficult to interpret as the study appeared not to use proper multiple-testing correction to determine significance of both individual genes and pathways.]

4.4 Organ toxicity

The toxicity of dichloromethane has been reviewed previously (Dhillon & Von Burg, 1995; WHO, 1996; Green, 1997).

4.4.1 Neurotoxicity

(a) Humans

Temporary neurobehavioural effects have been reported (Putz et al., 1979; Winneke, 1981), or not (Gamberale et al., 1975) after exposure to dichloromethane at doses as low as 200 ppm [694 mg/m^3]. Cerebral damage after exposure to dichloromethane has been reported (Barrowcliff & Knell, 1979).

(b) Experimental systems

Increase in concentrations of astroglial proteins S-100 and glial fibrillary acidic protein was found in the frontal and sensory motor cerebral cortex of gerbils exposed to dichloromethane at 210 or 350 ppm for 3 months (Rosengren et al., 1986). DNA concentration was also measured as

a possible index of astroglial proliferation. DNA concentration was not increased in the frontal and sensory motor cerebral cortex, but was decreased in the hippocampus at 210 and 350 ppm, and in the cerebellar hemispheres (Rosengren et al., 1986).

4.4.2 Liver

(a) Humans

An exposure-related increase in serum bilirubin was observed in workers exposed to dichloromethane, but no other sign of liver injury or haemolysis was reported (Ott et al., 1983)

(b) Experimental systems

A 2-year study of exposure to dichloromethane by inhalation in F344 rats reported that the incidence of some non-neoplastic liver lesions was significantly elevated in response to treatment when compared with concurrent controls (NTP, 1986). These liver lesions were haemosiderosis, focal necrosis, cytoplasmic vacuolization, and bile duct fibrosis in males, and focal granulomatous inflammation, haemosiderosis and cytoplasmic vacuolization in females. In the same study, liver cytological degeneration was observed in female B6C3F$_1$ mice.

A 2-year study of exposure to dichloromethane by inhalation in F344 rats reported that the incidence some non-neoplastic liver lesions (acidophilic, basophilic and vacuolated cell foci in males) was significantly elevated in response to treatment when compared with controls (JISHA, 2000a). In the same study, liver granulation and peripheral vacuolation were observed in male and female BDF1 mice.

Increased liver weight associated with glycogen accumulation in the hepatocytes, but no hepatotoxicity, was observed in another study of carcinogenicity in mice, in which an elevated incidence of hepatic tumours was observed (Kari et al., 1993). An experiment in female B6C3F$_1$ mice showed that the proportion of S-phase cells was frequently higher in altered foci than in cells from the areas of the liver with normal architecture, but similar to that in the altered foci from non-treated mice (Foley et al., 1993). Administration of dichloromethane to B6C3F$_1$ mice by gavage (1000 mg/kg, single dose) or inhalation (4000 ppm [13 900 mg/m^3] dichloromethane for 2 hours) did not induce DNA synthesis, as measured by the number of cells in S-phase ([^{3}H]thymidine incorporation) (Lefevre & Ashby, 1989). When female B6C3F$_1$ mice were exposed to dichloromethane at 1000, 2000, 4000, or 8000 ppm [3470, 6940, 13 900 or 27 800 mg/m^3] for 6 hours per day, 5 days per week, for up to 4 weeks, followed by a recovery period of 1–2 weeks (Foley et al., 1993), the hepatocyte labelling index was mostly decreased. There were, however, transient increases in the labelling index in the groups at 4000 and 8000 ppm at 2 weeks and in the group at 1000 ppm at 1 week.

In Sprague-Dawley rats, two doses of dichloromethane at 1250 mg/kg given by gavage for 4 and 21 hours, there was no effect on serum alanine aminotransferase levels, or hepatic GSH or CYP content, but hepatic ornithine decarboxylase activity increased in 3 out of 15 rats (Kitchin & Brown, 1989).

Hepatotoxic effects were seen after exposure to near-lethal concentrations of dichloromethane in mice (Gehring, 1968). Continuous exposure of mice to dichloromethane at 5000 ppm [17 400 mg/m^3] by inhalation caused swelling of the rough endoplasmic reticulum, fatty changes in the liver, and necrosis of individual hepatocytes (Weinstein et al., 1972). Slight liver damage was also observed after administration of dichloromethane (133–665 mg/kg bw) by gavage in mice (Condie et al., 1983).

Exposure of guinea-pigs to dichloromethane at 5200 ppm [18 000 mg/m^3] by inhalation for 6 hours increased hepatic concentrations of triglyceride (Morris et al., 1979). Exposure of guinea-pigs to dichloromethane at approximately 11 000 ppm [38 200 mg/m^3] for 6 hours

also increased hepatic concentrations of triglyceride, but concomitant exposure to ethanol at 21 400–24 100 ppm [40 200–45 300 mg/m^3] blocked this effect (Balmer et al., 1976).

4.4.3 Cardiovascular system

(a) Humans

Of four epidemiological studies on mortality from cardiovascular disease, two studies showed increased mortality from ischaemic heart disease in workers exposed to dichloromethane, compared with an internal reference group or a non-exposed cohort, although mortality did not increase compared with the general population (Tomenson et al., 1997; Tomenson, 2011).

(b) Experimental systems

No data were available to the Working Group.

4.4.4 Respiratory system

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Nasal cavity lesions of olfactory epithelium and hyperplasia of the terminal bronchiole have been reported in male and female BDF1 mice in a 2-year study of exposure to dichloromethane by inhalation (JISHA, 2000b). The incidence of eosinophilic changes in the respiratory epithelium was also elevated in female mice in this study.

F344/N rats were exposed to dichoromethane at a concentration of 0, 1000, 2000, or 4000 ppm by inhalation for 6 hours per day, 5 days per week, for 102 weeks. Squamous metaplasia of the nasal cavity was observed as a treatment-related non-neoplastic change in rats (Mennear et al., 1988).

The labelling index in bronchiolar epithelium (in two branches proximal to the terminal bronchiole and in the terminal bronchioles themselves) in female B6C3F$_1$ mice exposed to dichloromethane at 2000 ppm for 2–26 weeks decreased to 40–60% of the value for control mice. Exposure to dichloromethane at 8000 ppm led to a smaller decrease in labelling index. No pathological changes were found in the exposed lungs (Kanno et al., 1993). In male B6C3F$_1$ mice exposed to dichloromethane by inhalation (6 hours, single dose), vacuolation of bronchiolar cells was observed at exposure levels ≥ 2000 ppm [6940 mg/m^3], while no effect was observed at levels ≤ 1000 ppm [3470 mg/m^3] (Foster et al., 1994). Pretreatment with the CYP inhibitor piperonyl butoxide (300 mg/kg, administered intraperitoneally) 1 hour before exposure abolished the toxic effect in bronchiolar cells, while buthionine sulfoximine (1 g/kg, administered intraperitoneally), which decreased the pulmonary GSH content by 50%, had no protective effect. In Clara cells isolated after exposure to dichloromethane (≥ 1000 ppm), the proportion of cells in S-phase was increased.

4.4.5 Kidney

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In a 2-year study in female F344 rats exposed to dichloromethane by inhalation, kidney tubular degeneration was reported to be significantly elevated in response to treatment when compared with controls (NTP, 1986). In the same study, kidney tubule casts were observed in male and female B6C3F$_1$ mice.

In a 2-year study in female F344 rats exposed to dichloromethane by inhalation, the incidence of chronic nephropathy was significantly elevated in response to treatment when compared with controls (JISHA, 2000a). In a study in similarly exposed BDF1 mice, basophilic change, lymphocytic infiltration and proximal tubule vacuolation were observed (JISHA, 2000b).

After intraperitoneal administration of dichloromethane at near-lethal doses, hydropic degeneration was observed in the mouse kidney (Klaassen & Plaa, 1966), no kidney damage was observed after administration of dichloromethane at doses of 133–665 mg/kg bw by gavage (Condie et al., 1983). Slight calcification of the renal tubules in mongrel dogs was seen after intraperitoneal administration of dichloromethane at near-lethal doses (Klaassen & Plaa, 1967).

In rats, intraperitoneal administration of dichloromethane at 1330 mg/kg bw produced renal proximal tubular swelling (Kluwe et al., 1982). After a similar dose administered by gavage, a transient elevation in blood urea nitrogen levels and decreased urine output, coinciding with cloudy swelling of tubular cells, were observed (Marzotko & Pankow, 1988). Urinary flow was already decreased at the lowest dose tested (3.1 mmol/kg bw; 263 mg/kg bw). In F344/N rats exposed to dichloromethane at 0, 1000, 2000, or 4000 ppm by inhalation, for 6 hours per day, 5 days per week, for 102 weeks, treatment-related degeneration of kidney tubules was reported (Mennear et al., 1988).

4.4.6 Spleen

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

In F344/N rats were exposed by inhalation to dichloromethane at 0, 1000, 2000, or 4000 ppm, for 6 hours per day, 5 days per week, for 102 weeks, fibrosis of the spleen was observed as a treatment-related non-neoplastic change (Mennear et al., 1988).

4.5 Susceptible populations

4.5.1 Polymorphisms

(a) CYP2E1

The association between exposure to organic solvents including dichloromethane and NHL was investigated in relation to different genetic variations in four metabolic genes – CYP2E1, microsomal epoxide hydrolase (EPHX1), myeloperoxidase (MPO), and quinone oxidoreductase (NQO1) – using unconditional logistic regression models based on data collected from women in Connecticut, USA, in 1996–2000 (Barry et al., 2011). Overall associations between total NHL and dichloromethane (OR, 1.69; 95% CI, 1.06–2.69), carbon tetrachloride (OR, 2.33; 95% CI, 1.23–4.40), and methyl chloride (OR, 1.44; 95% CI, 0.94–2.20) were increased among women of genotype TT for rs2070673 in the CYP2E1 gene (dichloromethane: OR, 4.42; 95% CI, 2.03–9.62; *P* interaction < 0.01; carbon tetrachloride: OR, 5.08; 95% CI, 1.82–14.15; *P* interaction = 0.04; and methyl chloride: OR, 2.37; 95% CI, 1.24–4.51; P interaction = 0.03). In contrast, no effects of these solvents were observed among women of genotype TA/AA. Similar patterns were observed for dichloromethane and diffuse large B-cell lymphoma, follicular lymphoma, and marginal zone lymphoma (Barry et al., 2011). [The Working Group noted that the functional significance of this polymorphism was unknown.]

(b) GSTT1

GSTT1 polymorphisms may result in inter-individual variation in the ability to metabolize dichloromethane by GSH conjugation; some individuals (non-conjugators) completely lack GSH conjugation activity. Because GSH conjugation of dichloromethane leads to formation of reactive and genotoxic metabolites, it is plausible that diminished or lack of GSH conjugation activity will lead to reduced risk of carcinogenesis. For

instance, in the absence of GSTT1, exposure to dichloromethane did not lead to formaldehyde production in human erythrocytes (Hallier et al., 1994), and DNA–protein-cross-links were not detected in human liver cells (Casanova et al., 1997). This could be relevant to multiple target tissues that express GSTs, including the liver, kidney, brain, and lung (Sherratt et al., 1997, 2002).

Interindividual variation in the conjugation of dichloromethane with GSH by cytosolic GST in vitro was investigated in 22 samples of human liver (Bogaards et al., 1993). In nine of the liver samples, the α-, mu-, and pi-class GST subunits were quantified. In two of these samples, no activity was observed towards dichloromethane, while α-, mu-, and pi-class subunits were expressed in these human liver cytosolic samples, suggesting no relationship between enzymatic activities and dichloromethane with these classes of GST.

Hallier et al. (1993) found that dichloromethane induced sister-chromatid exchange in the human lymphocytes of non-conjugators donors lacking GST activity, but not in those of conjugators. However, Olvera-Bello et al. (2010) demonstrated that the group with high GSTT1 activity showed a larger increase in the frequency of sister-chromatid exchange induced by dichloromethane than did the groups with low and medium GSTT1 activity.

Garte et al. (2001) showed major and significant differences in the allele and genotypes frequencies between ethnic groups, especially between Asians and Caucasians (Table 4.6).

Table 4.6 Frequencies of *GSTT1*0* gene polymorphism in Caucasians and Asians

Ethnicity	No.	Homozygous	Range
Caucasians	5577	0.197	0.13–0.26
Asians	575	0.470	0.35–0.52

From Garte et al. (2001)

4.5.2 Life stage

Few studies have examined the influence of life stage on dichloromethane-induced toxicity or carcinogenesis. Most of the available studies related to potential differences in toxicokinetics across life stages, with no chemical-specific data on toxicodynamic differences. With respect to absorption and distribution, no age-dependent differences in the partition coefficient for mixtures of volatile organic solvents have been observed in rats (Mahle et al., 2007). No data on life-stage–dependent differences in elimination or excretion were available.

Although no direct data on life-stage–dependent differences in dichloromethane metabolism were available, based on information on the ontogeny of CYP2E1 and GSTT1, such differences are plausible. In humans, CYP2E1 activity is low during gestation and the early neonatal period (Choudhary et al., 2005), but no data were available on the ontogeny of GSTT1. Data in experimental animals suggested that both CYP2E1 (Choudhary et al., 2005) and GST (Cui et al., 2010) expression are low during gestation, and peak between 0 and 12 days after birth. Czekaj et al. (2010) found that CYP2E1 expression increases further in older adult rats. Although the qualitative patterns were similar, the available data were insufficient to estimate the magnitude of any differences in the proportion of oxidative metabolism versus conjugation during early life stages as compared with during adulthood. Therefore, there was inadequate evidence to conclude whether there are differences in susceptibility as a function of life stage as a result of changes in metabolism.

4.6 Mechanistic considerations

See Table 4.7

Two important metabolic pathways for the metabolism of dichloromethane have been

characterized in humans and experimental animals. One pathway is CYP2E1-mediated reductive dehalogenation, which ultimately generates CO and CO_2 as stable end products. One of the intermediates, formyl chloride, can react with nucleophiles. GSH conjugation, catalysed primarily by GSTT1, is the other important metabolic pathway of dichloromethane, resulting in the formation of reactive metabolites, including formaldehyde and *S*-chloromethyl GSH.

Supporting evidence for the GST pathway include in-vitro studies from human-derived tissue or cells, in-vivo studies in rodents, in-vitro studies in rodent-derived tissue or cells, in-vitro mutagenicity studies in microorganisms, and biochemical studies with purified enzymes. Humans are polymorphic for GSTT1, with a proportion of the population showing no activity towards dichloromethane. CYP2E1 catalytic activity predominates at relatively low concentrations of substrate, but there is ample evidence that GST-mediated metabolism eventually predominates at higher concentrations (Gargas et al., 1986; Clewell, 1995; Bos et al., 2006). Such higher concentrations of dichloromethane are readily observed in occupational settings and in some environmental exposures. Moreover, with continued exposure to dichloromethane, even at relatively low concentrations, CYP2E1 readily becomes saturated. Overall, evidence strongly supports qualitative similarities in both oxidative and GST-mediated metabolism of dichloromethane between humans and rodents.

Differences in activity levels and tissue and cellular distributions of GSTT1 exist across species. For instance, in the liver and lung, two sites where tumours are observed in mice in long-term bioassays (NTP, 1986), GSTT1 activity was greater in mice than in rats or humans (Reitz et al., 1989; Thier et al., 1998). Humans, however, have GSTT1 activity in erythrocytes that is comparable to that in the mouse liver, while neither rats nor mice exhibit GSTT1 activity in erythrocytes (Thier et al., 1998). Additionally, in the mouse liver, nuclear localization of GSTT1 was observed in hepatocytes, while in the human liver, nuclear localization of GSTT1 was observed in bile-duct epithelial cells (Quondamatteo et al., 1998; Sherratt et al., 2002). Thus, while the metabolic pathways are similar across species, the target tissues and cell types of GSTT1 metabolism differ across species.

Dichloromethane has been evaluated for genotoxicity in several test systems, both in the presence or absence of metabolic activation. In human cell lines or isolated cells, dichloromethane has been reported to induce micronucleus formation and sister-chromatid exchange (Hallier et al., 1993; Doherty et al., 1996; Olvera-Bello et al., 2010); but studies of DNA–protein cross-links, DNA single-strand binding proteins (SSBs), and unscheduled DNA synthesis have largely given negative results (Jongen et al., 1981; Graves et al., 1995; Casanova et al., 1997). In one study, the extent of sister-chromatid exchange was greater in cells from individuals without GST activity (Hallier et al., 1993). In another study, by contrast, the extent of sister-chromatid exchange was greater in cells from individuals with high GSTT1 activity (Olvera-Bello et al., 2010). In experimental animals, dichloromethane-induced genotoxicity also tended to correlate with GST activity, with positive results in cells derived from mouse liver and lung, which also exhibited the greatest GST activity (Graves et al., 1994b, 1995; Casanova et al., 1997). Similarly, after exposure to dichloromethane in vivo, although many studies gave negative results for genotoxicity, positive results in multiple measures of genotoxicity were reported in tissues with GST-mediated metabolism, such as the mouse liver and lung (Allen et al., 1990; Casanova et al., 1992, 1996; Graves et al., 1995; Sasaki et al., 1998). Finally, several studies in non-mammalian in-vitro systems showed evidence for mutagenicity, particularly in systems in which GST activity is present or exogenously enhanced (Jongen et al., 1978, 1982; Gocke et al., 1981;

Table 4.7 Relationship between the glutathione/glutathione *S*-transferase pathway and dichloromethane-induced genotoxicity

System	DNA damage without exogenous metabolic activation	Comments	Reference
Salmonella typhimurium TA1535 transfected with GSTT1 (GST5-5)	+	Increased number of revertants in transfected *GSTT1* strain compared with non-transfected strain	Thier et al. (1993)
Salmonella typhimurium TA100, reverse mutation	+	In strain NG54, GSH-deficient TA100, twofold reduction in the number of revertants was observed	Dillon et al. (1992)
Salmonella typhimurium TA100, reverse mutation	+	In strain NG11, GSH-deficient TA100, twofold reduction in the number of revertants was observed	Graves et al. (1994a)
Salmonella typhimurium TA1535 transfected with GSTT1	+	96–100% of mutations were GC → AT in TA1535 transfected with GSTT1 compared with 15% in *S. typhimurium* TA100 (homologue of TA1535 containing plasmid pKM101) without *GSTT1*gene	DeMarini et al. (1997)
Single-strand breaks, $B6C3F_1$ mouse and rat hepatocytes, in vitro	+	Pre-treatment of hepatocytes with BS decreased DNA damage	Graves et al. (1994b)
Single-strand breaks, $B6C3F_1$ Clara cells, in vitro	+	Cotreatment with BS, decreased DNA damage	Graves et al. (1995)
Single-strand breaks, $B6C3F_1$ mouse lung and liver, in vivo	+	Pre-treatment of mice with BS decreased DNA damage	Graves et al. (1995)
Sister-chromatid exchange, human peripheral blood mononuclear cells	+	Group with high GSTT1 activity group showed larger DCM-induced increase in frequency than groups with low or medium GSTT1 activity	Olvera-Bello et al. (2010)
Sister-chromatid exchange, human lymphocytes, in vitro	+	Positive results in lymphocytes from donors "non conjugators" lacking GST activity (not in lymphocytes from "conjugators") (type of GST, NR)	Hallier et al. (1993)
DNA–protein cross-links, $B6C3F_1$/CrlBR mouse liver, in vivo, inhalation	+	Mice (type, NR) formed DNA–protein cross-links in the liver	Casanova et al. (1992, 1996)
DNA–protein cross-links, human hepatocytes (expressing GSTT1), in vitro	–	RNA–formaldehyde adducts were detected in human hepatocytes expressing *GSTT1,* but not in those lacking *GSTT1*	Casanova et al. (1997)
DNA–protein crosslinks, murine GSTT1-transfected V79 cells, comet assay, in vitro	+	DNA–protein crosslinks not observed in parent V79 cell line	Hu et al. (2006)

+, positive;–, negative; BS, buthionine sulfoximine, a glutathione-depleting agent; DCM, dichloromethane; GST, glutathione *S*-transferase; GSTT1, glutathione S-transferase theta 1; NR, not reported

Green, 1983; Thier et al., 1993; DeMarini et al., 1997; Pegram et al., 1997). Overall, genotoxicity attributable to dichloromethane appears to be strongly associated with GST-mediated metabolism, consistent with the formation of reactive metabolites through this pathway. However, in two available studies in human cells, enhanced genotoxicity was observed without GSTT1 activity in one, and with high GSTT1 activity in another.

Increased liver weights and glycogen deposition were observed after long-term exposure to dichloromethane, but their relationship to carcinogenesis was not clear (NTP 1986; Kari et al., 1993). Several studies in mice have shown that liver cell proliferation does not increase with

exposure to dichloromethane, suggesting that proliferation does not play a role in hepatocarcinogenesis in the mouse (Lefevre & Ashby, 1989; Foley et al., 1993; Casanova et al., 1996). In the mouse lung, acute exposure to dichloromethane leads to vacuolization of Clara cells, but this effect appears to be transient (Foster et al., 1992), so is unlikely to be involved in carcinogenesis in the mouse lung. Mice exposed to dichloromethane for up to 26 weeks had no pathological changes in the lung, but exhibited a decrease in cell proliferation in this tissue. Neurological, renal, spleen, reproductive, and developmental toxicity have also been reported in humans or experimental animals, confirming the widespread distribution of dichloromethane or its metabolites.

Together, the relationship between GSTT1-mediated metabolism, formation of reactive metabolites, the association between GST activity and genotoxicity, and the presence of GSTT1 polymorphisms in the human population suggest that GSTT1 polymorphism may lead to differential susceptibility to dichloromethane-related carcinogenesis. However, no studies have directly investigated whether an association exists between GSTT1 polymorphism and the incidence of cancer. One study has reported an association between a CYP2E1 polymorphism and NHL in dichloromethane-exposed individuals (Barry et al., 2011). Whether this is due to differences in formation of CYP2E1-mediated metabolites, which may also be reactive, or to a shift in the proportion of GST-mediated reactive metabolites is unknown.

5. Summary of Data Reported

5.1 Exposure data

Dichloromethane is a chlorinated solvent that was first synthesized in the 1840s, and is produced by hydrochlorination of methanol or by direct chlorination of methane. Dichloromethane has been used in paint stripping, aerosol spray products, in the manufacture of polycarbonate plastic and hydrofluorocarbons, in the production of synthetic fibres, in metal cleaning, in printing-press cleaning, as an extraction solvent for certain foods, and in the production of refrigerants. Annual world production in 2005 to 2010 was estimated at between 764 000 and 814 000 tonnes.

The principal occupational exposures to dichloromethane have been from its use in paint stripping, spray painting, and metal and printing-press cleaning. Occupational exposures of more than 1000 mg/m^3 were measured in the paint, printing, and chemical manufacturing industries before 2000. More recently reported levels have been lower, except for some printing plants in Japan where values were estimated at being up to about 900 mg/m^3. The main current source of exposure to the general population is through the use of consumer products containing dichloromethane. Recent reports of ambient air concentrations around industrial areas in some countries are as high as 200 µg/m^3, and groundwater concentrations can remain high for many decades after spills. Several jurisdictions (including the USA, the European Union, and Japan) have moved to reduce the use and release of various volatile organic compounds, including dichloromethane. These measures have included reducing or banning dichloromethane use in paint strippers and cosmetics.

5.2 Human carcinogenicity data

Two cohort studies of workers exposed to dichloromethane (as well as acetone and methanol, but not 1,2-dichloropropane) in the USA reported findings for cancers of the liver and biliary tract, based on small numbers. One of the studies reported a positive association for cancer of the liver and biliary tract, while the other did not. Only one study reported a standardized mortality ratio separately for cancer of

the biliary tract (SMR, 20). Cancer of the biliary tract constituted three of the four liver cancers in the study with a positive association, and both of the liver cancers in the other. Given that cancer of the biliary tract normally represents a small proportion of cancers of liver and biliary tract combined, these proportions are very high. In a case series of cancer of the biliary tract (histologically identified as cholangiocarcinoma) among printing workers in Japan, most of the cases were exposed to dichloromethane, and all except one of these were also exposed to 1,2 dichloropropane. The high risk of this rare cancer in one cohort study of workers without exposures to other likely risk factors and among exposed printing workers in Japan is consistent with a causal association, but the number of exposed cases was small and the printing workers had other potentially confounding exposures, notably to 1,2 dichloropropane.

Two cohort studies and three case–control studies in several countries evaluated non-Hodgkin lymphoma (NHL), and all except one cohort study reported increased risks among workers exposed to dichloromethane. While positive associations for NHL were consistent among studies using different designs and in several countries, most subjects were exposed to several solvents (some of which have been previously associated with NHL) and the risk estimates were based on small numbers.

There were several studies that assessed other cancer sites, but these data were regarded as inadequate.

5.3 Animal carcinogenicity data

There were six studies of carcinogenicity with dichloromethane in mice: two studies of oral administration (one with drinking-water in males and females, and one by gavage in males and females), three studies of inhalation (two in males and females, one in females), and one study in which dichloromethane was injected intraperitoneally in males. Dichloromethane increased the incidence of hepatocellular carcinoma in three studies in male mice (two by inhalation, one in drinking-water), and in three studies of inhalation in female mice. Dichloromethane increased the incidence of hepatocellular adenoma or carcinoma (combined) in two studies of inhalation in male mice and three studies by inhalation in female mice. Dichloromethane increased the incidence of bronchiolo-alveolar carcinoma in two inhalation studies in male mice and three inhalation studies in female mice, and bronchiolo-alveolar adenoma or carcinoma (combined) in three inhalation studies in male mice and three inhalation studies in female mice. Dichloromethane increased the incidences of haemangioma of the liver and of all organs (including the liver) in one inhalation study in male mice, and may have increased the incidence of haemangioma or haemangiosarcoma (combined) in the liver in one inhalation study in female mice.

There were seven studies of carcinogenicity with dichloromethane in rats: two oral administration studies (one drinking-water study in males and females and one gavage study in males and females), five inhalation studies (four in males and females, one in pregnant females and their male and female offspring). Dichloromethane increased the incidence of fibroma of the subcutis in two inhalation studies in male rats and fibroma or fibrosarcoma of the subcutis in one inhalation study in male rats. Dichloromethane caused salivary gland sarcomas in one inhalation study in male rats (the sialodacryoadenitis virus was detected in these rats; the effect of this virus on carcinogenesis is unknown). Dichloromethane increased the incidence of mammary gland adenoma or fibroadenoma (combined) in two inhalation studies in female rats and one inhalation study in male rats. The incidence of mammary gland adenoma was also increased in another inhalation study in males and another one in females. Dichloromethane

caused a minimal increase (positive trend test) in hepatocellular adenomas and carcinomas (combined) in female rats in one oral administration (drinking-water) study.

There was one inhalation study on dichloromethane in male and female Syrian hamsters in which there was an increase in the incidence of malignant lymphoma in females.

5.4 Mechanistic and other relevant data

Dichloromethane is a volatile lipophilic compound that is readily absorbed after oral, inhalation, or dermal exposure, and distributed systemically. Two important metabolic pathways for the metabolism of dichloromethane have been characterized in humans and experimental animals. One pathway is CYP2E1-mediated, which ultimately generates carbon monoxide (CO) and carbon dioxide (CO_2) as stable end products. One of the intermediates, formyl chloride, is reactive with nucleophiles. glutathione conjugation, catalysed primarily by glutathione *S*-transferase theta-1 (GSTT1), is the other important metabolic pathway, and results in the formation of reactive metabolites, including formaldehyde and *S*-chloromethyl glutathione. CYP2E1-mediated metabolism is predominant at lower concentrations, but can be easily saturated, with glutathione *S*-transferase-mediated metabolism eventually predominating at higher concentrations.

Oxidative and glutathione *S*-transferase (GST)-mediated metabolism of dichloromethane are qualitatively similar between humans and rodents, but quantitative differences exist across species, tissues, and cell types, and among individuals. Differences in GSTT1 expression and localization may be important determinants of site-specific carcinogenicity caused by dichloromethane.

In human cells, dichloromethane induces micronucleus formation and sister-chromatid exchange, but not DNA–protein cross-links and DNA damage. In experimental animals, dichloromethane-induced genotoxicity is associated with the GST pathway. Studies in non-mammalian systems in vitro showed evidence of mutagenicity, particularly in systems with GST activity. Evidence for the role of GSTT1 in genotoxicity in humans is mixed. Overall, the genotoxicity of dichloromethane appears to be strongly associated with GST-mediated metabolism, consistent with the formation of reactive metabolites through this pathway.

Hepatic, neurological, renal, splenic, reproductive, and developmental toxicity have also been reported in humans or experimental animals.

There is little evidence for non-genotoxic mechanisms of carcinogenesis with dichloromethane.

No studies with dichloromethane in humans have investigated whether GSTT1 polymorphisms are associated with cancer. One study has reported an association between a CYP2E1 polymorphism and non-Hodgkin lymphoma in dichloromethane-exposed individuals; however, the functional significance of this polymorphism is unknown.

Overall, given the extensive evidence for genotoxicity, particularly in association with a metabolic pathway that is operative in humans, the Working Group concluded that the mechanistic evidence for dichloromethane carcinogenesis is *strong*.

6. Evaluation

6.1 Cancer in Humans

There is *limited evidence* in humans for the carcinogenicity of dichloromethane. Positive associations have been observed between exposure to dichloromethane and cancer of the biliary tract and non-Hodgkin lymphoma.

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

6.4 Rationale

The overall evaluation of Group 2A was based on *sufficient evidence* in experimental animals and *limited evidence* in humans. In addition, a Group 2A evaluation was also supported by *sufficient evidence* in experimental animals, and the *strong* evidence that the metabolism of dichloromethane via GSTT1 leads to the formation of reactive metabolites, that GSTT1 activity is strongly associated with genotoxicity in vitro and in vivo, and that GSTT1-mediated metabolism of dichloromethane occurs in humans.

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1,3-PROPANE SULTONE

1,3-Propane sultone was reviewed previously by the Working Group in 1973, 1987, and 1998 (IARC, 1974, 1987, 1999). New data have since become available, and these have been incorporated, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

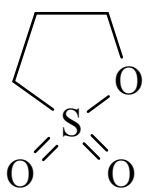
Chem. Abstr. Serv. Reg. No.: 1120-71-4

Chem. Abstr. Serv. Name: 1,2-Oxathiolane, 2,2-dioxide

IUPAC Systematic Name: Oxathiolane 2,2-dioxide

Synonyms: 3-Hydroxy-1-propanesulfonic acid sultone; 3-hydroxythietane-1,1-dioxide; 1,2-oxathiolane 2,2-dioxide; 1-propanesulfonic acid-3-hydroxy-gamma-sultone; propane sultone; propanesultone

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_3H_6O_3S$

Relative molecular mass: 122.14

1.1.3 Chemical and physical properties of the pure substance

Description: White crystalline solid or colourless liquid; foul odour above 31 °C (HSDB, 2014)

Boiling point: 180 °C at 40 HPa (30 mmHg) (Sigma-Aldrich, 2012)

Melting point: 30–33 °C (Sigma-Aldrich, 2012)

Specific gravity: 1.392 at 25 °C (Sigma-Aldrich, 2012)

Solubility: Readily soluble in ketones, esters and aromatic hydrocarbons; soluble in water (100 g/L) (HSDB, 2014); insoluble in aliphatic hydrocarbons (IARC, 1974)

Volatility: Vapour pressure, 0.27 mmHg at 25 °C; vapour density relative to air, 4.2 (NTP, 2011)

Stability: Hydrolyses to 3-hydroxy-1-propanesulfonic acid (IARC, 1974)

Octanol/water partition coefficient: log $k_{ow,}$ −0.28 (NTP, 2011)

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), 1 mg/m^3 = 4.99 ppm, calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm.

1.1.4 Technical products and impurities

One commercial-grade formulation of 1,3-propane sultone was reported to contain 99% active ingredient, 0.2% water, and 0.8% acid (as 3-hydroxy-1-propanesulfonic acid) (IARC, 1974).

1.1.5 Analysis

Two methods have been described for the determination of 1,3-propane sultone in workplace air. In the first, 1,3-propane sultone is preconcentrated from air in a wash bottle containing methyl-isobutylketone as absorbent. 1,3-Propane sultone is then determined by gas chromatography with sulfur selective detection. Alternatively, 1,3-propane sultone is collected by drawing air through an impinger, the inner wall of which being coated with 2-mercaptobenzothiazole (sodium salt) as a sink. In this way, 1,3-propane sultone is preconcentrated in the form of the sodium salt of 2-mercaptobenzothiazole-*S*-propanesulfonic acid, which is extracted by high-perfomance liquid chromatography (HPLC) with ultraviolet detection (Oldeweme & Klockow, 1986; Royal Society of Chemistry, 1989).

The United States Environmental Protection Agency (EPA) has developed a method (EPA-TO-15) for the analysis of a wide range of volatile organic compounds in air, including 1,3-propane sultone. The method involves sampling air, by vacuum or pumping, into an evacuated canister, which has a passivated, chemically inert inner surface. A known volume of sample is then directed from the canister through a solid multisorbent concentrator, where the analyte is concentrated before analysis by thermal desorption and analysis by gas chromatography/mass spectrometry (EPA, 1999).

1.2 Production and use

1.2.1 Production

1,3-Propane sultone can be produced commercially by dehydrating gamma-hydroxypropanesulfonic acid, which is prepared from sodium hydroxypropanesulfonate. This sodium salt can be prepared by the addition of sodium bisulfite to allyl alcohol (Li et al., 2013).

1,3-Propane sultone was produced in Germany and in the USA in the 1950s and 1960s (IARC, 1974). In 1974, the only producer of 1,3-propane sultone in the USA manufactured less than 500 kg annually (IARC, 1974). In 2009, 1,3-propane sultone was produced by one manufacturer each in Europe and China, and was available from 28 suppliers, including 13 suppliers in the USA (NTP, 2011). Reports filed in 1986, 1990, and 2002 under the Toxic Substances Control Act Inventory Update Rule of the EPA indicated that production plus imports of 1,3-propane sultone in the USA totalled 10 000 to 500 000 lb [~4.5 to 227 tonnes] (NTP, 2011).

1.2.2 Use

1,3-Propane sultone has been used as an intermediate to introduce the propylsulfonate group into molecules, and to confer water solubility and an anionic character (IARC, 1999).

Although the industrial use of 1,3-propane sultone was largely discontinued in the 1960s (Bolt & Golka, 2012), the compound has more recently been used for the manufacture of products for the galvanotechnical and photographic industry (Oldeweme & Klockow, 1986), and also for chemical synthesis in the laboratory (Geddes, 2000; Kirschner & Green, 2005; Smith & Zharov, 2008; Kumar et al., 2012).

A recent patent cites use of 1,3-propane sultone in the preparation of a pharmaceutical intermediate (Wei et al., 2012). 1,3-Propane sultone has also been proposed as an electrolyte additive to improve cyclability safety of lithium

ion batteries (Park et al., 2009; Han et al., 2012). It has also been used in the preparation of hydroxyl sulfonate surfactants for use in enhanced oil recovery, both in micellar polymer flooding and in foam treatment (Rist & Carlsen, 2005). 1,3-Propane sultone has been used to prepare ultrathin antifouling coatings with stable surface zwitterionic functionality (Yang & Gleason, 2012).

1,3-Propane sultone has been used as a chemical intermediate in the production of fungicides, insecticides, cation-exchange resins, dyes, vulcanization accelerators, and variety of other chemicals (IARC, 1999).

In the European Union, under the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulations, 1,3-propane sultone is registered for use as an additive for electrolysis, manufacture of fine chemicals, manufacture of bulk, large-scale chemicals, and formulations of preparations, substances, and mixtures (ECHA, 2015). 1,3-Propane sultone is also registered for the following uses at industrial sites: (a) as a transported isolated intermediate: 1,3-propane sultone is used as a pre-product in the manufacture of aqueous polyurethane dispersions, and of light-sensitive dyes for photographic and radiographic films; (b) as an onsite isolated intermediate: 1,3-propane sultone is used to manufacture sulfopropylated substances by complete conversion with amines, mercaptanes, alcoholates, and carboxylates; (c) in scientific research and development; and (d) in the manufacture of batteries and accumulators.

The only permitted consumer use of 1,3-propane sultone in Europe is in sealed batteries, where use does not infringe the restriction for use by non-professionals since batteries are articles with no intended release of the substance and not covered by the REACH regulations (ECHA, 2015).

1.3 Occurrence and exposure

1.3.1 Environmental occurrence

(a) Natural occurrence

1,3-Propane sultone is not known to occur naturally.

(b) Air, water, soil, or food

No data were available on levels of 1,3-propane sultone in air, water, soil, or food.

In moist air, 1,3-propane sultone will hydrolyse to form 3-hydroxy,1-propane sulfonic acid. In the atmosphere, it will react with photochemically produced hydroxyl radicals (half-life, 8 days) (NTP, 2011).

In water or a moist environment, 1,3-propane sultone will also rapidly hydrolyse to 3-hydroxy-1-propanesulfonic acid. 1,3-Propane sultone may occur in the waste streams of industrial facilities where it is manufactured or used, but is not expected to persist for long periods of time (IARC, 1974).

1.3.2 Occupational exposure

There are few data available on occupational exposure to 1,3-propane sultone. An exposure study of 1,3-propane sultone from 1972 by the Government of Japan shows a low level of exposure. Out of two reported companies with six types of jobs and 85 workers, 8-hour time-weighted average (TWA) exposure was less than 0.007 mg/m^3. In 2008, the Government of Japan required companies using ≥ 500 kg of 1,3-propane sultone to report the amount used and job types involved (MHLW, 2010).

The routes of potential human exposure to 1,3-propane sultone are ingestion, inhalation, and dermal contact (NTP, 2011). Workers involved in the formulation of compounds made from 1,3-propane sultone or the production of its end products are at the greatest risk of potential exposure (IARC, 1974).

1.3.3 Exposure of the general population

There were no data available on levels of exposure to 1,3-propane sultone in humans.

The Toxic Chemical Release Inventory of the EPA reported that 332 lbs [~150 kg] of 1,3-propane sultone was released to the environment in the USA in 2012 (TRI-Explorer, 2012).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists has recommended that occupational exposure by all routes to 1,3-propane sultone be carefully controlled to levels that are as low as possible (ACGIH, 2001).

The United States National Institute of Occupational Safety and Health has recommended that occupational exposures to 1,3-propane sultone be limited to the lowest feasible concentration (NIOSH, 2005).

In Ontario, Canada, under Regulation 833, occupational exposure by all routes to 1,3-propane sultone in the workplace is required to be carefully controlled to levels as low as possible (Ontario Ministry of Labor, 2013). There were no published limit values for 1,3 propane sultone in countries of the European Union, or elsewhere in the world.

The Scientific Committee on Occupational Exposure limits (SCOEL) of the European Commission has categorized 1,3-propane sultone in SCOEL Group A, as a genotoxic carcinogen without a threshold. Therefore, a health-based occupational exposure limit cannot be deduced. For humans, any contact with 1,3-propane sultone is to be avoided. Dermal absorption can contribute substantially to concern regarding health effects. Therefore, a skin notation is warranted (SCOEL, 2013).

In Japan, the Industrial Safety and Health Act requires enclosure systems during manufacturing, use, and waste treatment, as well as appropriate personal protective equipment to avoid skin contact; no exposure limit values have been established (MHLW, 2011).

2. Cancer in Humans

Only one study of cancer in humans exposed to 1,3 propane sultone was available to the Working Group. Bolt & Golka (2012) describe the occurrence of cancer among 55 employees at a factory in Germany that manufactured 1,3-propane sultone in 1952–1963; the last stocks were used as of 1977. A list of exposed workers was registered in 2007 as required by law, and those who developed cancer were eligible for compensation. As of 2010, cancer had been observed in 20 of the exposed workers. Among the 24 tumours reported were several rare cancers, including one cancer of the duodenum, and one malignant Schwannoma (a peripheral nerve sheath tumour, also known as neurosarcoma). The reported tumours included two glioblastomas (cancers of the brain were previously reported in experimental animals exposed to 1,3 propane sultone). Two cancers of skin (one basal cell, the other type unspecified) were also observed. No data on the expected numbers of all cancers were presented. [Without comparative data on the number of cancers expected, it is difficult to interpret the findings of this study, which is essentially a case series among an exposed population.]

3. Cancer in Experimental Animals

The carcinogenicity of 1,3-propane sultone in experimental animals was previously reviewed by the Working Group (IARC, 1999).

3.1 Mouse

1,3-Propane sultone was tested for carcinogenicity in one study in male and female mice treated by skin application, and in one study in female mice treated by subcutaneous injection.

See Table 3.1

Table 3.1 Studies of carcinogenicity with 1,3-propane sultone in mice

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Skin application				
CF1 (M) ≥ 78 wk Doak et al. (1976)	Single dose of toluene, single dose of 1,3-propane sultone 2.5% w/v in toluene, 10 doses of 2.5% w/v in toluene every other day, single dose of 25% w/v in toluene. Painted on shaved back skin, then observed for ≥ 78 wk 48 mice/group	*Skin tumours* 0/48, 0/48, 3/48 (6%), 29/36 (80%)* Benign: 0/48, 0/48, 1/48 (2%), 13/36 (36%)* Malignant: 0/48, 0/48, 2/48 (4%), 16/36 (44%)* *Systemic tumours* 29/48 (60%), 34/48 (71%), 47/48 (98%)*, 28/36 (78%) Lymphoreticular: 4/48 (8%), 10/48 (21%), 22/48 (46%)*, 12/36 (33%) Lung: 23/48 (48%), 27/48 (56%), 34/48 (71%), 24/36 (67%)	*[$P < 0.05$]	Purity, > 99.9% Most skin tumours were epidermal, but C3H mice developed mainly fibrosarcomas Systemic tumours were lymphoreticular, lung, mammary gland, uterine, and other sites
CF1 (F) ≥ 78 wk Doak et al. (1976)	Single dose of toluene, single dose of 1,3-propane sultone at 2.5% w/v in toluene, 10 doses of 2.5% w/v in toluene every other day, single dose of 25% w/v in toluene. Painted on shaved back skin then observed for ≥ 78 wk 48 mice/group	*Skin tumours* 0/48, 1/48 (2%), 2/48 (4%), 26/46 (56%)* Benign: 0/48, 0/48, 2/48 (4%), 18/46 (39%)* Malignant: 0/48, 1/48 (2%), 0/48, 8/46 (17%)* *Systemic tumours* 29/48 (60%), 42/48 (87%)*, 48/48 (100%)*, 36/46 (78%) Lymphoreticular: 9/48 (19%), 13/48 (27%), 36/48 (75%)*, 20/46 (43%) Lung: 17/48 (35%), 25/48 (52%), 32/48 (67%)*, 26/46 (56%)	*[$P < 0.05$]	Purity, > 99.9% Most skin tumours were epidermal, but C3H mice developed mainly fibrosarcomas Systemic tumours were lymphoreticular, lung, mammary gland, uterine, and other sites
CF1 (M) 56 wk Doak et al. (1976)	0 or 2.5% 1,3-propane sultone w/v in benzene painted on shaved back skin, 2 × per wk for 4 wk, then in toluene painted on shaved back skin, 2 × per wk for 52 wk 25 mice/group	*Skin tumours* 0/22, 15/21 (71%)* Benign: 0/22, 6/21 (28%)* Malignant: 0/22, 9/21 (43%)* *Systemic tumours* 7/22 (32%), 18/21 (86%)* Lymphoreticular: 0/22, 12/21 (57%)* Lung: 4/22 (18%), 9/21 (43%)	*[$P < 0.05$]	Purity, > 99.9% Most skin tumours were epidermal, but C3H mice developed mainly fibrosarcomas Systemic tumours were lymphoreticular, lung, mammary gland, uterine, and other sites

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CF1 (F) 56 wk Doak et al. (1976)	0 or 2.5% 1,3-propane sultone w/v in benzene painted on shaved back skin 2 × per wk for 4 wk, then in toluene painted on shaved back skin 2 × per wk for 52 wk 21–25 mice/group	*Skin tumours* 0/25, 3/24 (12%) Benign: 0/25, 3/24 (12%) Malignant: 0/25, 0/24 *Systemic tumours* 7/25 (28%), 18/24 (75%)* Lymphoreticular: 3/25 (12%), 17/24 (71%)* Lung: 4/25 (16%), 2/24 (8%) Uterine or mammary gland (combined): 1/25 (4%), 2/24 (8%)	*[$P < 0.05$]	Purity, > 99.9% Most skin tumours were epidermal, but C3H mice developed mainly fibrosarcomas Systemic tumours were lymphoreticular, lung, mammary gland, uterine, and other sites
C3H (M) 56 wk Doak et al. (1976)	0 or 2.5% 1,3-propane sultone w/v in benzene painted on shaved back skin 2 × per wk for 4 wk, then in toluene painted on shaved back skin 2 × per wk for 52 wk 25 mice/group	*Skin tumours* 0/25, 20/22 (91%)* Benign: 0/25, 2/22 (9%) Malignant: 0/25, 18/22 (82%)* *Systemic tumours* 13/25 (52%), 18/22 (82%) Lymphoreticular: 1/25 (4%), 2/22 (9%) Lung: 0/25, 5/22 (23%)*	*[$P < 0.05$]	Purity, > 99.9% Most skin tumours were epidermal, but C3H mice developed mainly fibrosarcomas Systemic tumours were lymphoreticular, lung, mammary gland, uterine, and other sites
C3H (F) 56 wk Doak et al. (1976)	0 or 2.5% 1,3-propane sultone w/v in benzene painted on shaved back skin 2 × per wk for 4 wk, then in toluene painted on shaved back skin 2 × per wk for 52 wk 25 mice/group	*Skin tumours* 0/21, 6/25 (24%)* Benign: 0/21, 2/25 (8%) Malignant: 0/21, 4/25 (16%) *Systemic tumours* 4/21 (19%), 17/25 (68%)* Lymphoreticular: 0/21, 2/25 (8%) Lung: 0/21, 1/25 (4%) Uterine or mammary gland (combined): 2/21 (9%), 18/25 (72%)*	*[$P < 0.05$]	Purity, > 99.9% Most skin tumours were epidermal, but C3H mice developed mainly fibrosarcomas Systemic tumours were lymphoreticular, lung, mammary gland, uterine, and other sites
CBah (Hairless strain) (M) 56 wk Doak et al. (1976)	0 or 2.5% 1,3-propane sultone w/v in benzene painted on shaved back skin 2 × per wk for 4 wk, then in toluene painted on shaved back skin 2 × per wk for 52 wk 25 mice/group	*Skin tumours* 0/24, 20/23 (87%)* Benign: 0/24, 2/23 (9%) Malignant: 0/24, 18/23 (78%)* *Systemic tumours* 2/24 (8%), 3/23 (13%) Lymphoreticular: 0/24, 2/23 (9%) Lung: 0/24, 0/23	*[$P < 0.05$]	Purity, > 99.9% Most skin tumours were epidermal, but C3H mice developed mainly fibrosarcomas Systemic tumours were lymphoreticular, lung, mammary gland, uterine, and other sites

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CBah (Hairless strain) (F) 56 wk Doak et al. (1976)	0 or 2.5% 1,3-propane sultone w/v in benzene painted on shaved back skin 2 × per wk for 4 wk, then in toluene painted on shaved back skin 2 × per wk for 52 wk 25 mice/group	*Skin tumours* 0/25, 18/25 (72%)* Benign: 0/25, 3/25 (12%) Malignant: 0/25, 15/25 (60%)* *Systemic tumours* 1/25 (4%), 6/25 (24%)* Lymphoreticular: 0/25, 2/25 (8%) Lung: 0/25, 0/25 Uterine or mammary gland (combined): 0/25, 5/25 (20%)*	*[$P < 0.05$]	Purity, > 99.9% Most skin tumours were epidermal, but C3H mice developed mainly fibrosarcomas Systemic tumours were lymphoreticular, lung, mammary gland, uterine, and other sites
Subcutaneous injection				
ICR/Ha Swiss (F) 63 wk Van Duuren et al. (1971)	1,3-Propane sultone at 0, 0.3 mg/0.05 mL in distilled water injected into the left flank 1 × per wk 30 mice/group	*Injection-site tumours* 0/30, 21/30 (70%)* Fibrosarcoma: 0/30, 7/30 (23%)* Epithelial tumours: 0/30, 9/30 (30%)*	*[$P \leq 0.01$]	Purity, 91% Injection-site tumours were: 1 papilloma, 7 adenoacanthomas, 1 undifferentiated carcinoma, 2 spindle cell sarcomas, 7 fibrosarcomas and 3 adenosarcomas

F, female; M, male; wk, week

3.1.1 Skin application

In a first experiment, groups of 48 male and 48 female CF1 mice (age, 6 weeks) were treated with a single dose of 1,3-propane sultone at 0 (toluene only), 2.5% weight/volume (w/v), or 25% w/v in toluene, or with 10 doses at 2.5% w/v every second day, and observed for up to 78 weeks. For each application, approximately 0.1 mL of the test solution was painted on the shaved back skin of mice. The incidence of malignant skin tumours of epidermal origin was significantly higher in male and female mice receiving a single dose at 25% w/v. The incidence of lymphoreticular tumours was significantly higher in male and female mice receiving 10 doses at 2.5% w/v. The incidence of tumours of the lung was significantly increased in female mice receiving 10 doses at 2.5% w/v (Doak et al.,1976)

In another experiment, groups of 20–25 male and female CF1, C3H, and hairless CBah Swiss mice (age, 6 weeks) were given 1,3-propane sultone (purity, > 99.9% [measurement method not reported]) at 0 (control) or 2.5% w/v in benzene by skin application, twice per week for 4 weeks. Beginning on week 5, 1,3-propane sultone was applied twice per week for 52 weeks (56 weeks in total) at concentrations of 0 (control) or 2.5% w/v in toluene. For each application, approximately 0.05–0.1 mL of the test solution was painted on the shaved back skin of mice. Benzene was replaced by toluene because of possible hazard to staff at the animal facility.

Most tumours of the skin observed were of epidermal origin in CF1 and CBah mice, while in C3H male mice the predominant tumour type at the painting site was dermal fibrosarcoma. The systemic tumours observed were lymphoreticular, lung, uterine, mammary gland (in females), and other sites In CF1 male mice, the incidences of malignant skin tumours and of lymphoreticular tumours (mainly lymphoreticular cell sarcomas [possibly malignant lymphomas]) were significantly increased. In C3H and CBah male mice, the incidence of malignant skin tumours was significantly increased. In C3H male mice, the incidence of lung tumours was significantly increased. [The Working Group noted that the biological behaviour of the lung tumours (i.e. benign versus malignant) was not indicated.] In female CF1 mice, incidence of lymphoreticular tumours was significantly increased, but the incidence of skin tumours was not. In female C3H mice, the incidence of skin tumours was significantly increased. In female CBah mice, the incidences of uterine or mammary gland tumours (combined) and skin tumours were significantly increased (Doak et al.,1976). [The Working Group noted the unusual grouping of tumours of the uterus and mammary gland.]

3.1.2 Subcutaneous injection

Groups of 30 female ICR/Ha Swiss mice (age, 6–8 weeks) were injected subcutaneously with 1,3-propane sultone (purity, 91%), at a dose of 0.3 mg/0.05 mL in distilled water in the left flank, once per week for 63 weeks. Controls received distilled water only. At 63 weeks, the incidence of benign and malignant tumours (combined) (21 out of 30: 1 papilloma, 7 adenoacanthomas, 1 undifferentiated carcinoma, 2 spindle cell sarcomas, 7 fibrosarcomas, 3 adenosarcomas) at the injection site was significantly increased compared with the control group (0 out of 30). The incidences of fibrosarcoma and epithelial tumours were significantly increased (Van Duuren et al., 1971).

3.2 Rat

1,3-Propane sultone was tested for carcinogenicity in two studies in male and female rats treated by gavage. [The Working Group considered two other studies by Gupta et al. (1981) and Druckrey et al. (1970) to be inadequate for the evaluation of the agent due to the lack of controls.]

See Table 3.2

Table 3.2 Studies of carcinogenicity in rats given 1,3-propane sultone by gavage

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance[a]	Comments
CD (M) 60–61 wk Ulland et al. (1971)	0, 28 mg/kg bw, 2 × per wk, for 60 wk (lower dose) or 56 mg/kg bw, 2 × per wk, discontinued at wk 32 (higher dose) 26 mice/group	Brain glioma: 0/6, 12/26 (46%)*, 16/26 (61%)**	*$P < 0.01$ **$P < 0.001$	Purity, NR The data for this study may have been included in the study by Weisburger et al. (1981)
CD (F) 60–61 wk Ulland et al. (1971)	0, 28 mg/kg bw, 2 × per wk, for 60 wk (lower dose) or 56 mg/kg bw, 2 × per wk, discontinued at wk 32 (higher dose) 26 mice/group	Brain glioma: 1/6 (17%), 15/26 (58%)*, 13/26 (50%)** Mammary gland: 0/6, 7/26 (27%), 13/26 (50%)*	*[$P < 0.01$] **[$P < 0.05$][a]	Purity, NR The data for this study may have been included in the study by Weisburger et al. (1981)
CD (M) 60 wk Weisburger et al. (1981)	0, 28 mg/kg bw, 2 × per wk for 60 wk (lower dose) or 56 mg/kg bw, 2 × per wk, discontinued at wk 32 (higher dose) 26 mice/group	Cerebrum, malignant glioma: 0/16, 10/26 (38%)*, 11/26 (42%)* Cerebellum, malignant glioma: 1/16 (6%), 6/26 (23%)*, 11/26 (42%)**	*$P < 0.05$	Purity, 91% Gliomas were described as astrocytomas by the authors
CD (F) 60 wk Weisburger et al. (1981)	0, 28 mg/kg bw, 2 × /wk for 60 wk (lower dose) or 56 mg/kg bw, 2 × /wk, discontinued at wk 32 (higher dose) 26 mice/group	Cerebrum, malignant glioma: 0/16, 12/26 (42%)*, 12/26 (42%)* Cerebellum, malignant glioma: 0/16, 8/26 (31%)**, 4/26 (15%) Mammary gland, adenocarcinoma: 0/16, 6/26 (23%)**, 13/26 (50%)*	*$P < 0.01$ **$P < 0.05$	Purity, 91% Gliomas were described as astrocytomas by the authors

[a] Fisher exact test

bw, body weight; F, female; M, male; NR, not reported; wk, week

Oral administration

In a first report, groups of 26 male and 26 female CD rats (age, 6 weeks) were given 1,3-propane sultone at a dose of 28 mg/kg bw by gavage, twice per week, for 60 weeks (lower dose), or 56 mg/kg bw (higher dose), twice per week, for 32 weeks. Control groups of 6 males and 6 females were given water for 61 weeks.

At 60–61 weeks, the incidence of brain glioma was significantly increased in males in the groups at the lower and higher doses. The incidences of tumours of the mammary gland, squamous cell carcinoma of the ear canal, leukaemia, adenocarcinoma of the small intestine, and other tumours were increased, but without statistical significance. In females, the incidence of brain glioma was significantly increased in the groups at the lower and higher doses. The incidence of tumours of the mammary gland was significantly increased in the group at the higher dose. The incidences of squamous cell carcinomas of the ear canal, leukaemia, adenocarcinoma of the small intestine, and other tumours were increased, but without statistical significance (Ulland et al., 1971). [The Working Group noted that the results of this report may also have been included in the report of the study by Weisburger et al. (1981) (see below).]

In a second report, groups of 26 male and 26 female weanling CD rats [specific age not reported] were quarantined for 7–10 days, and given 1,3-propane sultone (purity, 91%) at a dose of 28 mg/kg bw by gavage, twice per week, for 60 weeks (lower dose), or 56 mg/kg bw, twice per week for 32 weeks (higher dose). Two groups of 16 males and 16 females served as matched controls. Weighted mean doses were calculated as 28 mg/kg bw for the group at the lower dose, and 29.9 mg/kg bw for the group at the higher dose.

In males, at 60 weeks, the incidences of cerebrum malignant glioma [described as astrocytoma by the authors] and of cerebellum malignant glioma [described as astrocytoma by the authors] were significantly increased compared with controls at both doses. In females, the incidence of cerebrum malignant glioma was significantly increased at both doses, and the incidence of cerebellum malignant glioma was significantly increased at the lower dose. The incidence of adenocarcinoma of the mammary gland was significantly increased at both doses (Weisburger et al., 1981).

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

No studies were available on the toxicokinetics and metabolism of 1,3-propane sultone. [The Working Group noted that, in view of its chemical reactivity, it may be anticipated that 1,3-propane sultone is hydrolysed within the organism to 3-hydroxy-1-propane sulfonic acid.]

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

See Table 4.1

(a) In vivo

Groups of Sprague-Dawley rats were given 1,3-propane sultone as a single intravenous injection at a dose of 30.5 mg/kg bw Robbiano & Brambilla (1987). Within 1 hour after dosing, 1,3-propane sultone induced DNA fragmentation in the brain, indicated by increased DNA alkaline-elution rates.

To evaluate a new glycosylphosphatidylinositol *Pig-a* gene mutation assay in rats

Table 4.1 Genetic and related effects of 1,3-propane sultone

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA adducts, *N*-7 alkylation of guanosine and guanine in DNA, acellular system, in vitro	+	NT	24.5	Hemminki (1983)
DNA strand breaks, male Sprague-Dawley rat brain cells, in vivo	+	NA	30.5 iv × 1	Robbiano & Brambilla (1987)
Prophage, *umu* gene induction, SOS repair test, DNA strand breaks, cross-links or related damage	+	NT	16	Nakamura et al. (1987)
Gene mutation (*Pig-a*), male Sprague-Dawley rats, in vivo	+ [d]	NA	12.5 oral × 28 days	Dertinger et al. (2011a, b)
Salmonella typhimurium TA100, or TA1535 reverse mutation	+	NT	5 µg/plate	Simmon (1979a)
Salmonella typhimurium TA100, reverse mutation	+	NT	6	Bartsch et al. (1983)
Salmonella typhimurium TA1535, reverse mutation	+	NT	5 µg/plate	Simmon (1979a)
Salmonella typhimurium TA1536, TA1537, TA1538 or TA98, reverse mutation	–	NT	5 µg/plate	Simmon (1979a)
Saccharomyces cerevisiae, homozygosis by mitotic recombination or gene conversion	+	+	1000	Simmon (1979b)
Hordeum species (barley), mutation	+	NA	611	Kaul & Tandon (1981)
Hordeum species (barley), mutation	+	NA	975	Singh & Kaul (1985)
Hordeum species (barley), chromosomal aberrations	(+)	NA	611	Kaul & Tandon (1981)
Chromosomal aberrations, human lymphocytes, in vitro	+	NT	122	Kaul (1985)
Chromosomal aberrations, Chinese hamster Don lung fibroblasts, in vitro	+	NT	12	Abe & Sasaki (1977)
Chromosomal aberrations, Chinese hamster lung Don cells, in vitro	+	NT	63	Ishidate (1988)
Micronuclei in peripheral blood cells, male Sprague-Dawley rats, in vivo	+	NA	12.5 ip × 1	Torous et al. (2000)
Micronucleated reticulocytes, male Sprague-Dawley rats, in vivo	+ [c]	NA	25 oral × 28 days	Dertinger et al. (2011a, b)
Sister-chromatid exchange, human lymphocytes, in vitro	+	NT	61	Kaul (1985)
Sister-chromatid exchange, Chinese hamster Don lung fibroblasts, in vitro	+	NT	1.2	Abe & Sasaki (1977)
Cell transformation, human newborn foreskin epithelial cells, in vitro	+	NT	7.5	Milo et al. (1981)

Table 4.1 (continued)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cell transformation, C3H 10T½ CL8 mouse cells, in vitro	(+)	NT	50	Oshiro et al. (1981)
Cell transformation, Syrian hamster embryo cells, clonal assay, in vitro	–	NT	10	Pienta et al. (1977)
Poly(ADP-ribose)polymerase induction, primary human newborn foreskin fibroblasts, in vitro	+	NT	5	Sharma et al. (1994)
Host-mediated assay, *Salmonella typhimurium* TA1530 and TA1538 in Swiss-Webster mice, in vivo (alkaline elution assay)	+	NA	12 im × 1	Simmon (1979a)

[a] +, positive; (+), weak positive; –, negative; NA, not applicable; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw per day; im, intramuscular; ip, intraperitoneal

[c] LED was 20 mg/kg bw per day in a 3-day study

[d] LED was 80 mg/kg bw per day in a 3-day study

in vivo, Dertinger et al. (2011a, b) used 1,3-propane sultone as a positive control. Mutagenicity was assessed in male Sprague-Dawley rats treated with 1,3-propane sultone for 3 (doses: 0, 20, 40, or 80 mg/kg bw per day) or 28 consecutive days (doses: 0, 12.5, 25, or 50 mg/kg bw per day). 1,3-Propane sultone increased the frequencies of *Pig-a* mutation and of micronucleated reticulocytes in both the 3-and 28-day studies in a dose-dependent manner.

Groups of male Sprague-Dawley rats were given a single intraperitoneal dose of 1,3-propane sultone (12.5, 25, or 50 mg/kg bw) as a positive control to test whether modifications to a flow-cytometric scoring procedure for measuring micronucleated reticulocytes could be applied to enumerate micronuclei in rat peripheral blood (Torous et al., 2000). In circulating blood cells isolated from rats exposed to 1,3-propane sultone in vivo, there was a dose-dependent increase in the frequency of micronucleated reticulocytes 24 or 48 hours after dosing.

(b) In vitro

Hemminki (1983) reacted 1,3-propane sultone (200 mM) [24.5 µg/mL] with guanosine or DNA at physiological pH. The main reaction product was *N-7*-alkylated guanosine, accounting for more than 90% of total products. Two minor putative adducts were *N-1* and O^6 alkyl derivatives.

The genotoxicity of 1,3-propane sultone has been previously evaluated by IARC (1999). 1,3-Propane sultone caused DNA damage and mutation in bacterial and mitotic recombination in yeast. It induced mutations and chromosomal aberrations in plant cells. In cultured mammalian cells, 1,3-propane sultone induced chromosomal aberrations (including in human lymphocytes), sister-chromatid exchange (including in human lymphocytes), and cell transformation in all except one study.

In primary human neonatal foreskin fibroblasts exposed to propane sultone, there was a four- to eight-fold induction of poly(ADP-ribose)polymerase (PADPR) activity compared with untreated controls. Poly(ADP)ribosylation is considered to be involved in DNA repair and to represent an early response to DNA damage (Sharma et al., 1994). [The Working Group noted that the given concentration of 41 nM, equivalent to 5 ng/mL, was unusually low.]

(c) Interaction with proteins/histones

1,3-Propane sultone reacts with proteins, as demonstrated for histones (Wagner & Blevins, 1993). In human foreskin fibroblastic cells exposed to 1,3-propane sultone for 3, 12, or 24 hours, electrophoresis of the histone fractions resolved multiple forms of histones H1, H3, and H4. Propane sultone (0.1 mM) induced a broadening of the H2A and H2B bands after a 24-hour exposure, demonstrating histone modification.

4.3 Other effects relevant to carcinogenicity

Ippen & Mathis (1970) reported on cases of "protracted chemical burns" in chemical workers exposed to 1,3-propane sultone by dermal contact.

4.4 Mechanistic considerations

1,3-Propane sultone, as an inner hydroxyalkyl sulfonic acid ester, is a directly alkylating agent and does not require metabolic activation to induce genotoxicity. It reacts with DNA nucleosides and with proteins (Hemminki, 1983; Wagner & Blevins, 1993). Upon hydrolysis, 1,3-propane sultone is likely to be converted to the strongly acidic hydroxyalkyl sulffonic acid. The available data on 1,3-propane sultone demonstrate conclusively that it is a strong direct genotoxicant (Table 4.1). 1,3-Propane sultone has been used as a positive control for a variety of genotoxicity assays (Sharma et al., 1994; Dertinger et al., 2011a, b), indicating that it is widely recognized

as a genotoxic agent. Reactivity of 1,3-propane sultone with histones (Wagner & Blevins, 1993) suggests that additional epigenetic mechanisms may operate.

5. Summary of Data Reported

5.1 Exposure data

1,3-Propane sultone is an alkylating agent that has been produced in small quantities since the 1950s by the dehydration of gamma-hydroxypropanesulfonate. It is used as an intermediate to introduce the propylsulfonate group into molecules, and to confer water solubility and an anionic character. Although the industrial use of 1,3-propane sultone was largely discontinued in the 1960s, it has been used more recently in the manufacture of lithium batteries, and for chemical synthesis in the laboratory. Workers involved in the formulation of compounds made from 1,3-propane sultone are at the greatest risk of potential exposure.

5.2 Human carcinogenicity data

Only one case series among 55 employees at a factory in Germany that manufactured 1,3-propane sultone in 1952–1963 was available to the Working Group. The number of expected cancers was not presented, precluding interpretation of this study.

5.3 Animal carcinogenicity data

There were two studies of carcinogenicity with 1,3-propane sultone in mice: one study in males and females treated by skin application, and one study in females treated by subcutaneous injection. When administered by skin application, 1,3-propane sultone increased the incidences of benign and malignant skin tumours and lymphoreticular (lympho-haematopoietic system) tumours in males and females. When administered by subcutaneous injection, 1,3-propane sultone increased the incidences of fibrosarcoma and epithelial tumours.

There were two reports of studies of oral administration (gavage) in male and female rats. In these reports, 1,3-propane sultone increased the incidence of malignant glioma of the cerebrum and malignant glioma of the cerebellum in male and female rats. 1,3-Propane sultone also increased the incidence of adenocarcinoma of the mammary gland in female rats in one report, and of tumours of the mammary gland in the other report.

5.4 Mechanistic and other relevant data

1,3-Propane sultone is an alkylating agent that reacts directly with DNA and proteins. DNA reactivity is evident in a variety of assays for genotoxicity, including those in experimental animals and with human cells in vitro. A secondary non-genotoxic effect involving interaction with histones may also contribute to carcinogenicity. Because 1,3-propane sultone does not require metabolic activation and reacts directly with DNA and other macromolecules, it is likely that this mechanism operates both in experimental animals and in humans.

Overall, the mechanistic data for 1,3-propane sultone are *strong*, because the genotoxicity and mutagenicity of this compound are very well established, and are consistent across different experimental systems.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 1,3-propane sultone.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 1,3-propane sultone.

6.3 Overall evaluation

1,3-Propane sultone is *probably carcinogenic to humans (Group 2A).*

6.4 Rationale

In making this overall evaluation, the Working Group took into account that 1,3-propane sultone is a strong, direct-acting alkylating agent that reacts with DNA and proteins and that, as a result, is genotoxic in virtually all test systems examined, both in vitro and in vivo. Results of studies of cancer in experimental animals are consistent with this mechanism because tumours arose both at the site of exposure and at distant sites. In the absence of adequate data on cancer in humans, the overall evaluation of 1,3-propane sultone was upgraded from *Group 2B* to *Group 2A* on the basis of strong evidence for genotoxicity.

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LIST OF ABBREVIATIONS

2-AAF	2-acetylaminofluorene
8-OH-dG	8-oxo-2'-deoxyguanosine
AFB1	aflatoxin B1
AEGL	acute exposure guideline levels
ALT	alanine aminotransferase
AST	aspartate aminotransferase
bw	body weight
BBM	brush-border membrane
CAR	constitutive androstane receptor
CDNB	1-chloro-2,4-dinitrobenzene
CI	confidence interval
COHb	carboxyhaemoglobin
CCBL	cysteine conjugate β-lyase
CO	carbon monoxide
CO_2	carbon dioxide
coA	coenzyme A
CYP	cytochrome P450
DCVC	*S*-(1,2-dichlorovinyl)-L-cysteine
DEHP	di(2-ethylhexyl)phthalate
DNEL	derived no-effect level
ECF	electrochemical fluorination
EPA	United States Environmental Protection Agency
FTOH	fluorotelomer alcohol
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
GGT	gamma-glutamyltransferase
GSH	glutathione
GST	glutathione *S*-transferase
HR	hazard ratio
IL	interleukin
LC_{50}	median lethal concentration
LXRα	liver X receptor α
MEHP	mono(2-ethylhexyl)phthalate
MS	mass spectrometry

NAcTFEC	*N*-acetyl-*S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine
NADP(H)	nicotinamide adenine dinucleotide phosphate (reduced)
NAT	*N*-acetyltransferase
NHANES	National Health and Nutrition Examination Survey
NHL	non-Hodgkin lymphoma
NIOSH	United States National Institute of Occupational Safety and Health
NMR	nuclear magnetic resonance
OAT	organic anion transporter
OATP	organic anion-transporting polypeptide
OR	odds ratio
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
PPAR	peroxisome proliferator-activated receptor
ppbv	parts per billion by volume
ppm	parts per million
PXR	pregnane X receptor
ROS	reactive oxygen species
SIR	standardized incidence ratio
SLCO	solute carrier organic anion
SMR	standardized mortality ratio
T3	triiodothyronine
T4	thyroxine
TFEC	*S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine
TFEG	*S*-(1,1,2,2-tetrafluoroethyl)glutathione
TNFα	tumour necrosis factor α
TWA	time-weighted average
WHO	World Health Organization
w/v	weight per volume
ww	wet weight

M
IARC MONOGRAPHS

This volume of the *IARC Monographs* provides evaluations of the carcinogenicity of perfluorooctanoic acid, tetrafluoroethylene, 1,2-dichloropropane, dichloromethane, and 1,3-propane sultone.

Perfluorooctanoic acid is a fluorinated chemical that persists in the environment, having been detected in air, water, dust, and food. It is particularly important for the production of fluoropolymers such as polytetrafluoroethylene, which has a wide range of uses in industrial and consumer products, including non-stick coatings on cookware and waterproof clothing. Tetrafluoroethylene is a fluorinated monomer that is used mainly as an intermediate in the production of polytetrafluoroethylene. The chlorinated solvent 1,2-dichloropropane is used primarily as a production intermediate, but also in paint stripping and, until 2012, in printing-press cleaning in Japan. Dichloromethane is a chlorinated solvent that is used in paint stripping, aerosols, polycarbonate plastic and hydrofluorocarbon manufacture, metal and printing-press cleaning, and refrigerant production. Industrial use of the alkylating agent 1,3-propane sultone was largely discontinued in the 1960s, but it has been used recently in the manufacture of lithium batteries, and for chemical synthesis in the laboratory. Exposure to all five agents considered occurs in the general population as well as in different occupational settings.

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